In memory of Professor J Douglas Miller

Origin of Macrophages in Rat Syringomyelia

An investigative study using rat radiation bone marrow chimeras

by

Gabriel Y. F. Lee
Department of Neurosurgery
University of Adelaide

Submitted in fulfilment of requirements for the degree Master of Surgery, May 2001

TABLE OF CONTENTS

DECLARATION

This work contains no material that has been accepted for the award of any other degree or diploma in any university or tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent for this copy of my thesis to be made available for loan and photocopying when this copy is placed in the Barr Smith Library.

Gabriel Lee
May 2001

ACKNOWLEDGEMENTS

It has been my privilege to work under the supervision of Professor Nigel Jones over the last 12 months. I stand in awe of his brilliance in academic research and his prowess in operative neurosurgery. In particular, I am immensely grateful for his invaluable advice and support for my work throughout the year.

There is also no doubt in my mind that my research work would not have been completed without the help of a large number of individuals, many of whom have now become my friends. I would therefore like to offer my sincere thanks to the following people:

1. Professor Paul McMenamin (Department of Anatomy, University of Western Australia) who triggered many new ideas in my mind during the preparation phase of the study
2. Associate Professors Graham Mayrhofer (Department of Immunology, University of Adelaide) and Lesley Cleland (Department of Rheumatology, Royal Adelaide Hospital) for allowing me to use their newly established congeneic rats and providing helpful technical advice at many points during my research
3. Professor Ian Gibbons and Dr Michael Teo (Department of Anatomy, University of Flinders) for allowing me to perform immunofluorescence photography at the Flinders Medical Centre
4. Dr Stephen Lane and Miss Emma Slater (Serotec, UK) for ironing out some of the initial problems with the HIS41 antibody product and arranging for prompt replacements

5. Mr Peter Harman (Aust Lab Services) who liaised with Serotec to enable direct shipment of the HIS41 antibody to Adelaide

6. Miss Lori Gillette (Pharmingen, USA) for kindly arranging to have independent tests of the HIS antibody performed at the Pharmingen Laboratories

7. Miss Judy Bulua for providing technical assistance during the pilot study

8. Mr Rick Tochetti for allowing me to store my fresh frozen tissues in a departmental freezer at the Transfusion Services Department, Royal Adelaide Hospital

9. Mr Jim Manavis for assisting with initial attempts at using a combination of dye techniques to demonstrate co-localisation of the ED1 and RT7.2 antigens.

10. Mr Andrew Zanettino and Mr Tony Cambieri (Hanson Leukemia Research Laboratory) who were extremely helpful in my immunofluorescence work

11. MedVet services for allowing me to use their sublimator/freeze-dryer when I attempted to develop the methodology for freeze-drying

12. Dr David Wigg (Department of Radiation Oncology, Royal Adelaide Hospital) for his constructive criticisms of my irradiation protocol

13. Professor W.F. Hickey (UK) who was ever patient and helpful in his replies to many of my questions with regards to the technique of establishing radiation chimeras

14. Drs J Kampinga (Cambridge) and FGM Kroese (Netherlands), the clone originators of HIS41, who helped immensely to clarify some of the initial difficulties I encountered in using this particular antibody

15. Professor C Dijkstra (Netherlands), the clone originator of the ED1 antibody who was particular helpful in sharing her experiences with the use of the antibody

16. Mr Murray Schirmer (Head Radiographer, RAH) who assisted in irradiating the rats on 3 separate occasions

17. Mr Chris Brown for providing technical advice at various phases of the research work

18. Miss Lyn Cockram, Miss Melanie Smith and Mr Liqun Yang for sharing many delightful conversations and jokes in the office

19. Professor Richard Barnet (Department of Statistics, University of Adelaide) who advised on the statistical aspects of the study

20. Associate Professor P Reilly for taking a genuine interest in my progress

21. Associate Professor B Brophy for the many stimulating philosophical discussions pertaining to research, neurosurgery and life in general.

In addition, I would like to thank my wife, Grace for providing tremendous support for me particularly during the initial period of my research when only an endless series of apparently insurmountable challenges was in sight.

Finally but most importantly, I thank God because He has made all things possible.

"For by humility and fear of the Lord are riches, honour and life"

Proverbs 22:4

Financial support

The author was the recipient of the RACS/University Scholarship awarded by the Royal Australasian College of Surgeons.

ABBREVIATIONS

CNS	Central nervous system
CT	Computerized tomography
CSF	Cerebrospinal fluid
DAB	3, 3'- Diaminobenzidine
EDTA	Ethylenediamine tetraacetic acid
FITC	Fluorescein isothiocyanate
H&E	Haematoxylin and eosin
MRI	Magnetic resonance imaging
NRS	Normal rat serum
PBS	Phosphate-buffered saline

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Introduction:

Syringomyelia is an important condition in which a cystic cavity forms within the spinal cord. This leads to significant delayed neurological deterioration, which may be manifested as weakness, numbness or pain. The patho-physiology and mechanism of syrinx formation remains unclear. Human autopsy findings have demonstrated a prominent accumulation of macrophages in relation to the syrinx. Similar observations have also been made in a previously established rat model of syringomyelia. Little is known about the origin and precise functions of these cells.

Purpose:

The study was aimed at identifying the origin of macrophages in an experimental model of rat syringomyelia. The pattern and time course of macrophagic infiltration was also evaluated.

Method:

Syrinx formation was induced by intra-parenchymal injections of kaolin within the cervical spinal cords of 30 DA rat (RT7.1) radiation bone marrow chimeras reconstituted with bone marrow from RT7.2 congeneic donors. The distribution of macrophages was evaluated at survival times of 3 days, 1 week and 4 weeks. Immunostaining of fresh frozen spinal cord tissue was performed using specific antibodies against rat macrophage ED1

antigen and RT7.2 allele of CD45. This allowed donor-derived haematogenous macrophages to be distinguished from native cells.

Results:

Central canal dilatation was seen from 1 week. This was associated with extensive accumulation of $ED1^+$ macrophages within the spinal cord parenchyma. A large influx of bone marrow derived ($ED1^+$, $RT7.2^+$) macrophages was observed. However, a considerable proportion of resident microglia ($RT7.2^-$) also upregulated ED1. These activated microglia demonstrated distinct morphological features.

Conclusions:

Large numbers of macrophages were recruited from the bone marrow in kaolin-induced rat syringomyelia. However, a significant number of resident microglia upregulated their ED1 activity and appear to provide a substantial source of macrophages.

1 Syringomyelia

1.1 Introduction

"*It appears doubtful that syringomyelia can be regarded as a single entity either from the point of clinical presentation, from the view point of pathogenesis, or, most importantly, from the standpoint of rational therapy.*" [85]

Syringomyelia is a progressive myelopathy characterised pathologically by cavitation of the central spinal cord. [341] As a clinical entity, the varied and diverse symptomatology has posed significant diagnostic challenges. As a pathological entity, it is well recognised as a heterogeneous disease process of which the pathogenesis remains poorly understood.

The current work begins with a review of the literature on syringomyelia. Particular emphasis has been placed on clinico-pathological correlation and the pathogenetic theories.

1.2 Epidemiology

Although Schlesinger [348] considered syringomyelia to be one of the commonest spinal cord disorders in 1902, it is now widely accepted that this is a relatively uncommon disease . [102,376]

The prevalence and incidence figures are difficult to establish accurately. In a 1966 survey of neurological disease in an English city, Brewis *et al* [45] found a prevalence rate of 8.4/100,000 persons. Later estimates yielded similar figures.[287,292] Overall incidence figures have never been assessed. However, with increasing use of CT and MRI scanning, syringomyelia is increasingly diagnosed, often in asymptomatic patients.[20,110,376]

1.3 Clinical features

"Syringo-myelia, in which the symptoms are often numerous, so to speak, unforseen, so varied is the seat of the lesions which constitute that affection, is often attended by phenomena, which are more or less analogous to those occurring in tabes: these consist of trophic derangements, sensory disorders, pains, and at times the loss of the knee jerk."[252]

Syringomyelia usually begins during the second and third decades of life.[44,101,251,259] Affected patients may present with variable symptoms and signs, which were aptly highlighted by the neurologist, Marie late in the 18th century. Hence, it is not particularly surprising that in a recent review of 127 patients with Chiari malformation-

related syringomyelia, one third of the patients had previously been misdiagnosed or treated inappropriately. [223]

The classical manifestations of syringomyelia correspond to a high cervical cord syndrome and may vary depending on the extent of the syrinx and its associated abnormalities. [341] However, the most prominent feature at presentation appears to be motor impairment of the upper limbs. [14,251,388]Padovani et al presented a series of 29 patients with both 'hindbrain related' and 'non-hindbrain related' syringomyelia, in which all the patients demonstrated motor weakness. [303] Typically, a patient may initially notice the slow onset of wasting and weakness of one hand. [44] Wasting of the first dorsal interosseous muscle may occur relatively early in the course of the disease.[100,438] Alternatively, there may be loss of sensation over one hand and forearm leading to injuries, especially burns, which are often unnoticed. [44,388] The majority of patients present with unilateral symptoms. [341] In other patients, the symptoms may also be bilateral but asymmetric. [44,101]

Headaches or pain in the cervical area also occur commonly, particularly in patients with Chiari malformations. Certainly, this was the predominant complaint (65%) in a series of patients with Chiari malformation-related syringomyelia. [222]A similar finding was reported by Logue and Edwards. [241] Williams noted that the majority of these patients described an occipital "hindbrain related headache" which was exacerbated by straining activities.[438] However, this symptom is not confined solely to patients with Chiari malformations. [316]

Patients may also experience pain in the trunk or limbs. [14,223,241, 251,259,303,347,388,438,443,]This typically has an aching or burning quality and is lateralised to the face, neck, limbs or trunk within the area of pain and temperature impairment. [243] Other patients may experience radicular or localised spinal pains. [14,347] Straining manoeuvres such as coughing or sneezing can result in an electric shock-like, tearing or ripping pain which is often confined to a zone of the body but not usually to one dermatome. [241,438]

Examination is likely to demonstrate distal weakness, wasting and hypotonia in at least one upper limb. [44,295] More proximal muscle groups in the lower neck, shoulders and upper arms may also be affected. [341] Fasiculations are seen in the affected muscle groups although these may be subtle. [44,295] Tendon reflexes in the affected limb are often diminished or lost early in the course of the disease. [44,101] The lower limbs are characteristically spastic and extensor plantar responses are common. [295] Interestingly, Foster and Hudgson found that 'stiffness of the legs' consistent with spastic paraparesis was the commonest early complaint seen in 42% of their patients. [101]

There is associated loss of pain and temperature sensation in the affected limb. The distribution typically involves the ulnar side of the hand and forearm, before spreading to the radial side and onto the neck and chest although the precise dermatomal level often cannot be defined. [438] The preservation of light touch, vibration sense and postural stability leads to a pattern of "dissociated sensory loss". [291,341] This is relatively common although the classical 'cape-like' distribution of sensory loss over the nape of the neck, shoulders and upper arms is much less common. [14,101,223,438,443] When the syrinx extends to involve

the pons or medulla (syringobulbia), [341,376] a characteristic 'onion-skin' pattern of dissociated sensory loss which converges onto the nose and upper lip occurs.

Another cardinal clinical sign of syringomyelia is high thoracic kyphoscoliosis. [341] Trunk weakness commonly leads to spinal deformities and is associated with significant disability. [155] In one series, [251]scoliosis was clinically evident in 28% of cases but could be radiologically documented in 64% of patients at follow up.

Ocular sympathetic paralysis leading to ptosis and small pupils (Horner's syndrome) may be present. [101,223,291,438,] Other ocular signs such as nystagmus may be present in up to 47% of patients. [101, 223]

Less common clinical presentations include episodic dizziness, drop attacks, tongue weakness, dysarthria, palatal and vocal cord paralysis, tic douloureux, trigeminal hypalgesia, diplopia, urinary incontinence, Charcot joints, cauda equina syndrome, Lhermitte's phenomenon and decreased facial sweating. [14,101,225,251,341,347,443] Rarely, extremely acute presentations with paraplegia, respiratory failure and lateral medullary compression have also been reported. [451]

1.4 Progression of disease

The natural history of untreated syringomyelia is often unpredictable and variable. [224,241,251,296,341,347] The course is usually protracted over 10 to 15 years but possibly up to 50 years. [65,222,241,259,291]

For many patients, there is a gradual deterioration of symptoms. [65,259] In others, the disease may either be stationary or appear to progress before plateauing. [155,222,252,291] Curiously, the disease process may cease to progress at any time. [155] However despite the apparent stability in some patients, late neurological deterioration may still occur. [303] Deterioration of neurological deficits after coughing or sneezing has occasionally been noted by patients. [241,347]

While the progression is typically relentless, [44] syringomyelia rarely causes death directly unless it is associated with syringobulbia. [155]

1.5 Neuropathology in syringomyelia

Syringomyelia may be associated with tumours, vascular changes, infective processes, extramedullary compressive lesions and congenital anomalies. [54,94,154,172,341] However, with the exception of neoplastic syringomyelia, the histopathological findings are similar regardless of the associated condition. [376]

1.5.1 Definitions

The definition of syringomyelia is important in the neuropathological evaluation of this condition. Greenfield defined syringomyelia as a "tubular condition of the spinal cord extending over many segments". [155] On the other hand, Barnett and Rewcastle placed less emphasis on the dimensions of the cavity, defining it as a "cavitation in the spinal cord which has a wall largely composed of glial tissue". [18] Some authors further distinguish syringomyelia from 'hydromyelia', which is said to involve the dilation of the ependyma-lined central canal. In the current thesis, such a differentiation is not made.

1.5.2 Macroscopic features

Syringomyelia most commonly affects the lower cervical and upper thoracic spinal cord. [44,155,341,376] The cavity is usually the largest in the cervical region but rarely involves the first cervical segment. [155,259] This extends variably into the thoracic segments although the lumbosacral enlargement is rarely involved. [155]

When exposed at operation or autopsy, the spinal cord appears swollen (mainly in the transverse plane) and tense in the affected region. [44,155] It may fill the entire spinal canal. Externally the spinal cord appears otherwise normal and there is usually no leptomeningeal thickening. [155] Typically, the transverse section reveals a cavity containing clear (like CSF) or yellow fluid with a high protein content. [18,115,442] Occasionally, the cavity may reach massive proportions, occupying most of the cross section of the cord. [155] Upon release of the fluid from within the syrinx, the cord becomes flattened most commonly in an anterior-posterior diameter.

The shape and structure of the cysts are both variable and complex. Instead of a single cavity, each cyst may consist of several separate cavities which communicate variably with each other. [294] In the thoracic cord, the syrinx is often located unilaterally in the posterior horn. When present bilaterally, the cavities may communicate in a U-shaped manner in the grey commissure. [155]

Syrinxes usually extend over multiple segments of the spinal cord. [104] They may extend rostrally to the pons, midbrain and as far as the centrum semi-ovale. [300] While the syrinx may arise in continuity with the fourth ventricle, most commonly the syrinx involves a dilation of the central canal which is discontinuous with the fourth ventricle. Approximately 40% of these syrinxes demonstrate evidence of rupture paracentrally and dissection into the parenchymal tissues. [269] The cavity may reach the pial surface at the tips of the dorsal horns at any level. [155] Occasionally, the cavity may communicate with the

subarachnoid space. [172] In the remaining cases, the cysts are extra-canalicular and may be found at variable levels distal to the obex. [269]

1.5.2.1 Neuroanatomical considerations

Consistent with the resultant symptoms, the syrinx often occupies the central gray matter and frequently extends to involve the posterolateral tracts and the posterior gray commissure. [44,155,291] Thus fibres passing into the spinothalamic tracts may be involved at the posterior horns or as they cross the midline in the central commissure. Similar patterns of syrinx formation have been observed in experimental animal models following cisternal kaolin injection. [249,329,431]This has been attributed to the lack of connective tissue in this region of the spinal cord. [5,431]

The anterior white commissure is also often destroyed by pressure effects or direct midline extension of the cavity. [155] Expansion of the cavity/cavities and associated gliosis lead to compression of the anterior horns of the grey matter resulting in atrophy of the anterior horn cells and axonal degeneration. [44] Compression and degeneration of the long ascending and descending tracts occur late in the process with involvement of the pyramidal and spinocerebellar tracts and dorsal columns. [155]

1.5.2 Microscopic features

The syrinx is typically surrounded by a dense concentric wall up to 1-2 mm in thickness. [155] This usually consists of either fibrillary glial tissue or collagenous tissue although some parts of the cavity may be lined by ependyma. [94,291,292,294,376] The overall histological

appearances are variable and dependent on the part of the cyst studied. [155] The presence of a prominent layer of glial tissue has been variably attributed to intra-uterine chronic inflammation, [319] developmental defect of the glial cells [159] and ischaemia [72,245]. The cyst wall may also contain abberant nerve fibres. [155] Thicker strands of collagen or blood vessels with hyalinised walls may be seen passing across the cavity.

The pathological changes within the spinal cord parenchyma are less well studied. Vascular changes, oedema and haemorrhages may be observed. [376] Milhorat *et al* [269] recently performed a detailed study of 105 autopsy cases of nonneoplastic syringomyelia and observed evidence of chromatolysis and Wallerian degeneration in all cases. Neuronophagia and fat-laden macrophages were a prominent feature particularly in the parenchyma of spinal cords with syrinxes which either did not communicate with the fourth ventricle (central, noncommunicating) or were distinct from the central canal (extra-canalicular). In the latter group of syrinxes, haemosiderin-laden macrophages and microglial cells were also particularly common in the walls of the syrinxes suggesting previous trauma or haemorrhage.

1.5.3 Ultrastructural studies

Few ultrastructural studies of human syringomyelia are available. Ohama *et al* (quoted in Chakrabortty *et al* [55]) examined the autopsy material from 4 patients with Chiari Type I malformations and noted evidence of axonal degeneration, demyelination with nude nerve fibres as well as astrocytic proliferation. Hinokuma *et al* also observed the loss of neurons in the spinal cord parenchyma adjacent to tumours. [172]

1.6 Classification of Syringomyelia

One of the earliest classification systems used has been the designation of syringomyelia into 'primary' (or 'true') and 'secondary' (or 'acquired') forms. [18] In primary syringomyelia, the condition is thought to begin early in life, often in association with bony or soft tissue anomalies. Subsequently, syringomyelia became classified according to the associated lesions. Barnett [17] categorised the syndrome into five sub-types according to presumed causal pathophysiology:

1. Communicating syringomyelia
   a. Associated congenital anomalies at the foramen magnum.
   b. Associated acquired abnormalities at the skull base

2.Syringomyelia as a sequel of spinal arachnoiditis

3.Syringomyelia associated with spinal cord tumours

4.Syringomyelia as a late sequel of trauma (post-traumatic cystic myelopathy)

5.Idiopathic syringomyelia

In contrast, Williams felt that the principal separation is between "hindbrain-related" and "non hindbrain-related" (or "primarily spinal") syringomyelia. [438] Williams clearly

appreciated the complexity of the pathogenesis of syringomyelia. He pointed out that while the associated pathologies are likely to be causes of the syringomyelia, they were not necessarily so. [442] For example, he argued that while hydrocephalus may be regarded as a cause of syringomyelia when there is a wide communication between the fourth ventricle and the syrinx, it may also be regarded as additional evidence of disease of the CSF pathway. Thus the entire syndrome complex may be more accurately attributed to a primary disorder such as head injury or meningitis. [442] In addition, Williams also considered idiopathic syringomyelia to be extremely uncommon. When it occurs, it is not uncommonly associated with meningeal fibrosis.

Logue and Edwards classified syringomyelia into 2 main types depending on whether there was primary dilatation of the central canal. [241] More recently, Milhorat and colleagues [269] modified the classification to distinguish 3 types of spinal cord cavitations; 1) central canal syrinxes (communicating with the fourth ventricle) 2) central canal syrinxes (non-communicating) and 3) extracanalicular syrinxes (FIGURE 1). While the concept of communicating and non-communicating varieties of syringomyelia is not new, [103] Milhorat and his colleagues made important contributions to the understanding of syringomyelia by correlating the pathological/autopsy findings [271] with extensive ante-mortem MRI studies in subjects suffering from syringomyelia. [268] They also proposed an algorithm based on the MRI findings to assist in decision making during management of these patients. [268]

Other researchers have conceptualised syringomyelia in terms of the type of fluid present within the syrinx. For example, Bunge [364] distinguishes 2 types of syrinxes. In the first

type (which includes most cases of Chiari Type I and II malformations), there is free exchange between the CSF in the central canal and the subarachnoid space. In the second type, such a free communication does not exist and the fluid within the syrinx cavity differs in protein content from CSF.

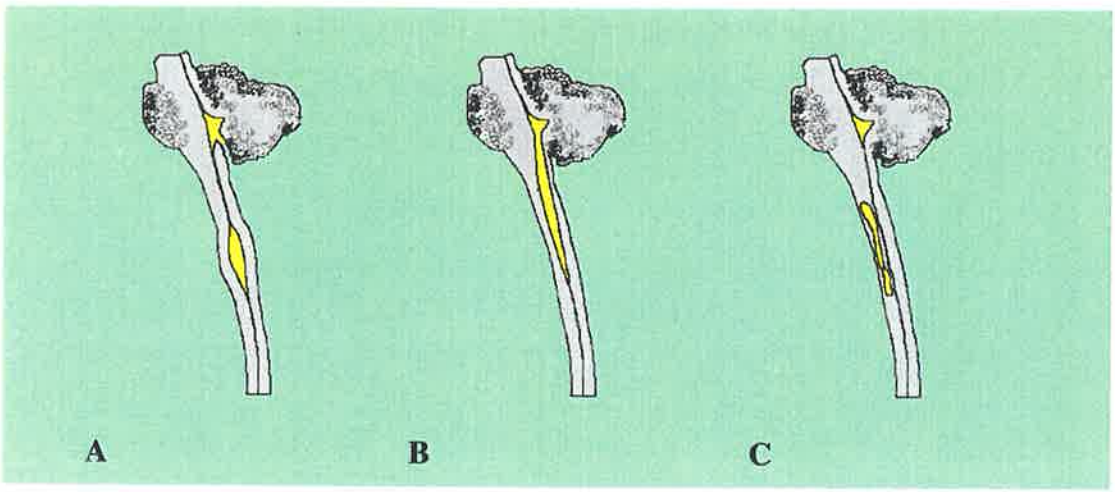

Figure 1. ***Milhorat classification of syringomyelia:*** *(A) non-communicating (central) (B) communicating (central) (C) extra-canalicular.*

1.7 Experimental models of syringomelia

The successful development of an experimental animal model will allow the clarification of the pathogenesis of cyst formation. It will also enable therapeutic interventions to be tested. [249] Unfortunately, despite extensive research, the quest for a clinically relevant animal model remains a major challenge. A brief overview of the available experimental techniques is included in the current work.

1.7.1 Arachnoiditis

The earliest investigators attempted to establish animal models of syringomyelia by injecting a variety of irritative substances into the cisterna magna of animals to induce chronic basilar arachnoiditis. This resulted in hydrocephalus as well as spinal cord cavitation. The agents used included combinations of fatty acids, sodium nucleinate, talc, ethyl iodophenylundecylate as well as kaolin. [261]

Later studies have largely utilised cisternal kaolin injections. Becker *et al* [25] demonstrated significant dilation of the central canals in feline spinal cords following cisternal kaolin injections. Occlusion of the obex and filum prevented syrinx formation which led the authors to lend support to the haemodynamic theory of Gardner. Similar experiments were subsequently reproduced in cats[329,330], dogs [152] and rabbits [60]. Histologically, there is evidence of ependymal flattening with loss of cilia and villi as well as subependymal oedema. These changes are associated with demyelination and axonal degeneration within the spinal cord parenchyma.[55]

In this model of experimental syringomyelia, it is clear that the central canal remains patent. [55,151] However, while hydrocephalus is necessary, it is not sufficient to result in syrinx formation alone. Consequently, it has been suggested that kaolin may possibly have other mechanisms of actions beyond simply producing hydrocephalus. James *et al* [190] performed intra-cisternal injections of silicone rubber instead of kaolin. This resulted in significant hydrocephalus without the characteristic spinal arachnoiditis. No syrinxes developed in any of the study animals. On the other hand, intradural or subarachnoid spinal injection of kaolin also does not lead to syrinx formation. [91]

Using the same animal model, Hall *et al* investigated the effect of varying ventricular pressure on the syringeal pressure by performing simultaneous in vivo measurements. Their results suggested the existence of a ventriculosyrinx valve effect, which they postulated may subsequently lead to progressive syrinx distension during transient increases of intra-cranial pressure. [152]

1.7.2 Post-traumatic models

In 1923, McVeigh created cavitary lesions within the spinal cords of dogs by crushing the cord between the vertebral bodies and his finger after performing a thoracic laminectomy. [262] However, Fehlings and Tator [93] credited Schmasus as the first to produce experimental degeneration and cavitation of spinal cords of rabbits by the application of blunt trauma.

Using a weight drop technique to induce severe spinal cord contusion in rats, Guizar-Sahagun *et al* more recently observed that cystic necrosis consistently led to well defined cysts at 3 to 4 weeks post-injury. [148] Similar findings have also been reported in the cat [63,423] as well as rabbits [60].

Cho et al [60] noted that the rabbits in which they performed an additional subarachnoid kaolin injection after inducing a traumatic injury to the spinal cord were more prone to form syrinxes. The explanation for this observation is not known. However, the authors proposed that the injected kaolin resulted in an adhesive arachnoiditis which altered the CSF circulation pathway although they failed to elaborate on their theory. This is believed to lead eventually to the extension and coalescence of multiple small cavities which were formed at the time of the initial injury. Other researchers have further suggested that the tethering of the spinal cord may lead to a reduction in local spinal cord blood flow. [370] This may lead to the release of excitatory amino acids, which result in tissue damage and subsequent formation of cavities within the spinal cord. [363]

1.7.3 Intra-medullary injections

Williams and Weller [431] injected small volumes of saline into the spinal cords of 5 beagle dogs. This was subsequently followed by sequential infusions of saline or cerebrospinal fluid through an implanted catheter. Williams and Weller concluded that their findings of spinal cord cavitation supported a haemodynamic concept for communicating syringomyelia in the human. This experimental technique induced significant morbidity in

the study animals which included limping and spasticity of hind limbs, pain on injection as well as scoliosis.

More recently, Milhorat et al [267] established an experimental model of noncommunicating syringomyelia in rats (FIGURE 2). They performed microinjections of kaolin into the dorsal columns and central gray matter of the C6 spinal cord. This led to reproducible dilatation of the central canals which did not communicate with the fourth ventricle. The central canal dilation was attributed to rostral obstruction by inflammatory cells and proliferation of the ependyma and fibrous astrocytes, which subsequently prevented rostral flow of CSF.

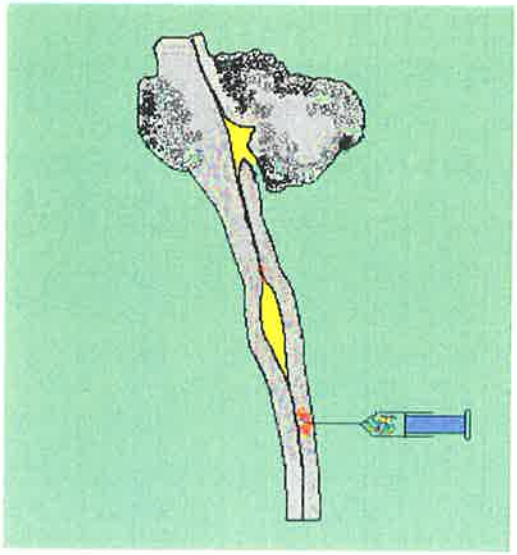

Figure 2. *Kaolin-induced model of non-communicating syringomyelia in the rat.*

1.8 Theories of pathogenesis

1.8.1 Theories of Congenital Origin

Charles Prosper Ollivier d'Angers (1796-1845) first coined the term "syringomyelia" in 1827. [302] However, this pathological entity had been well recognised for at least 2 centuries prior to this. [74]

According to Ollivier, syringomyelia arose from a developmental rest within the spinal cord. [302] Subsequent authors held the view that the syrinx developed as a consequence of incomplete fusion of the 2 folds of the primitive medullary groove which results in disruption of the germinal cell layer. [28,126,163,225,348]

John Cleland (1835-1925) noted that the central canal was dilated in a patient with hydrocephalus associated with spina bifida. [62] Consequently, he suggested that this was due to primary dysgenesis of the brain stem. Hassin [159] described "abiotrophy", a developmental anomaly of the glia. He suggested that the glial tissue initially proliferated but subsequently degenerated as it became walled off by proliferating connective tissue. Mackay and Favill [249] similarly theorised that abnormal glial proliferation was followed by degeneration and cavitation. Another view suggested that syringomyelia is a congenital malformation which occurs as a part of the spectrum of dysplastic conditions. [291] Tamaki and Lubin [387] later proposed that syringomyelia was a developmental anomaly caused by imperfect formation of the central canal by primitive medullary epithelium. The defective

cell rests subsequently undergo gliosis, with cavitation being a consequence of 'poor nourishment'. Chiari [59] in describing the abnormalities at the craniovertebral junction, postulated that syringomyelia represented a persistence of the physiological state of embryonic hydromyelia. More recently, Netsky proposed that syringomyelia begins as a congenital intramedullary vascular anomaly, which subsequently becomes occluded or ineffective. [291] This leads to tissue infarction and cavitation which is followed by reparative gliosis or connective tissue proliferation.

1.8.2 Theories of Acquired Origin

1.8.2.1 Hydrodynamic

The concept that hydrodynamic forces are significant in syringomyelia is not new. In 1862, Gull described a patient with "hydromyelus" which extended from the fifth cervical level down to the upper thoracic segments. [149] He attributed the atrophy of the spinal cord tissue to the distension of the cystic cavity as the fluid accumulated within the cyst.

Later researchers such as Taylor and his colleagues [390] suggested that CSF pressure may be a contributing factor in the formation of syringomyelia. Lichtenstein believed that in most cases of syringomyelia, the presence of congenital anomalies resulted in abnormal CSF drainage from the ventricles which led to central canal dilation. [228] However, Gardner and his colleagues [112,113,114,115,116,117,118,119,120] were among the first researchers to set out the hydrodynamic theory in a systematic fashion. They theorised that either a premature development of the choroid plexus or a delayed attenuation of the rhombic roof leads to a

pathological state of noncommunicating hydrocephalus. The obstruction may occur at the exit foramina of the fourth ventricle but may also occur at the foramen magnum in cases of tonsillar herniation. This leads to persistence of hydrocephalus and an enlarged central canal. When the anatomical relationships are normal, the pulse wave in the ventricular cerebrospinal fluid is conducted freely through the fourth ventricular foramina and is dissipated in the subarachnoid space. However, any obstruction to the outflow from the fourth ventricle will lead to pressure transmission down the central canal resulting in dilatation, with CSF entering the obex in an arterial dependent "water hammer" manner. With more severe forms, there is dissection of fluid into the parenchyma of the spinal cord. Gardner believed that this was applicable to all cases regardless of pathology.

However, this theory was considered deficient by later researchers. The theory of Gardner was challenged by Willams [430] who pointed out that the enormous dilation of cord cavities in the presence of "modest" hydrocephalus was not consistent with the idea that the arterial pressure wave was the prime distending force of the syrinx. Williams further pointed out that the syringomyelic cord was often collapsed at myelography and at surgery. Furthermore, he also argued that the foramina of Luschka were 'patent' in many cases

Since then, the lack of continuity of the syringomyelic cavities with the fourth ventricle in many patients has also been well recognised. [104,335,376,428] Even among patients with Chiari malformation-related syringomyelia, few patients (approximately 10%) maintain an opening at the obex. [428] Hinokuma and colleagues [172] studied 18 autopsy cases of

syringomyelia and concluded that the irregular and branching shape of the syrinx in Chiari I related cases could not be explained on the basis of Gardner's theory.

In contrast, Williams and his co-investigators [357,430,431,432,433,434,435,437,438,439,440,441,442] believed that the intermittent sharp rises in fluid pressure associated with central venous pressure alterations are of fundamental significance. They proposed that venous distension resulting from valsalva-type activities led to a pressure differential gradient between the central canal and the subarachnoid compartments. This results in a "suck" mechanism, which drives the CSF upwards from the spinal compartment past the foramen magnum into the intracranial cavity. In the setting of partial blockage of the subarachnoid space at the foramen magnum (as in hindbrain malformations), CSF is unable to 'return' to the spinal compartment and is consequently forced through a patent central canal into the syrinx. This requires all patients to have suffered at least a brief episode of hydrocephalus, producing the initial dilation of the central canal. Once a cavity has been formed, the cavity is said to enlarge by the process of "slosh", in which increased syrinx pressure during valsalva procedures leads to dissection of the spinal cord tissue. The importance of sudden changes in spinal venous pressure produced by coughing and straining in extending the syrinx after the cavity had formed was supported by Du Boulay *et al* [86].

While the anatomical basis of both Gardner's and Williams' theories appears identical, the fundamental difference rests in William's proposal that the distension of the central canal is an intermittent process produced by sudden increases in venous and CSF pressure. [104] In

contrast, Gardner's theory involves a steady rhythmical process associated with the 'water hammer' effect of transmitted arterial pulsation.

More recently, Oldfield *et al* performed dynamic MRI studies in patients suffering from syringomyelia associated with Chiari Type I malformations. [301] They observed abrupt downward movement of spinal CSF and syrinx fluid in the upper portion of the spinal canal during systole and upward movement during diastole. This led them to propose a "piston like" effect in which the movement of the impacted tonsils during systole is synchronous with sudden constriction of the cord and underlying syrinx. Consequently, CSF is forced into the spinal cord from the syrinx thus expanding the cavity. Terae *et al* [391] also studied the pulsatile movements of CSF in patients with Chiari malformations on MRI. They found greater pulsatile movement of the hindbrain and spinal cord in these patients in comparison with a control group. Subsequently, they have suggested that normal downward movement of CSF in systole is impeded and the increased intracranial pressure forces the medulla and tonsils downward. They postulated that the pulsatile movements of the spinal cord act as a "vacuum pump" that extends the size of the syrinx.

1.8.2.2 Ischaemia

Tauber and Langworthy [389] observed that cavities formed within the spinal cord after occlusion of the anterior spinal artery. McLaurin *et al* [261] performed cisternal injections of kaolin in 13 dogs. This induced both hydrocephalus as well as spinal cord cavitation. They postulated that the fibrosing arachnoiditis resulted in compression of meningeal blood

vessels which led to spinal cord ischaemia. This theory was subsequently refuted by other researchers. [16,17, 153,248,435]

Lichtenstein held the view that some cases of syringomyelia arose as a consequence of ischaemic necrosis due to distortions of the cervico-medullary junction within an abnormal posterior fossa. [228] Netsky believed that ineffective intramedullary vascular anomalies led to ischaemia and cystic necrosis within the spinal cord tissues. [291] More recently in the context of post traumatic syringomyelia, it has been suggested that haemorrhage into the spinal cord at the site of injury is usually followed by localised arachnoid adhesions which in turn leads to tethering of the cord, delayed ischaemia and impairment of CSF flow within the subarachnoid space. [16] Milhorat *et al* noted that many of the extracanalicular syrinxes were located in the watershed zone between the anterior and posterior spinal arteries and also suggested that there may be a vascular component to the injury. [269]

1.8.2.3 Tumours

A syrinx is associated with 15-58% of intramedullary spinal cord tumours. [10,319,361,446] These tumours may include astrocytomas, ependymomas, haemangioblastomas, nerve sheath tumours and less commonly metastases. [18,438] The proposed aetiologies of neoplasm-associated syringomyelia include faulty differentiation [319], spontaneous autolytic liquefaction [246], tumour haemorrhage [321], local arachnoiditis [18], interference with tissue fluid drainage [227] and secretion of fluid or an oedema-generating factor [94,367]. Tumours have also been suggested to interfere with arterial blood supply to or venous drainage

from the spinal cord. [172,176,291,322,316] Extramedullary compressive lesions may also lead to syringomyelia [18,295] although the mechanisms may be significantly different.

1.8.2.4 Inflammation

For many researchers, inflammation within the central nervous system is largely confined to immune-mediated diseases. [314] However, it has recently been implicated in the pathogenesis of a large variety of neurological conditions which include traumatic injury, ischaemia, AIDS-related dementia and Alzheimer's disease. [314]

The consideration of inflammatory factors in the pathogenesis of syringomyelia is a significant one. Certainly, syrinxes which form in association with diseases traditionally regarded to have an inflammatory basis (e.g. multiple sclerosis [268]) cannot be explained adequately by current theories of syringomyelia.

However, the concept that inflammation may play a significant role in at least some specific types of syringomyelia is not new. In 1945, Weil suggested that glioma-associated hydromyelia may occur as a consequence of "inflammations of the the spinal cord with an increased accumulation of fluid" or "the blocking of the upper medullary end of the central canal". [425] Feigin *et al* performed autopsy studies in 14 cases of syringomyelia and concluded that the condition resulted from "a variety of destructive processes among which edema is a major factor and inflammation, circulatory insufficiency and trauma additional factors". [94] In addition, they also noted that "microglial proliferation" was present in the majority of cases studied. More recently, Baldwin and Malone, [9] in

reviewing the pathogenesis of post-traumatic syringomyelia also suggested the possible role of inflammatory mechanisms but did not elaborate further on the issue. Limited but more direct evidence was provided by the study of Blagodatsky *et al.* [30] They detected elevated levels of immunoglobulin G, M or A in the syrinx fluid in 16 of 26 patients. They further observed that specific staining for Ig G was present in the pia mater during the early stages of the disease. The authors concluded that syrinx formation possibly resulted from the "synergic action of hydrodynamic and immunopathological mechansims". [30]

The predominant inflammatory cell type which has been most commonly implicated is the macrophage. Human autopsy studies have shown that fat and haemosiderin-laden macrophages are a common feature of non-communicating varieties of syringomyelia. [269] This is well supported by experimental data accumulated form animal research. In syrinxes which form after cisternal kaolin injections, many macrophages are seen at the margins of the syringomyelic cavity actively phagocytosing myelin. [56] Similarly, intra-parenchymal injections of kaolin into the rat spinal cord led to a florid local macrophagic inflammatory response and a significant dilation of the central canal rostrally. [269] Interestingly, similar intra-parenchymal injections of blood were associated with a less marked macrophagic response and did not lead to central canal dilation. [265] Guizar-Sahagun observed that macrophages in contused rat spinal cords persisted around the syrinxes up to 52 weeks post injury. [148] They suggested these macrophages exert "persistent destructive activity, although more selective and less intense than the initial necrosis".

2 Macrophages and inflammation

2.1 Definitions

The Dorland's Illustrated Medical Dictionary (27th Edition) defines a macrophage as "any of the many forms of mononuclear phagocytes found in tissues". [1] Consequently, it is necessary to stipulate the criteria which must be satisfied before a cell is called a 'mononuclear phagocyte'. [410,413] These requirements include morphological features, the presence of certain cytoplasmic enzymes and staining with specific monoclonal antibodies. [413] Other requirements include the presence of IgG Fc and C3 cell membrane receptors as well as demonstrable phagocytic and pinocytic activity. It is generally accepted that a cell must satisfy at least three of the above criteria before it is considered a mononuclear phagocyte. [413]

2.2 Mononuclear phagocyte system

In 1924, Aschoff assigned the monocytes and macrophages to the reticuloendothelial system. This system of classification was based on similarities in cellular morphology, properties, origin and functions. [6] However, such a classification system subsequently fell into disfavour as it became evident that cells such as fibrocytes and histiocytes are not as similar as first thought.

More recently, Van Furth and his colleagues proposed that monoblasts, promonocytes, monocytes and macrophages could be classified into the mononuclear phagocyte system

(MPS). [408,409] They felt that the concept of the MPS would promote research on the physiology of macrophages, monocytes, and their precursors under normal and pathological conditions, eventually leading to an improved understanding of certain diseases and their subsequent treatments. [411]

Since then, many more cells have been added to the list of mononuclear phagocytes based on studies of cell kinetics and specific monoclonal antibodies. [137,173, 180,181,413] While some authors consider the resting microglia to be cells of the mononuclear-phagocyte series, [413] this is controversial. [255]

2.3 Biology of the macrophage

2.3.1 Origin and kinetics

It is now almost universally accepted that tissue macrophages are formed in the bone marrow. [413] These cells are subsequently transported via the circulation to tissues where they are destined to perform their functions. [411,413] In the adult, the earliest recognisable macrophage precursor cell is the promonocyte. [407,413] Macrophage precursors subsequently undergo several cycles of proliferation and differentiation in the bone marrow (approx 8-10 days). They then circulate as monocytes for 1-2 days, [66,411] where they constitute 3-5% of the circulating leucocytes. [136] They eventually enter tissues where terminal differentiation occurs. [137]

Macrophages are detectable early in embryogenesis. [137] In the mouse, macrophages first appear in the yolk sac on Day 9 of embryonic life. [308] Haematopoiesis then shifts to the fetal liver, peaking at Day 14 and subsequently to the spleen and bone marrow just prior to birth. [308] They then accumulate in mesenchymal tissues during organogenesis as the circulation is established (from embryonic Day 10). The developing brain appears to be the first organ to be colonised with macrophages (and their precursors) at Day 12. [368] Consequently, significant numbers of macrophages may be found in various sites including gut, kidney and the central nervous system. [306]

2.3.2 Cellular turnover

Mature macrophages may be found in most tissues of the mouse; including haematopoietic, lymphoid organs as well as other connective tissues. [137] The turnover of these cells in different tissues is variable. [413] However, in the absence of any stimulus, these cells are relatively long lived (weeks to months). [413] They are continuously replaced by new haematogenous/bone marrow derived elements [136, 137] although there may be low levels of local replication [137].

The ultimate fate of macrophages is not well understood. It is not known whether they eventually die in the tissues of residence or migrate to another site. In the CNS, it has been suggested that macrophages may possibly migrate to the perivascular space [260] or to the brain surface and subarachnoid space [131] en route to the local lymph nodes [298,411]. While there is some evidence that macrophages can re-enter blood vessels[131] and reach the spleen

[340], recirculation of macrophages via peripheral blood does not appear to occur to any significant extent [413].

2.3.3 Identification of Tissue Macrophages

The morphological features observed on light microscopy are among the least reliable methods of identification. [191] Both the morphology and antigen expression of tissue macrophages vary in accordance with their state of activation and resident location. [49,191,220,312,414] However, regardless of location, most active macrophages display membrane ruffles and/or folds, pseudopodia, filopodia, and a rough or bubbly membrane surface. [164,191] Ultrastructurally, macrophages possess abundant microfilaments and granular endoplasmic reticulum, elongated mitochondria and numerous lysosomes and membrane-dense bodies. [164]

Consequently, a variety of histochemical and immunological techniques have been utilised to assist in the identification and characterisation of tissue macrophages. It has been suggested that the most reliable feature of macrophages is their phagocytic capacity. [191] A variety of agents such as dyes, colloids (e.g. Thorotrast) and enzymes (horseradish peroxidase) have been used for this purpose. [191,396,429] More recently, the technique of fluorescent spheres has also been utilised. [220, 392]

Histochemical techniques largely target the hydrolytic enzymes involved in phagocytosis. [191] These include acid phosphatase, non-specific esterase and aryl sulphatase. [164, 232,236]

Until recently, positive staining of mononuclear phagocytes for nonspecific esterase was considered by some to be the only characteristic which reliably differentiated between these cells and other mononuclear cells at sites of inflammation. [411]

The more recent development of monoclonal antibodies such as F4/80 which are considered specific for mononuclear phagocytes has also been particularly valuable. [174,215,411] Other techniques are aimed at the detection of receptors for the Fc fragment of IgG (Fc receptors), complement proteins (CR_1 and CR_3 receptors), lysosomal antigens (ED1-3) and acetylated low-density lipoprotein. [80,83,129,164,137,381,418] Several other membrane glycoproteins (MAC-1, MAC-2 and MAC-3) have also shown some promise as relatively selective markers for macrophages although they require further evaluation. [129,175,370,449]

	Amoeboid microglia	Resting microglia	Activated microglia	Reactive microglia	Peritoneal macrophages	Perivascular Cells
Phagocytosis	+++				+++	
Proliferation	+++				--	
Protease secretion	++				++	
Superoxide anion	+++				++	
GFAP	-					
Galactocerebroside	-					
Thy-1	-					
Laminin	-					
Factor VIII	-					
Histochemical						
Silver staining	++	++	++	++		
Enzymes						
Enolase	-					
Nonspecific esterase	+++	-		++	+++	
Peroxidase	-				++	
Thiamine pyrophosphate	++	++		++		
5'-Nucleotidase		-				
Antibodies						
MUC 101		+/-	+			
MUC 102		W++G+	++			
MAC-1	++	+		++	+++	
OX-42(CR-3)	++	+		++		-
Ox-41		-				-
OX-18(MHC I)	+	-		+		
OX-6(MHC II)	+	-		+		
ED1	++	-		++		+
ED2		-				+++
ED3		+/-				(+)
F4/80	++	+		++		
CD-4	+	+		++		
vimentin	++	-		++		
Ki-M2R		-				++
RMG 1 (rat microglia-1)		-	++			
RMG 2 (rat microglia-2)		++	++			
FA11				+		
beta-glucuronidase				+		
Lectin						
(Griffonia simplicifolia)						
B_4 isolectin	++	++		++		-
GSA-I-B_4		+				-
(Ricinus communis)						
agglutinin-120	++	++		++		
(Viscum albus)						
mistletoe lectin-1	?	++		?		
Receptor						
DIL-ac-LDL	+++	-		++	+++	
Fc	++	+		++		

Table 1. *Immunohistochemical characteristics of microglia and macrophages. The notation has been directly derived from the original papers. -: negative, -/+: inconsistent staining. (+): occasional staining, +: weakly positive, ++ or +++: strongly positive. (WM: white matter, GM: grey matter).* [121,130,277,289,395,418]

2.3.3.1 ED1 as a macrophage marker

The ED series (1-3) of monoclonal antibodies was first cloned by Dijkstra and her colleagues. [67,80,83] They are thought to recognise cells of the mononuclear-macrophage lineage in the rat exclusively. [80]

In particular, ED1 is generally regarded as a pan macrophage marker [83] although there are notable exceptions. Subpopulations within the lymphoid organs such as marginal macrophages in the spleen and subsinusoidal macrophages in lymph nodes either stain weakly or negatively. [80]

The ED1 monoclonal antibody appears to recognise a heavily glycosylated protein of 97,000 MW [80,81,83], expressed mainly on membranes of cytoplasmic granules such as phagolysosomes as well as on the cellular membrane [67]. Differential expression of the antigen may occur and appears to be correlated with the level of phagocytic/endocytic activity. [22,67,81] As the ED1 antigen shares many similarities with the human CD68 antigen, a role in antigen processing has been postulated. [81,83]

Within the CNS, macrophages may be reliably identified by ED1 expression. [84] More recently, it has become evident that resting microglia only rarely express ED1 immunoreactivity. [23,170] However, upon activation, synthesis of the antigen can occur leading to strong immunostaining. [23,122,212] While granulocytes with weak ED1 expression

have rarely been observed in peripheral blood, ED1 positive granulocytes have never been observed in tissues. [84]

2.3.4 Acute Inflammation

Large numbers of monocytes accumulate at sites of local inflammation and transform into macrophages. [136] Monocytes accumulate early in the presence of inflammation. [66] Both parabiotic and adoptive transfer experiments suggest that monocytes are the main source of recruitment into peripheral organs. [137,410,413,419] In short lived inflammation where the inciting stimulus is removed, macrophages are eliminated quickly (presumably either by death or migration into the lymphatic system). When inflammation persists, macrophages typically become the predominant cell type within 48 hours. [66] This involves the interplay of several processes which include:

(1) continued recruitment of monocytes from the circulation

(2) local proliferation of macrophages, and

(3) immobilization of macrophages. [66]

It is thought that a small, variable but significant component of the macrophagic response arises from the replication of local macrophages. [36,37,79,186,293,362,369,407,412,413,420,421] This process typically occurs after the second day. [413]

2.3.5 Chronic inflammation

The tissue macrophage is regarded as the "prima donna of chronic inflammation". [66] As the inflammation process extends into a chronic phase, the macrophages may interdigitate to form clusters or granulomas. [136]

Chronic inflammation can be classified into 2 types depending on the presence of granulomas. [413] In general, bone marrow derived monocytes are recruited to sites of persistent inflammation [413] with a small component arising from the local resident macrophages [68]. Irritant material which is not easily degradable results in the formation of granulomas. [39,57,68,69,127,382] In some cases, the macrophages may coalesce to give rise to mutinucleated giant cells. [136] These are formed by the fusion of newly arrived monocytes with existing macrophages rather than nuclear division of macrophages. [57,413]

2.3.6 Functional aspects of macrophages

Tissue healing generally involves macrophages. [66] Macrophages are believed to have a fundamental role in the maintenance, restoration and defense of damaged tissues (FIGURE 3). [314,351] Following an injury, macrophages are activated and migrate into the affected region. [351] While clearing up cellular debris and necrotic tissue, they secrete factors which are necessary for tissue healing to occur.

Macrophages are thought to have key roles in initiating immunological responses and antigen presentation (FIGURE 3). [426] Macrophages may become 'activated'. [66,426] This leads to increased cellular size, augmented production of lysosomal enzymes, more active

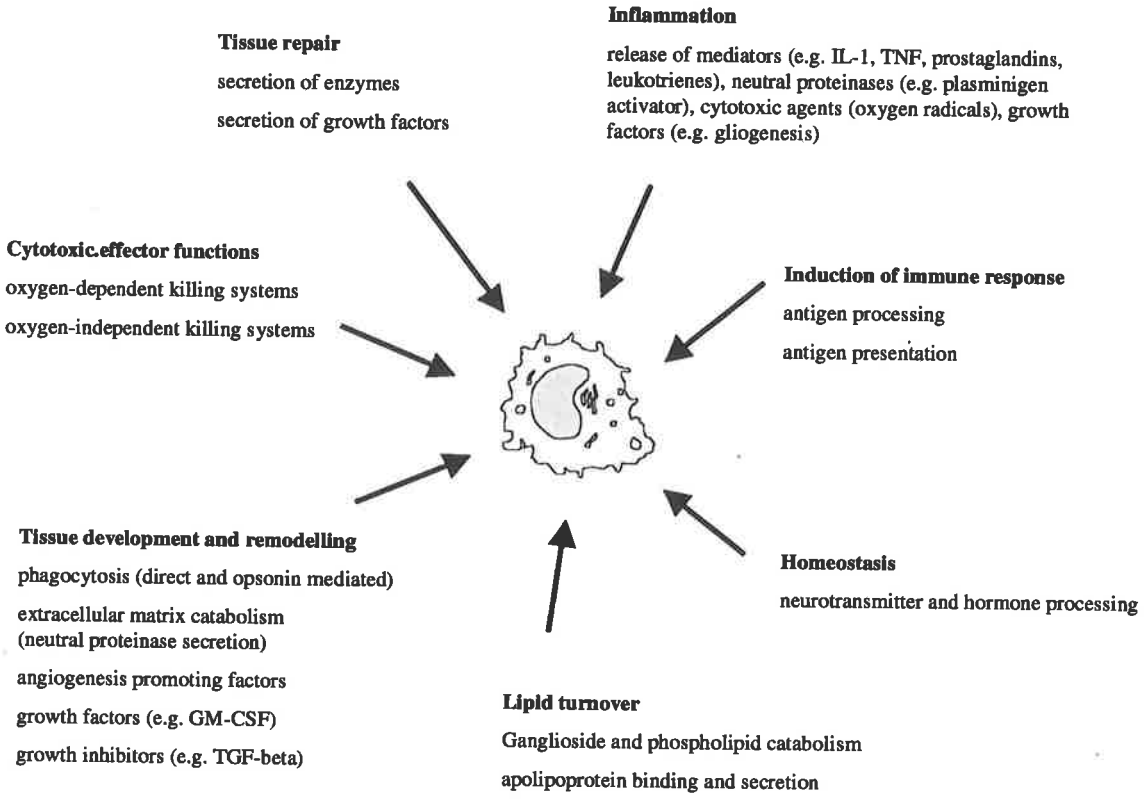

Figure 3. *Proposed functional roles of tissue macrophages.*

metabolism and phagocytic ability. [66] In vitro studies of resident macrophages isolated from the peritoneal cavity of the mouse demonstrate little microbicidal or tumouricidal activity.

However upon exposure to lymphokines produced by T-cells, macrophages upregulate their activity markedly and are subsequently capable of direct antimicrobial as well as tumoricidal functions. [426] The 'activation' process is complex and involves signals such as cytokines (e.g. IFN-gamma), bacterial endotoxins and ECM proteins like fibronectin. [66]

Macrophages express receptors which are capable of binding particles by both immunological and nonimmunological mechanisms. [136,324] As effector cells, they are capable of secreting products such as neutral proteases, acid hydrolases, growth factors (PDGF, EGF, FGF, TGF-beta), prostaglandins, oxygen free radicals , nitrogen oxide and inflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor-alpha. [71,90,157,290] Furthermore, macrophages can also act as antigen presenting cells to lymphocytes when they express MHC Class II molecules. [256,415]

The ability of macrophages to scavenge cellular debris and re-model the extracellular matrix has also been considered to be crucial to embryonic development. [308] These cells may potentially have widespread influences on angiogenesis, gliogenesis and neuronal development within the central nervous system. [308]

2.4 Macrophages in the Central Nervous System

The CNS is traditionally regarded as an immunologically privileged site. This has been attributed to the presence of a blood-brain barrier. [351] This is consistent with the very low numbers of 'classical' macrophages which are present within the parenchyma of the normal CNS.

However, some researchers hold the view that a population of resident macrophages exist in *all* tissues of the body. [314] In line with this view, they believe that the microglia are resident macrophages of the CNS which possess a highly differentiated morphology but markedly downregulated phenotype. [308,313,413]

Apart from the microglia, specific subsets of macrophages have also been described. These include the perivascular macrophages and macrophages of the choroid plexus and meninges, each with somewhat different phenotypic properties. [308,313] The role of these macrophages in the uninjured CNS remains poorly understood.

3 Microglia

3.1 Introduction

The microglia represent a class of glial cells within the CNS. They were first discovered independently by Nissl and Robertson in 1899.[13, 275] Nissl in particular described them as *Stäbchenzellen* or 'rod cells'.[146] Rio Hortega later added extensively to the body of knowledge in his classical work in 1932.[334]

The peak of microglial development during embryogenesis occurs in the first 2 post-natal weeks.[332] At maturity, microglia comprise up to 20% of all glial cells .[27] In humans, these cells constitute about 13% of the glial population .[160] This is comparable to the mouse, in which microglia constitute between 5-12% of all mouse CNS cells .[205, 391] Lawson *et al* estimated that the brain of the mouse alone contained 3.5 x 10^6 microglial cells.[214]

3.2 *"THE CELL"*

Microglial research has accumulated exponentially in recent years. Graeber estimated in 1993 that 50% of the published literature has been published after 1985 .[145] The most current MEDLINE search reveals that a total of 3142 microglia-related papers have been published since 1966. More than 90% of these occurred after 1985. Consequently, it is not surprising that some scientists have nominated the microglia as "THE CELL" in the *Decade of the Brain.*[145]

3.3 The microglial debate

The microglial cells have continuously provoked much debate and research since their discovery. Virtually every aspect, including their ontogeny, functions in both physiological and pathological environments have been subjected to controversy. Until very recently, even the very existence of these cells had been questioned by some scientists.[206] However, it is now widely accepted that this particular class of cells possesses unique features .[273]

Streit *et al*[367] highlighted 3 contentious but inter-related issues in the 'microglial debate'. These included: (1) the embryogenic origin of microglia (2) the microglial contribution to the macrophagic response in traumatic injury of the CNS (3) the origin of proliferative microglia following peripheral nerve injury. The former two points are particularly relevant in the current research and serve as a focal point for a literature review.

The current work does not set out in its primary aim to address the basic biology of microglia. However, these properties are intimately related to their proposed roles in the CNS under both normal and pathological conditions. An attempt has been made to provide an integrated view, with regards to their relationship to macrophages as well as their possible pathogenetic roles in pathological conditions of the CNS.

3.4 Nomenclature

One of the difficulties in discussing microglia has been the imprecise use of this term.[78] When del Rio-Hortega originally used this term, it was purely descriptive and hence

included other cell types such as oligodendrocytes and small astrocytes.[78] However in recent years, it has been considered to more specifically refer to intrinsic glial cells within the CNS with a characteristic morphological and antigenic phenotype.

3.5 Classification

The diverse nomenclature used in the literature has subsequently led to much confusion. However, it would appear that there are at least 2 sub populations of microglia. Based on morphological features, these cells may be classified into;

(1) *ramified microglia* (resting microglia), found predominantly in the white matter postnatally, and

(2) *amoeboid microglia* (brain macrophages, microgliocytes or precursor microglia) found in the grey and white matter perinatally[184, 275, 293, 394, 400]

The terms of 'ramified' and 'amoeboid' depict only the morphological subtypes of microglia. They do not describe the functional state of these cells.[84] Although the relationship of the amoeboid to ramified microglia remains unclear, it has been speculated that a proportion of the amoeboid microglia is transformed into ramified microglia during CNS development.[182, 235] Imamota and his colleagues initially suggested that the ramified microglia were derived from their amoeboid counterparts.[182, 183] Ling and his colleagues observed that during CNS development, the increase in ramified microglia was associated with a concomitant decrease in the amoeboid microglia. [231, 236] In a further set of

experiments, they noted that following intravenous injections of carbon-labelled mononuclear cells into early postnatal rats, carbon labelling was observed in both the earlier amoeboid as well as the later ramified microglial cell types. Interestingly, Suzumura *et al* demonstrated that cultured microglial cells may be chemically-induced to transform *reversibly* into either amoeboid or ramified forms in response to specific cytokines/factors.[379] This provides further support for the concept that the amoeboid and ramified microglia are essentially variants of the same cell type.

This simple classification of microglial cells was subsequently expanded upon by Flaris and Hickey 1993.[98] They further distinguished:

(3) *activated microglia* (CD11b/c++, CD4+, MHC II++), found in areas of secondary reaction as a consequence of nerve transection and CNS inflammation. In contrast, the ramified microglia are CD11b/c+, CD4-, MHC II-.

(4) *reactive (phagocytic) microglia*, found in areas of trauma, viral infection or neuronal degeneration

Murabe and Sano[283] further described another microglial sub-type which they considered to be restricted to the early postnatal period;

(5) a *round cell* form, which is smaller and exhibits pseudopodia but no other cellular processes.

These latter forms of microglia are also thought to correspond to variable morphological and functional states of a single cell type although the exact nature of their interrelationships is unclear.[191]

3.5.1 Ramified microglia

The ramified microglia form an even mosaic throughout the central nervous system.[51, 191, 275, 277, 278, 283] Rio-Hortega first described these cells in silver stained preparations at the light microscopic level in 1932.[334] On light microscopy, the ramified microglial cell possesses a small oval cell which is characterised by an elongated soma with 2 or more primary processes projecting from both poles of the cell.[275] Dense or inclusion bodies (lysosomes or phagosomes) may be found within the scanty cytoplasm.[51, 276, 283, 284, 285] Ultrastructurally, the small or angular nucleus contains coarse chromatin at the periphery and a small nucleolus .[31] Small granules and rough endoplasmic reticulum may be found within the scanty cytoplasm at either pole.

There is conflicting evidence with regards to the precise distribution of ramified microglia within the central nervous system. Some regard the microglia as a uniform population of cells with equal density in both the white and grey matter.[27] Others however have shown that regions with the highest density of microglia such as substantia nigra and the ventral pallidum demonstrate a 5 to 6 fold difference from those in the brain stem and cerebellum .[214, 313] It is not entirely clear whether this relates to variations between species of animals.

However, there is general agreement that the dendritic fields of microglia do not overlap and the entire neuropil is uniformly occupied by the ramified processes of microglia .[27, 124]

3.5.2 Amoeboid microglial cells

The amoeboid microglial cells occur transiently during embryogenic CNS development .[187, 397] In rats, they arise in the last week of gestation in rats and disappear during the second postnatal week.[130, 191, 270] Similar findings have also been reported in mice.[172] They are typically located in clusters within the white matter of the developing telencephalon .[184, 187] In particular, Ling and Tan[231] noted that these round amoeboid microglial cells are particularly prominent in the corpus callosum of early postnatal rats.

The functional differences of amoeboid cells are not entirely understood. However, they are believed to be involved in the phagocytosis of apoptotic or degenerating cells within the developing CNS.[95, 308, 313] It has further been suggested that amoeboid microglia may release peptides (including IL-1,GPF-2 and GPF-4) which influence the growth and proliferation of astroglia.[130]

3.6 Perivascular microglia

The term 'perivascular microglia' deserves special attention as it appears to have various applications in the scientific literature.[24] Hickey and Lassmann considered that the "perivascular microglia", "pericytes", "fluorescent granular perithelial cells" and "perivascular monocytes" may possibly refer to the same cell type.[170] Bauer *et al* shared

this view but further suggested that the 'perivascular cell' is also the same cell.[24] Risau and Wolburg proposed that these cells may be an integral part of the blood-brain barrier.[339]

In contrast, other researchers believed that this was tantamount to "indiscriminate use of terminology".[142] Graeber and Streit[142] strictly defined the 'perivascular microglia' as true microglial cells which were located in the proximity of blood vessels outside the basal lamina. They considered these cells to be distinct from 'pericytes' which form part of the vascular wall within the basal lamina. Furthermore, these cells are also distinct from the 'perivascular cells', which may morphologically resemble 'pericytes' but are better understood as a distinct population of CNS resident macrophages. These views were supported by Berry and Butt.[27]

3.6.1 Perivascular cells

The 'perivascular cells' are located outside of the CNS parenchyma proper and are separated by the basement membrane and the glial lamina limitans.[27] They are relatively large, spindle shaped cells which are not in direct contact with neurons.[27] These cells may be found either in the Virchow-Robin space[170] or along capillaries and small venules between the endothelium and basal lamina of blood vessels.[27, 142, 170] They can be identified positively by the monoclonal antibodies ED1, ED2[24, 141, 142] as well as OX-1 and OX-42[170]. Hickey and Kimura demonstrated that in rat radiation bone marrow chimeras, these cells are continually renewed from the bone marrow every few days. Consequently, they also suggested that the perivascular cells may be considered as part of the mononuclear phagocyte system.[168]

While perivascular cells may express MHC class II antigens in the absence of pathology,[168] those which do not express these antigens constitutively may become MHC class-II positive after intravenous administration of interferon-gamma.[210] Consequently, they have been implicated as antigen presenting cells within the central nervous system. When stimulated under certain situations, they may also be positive for IL-1,[406] intercellular adhesion molecule-1 and leucocyte function antigen-1,[24] allowing them to interact with leucocytes as they enter the perivascular space. Furthermore, these cells may also express CD4.[280]

3.7 Origin of microglia

The ontogeny of microglia is controversial. Rio Hortega (1932) initially proposed that microglial cells are of mesenchymal origin.[338] He hypothesized that they were derived from the invasion of mesodermal pial elements during embryogenesis. This theory was widely accepted by many later investigators.[40, 51] However since then, a number of alternative theories have been put forward to explain the origin of microglia.

3.7.1 Neuroectodermal theory

Another long standing opposing theory states that the microglia are derived from neuroectodermal tissues.[224] Rydberg believed that ramified microglia were derived from the subependyma of the lateral ventricles.[343] In studying gliogenesis in the optic nerve, Vaughan and his colleagues concluded that the ramified microglia were derived from

neuroectodermal matrix cells.[360, 416, 417] Fujita and Kitamura[109] used the silver carbonate method to stain tissue sections of both rabbit and human brains. They observed staining of the glioblasts with their intermediate forms up to the so called microglia, oligodendroglia and astrocytes. Consequently, Fujita and Kitamura believed that these cells originated from the subependymal glioblasts. This view has also been supported by more recent researchers. [92, 109, 200, 346]

On the other hand, some notable investigators[237, 377] have considered this theory to be unacceptable, arguing that common markers between these glial elements have not been demonstrated by immunohistochemical methods.[237]

3.7.2 Pericytal origin

Yet another view suggests that microglia are of pericytal origin.[19, 278] Mori and Leblond postulated that the pericytes associated with blood vessels migrate into the neuropil due to the discontinuous nature of the overlying basement membrane.[278] On the other hand, while Boya *et al* considered that microglia are largely derived from "the meningeal membranes and the vascular adventitia", these cells may also arise from the pericytes.[42] Interestingly, Ling and Wong pointed out that the pericytal theory may also be consistent with a moncytic origin of microglia as the pericytes are themselves possibly derived from migrating monocytes.[237]

3.7.3 Monocytic origin

The theory of a haematopoietic/monocytic origin was first put forward by Juba and his colleagues[192, 344] after they noted that the appearance of ramified microglia coincided with the vascularisation of the brain. It represents the most widely accepted theory to date.

Several criteria are expected to be fulfilled to establish the monocytic origin of microglia.[307, 308] These include evidence that:

(1) bone marrow derived cells enter the CNS and adopt the morphology of microglia

(2) monocytes transform into microglia

(3) microglia express antigens which may be restricted to the monocytic lineage.

A review of the literature suggests that all of these criteria have been satisfied.[307]

3.7.3.1 Developmental studies

A number of developmental studies have lent support to the theory of monocytic origin of these cells. Dunning and Forth observed that cultured monocytes were capable of transforming into cells which resembled ramified microglia.[87] Imamoto and Leblond described amoeboid microglial cells in the postnatal corpus callosum of the rat which share similar ultrastructural features to monocytes.[182]

Ling injected carbon particles into the circulation of 3 to 5 day old rats and demonstrated the labelling of amoeboid microglia.[233, 235] It was thought that the monocytes had

phagocytosed the carbon particles prior to infiltrating the corpus callosum. Ling[238] subsequently directly injected carbon labelled monocytes from donor Lewis rats into early postnatal syngeneic rats. Similarly they found carbon labelled microglia in the postnatal corpus callosum.[233, 235] These cells disappeared within 2 weeks postnatally. This was associated with a concomitant increase of ramified microglia suggesting a precursor and progeny relationship.[238] While it has been suggested that the apoptosis in neonatal brains may lead to chemotactic signals for the entry of monocytes into the CNS, direct evidence for this is lacking.[313]

Histochemical studies using the rat monoclonal antibody F4/80 and other antibodies directed against the Fc and complement type 3 receptors (present on monocytes and macrophages) have documented large numbers of macrophages in the mouse brain prenatally which progressively developed morphological features typical of ramified microglia.[215, 226] In combination with autoradiographic techniques, Lawson *et al* further observed that even in the adult mouse brain, the slow turnover of microglia is maintained by division of resident microglial cells as well as entry of monocytes into the CNS .[215] Other researchers have made similar observations using less specific staining methods.[40, 61, 285]

3.7.3.2 Chimeric studies

Another experimental approach has involved the use of rat radiation bone marrow chimeras. Irradiation of animals at high doses eliminates the haematopoietic precursors within the bone marrow. Subsequently, they are reconstituted with bone marrow cells

harvested from congeneic donor animals which carry a cellular marker distinct from the recipient. Using this technique, small numbers of bone marrow-derived cells have been demonstrated within the brain and spinal cord.[73, 168, 170] Hickey and his colleagues further observed that some of these cells subsequently adopt the morphology of microglia.[168, 170] In contrast, Matsumoto and Fujiwara were unable to reproduce these findings in their chimeric rats over a study period of 24 weeks.[257] However, Matsumoto and Fujiwara cautiously pointed out that their results did not exclude the bone marrow origin of microglial cells as the influx of haematogenous precursor cells may be largely confined to the perinatal period. While definite evidence is lacking, there is general agreement that the microglial turnover in the postnatal period appears to be very low.

3.7.3.3 Immunohistochemical studies

Much of the initial opposition to the monocytic origin of microglia had been centred on the apparent lack of antigens which are common to monocytes, macrophages and microglia.[297, 402, 444] This no longer appears to be a valid criticism. It is known that the microglial cells are capable of upregulating their expression of certain antigens depending on their state of activation. There is also accumulating evidence that a large panel of antigens are shared by microglia, monocytes and macrophages.[98, 308] Certainly, microglia express most of the antigens which are expressed by monocytes.[275, 286, 308, 309, 404]

3.7.3.4 Entry into the CNS

At present, the site and mode of entry of haematogenous precursors into the central nervous system is poorly understood. It is unclear whether monocytes enter through

specific blood vessels or migrate within the parenchyma after these cells have left the circulation. Ling and Wong have suggested these cells may enter through; (1) the endothelial wall of blood vessels (2) transependymally and (3) through the pial surface.[237]

Perry *et al* noted that the initial influx of $F4/80^+$ cells into the embryonic mouse brain is closely associated with the blood vessels.[306] Others have suggested that monocytic entry may occur specifically through local blood vessels within the corpus callosum and other regions of the developing brain where cell death naturally occurs.[7, 95] Other sites of entry suggested have included the transitory cavum septum pellucidum or subependymal cysts.[195, 401]

Boya and colleagues[42, 43] described the immigration of macrophagic meningeal cells into the brain parenchyma to become ramified microglia. While this may initially appear to contradict the monocytic theory, meningeal macrophages are also known to be derived from monocytes.[263] More recently, Kurz and Christ[207] studied chick-quail blood chimeras using a parabiotic technique. They concluded that the phagocytic haematopoietic cells originate largely from the yolk sac and invade the embryonic pial surface directly without involvement of the vascular circulation.

The prevailing view states that monocytes enter the CNS during a confined perinatal period to form microglia. Beyond the fetal period, these cells become macrophages and do not transform into microglia.[27]

3.8 Functions of microglia

3.8.1 Normal conditions

The in vivo functions of resting microglia in the normal adult CNS remain enigmatic.[124, 138] A number of theories have been proposed.

3.8.1.2 Immune surveillance

The combination of macrophage-like immunophenotype, highly branched morphology and distribution within the CNS has led some to suggest that microglia form a network of immuno-competent cells which are adapted to the architecture of the CNS.[13, 142] These researchers regard the microglia as a network of immune alert resident macrophages which are capable of immune surveillance and 'first line defence'.[204, 205] The early microglial activation which follows an injury to the central nervous system is consistent with this hypothesis.

3.8.1.3 Tissue maintenance

It has also been proposed that the microglia may also have a constitutive role in "cleansing" extracellular fluid in the CNS and monitoring changes in the extracellular milieu.[205, 327, 395] This suggestion is largely based on observations of high levels of pinocytosis[327] and observed patterns of motility.[395, 449]

Ramified microglia may contribute to tissue maintenance by removing cellular metabolites and waste by-products and inactivating harmful agents which have been released from

damaged cells.[399] They may also limit the spread of diffusable neurotransmitters or modulators within the extracellular fluid.[395] A role of microglia in neurotransmitter metabolism has similarly been suggested by other investigators. However, the supportive evidence for these microglial functions is lacking.

3.8.1.4 Neuronal/Glial support

The ability to produce growth factors such as transforming growth factor-beta, platelet derived growth factor, epidermal growth factor, insulin-like growth factors and basic fibroblast growth factor suggests that microglia play a potentially trophic/supportive role for glia and neurons. [89] Cultured microglia cells are also capable of secreting NGF, upon challenge/stimulation with LPS, cytokines interleukin-1beta and tumour necrosis factor.[162, 250, 273] These further support a major neurotrophic role during development and neuronal regeneration.

3.8.2 Pathological conditions

3.8.2.1 Effector functions

Rio Hortega noted that microglia had the ability to transform into macrophages.[338] While this is somewhat controversial, it is now widely accepted that activated microglia actively phagocytose cell and tissue debris which may include myelin.[277, 395, 449]

Microglia can participate in both antibody-dependent cellular cytotoxicity via their Fc gamma-receptors as well as complement-mediated endocytosis.[306, 403, 418] During the process of antibody-mediated demyelination, activated microglia are capable of lysing antibody coated target cells via interaction of Fc[365] and complement receptors with immune complexes and complement opsonised antigens.

Microglial cells are well placed to act as effector cells within the central nervous system.[272] When exposed to IFN-gamma and endotoxin, the microglial cells produce tumour necrosis factor alpha.and develop tumour cell cytotoxicity.[106, 452] Other in vitro studies have further demonstrated that microglia contain superoxide anions and nitric oxide which may be released upon interferon-gamma (IFNgamma) stimulation.[38, 58, 64, 193, 446] Futhermore, they are capable of secreting proteases, arachidonic-acid derivatives, excitatory amino acids, quinolinic acid and cytokines.[12, 167, 177, 272] Some of these liberated agents have a direct cytocidal effect. For example, free oxygen radicals released by microglia have a neurotoxic effect in co-cultures of neurons and microglia.[393] Other neurotoxins are less well defined.[133, 134]

3.8.2.2 Local vascular regulation

.Cultured microglial cells provide a rich source of prostaglandin E_2, prostaglandin D_2, and thromboxane A_2 when challenged with lipopolysaccharide. This may further be augmented by beta-adrenergic activation.[271] It has been suggested that thromboxane secreted by activated microglia may help to control local vascular blood flow at the regions of acute CNS injury.[135]

3.8.2.3 Tissue modelling

Microglial involvement in tissue healing and modelling has also recently been proposed based on limited evidence. TGF-ß1 is produced by macrophages and microglial cells after a penetrating injury.[229] It strongly inhibits astrocytic proliferation and suppresses the mitotic effect of FGF and EGF on astrocytes. This may promote tissue repair by reducing astrocytic scar formation. Plasminogen which is also secreted by microglial cells leads to neurite outgrowth in vitro,[185, 289] thus further implicating a role for microglia in proteolytic processes involved in tissue remodelling.

3.8.2.4 "Synaptic Stripping"

Following facial nerve axotomy, the microglia proliferate, particularly in a perineuronal position. They subsequently ensheath the injured motor neuron, interposing processes between afferent synaptic terminals and the neuronal surface.[35] This process is now known as 'synaptic stripping'.[27] The physiological significance is unclear although it has been suggested that neuronal energy expenditure is minimised by cessation of the "transmission" function.[289] 'Synaptic stripping' may also influence synaptic reorganisation of injured motor neurons.[27]

3.8.2.5 Neuroprotective functions

Microglia may potentially exert neuroprotective functions on neurons although this has been particularly difficult to evaluate in vivo. Indirect supportive evidence cited has largely been based on the observation of potentially neuroprotective agents which are released by

microglial cells. For example, the production of nitric oxide delays neuronal death in ethanol-induced necrosis of the rat striatum.[386] Other agents such as PGE2 may potentially have multiple effects on APCs and T lymphocytes which eventually result in neuroprotection.

He *et al*[161] performed sciatic neurectomies in athymic mice which congenitally possess a significantly lower number of microglia. They found that the loss of motor neurons occurred faster than standard BALC/c mice which had undergone the same procedure. They concluded that these results suggest a neuroprotective function for microglial cells although the extent of motor neuron cell loss were no longer significantly different by day 15. On the other hand, when the microglial proliferation following a crush injury to the hypoglossal nerve was aborted by intraventricular infusion of cytosine-arabinoside, there was no observed difference in axonal regeneration.[384]

3.8.2.6 MHC II upregulation

The low level of MHC II expression within the central nervous system is traditionally thought to be consistent with the view that the brain and spinal cord are immunologically privileged sites.[27]

Following both inflammatory and non-inflammatory lesions, MHC class II antigens may be rapidly upregulated in activated microglia.[209, 276, 354, 379] Systemic or intrathecal infusion of interferon-gamma leads to upregulation of MHC class II antigens in microglial cells.[147] Similarly, newly cultured microglial cells which are negative for class II antigens of the

major histocompatibility antigen complex also became Ia^+ when treated with IFN-gamma.[106]

However, MHC class II antigen expression is complex and varies even between different strains of rats. In the brains of Lewis rats, the MHC II antigen expression is usually low in normal conditions and is mainly found in macrophages in the leptomeninges and choroid plexus, perivascular cells, white matter of cerebellum and the white matter of the lumbar spinal cord.[27] In contrast, the microglia of adult Brown Norway rats are constitutively MHC Class II positive.[353]

MHC class II expression by APCs is essential for the activation of $CD4^+$ T cells.[358] Typically, these antigens are presented with the processed antigens in conjunction with other surface co-stimulatory molecules such as the B7 molecule found on antigen presenting cells.[27] This leads to the stimulation of antigen-specific T lymphocytes. However, whether the upregulation of MHC class II antigen expression on microglia reflects an increased capability of antigen presentation remains unknown. In contrast, it has also been suggested that MHC class II upregulation in the absence of co-stmulatory molecules may protect the CNS against immune mediated injury.[13, 27, 75]

The current literature is conflicting. On one hand, microglia derived from neonatal brains present antigens effectively in experiments simulating in vivo conditions.[2] On the other hand, while adult microglia also express costimulatory molecules, B7.2, ICAM-1, and CD40 and could be induced to express MHC class II antigens, they failed to present

antigen.[52] It is possible that the maturity/age of the microglial cells have an impact on their ability to present antigens although further studies are required to clarify this.

Peripheral nerve axotomy similarly leads to rapid upregulation of MHC class II antigens. This is predominantly restricted to areas of primary projection.[379] It has therefore been suggested that this high state of immune alertness may facilitate delayed hypersensitivity reactions rather than antigen presentation functions.[27]

3.9 Microglial response in experimental paradigms

3.9.1 Introduction

Microglial cells are widely regarded as the resident macrophages of the CNS.[307, 308] However, in many neuropathological conditions , it is not known whether the increase of macrophages is due to proliferation of the resident microglia or to the influx of monocytes.[309] This is because the respective cell types cannot be differentiated based on morphological characteristics and cell surface antigens.[289] Consequently, some researchers have even resorted to a cautious approach using terms such as "microglia/Mø" to describe the inflammatory infiltrate within the central nervous system.

3.9.2 Cranial nerve lesions

3.9.2.1 Facial nerve axotomy

The study of the microglial response to CNS injury has been a challenge. Experimental animal models which involve penetrating injuries are by definition, associated with a disrupted blood brain barrier. Hence, it becomes difficult subsequently to delineate the local microglial responses from the peripheral macrophagic/monocytic component.[277]

The facial nerve axotomy model is thought to allow the examination of the microglial activation/proliferation in the absence of haematogenous cells by maintaining an intact blood brain barrier.[205] This model involves the transection of the facial nerve, which results in a retrograde, non-lethal injury of cell bodies within the facial nerve nucleus.[138, 205] Facial nerve compression leads to similar but possibly less marked changes.[342] In addition to the preserved blood barrier, this model has the added advantage of creating a well defined and reproducible lesion at the level of the brain stem which is away from the site of direct injury.[277]

Following facial nerve transection, the resident microglia proliferate[139, 205] with little contribution from haematogenous phagocytes.[296] They begin to express macrophage related antigens such as complement receptor 3, vimentin, MHC-I and II, MUC 101, MUC 102 epitopes and co-stimulatory molecules such as B7-1TNF-alpha and several cell adhesion molecules including thrombospondin.[140, 205, 379] The microglia also begin to upregulate cytokine production.[107, 108, 277] This process is blocked by simultaneous

administration of the cytostatic agent adriamycin which is thought to inhibit microglial differentiation and proliferation.[140, 274]

In this model, the facial motor neurons eventually regenerate the injured axons. The microglial cells become activated but not phagocytic.[35, 205] In contrast, when neuronal cell death is induced by the injection of ricin into the facial nerve, the microglia rapidly transform into macrophages to remove neuronal debris.[377, 378,379, 380] This is accompanied by further increase in MHC Ia antigen expression and de novo of ED1 and ED3 macrophage antigens.[144, 395]

Hence, the process of microglial activation appears to occur in graded phases which are under strict control in vivo.[205] While cell death results in significant transformation of microglia into brain macrophages, the precise triggers and signals which influence this process remain poorly understood.

3.9.2.2 Other similar models

George and Griffin transected the lumbar dorsal roots just proximal to the dorsal root ganglia in rats.[125] Following this procedure, early microglial activation was readily detected; peaking at 3 days within the dorsal columns. In contrast, round macrophages were not detected until days 18-21. Importantly, they believed that these latter cells had originated largely from endogenous microglia. A similar activation response in microglia is also observed following sciatic neurectomy[196, 280] and hypoglossal nerve transection.[332, 359]

In the latter model, Svenson and his colleagues demonstrated that the infusion of cytosine-arabinoside into the fourth ventricle or cisterna magna leads to cessation of microglial proliferation (and secondary astrocytic activation) following hypoglossal nerve transection.[384, 385]

3.9.3 Optic nerve lesions

Schnitzer and Scherer demonstrated that within several days of transecting the optic nerve in rabbits, there was increased number of nucleoside diphosphatase (NDPase) labelled microglial cells within the inner plexiform layer, nerve fiber layer as well as the outer plexiform layer.[350] Using a combination of [^{3}H]-Thymidine labelling and X-irradiation (to exclude the participation of haematogenous cells), Lawson *et al* concluded that the majority of macrophages responding to Wallerian degeneration were of endogenous microglial origin.[216]

3.9.4 Ischaemic lesions

Following an ischaemic injury, the microglia also respond rapidly. Transient forebrain ischaemia (4 vessel occlusion for 25 minutes) in the rat led to microglial activation within minutes as evidenced by an upregulation of GSA-I-B_4 lectin-binding sites.[279] This became very pronounced at 24 hours and was maximal at 4 to 6 days. In the hippocampus, a similar response was observed within hours. Activated microglial cells persisted up to 4 weeks.

Ischaemia induced in other regions of the brain by middle cerebral arterial (MCA) occlusions also result in a microglial response in the affected cerebral cortex within 18 hours.[279] In contrast, the recruitment of haematogenous cells occurs later. Garcia and Kamijyo described a large influx of monocytes at 7 and 16 days after permanent MCA occlusion in primates.[111]

Such a pathological response also extends beyond the region of primary insult.[277] Regions of the brain which suffer from retrograde injury similarly demonstrate microglial activation.[277] Following unilateral MCA occlusion, progressive microglial activation was subsequently noted in the contralateral cortex and hippocampus and the spinal cord (cortico-spinal tracts).[281] However, this secondary response is slower, of a lesser magnitude and possibly non-fatal.[277, 281]

Despite the clear demonstration of microglial activation following ischaemia, whether such a response is harmful or potentially beneficial remains inconclusive. While the excitatory amino acids released may facilitate post-ischaemic injury[166, 254, 445] or delayed neuronal death[221], other factors may in apparent contradiction protect against neuronal death[221].

The use of immunosuppressive interventions in animal ischaemic models have yielded interesting results[445];

(1) Guilian and Robertson administered chloroquine and colchicine after inducing ischaemic injury in rabbit spinal cords.[132] They observed a reduction in the number of mononuclear phagocytes in the grey matter which was associated with improved hindlimb and bladder function. It is thought that both these agents administered primarily led to the inhibition of microglial proliferation.

(2) Cyclosporin A is capable of down regulating activated microglia .[345, 445] Administration in transient brain ishaemia leads to reduced neuronal and muscarinic receptor losses in the gerbil hippocampus.[299]

(3) IL-1RA is believed to antagonise the actions of IL-1alpha and IL-1beta. Relton et al recently demonstrated that peripheral treatment with IL-1RA has a neuroprotective effect in rat focal cerebral ischaemia.[333, 334] It has also recently been suggested that recombinant IL-1RA has neuroprotective effects in experimental settings of excitotoxicity and trauma as well.[304]

(4) Cycloheximide may possibly exert neuroprotective functions by inhibiting the synthesis of proteins that promote cell death.[356, 397] The precise mechanisms remain unclear.[240]

3.9.5 Penetrating lesions

Stab lesions or freezing injuries of the brain lead to a large influx of haematogenous cells, which subsequently contribute to a large proportion of macrophages within the lesion.[77, 182, 199, 202, 340, 346, 371, 402] Some researchers maintain that macrophages are ONLY derived

from blood monocytes[198] and that the "resting branched microglia of Hortega" do not play a significant role[199]. This has not been particularly surprising in view of the extensive vascular damage associated with these injuries.

However, the major deficiency of these studies has been the inability to distinguish the haematogenous macrophages from the other cells of microglial origin. This difficulty is highlighted by the ambiguous observations made in one such study by Giordana *et al*[128] who described the inflammatory cells around the stab wound as having "features of amoeboid activated microglia and possibly originate from the blood stream, but a local origin cannot be excluded". More recently, the resident microglial cells are also thought to contribute significantly to the macrophagic pool in clearing the cellular debris.[41, 277, 377] However, the relative contributions of haematogenous/endogenous macrophages remain unclear.

3.9.6 Demyelination

Multiple sclerosis (MS) is a multifocal disease with scarring plagues distributed within the cerebral white matter and spinal cord.[277] It is further characterised by neurological symptoms due to impaired nerve conduction.[24] Clinical exacerbations are often followed by temporary remissions although these become less frequent with progression of time.[24] In MS, it is believed that T-cell mediated activation of effector cells such as macrophages lead to destruction of the myelin.[24, 82, 209]

Experimental autoimmune encephalomyelitis (EAE) provides a useful animal model for the study of multiple sclerosis in view of the comparable clinical manifestations and pathological findings.[24, 82, 326] EAE can be induced in rodents and primates in 2 main ways.[24, 253] Firstly, active immunisation may be performed by subcutaneous or intracutaneous innoculation of an encephalitogenic emulsion in the flank or foot pads of animals. This may consist of whole spinal cord, purified myelin or specific myelin proteins such as myelin basic protein or proteolipid protein with Freund's complete adjuvant and killed *Mycobacterium tuberculosis.* Alternatively, this can be achieved by passive transfer of $CD4^+$ T-cells that have been derived from animals previously sensitised against CNS antigens such as myelin basic protein.

Pathologically, the perivascular lesions are characterised by both lymphocytes and macrophages.[305, 317] It is postulated that macrophages may play various roles in the pathogenesis of EAE. Initially, macrophages may release factors such as radical oxygen species[201] which disrupt the basal lamina of the glia limitans[24]. This may subsequently promote further recruitment of lymphocytes from the peripheral circulation. During the clinical phase, neuronal damage may result from various inflammatory mediators although the exact mechanisms are still poorly understood. These include proteolytic enzymes, radical oxygen species, NO, complement and cytokines such as tumour necrosis factor-alpha.[46, 197, 239, 264, 357] Local TNF production, for example, can lead to a damaged blood brain barrier, induction of adhesion molecules on the CNS vasculature[158], direct damage to myelin and oligodendrocytes[355] and induction of other inflammatory cytokines[96, 454]. Macrophages further phagocytose damaged myelin sheaths[208, 305, 325].

Depletion of macrophages prior to the onset of the clinical signs in EAE significantly reduces both the clinical[47, 178, 179] and pathological [400] manifestations of the disease. Berger *et al* further showed that disease severity is correlated with the absolute number of macrophages invading the CNS parenchyma.[26] Similar results have been obtained using newer chemotherapeutic agents that inhibit macrophage activation.[253]

The resident microglial cells within the central nervous system are also thought to play an important role in the immunopathology of EAE[123, 256, 415] although this is less well studied. Myelinophagic microglial cells appear at early stages in the disease process.[277] The presence of microglial cells in demyelinating plaques suggest a phagocytic role although they may have other roles such as antigen presentation and/or destruction of myelin and oligodendrocytes.[277] Parenchymal microglial cells may upregulate the expression of MHC class II antigens during EAE.[123, 256, 417] In both acute EAE and the first attack of chronic relapsing EAE, activated microglial cells demonstrate phagocytosed myelin structures.[22, 337] Interestingly, while this population of cells makes up 7 to 15% of immunocompetent cells within the inflammatory infiltrates, the activated microglial cells appear to be more effective in the removal of myelin debris then their haematogenous counterparts.[337] This is supported by in vitro studies demonstrating that cultured microglia appear to phagocytose myelin more actively than peritoneal macrophages. However, while activated microglial cells may share many macrophagic capabilities, the microglial cells do not appear to take over as effector agents when peripheral macrophages are depleted.[23]

AIMS AND HYPOTHESES

Hypotheses

1. Macrophages in experimental rat syringomyelia are derived from the bone marrow rather than resting microglia.
2. Macrophages that accumulate within the spinal cord parenchyma, subsequently migrate towards the central canal of the spinal cord.

Aims

1. To establish rat radiation bone marrow chimeras.
2. To determine the origin of macrophages in a previously established rat model of noncommunicating syringomyelia.
3. To study the time course and pattern of macrophagic infiltration.

METHODS

1 General aspects

1.1 Ethics Approval

The current study was reviewed and approved by both the Animal Ethics Committee of the Institute of Medical and Veterinary Science/Royal Adelaide Hospital (Project 79/98) and the University of Adelaide (Project M/71/98).

1.2 Animals used

The F1 hybrids (DAxPVG/c) that served as bone marrow donors were generated in an animal colony at IMVS under the direction of Professors G Mayrhofer and L Cleland (Adelaide, South Australia). Adult DA (RT7.1) rats were supplied by the IMVS Animal Breeding Facility (Gilles Plains, South Australia) while PVG/c (RT7.2) rats used for the initial cross-breeding purposes were acquired from the Animal Resource Centre (Perth, Western Australia).

1.3 Special facilities

RT7.1 DA rats that were irradiated were transported to the IMVS animal barrier room (Frome Road, Adelaide) approximately one week prior to the commencement of the experiments. They were then kept behind barrier continuously until the time of surgery at

about 6 weeks. A constant laminar air flow was maintained in the barrier room under constant positive pressure.

2 Generation of rat radiation bone marrow chimeras

2.1 Pilot Study

This was performed to ensure that rat radiation bone marrow chimeras could be established successfully (FIGURE 4)

2.1.1 Animals used

12 male RT7.1 DA rats (194-212 gms) were used in this initial experiment. These animals had been reared in a pathogen-free environment and were transported to the IMVS barrier room 4 days prior to the radiation treatment. Donor marrow was harvested from 2 RT7.2 congeneic DA rats.

2.1.2 Antibiotic prophylaxis

The antibiotic regimen was advised by the Dr Tim Kuchel, veterinarian, IMVS. Accordingly, the rats received subcutaneous injections of gentamicin (Gentamicin 50, RWR Veterinary Products Pty Ltd) twice daily commencing 4 days prior to the irradiation date and continued for 10 days afterwards. The total daily gentamicin dosage was calculated at 0.2 mg/kg for each rat.

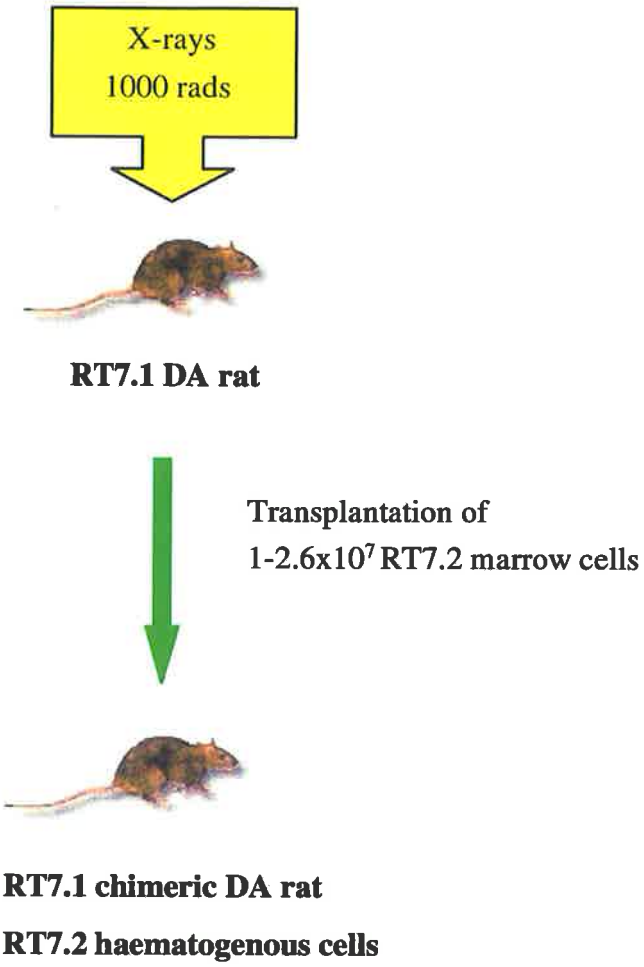

Figure 4 . *Protocol for establishing rat radiation bone marrow chimeras.*

2.1.3 Irradiation protocol

A Philips Deep X Ray Unit (250 Kilovolts/15 milli-amps) on free control, without added filter was utilised (half value thickness of 0.7 mm of Copper) to administer the irradiation. The dosage was calculated at approximately the mid point of the rat. A total of 10.00 Gray was administered over 10.51 minutes.

Each rat was transported from the barrier room to the Radiotherapy Unit at the Royal Adelaide Hospital in individual standard perspex containers (dimensions 10x28x8 cm) on the morning of the irradiation treatment. Each container had a glass lid with ventilation holes which were subsequently overlaid and sealed with 2 layers of filter paper thus preventing contamination of pathogens during transfer.

The 12 rats were irradiated in pairs in 6 consecutive treatment sessions. During each treatment, 2 perspex boxes (housing 2 rats) were placed alongside each other within the radiation field of 28x28 cm. The animals were promptly returned to the barrier room at the completion of the radiation treatment.

2.1.4 Preparation of donor bone marrow

The RT7.2 congeneic donor rats were anaesthetised prior to being euthanased with a lethal intra-peritoneal injection of barbiturate.

The long bones of the hind limbs were then dissected cleanly from the attached muscles and soft tissues.The ends of the femurs and tibias were excised. Each bone was then flushed with PBS/5% fetal calf serum to remove the bone marrow. A cell suspension was then created by repeated gentle aspiration and flushing through a 22 G syringe into a petri dish. The suspension was then filtered through a ball of cotton wool to remove any bony fragments. Subsequently, the test-tube was centrifuged at 1000 rpm (500G) for 10 minutes. The supernatant was discarded and the pellet re-suspended in fresh PBS/5% fetal calf serum solution to make up 10 ml of marrow cell suspension in all.

A manual cell count was finally performed under direct microscopy.

2.1.5 Intravenous transfer of donor cells

Approximately 2 hours following the irradiation procedure, each irradiated RT7.1 DA rat was anaethetised prior to injection of the cell suspension via the ventral tail vein. Each rat received 0.8 mL of injected suspension (equivalent to 1×10^7 bone marrow cells).

2.1.6 Subsequent care

Upon recovery from anaesthesia, each animal was restored to its cage. The rats were initially housed in individual cages but were subsequently housed in groups of 3 from Day 5. The rats were carefully examined and weighed daily for 3 weeks. Detailed records were maintained using standard post-irradiation IMVS AEC clinical record sheets. Thereafter,

the animals were reviewed weekly. The animals were maintained behind barrier until the time of surgery.

2.2 Main study

2.2.1 Animals used

30 male RT7.1 pathogen-free DA rats (231-284 gms) were used in the main study. The irradiation experiments were carried out on 2 separate days, involving 12 and 18 rats respectively. A total of 10 donor RT7.2 DA rats provided bone marrow for transplantation. Each irradiated rat received bone marrow which had been harvested from the long limbs of 3 donor rats.

2.2.2 Antibiotic prophylaxis

A change in antibiotic regimen was advised by Dr Tim Kuchel, veterinarian, IMVS. For 2 days prior to irradiation, soluble oxytetracycline (10 gm/Litre,Tetravet 100) was administered in drinking water. Subcutaneous injections of oxytetracycline (20 mg/Kg body weight, Terramycin L/I, Pfizer Animal Health) were subsequently performed on Day 1 and Day 4 following the irradiation procedure. From Day 7, soluble oxytetracycline was again administered in drinking water for a further 7 days.

2.2.3 Animal handling and transportation

New perspex containers with improved lids were used. These allowed for easier handling and transportation. Each container was further divided into two equal halves by a plastic separator, thus confining one rat to each compartment. This modification of the perspex container minimised movement of the animals during the radiation treatment. The animals

were irradiated in pairs over 6 and 9 consecutive treatment sessions respectively over 2 separate days.

2.2.4 Irradiation protocol

The dosage of irradiation was re-defined and calculated at the exit point of the rat. This ensured that the radiation administered to each rat did not exceed 1000 rads in any part of the animal. Irradiation protocol details were otherwise as previously described (section 2.1.3).

2.2.5 Preparation and transfer of donor bone marrow

Bone marrow was harvested from 10 RT7.2 donor rats and infused intravenously as previously described. Each recipient in the initial group (12 rats) received 2×10^7 bone marrow cells. The later group of 18 rats received 2.6×10^7 marrow cells each.

2.2.6 Subsequent care

Daily subcutaneous injection of sterile isotonic saline was performed (5 mLs in each flank) for a total of 4 days following the irradiation procedure. The animals were examined and weighed daily for 3 weeks and weekly thereafter. Animals that became obviously unwell were euthanased. Animals that experienced a weight loss of more than 20% of their original weights in the first 5 days were closely scrutinised by the researcher in conjunction with experienced animal care workers and the IMVS veterinarian.

3 Intraparenchymal kaolin model of syringomyelia

3.1 Animals used

Thirty DA chimeric rats (6 weeks post-irradiation) were used (FIGURE 5) Kaolin was injected into the spinal cords of 30 rats. A needle puncture (injection without kaolin) was performed in 2 rats. The remaining 3 rats received no injection and were used as control rats.

Fifteen standard, non-irradiated RT7.1 DA rats were also used (FIGURE 6) Kaolin injections were performed in 14 rats. One rat received an injection without kaolin.

3.2 Kaolin preparation

The kaolin suspension used for intra-parenchymal spinal cord injection was prepared by adding 2.5 gms of non-autoclaved Georgia kaolin or hydrated aluminium silicate (Sigma Chemical Corporation) to 10 mLs of normal saline. The suspension was prepared freshly each morning.

3.3 Kaolin injection

Anaesthesia was induced by placing each rat in a perspex chamber that had been pre-saturated with a mixture of 4% isoflurane and oxygen at 2 Litres/min. Each rat then continued to self ventilate through a nose cone on a 2.5% isoflurane/oxygen mixture.

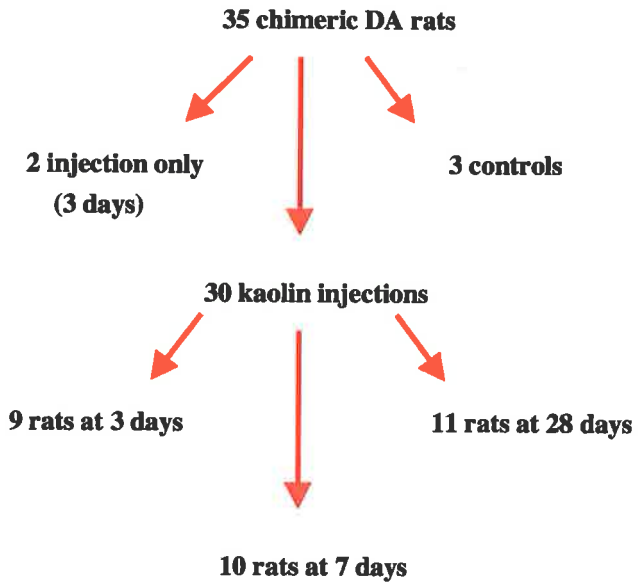

Figure 5. *Use of RT7.2 chimeric DA rats (3, 7 and 28 days indicate sacrifice intervals).*

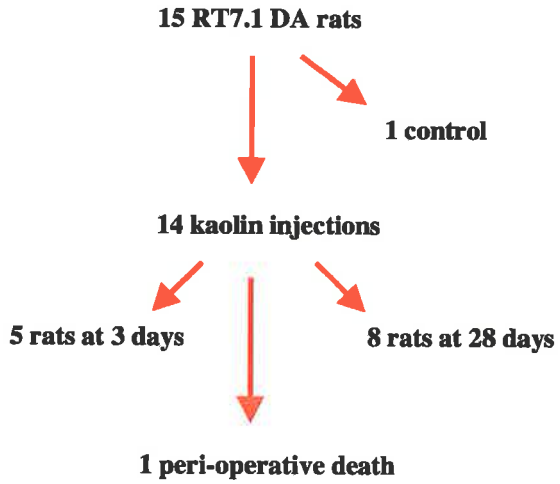

Figure 6. *Use of commercially available RT7.1 DA rats (3 and 28 days indicate sacrifice intervals).*

Adequate depth of anaesthesia was assessed by performing the pedal withdrawal test at the start of the procedure. During surgery, the depth of anaesthesia was monitored by frequent assessments of inspiration depth and frequency.

The animal was supported across the thorax by a small soft drape roll. This produced a gentle flexion of the cervical spine which made subsequent surgery easier.

Aseptic surgical technique was used throughout the operation. Having prepared the shaved skin over the cervical region with betadine, the operative field was draped appropriately. A vertical mid-line skin incision of approximately 2 cm was performed to end caudally just beyond the palpable vertebrae prominens. Having also incised the midline raphe of the posterior cervical muscles, a relatively bloodless plane could be identified in the midline. This allowed the para-spinal muscles to be dissected off the C5 to C7 spinous processes and lamina with relative ease. Dissection was extended to the level of T1 only if deemed necessary during the operation. Upon adequate exposure of the laminae, a laminectomy were performed at C6 to expose the dura overlying the spinal cord under microscopic vision. Haemostasis was achieved without electro-cautery.

The stereotactic frame which had been mounted with a 10 μL Hamilton syringe with a 30 gauge point style 4 needle was then brought into the operative field. The needle tip of the syringe was then localised over the ideal site (in the dorsal columns) and lowered onto the dura without making any attempt to puncture it. A 29G needle on a 0.5 mL syringe (B-D, Ultra-fine) was then used to cautiously puncture the dura and underlying arachnoid at this

point. The needle of the Hamilton syringe was inserted to an approximate depth of 1 mm into the spinal cord, with the bevel pointing laterally. 1.5 μL of kaolin suspension was gradually injected over 60 seconds while ensuring that there was no significant reflux into the subarachnoid space.

The needle was finally removed and the stereotactic frame swung out of the operative field. The wound was closed in 2 layers using 3/0 Vicryl sutures.

3.4 Post-operative care

Following the cessation of anaesthesia, each animal was left to recover while breathing oxygen at 2 Litres/min for approximately 2 minutes prior to restoring the animal into its individual cage in the lateral position. Subcutaneous butorphanol tartrate (10 mg/mL Dolorex, Intervet (Australia) Pty Ltd, Castle Hill, NSW) was administered for post-operative analgesia.

3.5 Post-operative review

Each animal was reviewed clinically on a daily basis for the initial 2 weeks following the operation. Close attention was also paid to mobility and feeding behaviours of each animal. Daily weighings were also carried out until they were sacrificed at the pre-determined sacrifice time intervals or their pre-operative weights were restored.

4 Tissue removal from the rats

Under deep anaesthesia (isofluorane), the old incisional wound was re-opened. The skin incision was then extended linearly over the dorsal aspect of each rat to expose the whole spinal column. Laminectomies were subsequently performed at all cervical and thoracic levels to expose the spinal cord. When adequate exposure was obtained, the nerve roots were cautiously divided bilaterally. Finally the spinal cord was transected distally and then proximally at the base of the skull.

The animal was then immediately turned over and a laparotomy performed. The spleen was dissected free from its mesenteric attachments and harvested.

5 Tissue processing in developmental phase of study

5.1 Freeze Drying

Neural and splenic tissues were harvested as described previously (section 4). These tissues were placed on a stainless steel tray (10x10x1 cm) and frozen by flotation of the tray on liquid nitrogen. The tissues were then stored at -70 degrees Celsius until freeze drying was performed.

The frozen tissue samples were placed inside the cavity of a sublimator (Modult, Edwards) and maintained at -40 degrees Celsius throughout the freeze drying period. The cavity vaccuum was kept at $<10^{-2}$ mTorr for 3 days. The refrigeration was then switched off and the chamber temperature allowed to equilibrate to ambient room temperature while still under vacuum. The tissue samples were collected immediately following the release of vacuum and embedded in paraffin. The tissue specimens were immersed in paraffin wax for 30 mins, 1 hour and 2 hours respectively under normal atmospheric pressure.

5.2 Paraffin sections

Paraffin sections were only used during the initial developmental phase of the project for both histology and immunohistocytochemistry.

5.2.1 Tissue preparation

The spleens from the two RT7.2 donor DA rats (in the pilot study) were harvested and fixed in 4% paraformaldehyde for 5 days. The tissues were subsequently processed through a series of graded alcohol to paraffin with xylene as a clearing agent. The blocks were then embedded in paraffin wax. Six μm tansverse sections were cut using a microtome prior to mounting on glass slides.

5.2.2 Histology and immunohistochemistry

Tissue sections were either stained with haematoxylin and eosin in the standard manner or prepared for immunostaining. Paraffin sections destined for immunohistochemical studies were first de-paraffinised and rehydrated. Immunostaining procedures were then carried out using the method described in Section 6.1.3.1. The primary antibodies used were directed against RT7.2 (HIS41) and ED1.

6 Tissue processing in the main study

6.1 Fresh frozen sections

As immunohistochemical studies performed during the developmental phase of the study demonstrated that the RT7.2 antigen was not stable to paraformaldehyde fixation, fresh frozen sections were used for the main study.

6.1.1 Freezing techniques

Both the spinal cord and the splenic tissues were sectioned into longitudinal blocks each measuring approximately 10 mm. Tissue segments were embedded in OCT contained in 15x15x5 mm disposable vinyl specimen molds (Tissue-Tek, Miles). Each mold was then promptly immersed in a bath of octapentane precooled within a chamber of liquid nitrogen.

The tissue/OCT blocks were then stored at -70 degrees Celsius until ready for use.

6.1.2 Histology

Cryostat sections of 5µm thick were cut, mounted on slides and air-dried. Sections for light microscopy were stained with haematoxylin and eosin in the standard manner.

6.1.3 Immunohistochemistry

6.1.3.1 Immunoperoxidase staining procedure

Tissue sections destined for immunostaining were fixed in acetone for 10 minutes and air-dried overnight. Primary antibodies ED1 and HIS41 were obtained from Serotec (Oxford, UK). The secondary species-specific peroxidase linked anti-mouse immunoglobulin was from Amersham International (Buckinghamshire, England). Normal rat serum (10%) was added to all antibodies to minimise non-specific staining.

Slides were initially re-hydrated in PBS after wax circling the tissue sections with a PAP PEN (Zymed, San Francisco, CA, USA0. These were then exposed to 3% hydrogen peroxide/PBS for 5 minutes. The tissue sections were then washed in PBS and incubated with the appropriate primary antibody for 1 hour.

Antibody	Use	Dilution
ED1 (Serotec)	immunohistology	1/400
HIS41 (Serotec)	immunohistology	neat
HIS41 (Pharmingen)	flow cytometry	1/20
Secondary (Amersham)	immunohistology	1/25
Secondary (Pharmingen)	flow cytometry	1/50

Table 2. Antibodies used for immunohistochemistry.

After two further washes in PBS, the secondary antibody was applied at a dilution of 1/25 and incubated for a further 1 hour, prior to visualisation with nickel 3,3'-diamino-benzidine (DAB).

6.1.2.1 Indirect Immunofluorescent Staining

Cryostat sections of 5 μm stored at -70 degrees Celsius were thawed. They were then fixed in acetone for 10 minutes and allowed to air dry overnight.

Slides were initially re-hydrated in PBS after wax circling the tissue sections with a PAP PEN (Zymed, San Francisco, CA, USA0. These were then exposed to 3% hydrogen peroxide/PBS for 5 minutes. The tissue sections were first incubated for 1 hour with ED1 (dilution 1/400) (Serotec). The slides were then washed twice in PBS after which they were incubated with the secondary donkey anti-mouse-specific-Texas Red (dilution 1/100)

(Rockland Laboratories, US) for another hour. Following 2 further washes, the sections were exposed to 50% normal mouse serum for 30 minutes to block the free valencies present on the secondary antibodies previously applied. Finally, the second primary antibody HIS (directly conjugated to FITC) (dilution 1/50 in 50% normal mouse serum) (Serotec, UK) was added and incubated for another hour. After 3 final washes in PBS, the slides were mounted using 'antifade' aqueous mountant solution.

6.1.2.2 Assay of chimerism

The spleens of all chimeric rats were removed at the time of sacrifice and stored at -70 degrees Celsius. Chimerism was assessed by examining splenic tissue sections derived from 3 randomly selected chimeric rats. Immunostaining was performed using monoclonal antibody HIS41 against RT7.2. In the chimeras, the transplanted cells were $RT7.2^{+}$ whereas the host cells were $RT7.2^{-}$.

6.1.2.3 Counting of immunoreactive cells

6.1.2.3.1 Spinal cord tissues

The distribution of immunoreactive cells within the parenchyma of the spinal cord was carefully studied. Typically, the kaolin droplet was visible to the naked eye on 3 to 4 transverse sections. A representative slide was chosen in which the cross sectional area of the kaolin droplet was the largest. Manual counts of ED1 and HIS41 positive cells were then performed on separate but nickel DAB- stained sections at the same level of chimeric

rats. This proved to be reliable on comparison with the double-labelling immunofluorescence studies.

In the 3-day group, evaluation was performed directly on the immunofluorescence slides. This was because there was a relatively small but nonetheless significant component of $RT7.2^+$ but $ED1^-$ cells which represented a combination of neutrophils and lymphocytes. Counts performed on the nickel DAB stained sections would have led to an overestimation of $RT7.2^+$,$ED1^+$ cells. Manual counts of ED1 positive cells in tissue sections from non-chimeric rats were performed only on Nickel DAB sections.

Two methods of manual counting were used. Firstly, the *total* number of ED1+ cells within the entire selected spinal cord section was evaluated. Secondly, cell counts were performed within a fixed area at the edge of the kaolin lesion under high magnification (100Xs). This was achieved by using an objective microscopic eyepiece which had been pre-embedded with a small grid in the centre of the visual field. At least 3 different areas were counted in each case and an average derived.

6.1.2.3.2 Splenic tissues

The RT7.2 immunostaining patterns in splenic tissue sections were carefully studied. Cell counts of immunoreactive cells in both myeloid and lymphoid regions were performed under high magnification (X100) in 3 randomly selected fields from which an average count was derived.

6.1.2.4 Statistical analysis

Statistical comparisons of cell counts between chimeric and non-chimeric rats were performed using 2-tailed Student t-tests ($p<0.05$). Calculations were performed using Microsoft Excel Software.

6.1.2.5 Photography

Conventional photomicrographs were taken using a Nikon F601 camera mounted on a BX50 Olympus microscope. The film used for conventional photography was Kodak Ektachrome EPY 135-36.

Immunofluorescence analysis of tissue sections was performed using an Olympus BX50 fluorescent microscope. Immunofluorescence photography was carried out using a digital camera (Sony, SSC-M370CE B&W CCD) set up which was attached to a 7600/200 Power Macintosh computer. The software utilised was NIH Image v1.60b7. Images (Grey scale) were viewed in real time and captured as TIFF files. For illustrations in the current thesis, the pictures were colourised using Adobe photoshop, Version 5.0 Software to reflect the true colour under direct microscopic vision.

6.2 Flow Cytometric Analysis

Flow cytometry on peripheral blood was performed only during the pilot study. The nine surviving rats in the pilot study were tested at 3 weeks post-irradiation/bone marrow transplantation.to confirm that successful chimerism was being established with the existing study protocol.

6.2.1 Collection of blood specimens

Under anaesthesia, 400 µL of blood was collected from the ventral tail vein of each of the 9 rats using a heparinised 23G needle and temporarily stored in plastic containers containing EDTA. Blood was also collected from RT7.2 non-irradiated DA rats and RT7.1 DA rats. These served as both positive and negative controls respectively.

6.2.2 Indirect labelling of cells with monoclonal antibodies

All labelling steps were performed at 4°C. Fifty µL of primary antibody (either HIS41 or OX41(control)) containing 10% (v/v) heat inactivated NRS was added to 100 µL of whole blood in each FACS tube (Falcon, Becton Dickson Labware, New Jersey, USA, Cat. No. 2008). The cells were then incubated on ice for 1 hour. Following this incubation, the cells were washed twice with immunoflorescence buffer (1L PBS, 20 ml FCS, 10 mL 1M Azide) and resuspended in 50 µL of FITC-conjugated secondary antibody (1/50 dilution, FITC conjugated goat anti-mouse polyclonal Ig in 10% NRS). After a further incubation of 1 hour, the cells were then washed again with IF buffer. The cells were then stored at 4°C prior to flow cytometric analysis

6.2.3 Flow cytometry

Labelled cells were analysed using a FACSan (Becton Dickinson, San Jose, California) equipped with CellQuest software (versions 1.2 and 3.1f). Fluorescence and forward light scatter (FLS) profiles were accumulated from 1 x 10^5 cells. The proportion of cells specifically labelled was estimated as those with fluorescence intensity greater than 98% of control cells labelled with an appropriate irrelevant immunoglobulin.

RESULTS

1 Flow cytometry

Flow cytometry studies were only performed in the initial pilot study. Tests performed at 3 weeks post irradiation/marrow transplantation showed that RT7.1/RT7.2 chimerism was successfully established in all nine surviving study rats in the pilot study. The results of labelling with HIS41 are displayed in Table 3.

	HIS41 positivity,%
1.	65.3
2.	56.7
3.	70.3
4.	51.2
5.	66.0
6.	71.5
7.	29.7
8.	71.5
9.	47.6

Table 3. *Flow cytometric results from testing of peripheral blood in pilot study.*

2 Outcome in establishing rat radiation chimeras

2.1 Pilot study

One rat was euthanased on Day 5 as it appeared clinically unwell. Two other rats were subsequently found dead on Day 6 post-irradiation.

Autopsies on the three deceased rats were performed by Dr J W Finnie, Veterinary Pathologist, Veterinary Services Division, IMVS. The salient pathological findings were those of radiation-induced bone marrow depression and gastro-intestinal haemorrhage.

All the other nine surviving rats sustained a weight loss of 15-20% over the first 5 days. While most of the rats displayed ruffled fur and showed evidence of reduced drinking and eating during this time, all of them remained reasonably active. They began to regain their weights after Day 6 and the majority of animals were near to or had surpassed their initial weights at two weeks.

2.2 Main study

The rats were irradiated in groups of 12 and 18 respectively on separate days.

In the first group of 12 animals, there were no deaths. All rats were mildly dehydrated for several days post-irradiation but remained well and active.

In the second group of 18 animals, 3 rats were euthanased between Days 4-6 because they were clinically unwell. One rat was found dead in the cage. Thus the overall mortality was 13%. A similar pattern of initial weight loss and subsequent gain in the second week was observed. Similarly, the surviving rats remained active throughout the period of observation.

3 Developmental issues in tissue processing

3.1 Freeze drying

This technique of tissue processing was not utilised in the main study. The limited preliminary results obtained during the developmental phase of the study showed that the paraffin wax did not penetrate the tissue blocks adequately. Beyond the most superficial 3-4 mm, the internal architectural structure of the tissues (both spleen and spinal cord) was severely disrupted when the cut surface of the tissue block was examined under a 10xs magnification dissecting microscope. It was also not possible to cut tissue sections using the microtome in the conventional manner.

3.2 Problems encountered in initial immunostaining testing of HIS41

Initial immunological testing was performed on paraffin sections of splenic tissues of RT7.2 DA rats in accordance with recommendations of the antibody supplier (Serotec). Immunostaining using HIS41(neat) revealed only minimal patchy staining of clumps of nucleated cells.

Parallel testing on paraffin sections of spleen was also carried out using a sample of HIS 41 (purified antibody at 0.5 mg/ 0.5mL) from a different manufacturer (Pharmingen). Similarly, no evidence of immunostaining was observed at dilutions of 1/20, 1/40, 1/80.

4 Main study

4.1 Surgical outcome

One non-chimeric rat died from anaesthetic related problems. It suffered a cardio-pulmonary arrest at the end of the surgical procedure. The autopsy of the animal did not reveal any surgical complications. No neurological sequelae were observed in all other rats that underwent surgery.

4.2 Exclusion of tissues from analysis

The crucial tissue blocks of spinal cord in a RT7.1 DA rat suffered severe fractures during the freezing process, thus precluding any further tissue anaysis. Cell counts were not performed in one chimeric DA rat because the volume of kaolin injected was clearly disproportionately small and deemed insufficient. This was attributed to mechanical problems with the Hamilton syringe/needle encountered during the operative procedure in this particular rat. The tissues derived from the rat that died peri-operatively were also excluded from the study.

4.2 Histology

4.2.1 Control chimeric rats

Each cervical spinal cord contained a short segment of central canal in which a small ovoid or slit like lumen could be identified readily. Extending caudally beyond this segment, the central canal was collapsed without a distinct lumen. The ependymal epithelium mostly consisted of 2 layers of cells in close apposition (FIGURE 7). In some sections, the ependymal epithelium was folded.

4.2.2 Kaolin injections in chimeric rats

4.2.2.1 Central canal

At 3 days following the intraparenchymal injection of kaolin, subtle abnormalities of the central canal were observed. These changes included distortions of the ependymal epithelium and alteration of the shape of the central canal. However, none of the central canals appeared to be abnormally enlarged.

Dilated segments of the central canal were seen at one week and 4 weeks following the intra-parenchymal kaolin injection (FIGURE 8). Although this typically occurred rostral to the site of injection, the dilated canal occasionally extended caudally past the point of kaolin injection. The location and length of the syrinx was variable and unpredictable. Synechiae and occasional cells were seen within the central canal.

At 4 weeks, the enlarged central canal was occluded by large aggregates of kaolin particle-laden macrophages in some animals (FIGURE 9). This reached massive proportions at some segments and extended over many segments of the spinal cord. In the other animals, such a macrophagic inflammatory infiltrate was not observed within the central canals of their spinal cords.

Massive central canal distension was not observed in any of the animals studied.

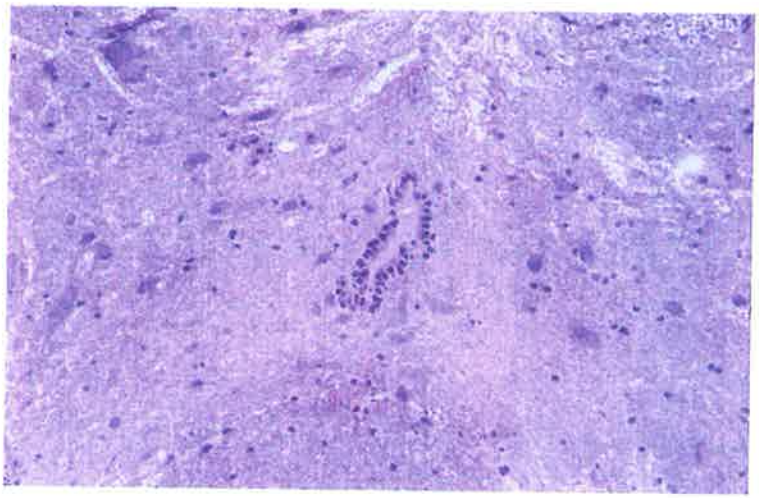

Figure 7. *Photomicrograph of transverse spinal cord section demonstrating the appearance of a normal central canal at C6 in a control chimeric rat. H&E, X50.*

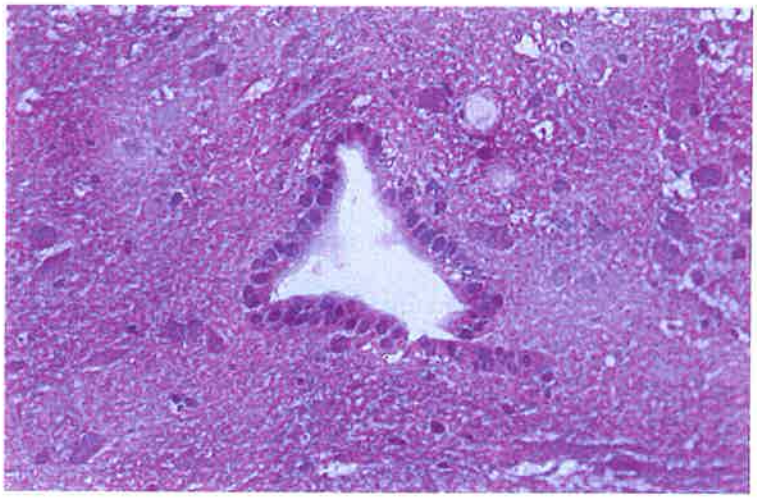

Figure 8. *Photomicrograph of a transverse spinal cord section from a chimeric rat sacrificed at 1 week following kaolin injection. The central canal is significantly dilated. H&E, x100.*

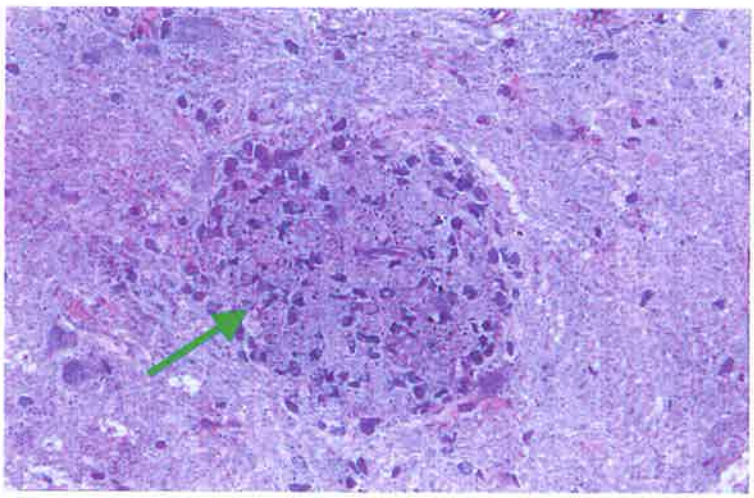

Figure 9. *Photomicrograph of a transverse spinal cord section from a rat sacrificed at 4 weeks. The central canal has been totally occluded by kaolin-laden macrophages (arrow). H&E, x100.*

4.2.2.2 Site of injection

At the level of injection, the kaolin droplet was readily detected in the dorsal columns and the central grey matter of the spinal cord (FIGURE 10). This was visible to the naked eye and typically occupied 3 to 4 levels (each 250μm apart) of tissue sections. At 3 days, an inflammatory infiltrate could be seen on the edge of the kaolin lesion. By 1 week, a relatively mature granuloma had formed with a distinct wall of mononuclear cells. At 4 weeks, extensive mononuclear migration had occurred into the kaolin droplet. Free kaolin was no longer observed.

4.3 Immunohistochemistry in spinal cord tissues of chimeric rats

4.3.1 ED1

4.3.1.1 Morphological features

Immunostaining of ED1 yielded an intense cytoplasmic staining pattern within the majority of immunoreactive cells (FIGURE 11). Although cytoplasmic membrane staining was not evident with routine dye (nickel DAB) technique, this was visualised under immunofluorescence (FIGURE 12).

The morphological appearances of $ED1^+$ cells were varied. The immunoreactive cells fell into 2 broad categories. Firstly, large, round (or ovoid) cells with foamy cytoplasm

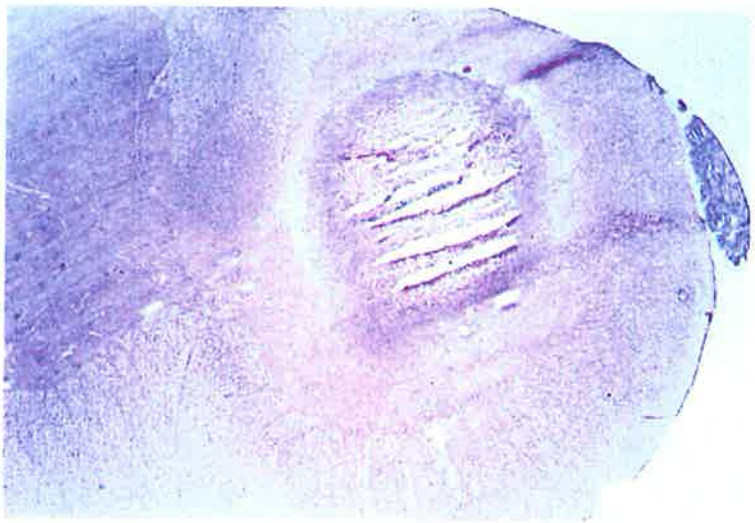

Figure 10. *Photomicrograph of transverse spinal cord section at the level of C6. The kaolin droplet has been injected into the dorsal columns and the central grey matter of the cord parenchyma. H&E, X25.*

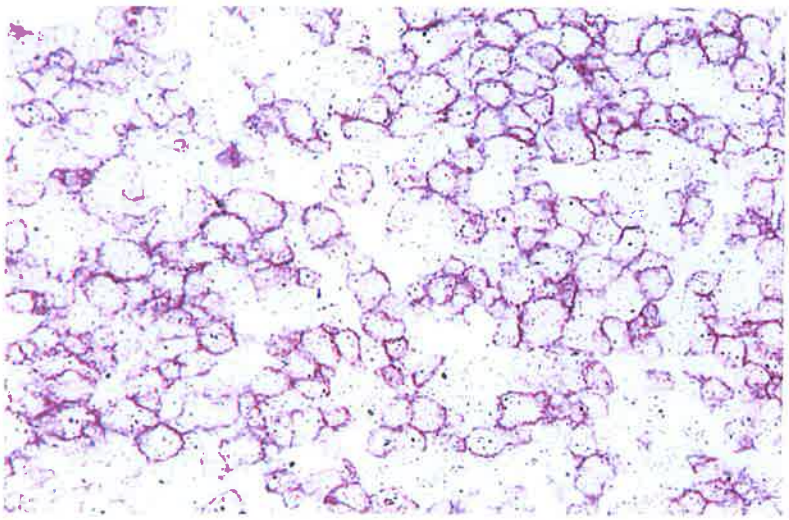

Figure 11a . *Photomicrograph of RT7.2 positive cells within the kaolin droplet at 1 week. The cytoplasmic membrane immunostaining pattern is clearly demonstrated. Nickel DAB, x100.*

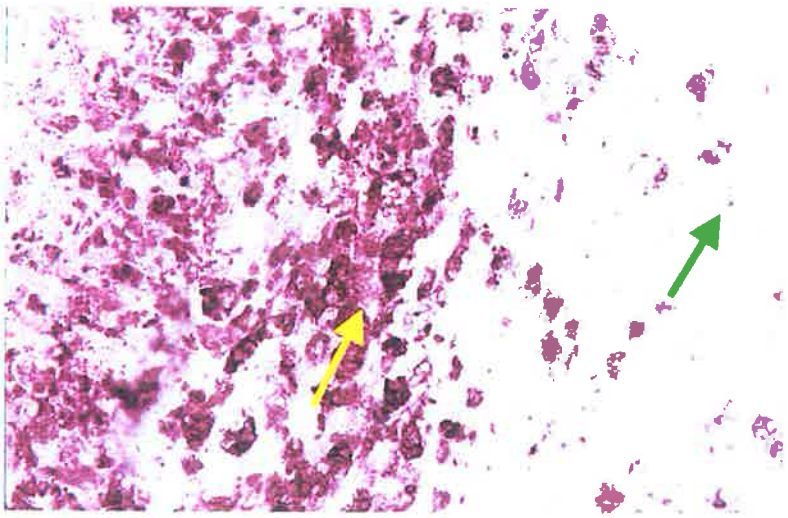

Figure 11b. *Photomicrograph of ED1^{+} cells on the edge of the kaolin droplet on a rat sacrificed at 1 week. The larger cells with intense cytoplasmic staining (yellow arrow) are contrasted with smaller cells more distant from the lesion (green arrow). Nickel DAB, x100.*

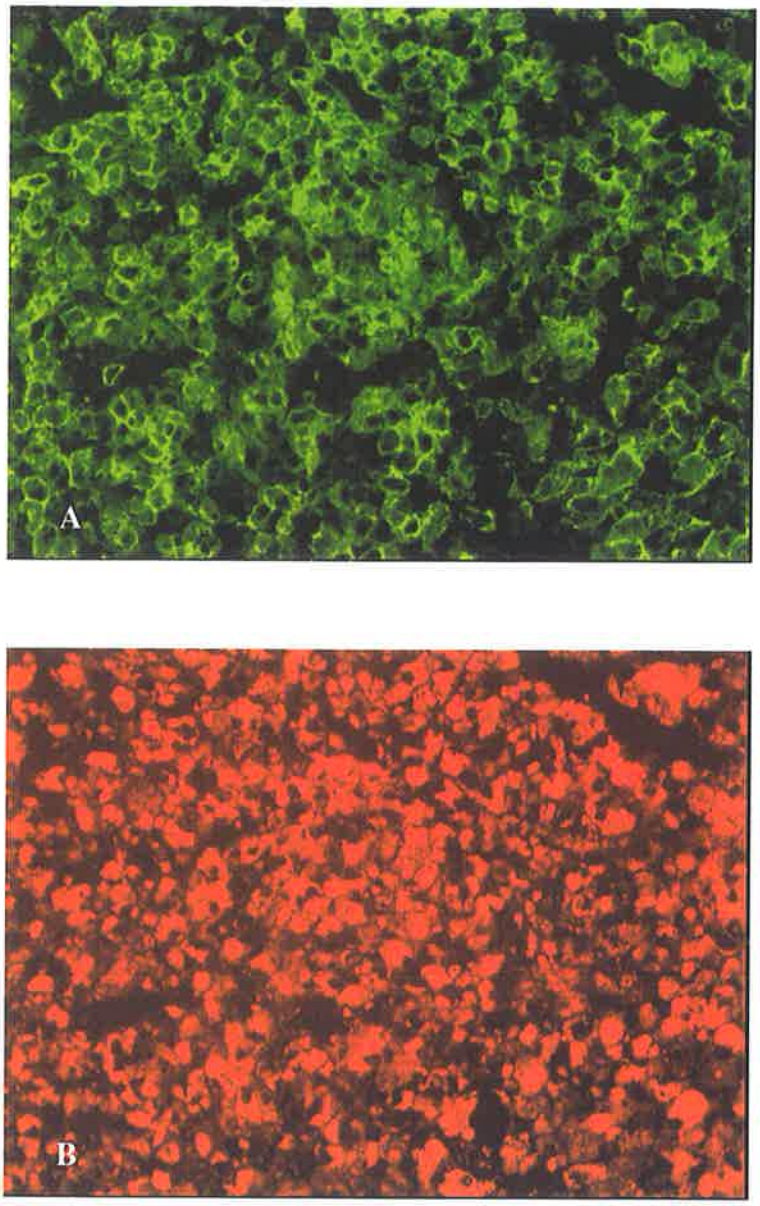

Figure 12. *Immunofluorescence patterns of RT7.2 and ED1 antigens. The typical cytoplasmic membrane staining pattern of RT7.2$^+$ cells is well seen in (A).FITC, X100.This is contrasted with the more intense, cytoplasmic staining in ED1$^+$ cells in (B). Texas Red, X100.*

resembling classical macrophages could be identified. These were easily distinguished from other smaller, microglial-like cells with denser staining cytoplasm and processes which were scattered within the spinal cord parenchyma. However, many cells displayed intermediate morphological features and these could not be readily classified into either of the above categories.

4.3.1.2 Pattern of immunoreactivity

Large numbers of ED1 immunoreactive cells were seen within the spinal cord parenchyma at the level of the kaolin injection even at Day 3. Many large cells with intense cytoplasmic staining had accumulated around the kaolin droplet. These cells were also seen along the needle track extending from the pia into the kaolin lesion. Away from the kaolin lesion, progressively fewer immunoreactive cells were observed in the parenchyma. The immunoreactive cells located furthest away from the kaolin droplet were typically small and thin cells with occasional cytoplasmic processes.

The extent of inflammation was most marked at 1 week (FIGURE 13). A dense rim of $ED1^+$ cells had walled off the kaolin droplet. In some rats, the $ED1^+$ cells appeared to extend centripetally inwards (toward the central canal) within the cord parenchyma. The majority of these cells (towards the central canal) were of the smaller, microglial-like cell type.

By 4 weeks, three distinct patterns of macrophage accumulation were evident.

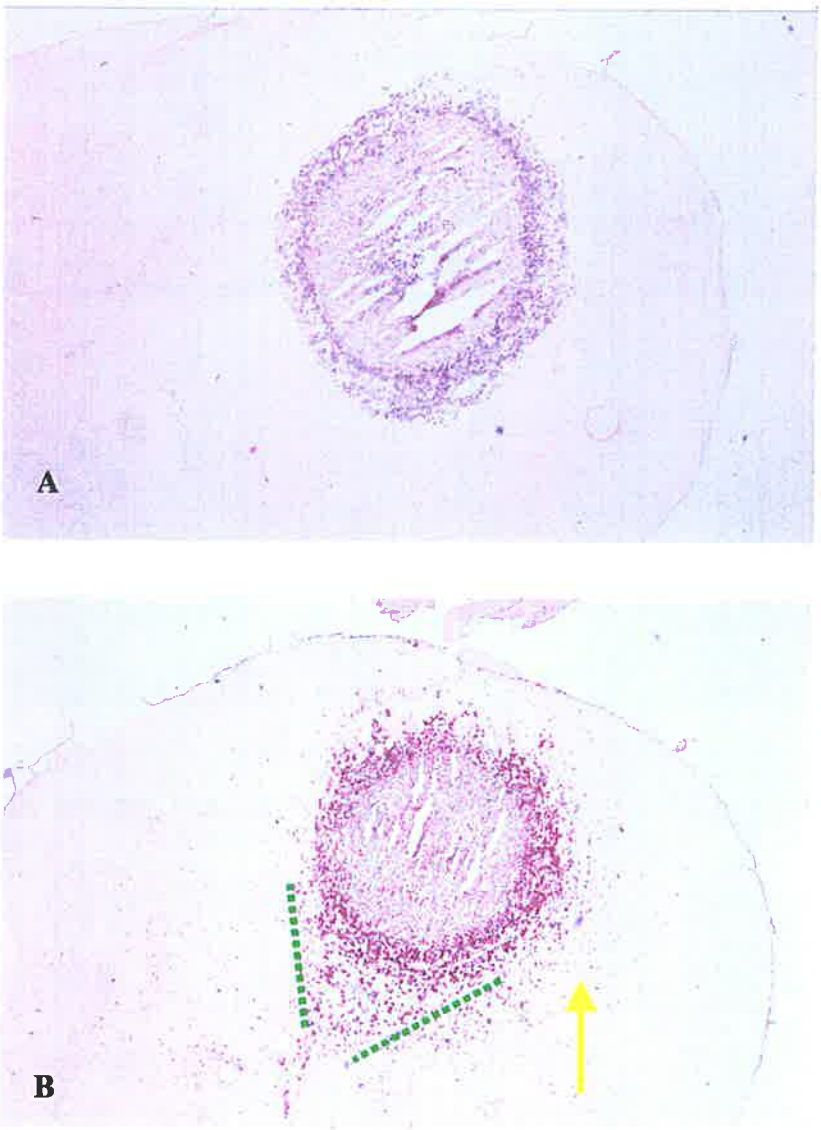

Figure 13 . *Photomicrographs of spinal cord sections from a chimeric rat sacrificed at 1 week following the kaolin injection. (A): Transverse section at C6 demonstrating the 'walling' effect of RT7.2 positive cells around the kaolin droplet. Note the absence of immunoreactive cells within the parenchyma away from the kaolin droplet (B): Transverse section showing the more florid response of ED1 positive cells, particularly in the parenchyma of the spinal cord (yellow arrow). In addition, the centripetal pattern of ED1 immunoreactivity towards the central canal is noted (green lines).*

Firstly, the kaolin droplet was totally engulfed by ED1 positive cells although the staining intensity of each cell had diminished quite markedly in comparison with previous time intervals (FIGURE 14). Around the granuloma, there was an obvious rim of strongly positive cells which had the morphological features suggestive of classical macrophages (large, round with foamy cytoplasm). Beyond this, a large number of progressively smaller ED1 positive cells with intermediate morphological features were seen scattered throughout the cord parenchyma. These cells extended rostrally and caudally for multiple levels beyond the lesional site (FIGURE 15).

Secondly, in some animals, the enlarged central canal was totally occluded by an extensive accumulation of large, round ED1 positive cells. These cells also exhibited less marked staining intensity.

Lastly, it was observed that away from the kaolin injection site the ED1 positive cells appeared to be preferentially located within the white matter of the spinal cord (FIGURE 16). This is particularly evident in some tissue sections (both rostral and caudal to the kaolin droplet) where these immunoreactive cells were only confined to the white matter of the cord contralateral to the site of injection.

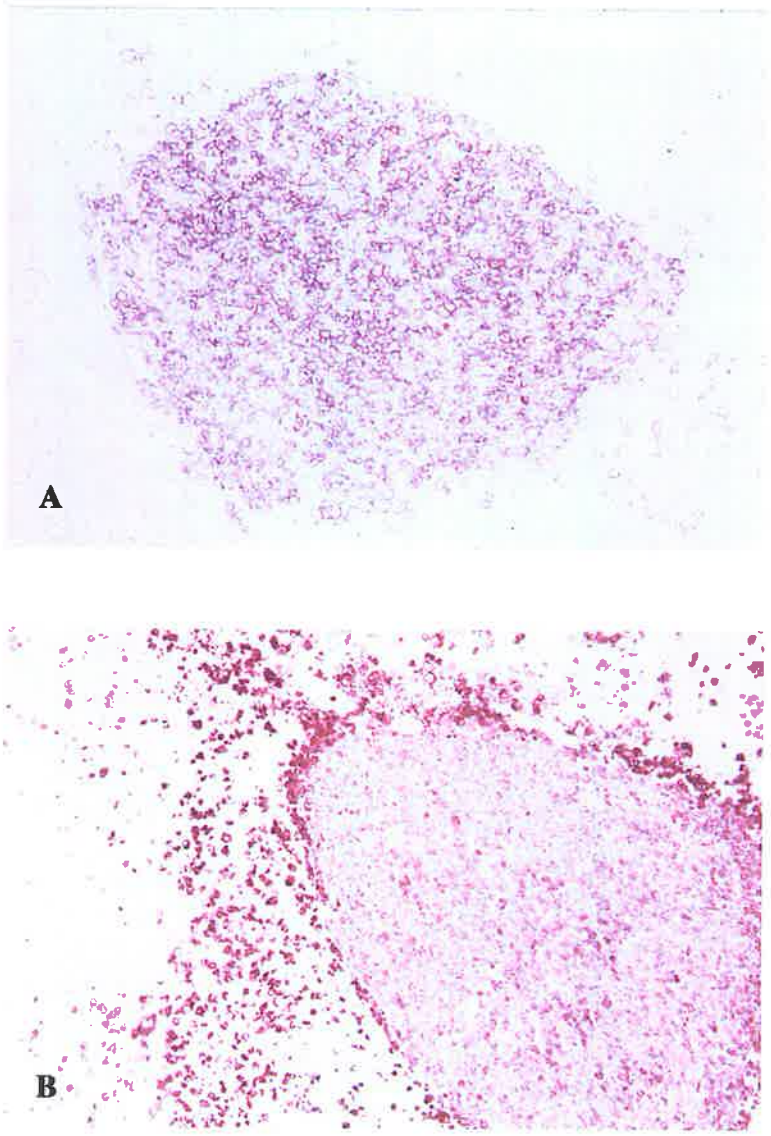

Figure 14 . *Photomicrographs of spinal cord sections from a chimeric rat sacrificed at 4 weeks.Nickel DAB, X50 (A) The kaolin droplet has been engulfed largely by bone marrow-derived (RT7.2$^+$) cells (B) The inflammatory infiltrate of ED1$^+$ is extensive and not confined to the kaolin droplet. ED1$^+$ cells within the kaolin droplet stain much less intensely than those cells at the boundary of the lesion. This probably reflects the cytotoxic effects of kaolin particles on macrophages. Cells within the parenchyma stain strongly.*

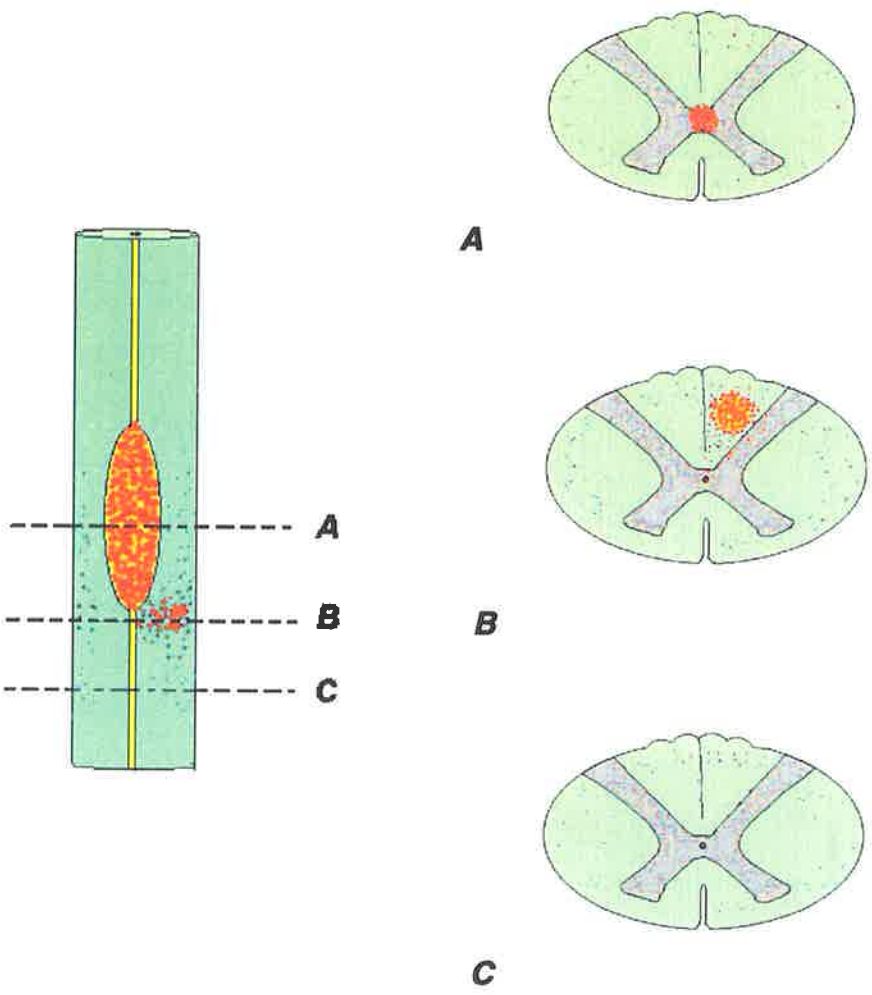

Figure 15. *Schematic representation of transverse spinal cord sections sampled from different levels in a rat sacrificed at 4 weeks. ED1⁺ cells are present at multiple levels both rostral and caudal to the site of injection.*

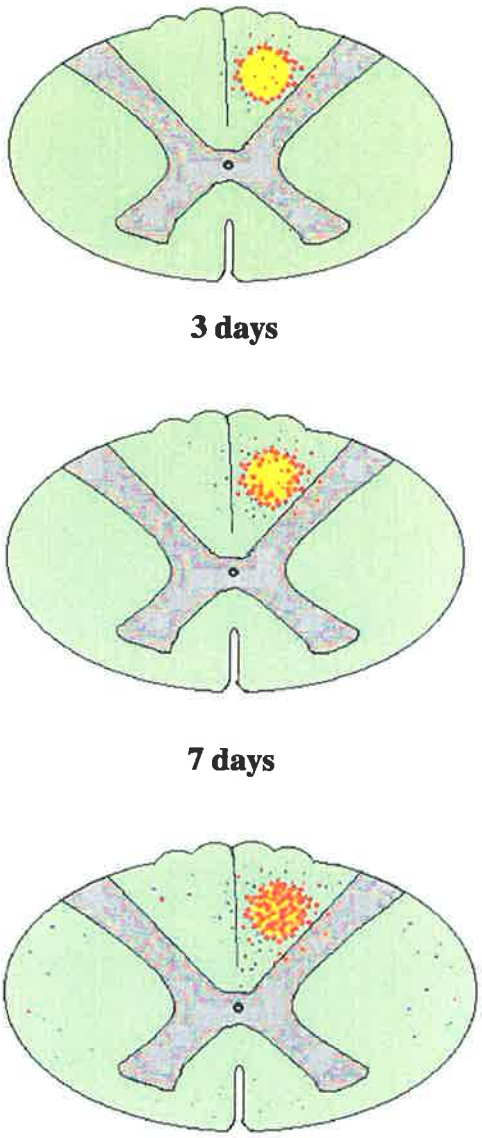

Figure 16. *Schematic representation of transverse spinal cord tissue sections at level of kaolin droplet from rats of varying survival times. Microglial activation occurs early and becomes progressively more marked. Haematogenous macrophages migrate primarily towards the kaolin droplet. In some animals, the haematogenous macrophages were also seen within the dilated central canal at 28 days.*

4.3.2 RT7.2

4.3.2.1 Morphological features

In contrast to ED1, the RT7.2 allele of CD45 yielded a distinct membrane staining pattern (FIGURES 11 and 12). The majority of immunoreactive cells appeared to share morphological features with the large round ED1 positive cells described above.

4.3.2.2 Pattern of immunoreactivity

$RT7.2^+$ cells progressively accumulated as a visible rim around the kaolin droplet (3 days and 1 week) (FIGURE 13). Some immunoreactive cells could be seen perivascularly within the parenchyma of the spinal cord. These perivascular cells appeared morphologically distinct (FIGURE 17). Typically they were smaller, thinner and appeared to stain more intensely. The $RT7.2^+$ cells were not observed within the kaolin droplet at 3 days. However, by 4 weeks, these cells could *only* be found within the kaolin droplet accounting for the large majority of cells within the granuloma.

4.3.3 Co-localisation of ED1 and RT7.2

4.3.3.1 Pattern of immunoreactivity

The double-labelling immunofluorescence studies allowed cells which were $RT7.2^+$, $ED1^+$ to be easily identified (FIGURE 18). These antigens appeared to be co-localised on the large majority of cells that had accumulated around and within the kaolin droplet (FIGURE 19). At 4 weeks, most of the $ED1^+$ cells found within the granuloma were also

RT7.2^{+} (FIGURE 20). Hence, the bone marrow-derived macrophages were therefore largely responsible for the granuloma formation. In addition the macrophages within the syrinxes seen at 4 weeks were also doubly labelled (ED1+,RT7.2+).

In contrast, the RT7.2^{-},ED1^{+} cells of presumed microglial origin accounted for the other immunoreactive cells observed within the parenchyma of the spinal cord. These cells extended to the boundary of the kaolin granuloma. Certainly, these particular cells near the kaolin-cord interface could not be distinguished from their haematogenous counterparts on morphological features alone and without the aid of immunohistochemistry (FIGURE 21).

Notably at 3 days, a small but significant number of RT7.2+ED1- cells was present within the inflammatory infiltrate. These are most likely to represent neutrophils (or lymphocytes) which form a part of the early inflammatory response. They were rarely observed beyond this time interval.

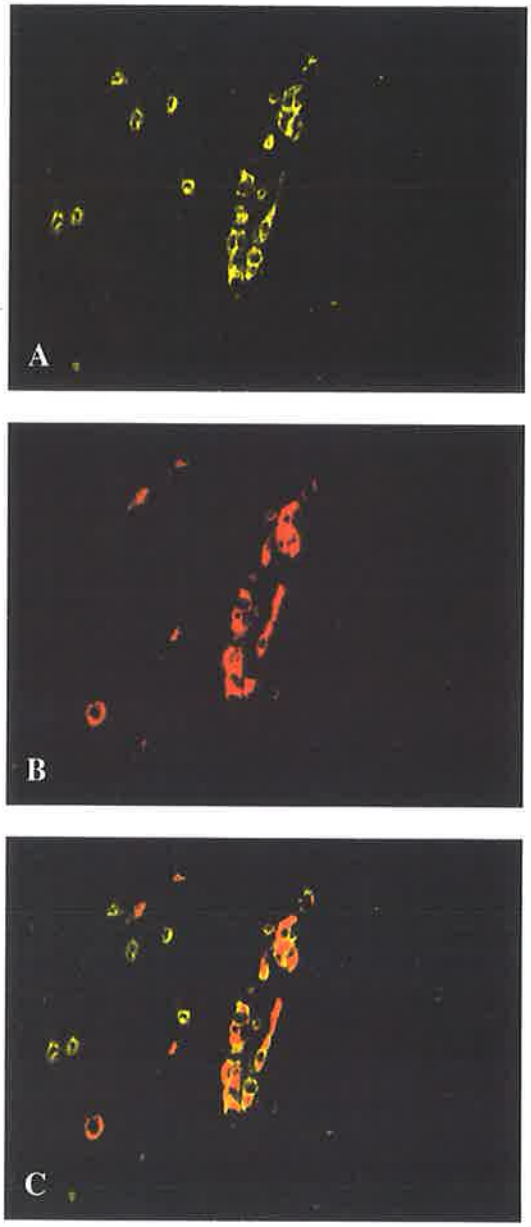

Figure 17. *Photomicrographs of perivascular cells in a chimeric rat sacrificed at 4 weeks. (A) All the perivasular cells are derived from haematogenous (RT7.2$^+$) in origin. FITC, X100. (B) These cells also express the ED1 antigen intensely. Texas Red, X100. (C) A mixture of activated microglia and other haematogenous cell types (such as neutrophils and lymphocytes) may be seen nearby. FITC, Texas Red, X100.*

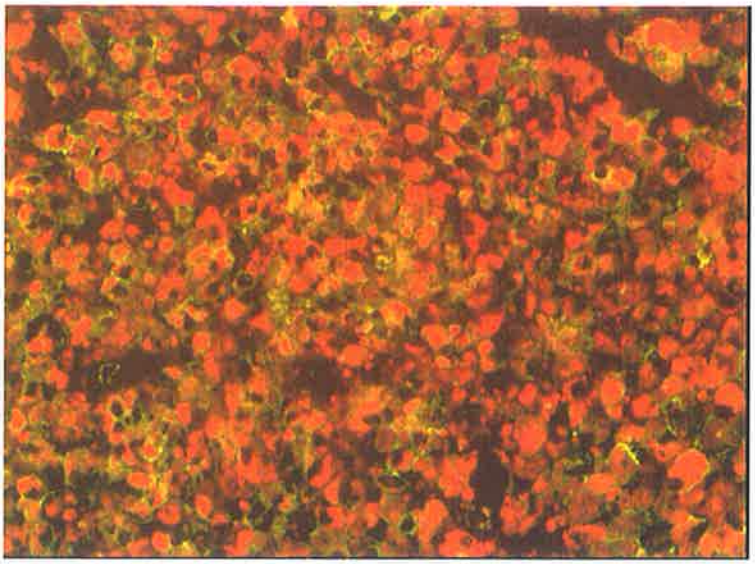

Figure 18 . *Immunostaining features of doubly-labelled cells. X100. The co-localisation of ED1 (Texas Red) and RT7.2 (FITC) yields an orange colour on immunofluorescence. Some cells also exhibit a greenish-yellow 'halo' effect, as illustrated here. This is not as easily perceived at low magnification.*

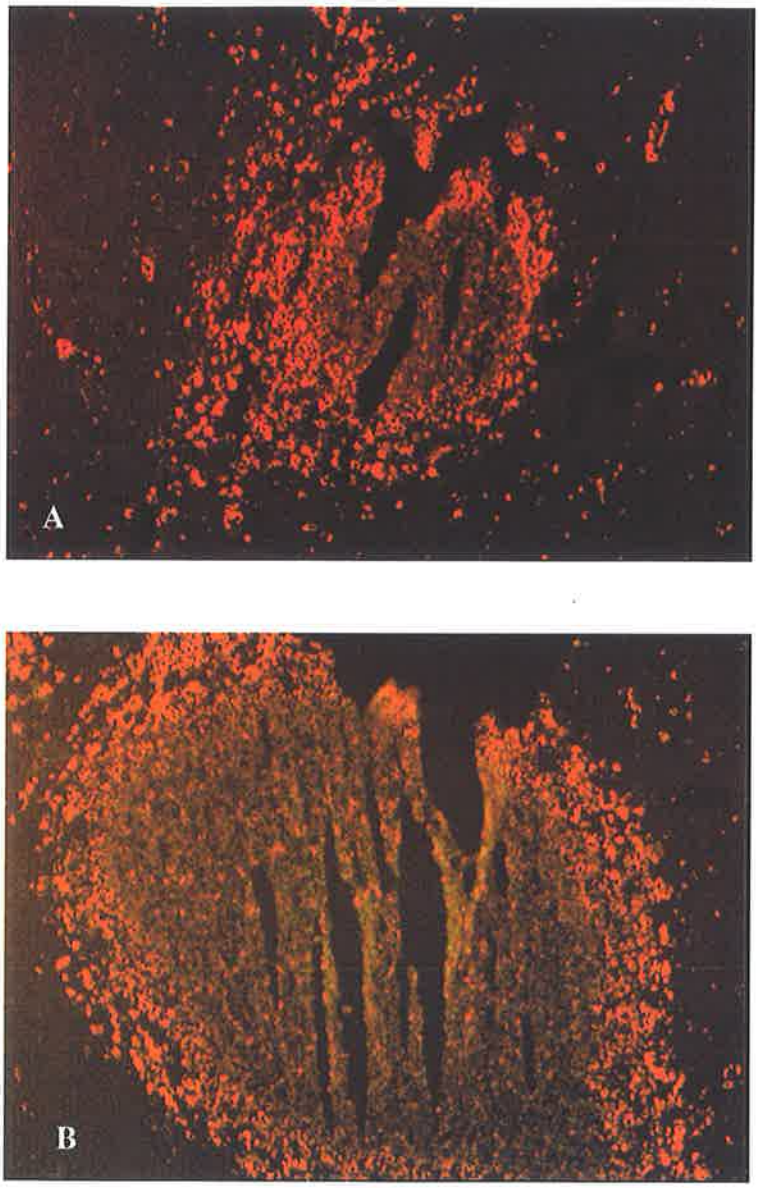

Figure 19. *Photomicrographs of spinal cord sections in a chimeric rat sacrified at 1 week. (A) Significant accumulation of ED1$^+$ cells within the surrounding parenchyma around the kaolin droplet, Texas Red, x 25. (B) The majority of the macrophages migrating into the kaolin droplet are bone marrow-derived (co-localisation of ED1 and RT7.2 antigens). FITC and Texas Red, x50.*

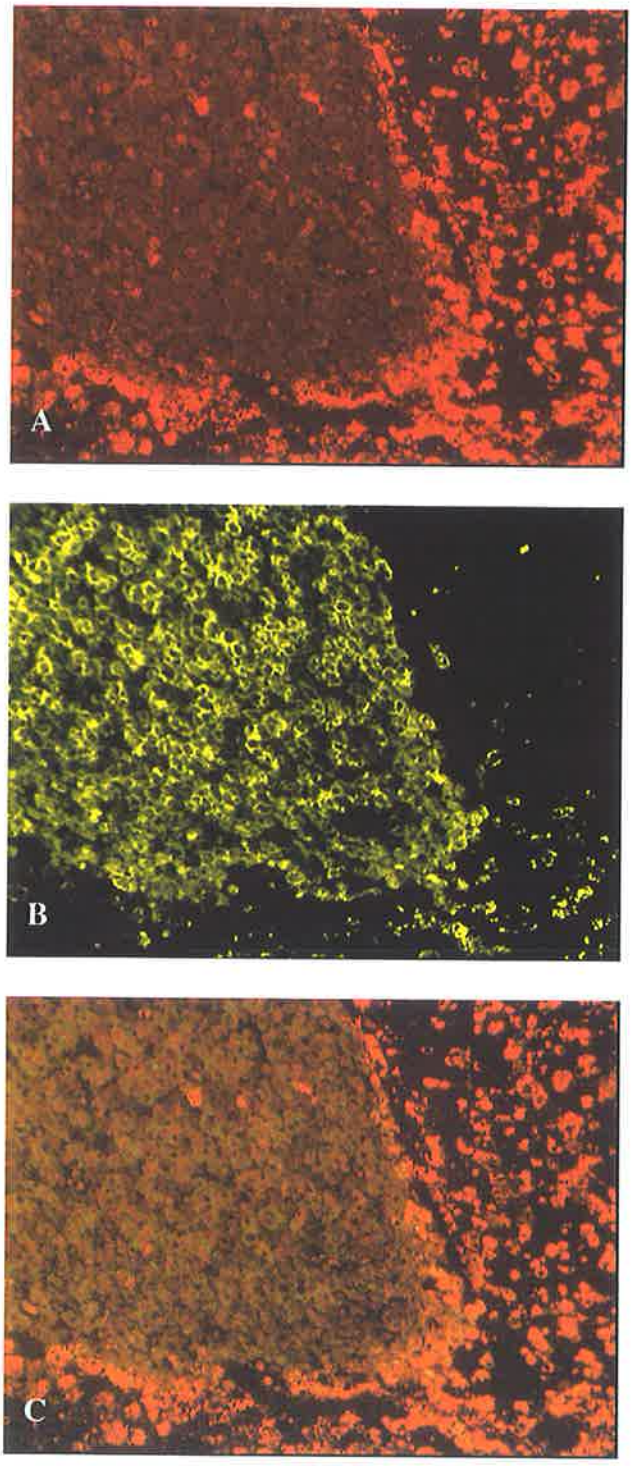

Figure 20. *Photomicrographs of spinal cord sections in a chimeric rat sacrificed at 4 weeks, X50. (A) Large numbers of ED1$^+$ cells are seen both within the kaolin droplet and the surrounding parenchyma. Texas Red (B) RT7.2$^+$ cells derived from the bone marrow are confined to the vicinity of the kaolin droplet. FITC. (C) Haematogenous macrophages (RT7.2$^+$) demonstrate an affinity for the kaolin droplet while microglial-derived macrophages (RT7.2 are largely seen in the surrounding spinal cord parenchyma.*

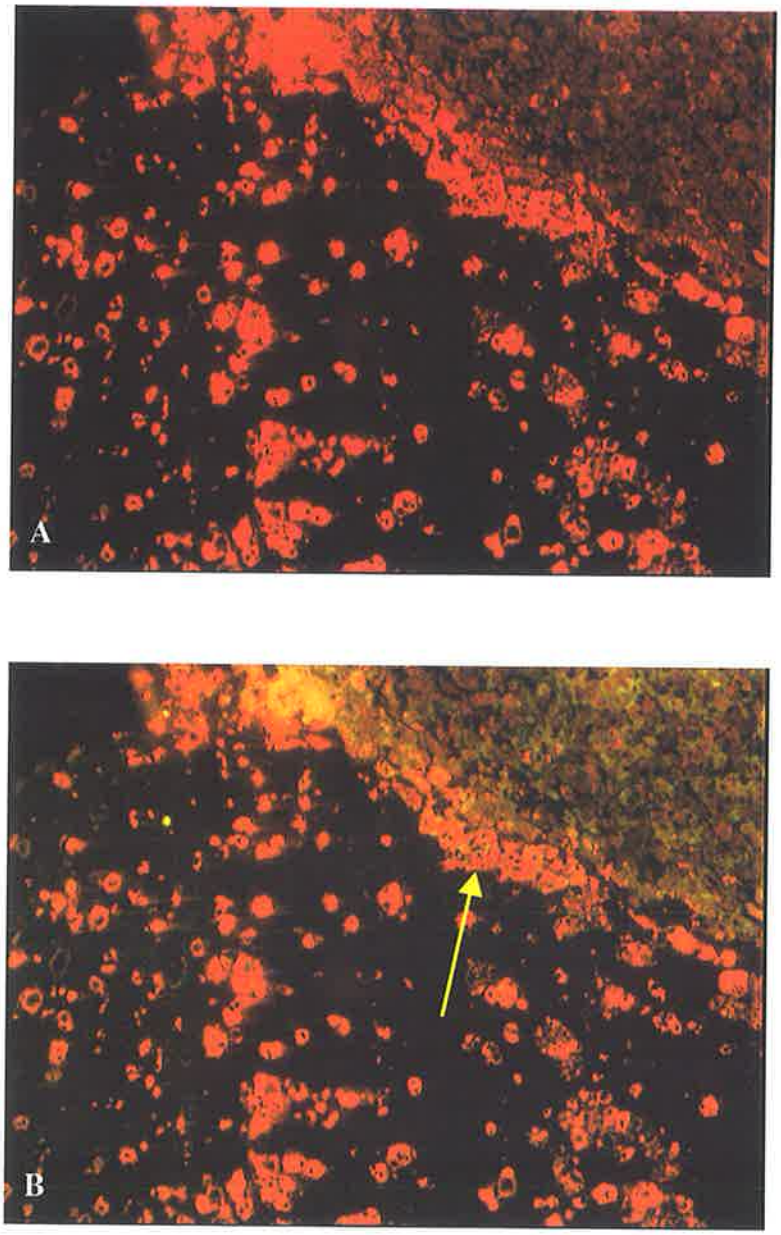

Figure 21. *Photomicrograph of transverse spinal cord sections in a chimeric rat sacrificed at 4 weeks. (A) It is not possible to distinguish the origin of ED1^{+} macrophages based on morphological features alone. Texas Red, X100. (B) Co-labelling of the RT7.2 antigen (arrow) by using the monoclonal antibody HIS41 allows this differentiation to be made with relative ease. FITC, Texas Red, X100.*

Counts of $ED1^+$ and $RT7.2^+$ cells on sections of transverse spinal cord sections sampled at the level of the kaolin droplet are listed in Table 4. Proportions of $ED1^+/RT7.2^+$ and $RT7.2^-/ED1^+$ cells are subsequently presented in Table 5.

	3 days	*1 week*	*4 weeks*
no. of animals	9	10	10
ED1+ cells	3625	5048	4375
RT7.2+ cells	1862	2201	2042
ED1+/RT7.2+	51±7%	45±12%	48±12%

Table 4. *Counts of $ED1^+$ and $RT7.2^+$ cells at sacrifice intervals of 3 days, 1 week and 4 weeks.*

4.4 Immunohistochemistry in spleens of chimeric rats

The spleens of 3 chimeric rats were examined. More than 93% visualised mononuclear cells stained positively for RT7.2 in all sections examined (FIGURE 22). The endothelial cells, vessel wall, connective tissue and capsule, all of which are of host origin remained unstained.

4.5 Histology and immunohistochemistry in RT7.1 DA rats

The RT7.1 DA rats were sacrificed at 3 days (n=5) and 4 weeks (n=7) following the

Time interval	*ED1⁺/RT7.2⁺ (haematogenous)*	*ED1⁺/RT7.2⁻ (microglial)*	*+/- SD*
3 days	51.3%	48.7%	6.6%
7 days	45.5%	54.4%	12.5%
28 days	47.6%	52.4%	12.1%

Table 5. *$ED1^+$ cells of microglial ($RT7.2^-$) and bone marrow ($RT7.2^+$) origin in chimeric rats sacrificed at 3 days, 7 days and 28 days. Cell counts were performed on transverse spinal cord tissue sections, in which the cross-sectional area of the kaolin droplet was the largest.*

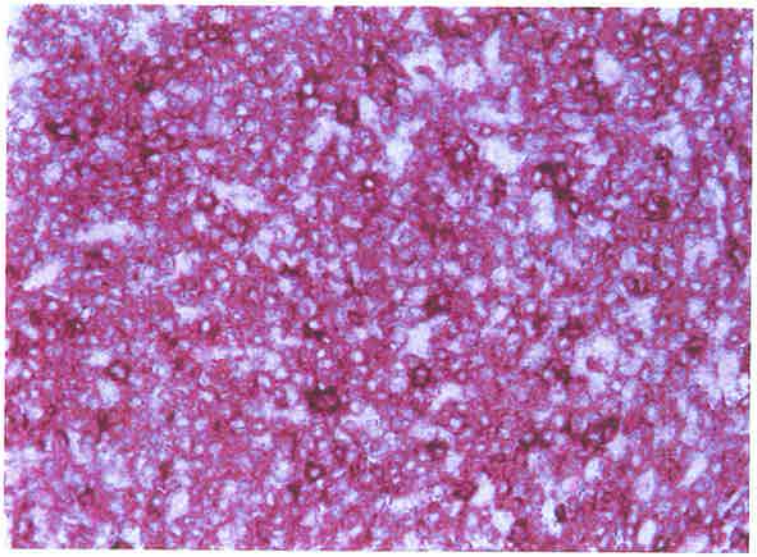

Figure 22. *Photomicrograph of splenic tissue section from a chimeric rat sacrificed at 6 weeks post-irradiation and bone marrow transplanation. The spleen has almost been completely re-constituted by RT7.2^{+} lymphoid and myeloid cells. Nickel DAB, x50.*

kaolin injections. At both time intervals, the histological appearances were identical to those observed in chimeric rats sacrificed at the same survival intervals. Immunostaining of spinal cord sections was only performed with the antibody against ED1. Similarly, no differences in the distribution and pattern of $ED1^+$ cells were observed.

The extent of macrophagic inflammation, as represented by counts of $ED1^+$ cells in non-chimeric rats were not statisically different from the chimeric rats at 3 days ($p=0.19$) and 4 weeks ($p=0.29$) respectively.

DISCUSSION

1 Rat radiation chimeras

1.1 Historical perspective

In 1951, Jacobson and his colleagues[189] discovered that the implantation of autologous spleen could prevent the death of mice which had been lethally irradiated. Lorenz *et al* [242] reported in the same year that the administration of isologous bone marrow had a similar protective effect. Subsequently it became clear that the injected bone marrow cells acted as a transplant or graft[99, 230, 422], repopulating both haematopoietic as well as lymphoid tissues. These were named radiation chimeras.

1.2 Relevance to current study

At present, there is no histological feature or cellular marker that reliably distinguishes microglia-derived macrophages from those that have been derived from the bone marrow. In an attempt to circumvent this problem, a number of experimental techniques have been tried.

In the past, autoradiography was most commonly used for this purpose. In this technique, cells in the S phase are labelled following a single pulse of [^{3}H]thymidine. If a short survival time (e.g. less than 1 hour) is used, only the locally dividing cells will be labelled in the tissue section. However, if the survival time is longer e.g. 24 hours, there will also

be a contribution of labelled cells from the circulation.[312] In general, the deficiencies of autoradiographic studies have been clearly highlighted by Matsumoto and Fujiwara.[257] The use of DNA-binding radioactive agents to label a highly proliferative cell population implies that the level of radioactivity may diminish after a number of cellular divisions. Subsequently, the possibility of unlabelled blood-derived cells in the CNS cannot be excluded. Furthermore, this technique has also been criticised due to the technical difficulty and consequent interpretation variations.

Other potentially helpful experimental techniques such as carbon labelling of peripheral monocytes are only suitable for studies with short sacrifice times.[233, 234] The current study required animal survival times of up to 4 weeks. This would have introduced the possibility of carbon being re-utilised or ingested by other macrophages, as the original macrophages die.

The concept of rat radiation bone marrow chimeras provides an elegant method of unequivocally identifying macrophages of bone marrow origin in the current experimental model of noncommunicating syringomyelia. Recently, this technique has also been used extensively to study the role of macrophages/microglia in the setting of EAE.[168, 211, 257]

In the current study, commercially available RT7.1 Dark Agouti rats were irradiated to ablate their native bone marrow. They were then transplanted with bone marrow previously harvested from RT7.2 congeneic DA rats. The animals were then allowed to

mature over 6 weeks as the haematogenous cells repopulated the somatic tissues. It is known that this process is complete by 6 weeks from previous studies.[257]

1.3 Technical aspects

The mortality of animals in the pilot study was 25% (3 of 12 animals). A number of modifications were subsequently made to the experimental protocol for the main study. These included:

(1) subcutaneous supplementation of normal saline on the first 4 post-irradiation days as recommended by Dr Tim Kuchel, veterinarian, IMVS.

(2) prescribing the maximal dose of irradiation (1000 rads) at the exit point of the animal's body on the ventral aspect

(3) doubling the quantity of bone marrow infused into the recipients.

This resulted in a mortality rate of 13 % in the main study (4 of 30 animals). While it is not possible to draw any firm conclusions from the comparison of these results, the increased quantity of bone marrow transplanted was most likely to have imparted the greatest protective benefit.

While death after whole-body exposure to doses injurious to haematopoietic and gastrointestinal tissues is often thought to be caused by infection[4], gastro-intestinal haemorrhage was the predominant cause of death in our group of animals, as defined at autopsy.

Our results are comparable to the experience of Mayrhofer who obtained a mortality rate of approximately 10% using a similar study protocol[258]. In contrast, Hickey and colleagues reported no mortality and minimal morbidity (no evidence of bleeding diathesis) in their series of DA radiation chimeras[171]. They administered 10 Gy over 5 minutes, resulting in a significantly higher dosage rate. The reasons for these differences are not entirely clear. One possible explanation may be that Hickey and his colleagues administered significantly more donor marrow cells ($1x10^8$) into each recipient animal. Clearly, further research is required to clarify this.

The variability of survival outcomes following lethal irradiation (even with bone marrow rescue) has long been noted by other researchers. Lorenz et al injected homologous bone marrow into lethally irradiated mice. While this offered "excellent protection", the 21-day mortality figures in different study groups ranged from 0 to 32%.[243] Typically, when mortality occurs in mice, it occurs within 7-10 days when the haematopoietic depression is most marked.[243] All deaths in the current study occurred within the first week.

Furthermore, there is also inter-species variation in terms of both radiation susceptibility and requirements for a bone marrow "rescue".[150, 243, 407] The mean lethal radiation dose

(LD_{50}) varies from 3.7 Gy in dogs to 7.75 Gy in the rat [150]. In contrast, the LD_{50} for humans is thought to be approximately 4 Gy.[150]

The optimal dosage for ensuring ablation of the bone marrow while minimising mortality is difficult to establish. The differences in the X-ray machines utilised and precise prescription of treatment may potentially result in variations of the dosage administered. These details are often not stated in previously published studies. While these variations are usually considered to be minor, they may theoretically become significant if they fall onto the steep part of the dose-mortality curve. Previous studies have utilised radiation doses of between 900-1000 rads depending on the source of radiation (deep X-rays or gamma rays from a cobalt source) with varying dosage rates.[29, 168, 170, 211, 213, 257] While it would be expected that the mortality rate would be reduced with a reduction of the radiation dose, this would compromise the aims of the current study as a result of incomplete ablation of the native bone marrow due to incomplete chimerism.

1.4 RT7 allellic system

The RT7 antigen is polymorphic and expressed as a diallelic system, either as RT7.1 or RT7.2.[244] The RT7.1 strain of rats include LEW, COP, DA, BN, F344, M520 and WF/fz while the RT7.2 strain includes WF, BUF, NBR and BH.[244] The HIS41 antibody reacts specifically with CD45 (Leukocyte Common Antigen) expressed on both myeloid and lymphoid cells (to the exclusion of erthrocytes) of the RT7.2 allotype and not the RT7.1 allotype.[194]

CD45 is a member of the Protein Tyrosine Phosphatase (PTP) family. It is a heavily glycosylated glycoprotein with an Mr 180,000 to 240,000.[15, 188] The extracellular domain of CD45 varies between 391 and 552 amino acids long with 11-16 N-linked carbohydrate attachment sites.[15, 188] It is highly variable as a result of alternative splicing of exons 4,5, and 6 and differing levels of glycosylation. The cytoplasmic domain contains 700 amino acids, with two PTP catalytic domains. The CD45 isoforms are believed to play complex roles in T-cell and B-cell antigen receptor signal transduction.

1.5 Assay of chimerism

The optimal way of assessing the extent of chimerism in radiation chimeras is unclear. In the initial pilot study, it was necessary to verify that chimerism had been established in the DA rats. Flow cytometric studies performed on peripheral blood was a relatively simple technique that allowed such an aim to be realised. The detection of RT7.2 haematogenous cells in the peripheral blood of all nine rats in the pilot study confirmed that chimerism had been successfully achieved. However, the wide variability of flow cytometric results was somewhat surprising. One probable explanation is that the cytometric studies were performed at 3 weeks, prior to the establishment of stable chimerism. This process is known to be complete only after 4 weeks in rats.[257]

Another difficulty encountered is the interpretation of the test results. Currently, there are no studies that have directly compared the chimerism assays obtained from peripheral

blood with those derived from somatic tissues such as spleen and lymph nodes. Hence the most accurate or 'ideal' method of assaying chimerism is not known. Recent studies using radiation chimeras have assessed the extent of chimerism by the analysis of cellular populations in somatic tissues such as the spleen.[168, 169, 170, 171, 212, 213, 257] This approach was therefore adopted in the current study.

2 Developmental issues

2.1 Initial immunohistochemistry testing

The initial testing of HIS41 was hampered by the supply of a defective product from the manufacturer. Subsequently, difficulties were also encountered in establishing successful immunostaining on *paraffin* sections of RT7.2$^+$ rat splenic tissues in accordance with the manufacturer's data sheets. No significant immunostaining was observed in these tissue sections.

Personal communications with the clone originators, Drs J Kampinga (Quadrant Research Foundation, Cambridge Research Laboratories) as well as FGM Koese (Department of Histology and Cell Biology, University of Groningen) confirmed that they had not performed any immunological work on formaldehyde-fixed, paraffin-embedded tissues previously. Independent testing of HIS41 was requested and kindly performed concurrently by Pharmingen laboratories (USA). Their results confirmed that only "unsatisfactory patchy staining" was seen on *paraffin* sections of RT7.2 DA rat spleen.

As it became evident that the RT7.2 antigen was unstable to formaldehyde fixation (in contradiction to the manufacturer's initial claims), it was concluded that the application of HIS41 on paraffin sections did not constitute a sufficiently reliable and reproducible basis for use in the main study. Hence fresh frozen tissues were utilised in the main study.

2.2 Freeze-drying tissue processing

The technique of freeze-drying tissues possesses theoretical benefits of optimising antigen preservation while maintaining superior morphological detail. The possibility of utilising this technique was initially explored in view of these perceived advantages. Unfortunately, the initial results were disappointing. The main difficulties encountered were largely due to the lack of suitable equipment (e.g. sublimator, vacuuum wax embedder etc).

Initial freeze-drying tests were performed using a sublimator which served the food processing unit at RAH. As this was shared equipment, it was not practical to perform multiple tests due to the long duration of freeze drying required by the freeze-drying process (up to 7 days). On each occasion of testing, the machine could not be used for its original intended purposes.

Consequently, it was felt that a much longer time frame (at least several months) was required for optimising various aspects of the freeze drying methodology. This was not considered to be feasible in the current study.

2.3 Modification to operative technique

In previous departmental studies, the kaolin injections were performed using a manually sharpened 30 gauge needle which was attached to a Hamilton syringe. Despite cautious sharpening, the needle point invariably deformed the dura and the underlying spinal cord to a significant extent prior to penetration. On occasions, several attempts were required to achieve penetration of the cord tissue. This was deemed unsatisfactory in the current study. This problem was overcome by the initial use of a commercially available 29G tuberculin/insulin syringe which was sufficiently sharp to pierce the dura and underlying arachnoid with minimal cord indentation. The kaolin suspension was then injected using the Hamilton syringe and needle with ease.

3 Results of the current research

3.1 Histological findings

The histological features in the current model of rat syringomyelia at the light microscopy level have already been extensively studied in Sprague Dawley rats.[267, 375] Similar findings have been reproduced in the current study using Dark Agouti rats with several notable differences.

Firstly, in contrast to results reported by Milhorat and colleagues[267], massive dilation of the central canal was not observed in the current study. Secondly, in some rats (both

chimeric and non-chimeric) sacrificed at 4 weeks, the syrinx appeared to be totally occluded by macrophages over multiple spinal cord levels. While Stoodley *et al*[375] reported similar observations of macrophages within the central canal in rats 6 weeks after kaolin injection, these were apparently quite localised. The reasons for these differences are unclear. However, it is possible that the different strain of rats may account for some of the observed differences. DA rats were selected for the current study because of the availability of a congeneic RT7.2 strain which allowed the production of radiation bone marrow chimeras.

3.2 Origin of macrophages

The extensive macrophagic inflammation and microglial activation at 3, 7 and 28 days following intra-parenchymal kaolin injection had been well demonstrated by immunohistochemical methods (ED1) in the current study. Importantly, the extent of inflammation was significantly underestimated on initial routine light microscopic examination.

Giulian observed that following a stab injury to the rat brain, 2 apparently distinct populations of Dil-ac-LDL(+) cells could be observed as early as 5 hours.[130, 131] They concluded that the round cells were "extrinsic mononuclear phagocytes" which had invaded the site of the stab wound. In contrast, the ramified cells with newly expressed ac-LDL receptors were thought to reflect activated microglial cells distant to the imediate site of injury. This interpretation appears to be relatively accurate on the basis of our findings.

However, many ED1^{+} cells were observed in the current study that could not be classified into either morphological categories.

When cell counts were performed, approximately half of the ED1 positive cells were derived from the bone marrow (RT7.2^{+}) at all sacrifice intervals (FIGURE 23). These were typically large, round cells with foamy cytoplasm which fitted the classical description of macrophages. Conversely, an equally large proportion of *ED1 positive cells* (instead of ED1 positive macrophages) are therefore of presumed microglial origin. This cautious and qualified conclusion is made because it is recognised that both macrophages and 'activated'

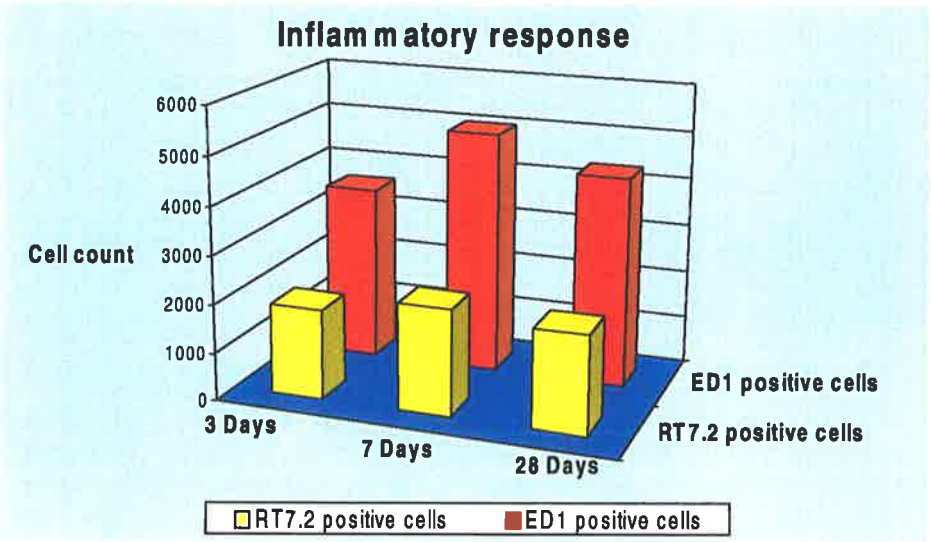

Figure 23. *Counts of RT7.2 and ED1 immunoreactive cells in chimeric rats sacrificed at 3 days, 7 days and 28 days. The ED1 inflammatory reponse was most marked at 7 days. At all time intervals, approximately half of the ED1$^+$ cells were derived from the bone marrow (RT7.2$^+$). The rest of the ED1$^+$ cells are of presumed microglial origin.*

microglial cells upregulate ED1 expression. These cells of microglial origin ($RT7.2^-$, $ED1^+$) demonstrate a continuous spectrum of morphological features, ranging from 'classical' macrophages near the kaolin/cord interface to other smaller and more distant cells which are more typical of microglia. In a large number of cells, the origin could not be determined based on morphological features alone.

3.3 Differential Functional Roles of Macrophages

In the rat spinal cord, the microglia-derived $ED1^+$ cells ($RT7.2^-$) were largely distributed *around* the kaolin droplet at 3 days and 1 week. By 4 weeks, significant numbers of activated microglia were seen throughout spinal cord tissue sections at multiple levels both rostral and caudal to the kaolin lesion. Overall, these cells were largely confined to the parenchyma of the spinal cord, with a predilection for the white matter. In contrast, the bone marrow derived macrophages appeared to be directed towards the kaolin particles. By 4 weeks, these cells were totally confined within the kaolin droplet or within the dilated segments of central canal. It is unclear whether the latter observation represents direct migration of kaolin-laden macrophages into the canal or secondary recruitment of macrophages to kaolin particles already present within the central canal.

While there is some evidence to suggest that microglial-derived macrophages demonstrate different phagocytic potential to their haematogenous counterparts, the apparently different roles of macrophages postulated has not previously been reported. In the current study, the haematogenous macrophages appear to have a primary role in phagocytosing

material foreign to the neural tissue. Theoretically, this may also include other 'foreign' material such as blood, infective agents etc. On the other hand, macrophages of microglial origin appear to be most active within the neural tissue, possibly playing a key role in phagocytosing myelin. This is consistent with previous studies which have shown that microglia possess a significantly greater myelinophagic capacity[22]. However, ultrastructural studies were not performed and would be required to confirm this hypothesis.

3.4 Microglial activation

3.4.1 Microglial response in animal models

The phenomenon of microglial activation has been well demonstrated in experimental animal models. It occurs in response to brain trauma, T cell-mediated inflammation, global and focal ischaemia, transplants into the brain, experimental gliomas and viral and bacterial infections[27].

3.4.2 Microglial response in spinal cord injury

The pattern of microglial activation following spinal cord injury has been evaluated in a number of recent studies.

Koshinaga and Whittemore utilised the photosensitising dye rose bengal and an argon dye laser to induce a spinal cord lesion at T8, thus severing axons of the dorsal columns and corticospinal tracts.[203] This led to rapid microglial activation. Interestingly, the pattern of activation appeared to be both temporally and topographically specific. For example at T4-5 two days post-lesion, they observed striking accumulation of cells with intense CR3 receptor immunoreactivity in the fasiculus gracilis, but little change in the corticospinal tracts and fasiculus cuneatus. The explanation for this observation is not known although the authors suggest that this may be related to differences in axonal properties. Watanabe *et al*[425] reported similar findings after either totally transecting or severely compressing the spinal cords of Wistar rats. They observed at least 2 different spatial and temporal patterns of microglial activation. Interestingly, Watanabe *et al* postulated that the early pattern possibly involves the blood borne complement activating system while the later response accompanies Wallerian degeneration and is independent of the complement system. Dusart and Schwab further observed that a significant microglial/macrophagic reaction (using ED1 and OX42) may persist for at least 3 months after transecting the dorsal one half to two-thirds of the rat spinal cord .[88]

3.4.3 Mechanisms of microglial activation

The current study has provided evidence of early microglial activation with $ED1^+$ expression within the spinal cord parenchyma in rat syringomyelia. In fact, the microglial cells were the earliest cells to migrate into the kaolin droplet (at 3 days). They were subsequently replaced by their haematogenous counterparts at later time intervals. These

findings are consistent with Kreutzberg's theory that the microglia act as "sensors" in the first line defence of the central nervous system.

The activation of remote microglial cells following relatively mild injuries suggest that fast activation pathways are operating. Thomas monitored living microglial cells using time-lapse video recording and found that individual cells were capable of assuming a different morphology within 5 minutes.[394]

The potential routes and mechanisms of these processes are difficult to delineate.[314] Recently, it has been proposed that the presence of an inward rectifying K^+ channel without an outward channel may account for the microglial sensitivity to the ionic environment.[11, 424] These microglial channels are probably linked to P_2 purinoreceptors which may become activated by ATP release.[27] Cytokines such as macrophage colony stimulating factor (M-CSF) and granulocyte macrophage colony stimulating factor (GM-CSF) may influence the expression of K+ channels in macrophages.[97]

Binding of antibodies to surface receptors such as complement receptor 3[331] may also lead to rapid microglial activation. ATP which is released during cellular damage can lead to the same process by binding to membrane receptors.[424]

The function of microglial 'activation' is unknown. Whether such a response is purposeful or a non-specific reaction to neural injury remains poorly understood. It is possible that the activated microglia may assist in further recruitment of haematogenous macrophages

to the site of neural injury. Certainly, our observations of early microglial migration into the kaolin droplet which precedes the influx of haematogenous macrophages does not contradict this hypothesis. However, it is possible that the "activation" process may be an inevitable part of the transformation process into macrophages.

4 Macrophages in CNS

4.1 Response to CNS injury

Injury to the central nervous system results in tissue destruction at the site of injury. Inevitably, this also leads to retrograde degeneration and Wallerian degeneration of axonal fibres.[310, 314] In particular, the process of retrograde degeneration is associated with a prominent mononuclear phagocytic response that has largely been attributed to the proliferation of activated microglial cells.[216, 380] In contrast, injury to the peripheral nervous system leads to a delayed but extensive recruitment of monocytes.[310, 315]

Within the central nervous system, haematogenous macrophages are generally considered to be the main source of cells that phagocytose myelin.[309] Similarly, they also assist in the subacute and delayed phases of myelin breakdown when peripheral nerves undergo Wallerian degeneration.[8, 373] Phagocytosis by macrophages occurs after binding of the Fc receptor to antibodies on the myelin surface[352, 365] or by binding to complement via complement receptor 3.[48]

4.2 Role of macrophages in the uninjured CNS

The role of macrophages in the central nervous system is controversial.

On one hand, macrophages are deemed to be beneficial to neuroanatomical repair. It has also been suggested that these cells may modify the nonpermissive nature of the adult CNS although the mechanisms have not been clearly defined.[70] Macrophages may remove myelin debris, which is known to inhibit axonal regrowth[50, 349], recycle lipid degradation products[156, 374, 382] or secrete cytokines and growth factors.[165] Recent research has further shown that the direct implantation of activated microglia/macrophages into the site of neural injury promotes axonal regeneration.[105, 217, 320, 323, 328, 351]

On the other hand, macrophages have also been thought to be key effectors of cellular damage in neural injury and demyelination.[24, 32, 33, 82, 209] In animal models of both inflammation (EAE) and spinal cord injury, macrophage depletion or deactivation leads to either amelioration of symptoms or improved neurological outcome.[22, 34, 47, 178, 251, 318, 400] It has been postulated that the absence of macrophages may limit chondroitin sulfate proteoglycan deposition and reduce release of injurious agents such as superoxide radicals, hydrogen peroxide, hypochlorous acid, quinolinic acid as well as other proteolytic enzymes.[318] Furthermore, the use of anti-inflammatory agents such as methyprednisolone following induced spinal cord injury in rats reduced the extent of damage and prevented injury induced hypothermia.[450] More recently, these benefits have been reproduced in the clinical context. The administration of methyl prednisolone within 8 hours of the human spinal cord injury had been shown to improve neurological outcome.[3, 450]

Such apparently contradictory data may be partially reconciled by an understanding of the following factors:

(1) In the post-injury phase, it is important to distinguish 2 complementary processes: *neuroprotection,* the rescue of spared axons and neural *regeneration* which involves the regrowth of transected nerves.[353] These processes vary depending on the type and extent of CNS injury studied.

(2) The microenvironment in which microglial and macrophagic activation occurs is known to influence their neurotrophic or neurotrophic expression.[289, 453] Lazarov-Spiegler and colleagues demonstrated that transplanted macrophages preincubated with peripheral (sciatic) nerve were endowed with greater phagocytic activity and induced superior axonal regrowth within the central nervous system in comparison with macrophages which were pre-incubated with optic nerve segments.[217, 218] The researchers conclude that an 'immune privilege factor' inhibits macrophagic migration into the central nervous system.[219] This is believed to lead to a broad range of effects which either make the CNS environment growth-supportive or growth-inhibitory.[217]

(3) The role of other non-neuronal cells within the central nervous system may also be important. For example, the astrocytes are known to have many important functions in development, homeostasis and response to CNS injury.[308]

Clearly, a better understanding of the signals controlling macrophage activation is required.

5 Contribution to pathogenesis of syringomyelia

5.1 Pathological basis of syringomyelia

Syringomyelia is a heterogeneous pathological entity associated with varied aetiologies. It is therefore highly unlikely that an all-embracing pathogenetic theory can account for the formation of all forms of syingomyelia.

In the communicating form of syringomyelia, co-existing hydrocephalus appears to be the key feature. This is reflected in both MRI scanning[266] and also post mortem autopsy studies[269]. The hydrodynamic theories of both Gardner and Williams are likely to be relevant. As the ventricles enlarge in the setting of impaired CSF outflow from the fourth ventricle, the central canal enlarges as a compensatory mechanism. Occasionally the CSF may dissect into the spinal cord parenchyma.

However, MR imaging has demonstrated that less than 10% of cavities communicate directly with the fourth ventricle although the compressed or distorted segment of the central canal may be anatomically patent at the time of autopsy.[267] The largest autopsy series further confirmed that the majority of syrinxes are either of the non-communicating or extra-canalicular varieties.[269] In these cases, ventricular CSF pressure cannot be

transmitted directly from the fourth ventricle into the syrinx. Other factors must therefore come into play.

We hypothesize that macrophages/activated microglia may play an important role in the pathogenesis of at least some forms of syringomyelia- in particular the non-communicating and possibly the extra-canalicular varieties. This is largely based on pathological evidence showing that macrophages are a particularly prominent finding in the spinal cord parenchyma of both these forms of human syringomyelia. The role of these cells in syringomyelia has not been evaluated systemically to date.

5.2 Rat model of non-communicating syringomyelia

In the past, cisternal injection of kaolin was one of the most widely used approaches to induce hydrocephalus and concomitant syringomyelia. However, this is less clinically relevant as syringomyelia is rarely associated with overt hydrocephalus in clinical practice.

Milhorat et al first described a reproducible experimental rat model of non-communicating syringomyelia which involves an intraparenchymal injection of kaolin into the cervical spinal cord. This model has also been particularly valuable for the study of CSF dynamics in relation to the syrinx.[377, 378]

The pathology of kaolin-induced noncommunicating syringomyelia is thought to occur in step-wise fashion as follows[267]: (1) initiation of a localised inflammatory reaction at the

kaolin injection site (2) drainage of inflammatory products rostrally within the central canal (3) occlusion of the central canal by proliferating ependymal cells and astrocytes and (4) dilation of the obstructed segments of the canal.

Inflammatory mediators produced by activated microglia and accumulating macrophages may be driven by CSF flow into the central canal . This may induce ependymitis and ependymal proliferation rostral to the site of injection. The obstruction to "normal" upward CSF flow within the central canal eventually results in distension of the central canal. While convincing evidence of CSF flow trans-parenchymally from the subarachnoid space into the central canal has recently been presented, a rostral pattern of normal flow in the central canal still remains to be proven.[21]

In an earlier work. Milhorat and his colleagues performed similar intraparenchymal injections of blood into the dorsal columns of rat thoracic spinal cords (T5-6).[265] This resulted in a significant microglial reaction locally at the site of injection. While erythrocytes, cellular debris and fibrin were observed within the central canal, neither central canal occlusion or syrinx formation was observed. This suggests that certain specific characteristics of the kaolin induced inflammation are important for syrinx formation. Unfortunately, these are not known.

5.3 Sink action hypothesis

In the current study, the macrophages and activated microglia appear to converge centripetally onto the central canal. This was particularly so at 4 weeks. It is not known

whether this represents the direct migration of $ED1^+$ cells from the kaolin droplet toward the central canal or 'static' by-stander cells that have been activated by inflammatory mediators carried by the inward CSF flow. Despite this, these observations appear to provide support for the 'sink action hypothesis' of the central canal. It is consistent with current understanding of transparenchymal CSF flow from the subarachnoid space. Subsequent CSF flow into the central canal may occur through enlarged Virchow-Robin spaces or the dorsal roots.[10, 267] Other possible sources of a net increase in syrinx fluid include inflammatory products, ependymal secretions and interstitial fluid derived from water during metabolism.[267]

Some animals in the 4 week study group demonstrated marked macrophagic aggregation within enlarged central canals. We speculate that this may potentially lead to further enlargement of the cystic cavity in some animals (FIGURE 24). It is likely that as the macrophages die or disappear, a large cystic cavity results. Continued CSF egress may lead to further extension of the cavity. Such a pattern of macrophage-filled syrinx may not be readily observed in human pathology because these studies are not typically performed in the acute/subacute stages of the disease. This hypothesis remains speculative at present and further research is necessary to clarify this.

5.4 Other forms of syringomyelia

Macrophages may also play a significant role in the initiation of extra-canalicular syringomyelia associated with conditions such as trauma and ischaemia. Multiple microcystic cavities may initially form as a consequence of macrophagic phagocytic

activity. These may subsequently coalesce to form a typical syrinx. Continued CSF flow into the cyst may then lead to either persistence or progressive enlargement of the cyst.

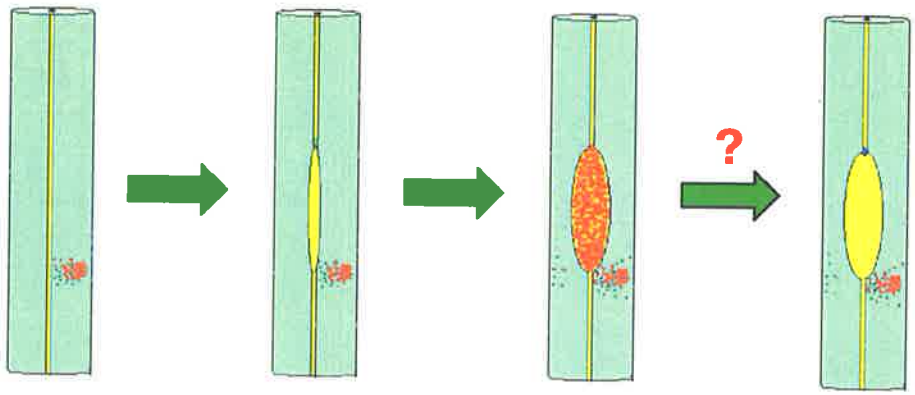

Figure 24. ***Proposed pathogenetic mechanisms in non-communicating rat syringomyelia.*** *A local macrophagic inflammatory infiltrate occurs early within the spinal cord parenchyma at the site of kaolin injection. Inflammatory mediators secreted by macrophages and activated microglia are carried by inward transparenchymal CSF flow into the central canal.This leads to ependymitis and canal occulsion rostral to the site of injection, resulting in an "obstruction" to upward flow of CSF. The central canal subsequently dilates. In some cases. macrophages further accumulate within the central canal causing further dilation. It is postulated that as these macrophages eventually disappear, a large syrinx results. Continued CSF egress may maintain or cause further expansion of the syringomyelic cavity.*

6 Limitations of the current research

Several potential limitations of the current study must be considered:

(1) The kaolin-induced model of non-communicating syringomyelia reproduces many of the pathological features (including macrophages) seen in human syringomyelia. However, it involves an intra-parenchymal injection of a foreign substance (kaolin) into the spinal cord substance. While this is not ideal, the current animal model represents the only reproducible model of noncommunicating syringomyelia to date.

(2) The irradiation of spinal cord tissues in the RT7.1 DA rats may theoretically alter the behaviour and activation of endogenous microglial cells. However, there is no evidence of ED1 upregulation in the spinal cords of control chimeric rats. Furthermore, the pattern and magnitude of ED1 immunoreactivity of chimeric rats were not significantly different from the non-irradiated rats at 3 days and 4 weeks.

(3) There is a theoretical possibility that some haematogenous cells may alter or down regulate their expression of the RT7.2 antigen as they enter the central nervous system. There is no literature to suggest such a "down regulation" phenomenon has ever been observed and is therefore considered to be extremely unlikely.

(4) The counting of immunoreactive cells (as performed in the current study) has well recognised inherent flaws. The number of immunoreactive cells on a particular tissue

section depends on the orientation of the section (transverse or longitudinal) and the precise level of the spinal cord where the section was obtained. In the current study, cell counting was performed on transverse tissue sections at the level where the cross sectional area of the kaolin droplet was the largest. It is the author's opinion that the cell counts when coupled with descriptive observations, provides a balanced view of the relative contributions from macrophages of different origins in the current rat model of non-communicating syringomyelia.

(5) Another criticism of the current proposed pathogenetic theory is that it requires a patent central canal. The critics argue that progressive stenosis of the central canal occurs in humans with increasing age. It has been widely accepted that in 71% to 80% of normal subjects the central canal of the spinal cord closes and becomes a vestigial structure represented by a core or nests of ependymal cells.[65] However, the implications of these findings are not entirely clear. For example, it is not known whether humans whose spinal cord central canals remain patent throughout life are more prone to syringomyelia. Furthermore, apparently stenosed or occluded central canals may still be functionally patent despite their histological appearances.

7 Conclusion

A large proportion of the macrophages has been derived from the bone marrow in the current rat model of non-communicating syringomyelia. At all time intervals, this represents nearly 50% of all $ED1^+$ cells. In addition, there has also been significant microglial activation and contribution to the pool of macrophages. The current research

has also provided evidence that the haematogenous macrophages appear to have different functional roles from their microglial derived counterparts.

8 Future research

The use of rat radiation bone marrow chimeras has provided an answer to the origin of macrophages in a well established noncommunicating model of rat syringomyelia. This is both a fundamental neurobiological as well as pathophysiological question that has not previously been addressed.

We have postulated that macrophages play a central pathogenetic role in the initiation of noncommunicating syringomyelia although this hypothesis has not been tested directly in the current work. A logical extension of the current work would therefore be to study the effect of macrophage depletion on rat syringomyelia.

Our results suggest that to achieve this, the therapeutic agent possibly needs to deplete both microglial as well as bone marrow derived macrophages effectively. Furthermore, the agent must be sufficiently specific in its actions, so that any observed result cannot be attributed to other immunosuppressive functions. Such an agent has recently become available.[178, 318] While it probably does not cross the blood brain barrier, it appears to deplete both exogenous and endogenous macrophages effectively.

Previous departmental work has shown that post-operative administration of dexamethasone and indomethacin reduces early syrinx formation at one week but not at 6 weeks. While these results appear to contradict the proposed inflammatory hypothesis, two factors must be considered. Firstly, the doses of the inflammatory agents used may not be optimal. Secondly, these agents may not be the ideal agents in targeting mononuclear phagocytes. While glucocorticoids appear to inhibit proliferative and secretory functions of monocytes/macrophages in vitro[429], Giulian et al observed that dexamethasone (0.8 mg/kg) in vivo neither inhibited the phagocytic activity in mononuclear phagocytes nor influenced astrogliosis or neovascularisation at the sites of brain injury[130]. Furthermore, both the activation and proliferation of microglia also did not appear to be affected by glucocorticoids.[53]

The other possibilities that stem from this work include;

(1) the application of rat radiation bone marrow chimeras to determine the origin of macrophages in other experimental models such as stab injuries and fat embolism to the brain.

(2) specifically studying the response of microglial cells in conditions such as experimental head injury, hypoxic brain injury and brain tumours.

(3) studying the spinal cords of rats with longer periods of survival (e.g. 6 to12 months) following kaolin injection into the spinal cord.

(4) studying the functional roles of macrophages in spinal cord trauma.

PRIZES

1. ***Justin Miller Memorial Prize 1999***

   - best paper delivered at the Annual Scientific Meeting

   (SA and NT), Wirrina Cove Paradise, South Australia.

2. ***NimmoResearch Prize 2001***

   - *best paper (full time category)*

   - *Royal Adelaide Hospital, South Australia*

SCIENTIFIC PRESENTATIONS

1. RACS Annual Scientific Meeting

   (South Australia and Northern Territory)

   Wirrina Cove Paradise,

   South Australia.

   August 7, 1999.

2. Neurosurgical Society of Australasia/

   Neural Injury Society of Australia

   Annual Scientific Meeting

   Hamilton Island,

   Queensland.

   October 9, 1999.

3. Spinal Society of Australasia

   Annual Scientific Meeting

   Fremantle,

   Western Australia.

   April 29, 2001.

References

1 Anonymous. Dorland's illustrated medical dictionary, Philadelphia:WB Saunders, 1988.

2 Aloisi F, Ria F, Penna G, Adorini L. Microglia are more efficient than astrocytes in antigen processing and in Th1 but not Th2 cell activation. **J Immunol** 1998;160 **(10)**:4671-4680.

3 Amar AP, Levy ML. Pathogenesis and pharmacological strategies for mitigating secondary damage in acute spinal cord injury. **Neurosurgery** 1999;**44(5)**:1027-1040.

4 Anderson RE. Ionising radiation and the immune response. **Adv Immunology** 1976; **24**:215-335.

5 Anton HA, Schweigel, JF. Posttraumatic syringomyelia: the British Columbia experience. **Spine** 1986;**11(9)**:865-868.

6 Aschoff L. Das reticulo-endotheliale System. **Ergebn.Inn.Med.Kinderheilk** 1924;**26:**1-118.

7 Ashwell K. Microglia and cell death in the developing mouse cerebellum. **Dev Brain Res** 55:219-230, 1990.

8 Avellino AM, Hart D, Dailey AT, MacKinnon M, Ellegala D, Kliot M. Differential macrophage responses in the peripheral and central nervous system during wallerian degeneration of axons. **Exp Neurol** 1995;**136(2)**:183-198.

9 Baldwin NG, Malone DG. Posttraumatic syringomyelia. In: Anson JA, Benzel EC, Awad IA, eds. Syringomyelia and the Chiari Malformations. USA:AANS Publications Committee, 1997:105-112.

10 Ball MJ, Dayan AD. Pathogenesis of syringomyelia. **Lancet** 1972;**2(7781)**:799-801.

11 Banati RB, Hoppe D, Gottmann K, Kreutzberg GW, Kettenmann H. A subpopulation of bone marrow-derived macrophage-like cells shares a unique ion channel pattern with microglia. **J Neurosci Res** 1991;**30**:593-600.

12 Banati RB, Gehrmann J, Schubert P, Kreutzberg GW. Cytotoxicity of microglia. **Glia** 1993;**7(1)**:111-118.

13 Banati RB, Graeber MB. Surveillance, intervention and cytotoxicity: is there a protective role of microglia? **Dev Neurosci** 1994;**16(3-4)**:114-127.

14 Barbaro NM, Wilson CB, Gutin PH, Edwards MS. Surgical treatment of syringomyelia. Favorable results with syringoperitoneal shunting. **J Neurosurg** 1984;61 (3):531-538.

15 Barclay AN, Jackson DI,Willis AC, Williams AF. Lymphocyte specific heterogeneity in the rat leucocyte common antigen (T200) is due to differences in polypeptide sequences near the NH2-terminus. **EMBO J** 1987;**6(5)**:1259-1264.

16 Barnett HJ, Jousse AT, Ball MJ. Pathology and pathogenesis of progressive cystic myeopathy as a late sequel to spinal cord injury. In: Barnett HJ, Foster JB, Hudgson P, eds. Syringomyelia, London:WB Saunders, 1973:261-301.

17 Barnett HJM. The epilogue. In: Barnett HJM, Foster JB, Hudgson P, eds. Syringomyelia, London:W.B. Saunders, 1973: 302-313.

18 Barnett HJMand Rewcastle NB. Syringomyelia and tumors of the nervous system. In: Barnett HJM, Foster JB, Hudgson P, eds. Syringomyelia, London:WB Saunders, 1973: 261-301.
19 Baron M, Gallego A. The relation of the microglia with the pericytes in the cat cerebral cortex. **Z Zellforsch Mikrosk Anat** 1972;**128**:42-57, 1972.
20 Batzdorf U. Chiari I malformation with syringomyelia. Evaluation of surgical therapy by magnetic resonance imaging. **J Neurosurg** 1988;**68(5)**:726-730.
21 Batzdorf U. Pathogenesis and development theories. In: Anson JA, Benzel EC, Awad IA, eds. Syringomyelia and the Chiari Malformations, USA:AANS Publications Committee, 1997: 35-40.
22 Bauer J, Sminia T, Wouterlood FG, Dijkstra CD. Phagocytic activity of macrophages and microglial cells during the course of acute and chronic relapsing experimental autoimmune encephalomyelitis. **J Neurosci Res** 1994;**38(4)**:365-375.
23 Bauer J, Huitinga I, Zhao W, Lassmann H, Hickey.W.F., Dijkstra CD. The role of macrophages, perivascular cells, and microglial cells in the pathogenesis of experimental autoimmune encephalomyelitis. **Glia** 1995;**15**:437-446.
24 Bauer J, Ruuls SR, Huitinga I, Dijkstra CD. The role of macrophage subpopulations in autoimmune disease of the central nervous system. **Histochem J** 1996;**28(2)**:83-97.
25 Becker DP, Wilson JA, Watson GW. The spinal cord central canal: response to experimental hydrocephalus and canal occlusion. **J Neurosurg** 1972;**36(4)**:416-424.
26 Berger T, Weerth S, Kojima K, Linington C, Wekerle H, Lassmann H. Experimental autoimmune encephalomyelitis: the antigen specificity of T lymphocytes determines the topography of lesions in the central and peripheral nervous system. **Lab Invest** 1997;**76(3)**:355-364.
27 Berry Mand Butt AM. Structure and function of glia in the central nervous system. In: Graham DI, Lantos PL, eds. Greenfield's Neuropathology, New York:Arnold, 1997:78-79.
28 Bielschowsky M, Unger E. Syringomyelie mit Teratom-und extramedullarer Blastombildung. **J Psychol Neurol** 1920;**25**:173-218.
29 Bjornson CRR, Rietze RL, Reynolds BA, Magli MC, Vescovi AL. Turning brain into blood: A hematopoietic fate adopted by adult neural stem cells in vivo. **Science** 1999;**283**:534-537.
30 Blagodatsky MD, Larionov SN, Manohin PA, Shanturov VA, Gladyshev YV. Surgical treatment of "hindbrain related" syringomyelia: new data for pathogenesis. **Acta Neurochir Wien** 1993;**124(2-4)**:82-85.
31 Blakemore WF. The ultrastructure of normal and reactive microglia. **Acta Neuropath Berl** 1975;**VI(suppl)**:273-278.
32 Blight AR. Delayed demyelination and macrophage invasion: A candidate for secondary cell damage in spinal cord injury. **Central Nervous System Trauma** 1985;**2(4)**:299-315.
33 Blight AR. Macrophages and inflammatory damage in spinal cord injury. **J Neurotrauma** 1992;**9(Suppl 1)**:83-91.

34 Blight AR. Effects of silica on the outcome from experimental spinal cord injury: implication of macrophages in secondary tissue damage. **Neuroscience** 1994;**60(1)**:263-273.
35 Blinzinger K, Kreutzberg G. Displacement of synaptic terminals from regenerating motoneurons by microglial cells. **Zeitschrift fur Zellforschung** 1968;**85**:145-157.
36 Blusse van Oud Alblas A, van der Linden-Schrever B, van Furth R. Origin and kinetics of pulmonary macrophages during an inflammatory reaction induced by intravenous administration of heat-killed BCG. **J Exp Med** 1981;**154**:235-252.
37 Blusse van Oud Alblas A, van der Linden-Schrever B, van Furth R. Origin and kinetics of pulmonary macrophages during an inflammatory reaction induced by intra-alveolar administration of aerosolized heat-killed BCG. **Am Rev Respir Dis** 1983;**128**:276-281.
38 Boje KM, Arora PK. Microglial-produced nitric oxide and reactive nitrogen oxides mediate neruonal cell death. **Brain Res** 1992;**587**:250-256.
39 Boros DL. Granuolmatous inflammations. **Prog Allergy** 1978;**24**:183-267.
40 Boya J, Calvo J, Prado A. The origin of microglial cells. **J Anat** 1979;**129(1)**:177-186.
41 Boya J, Calvo J, Carbonell AL, Garcia ME. Nature of macrophages in rat brain. A histochemical study. **Acta Anat Basel** 1986;**127(2)**:142-145.
42 Boya J, Carbonell AL, Calvo J, Borregon A. Ultrastructural study on the origin of rat microglia cells. **Acta Anat.Basel.** 1987;**130(4)**:329-335.
43 Boya J, Calvo JL, Carbonell AL, Borregon A. A lectin histochemistry study on the development of rat microglial cells. **J.Anat.** 1991;**175**:229-236.
44 Brain R. Brain's Neurology, 1960.
45 Brewis M, Poskanzer DC, Rolland C, Miller H. Neurological disease in an English city. **Acta Neurol Scand** 1966;**42(Suppl):**42:89.
46 Brosnan CF, Cammer W, Norton WT, Bloom BR. Proteinase inhibitors suppress the development of experimental allergic encephalomyelitis. *Nature* 1980;**285**:235-237.
47 Brosnan CF, Bornstein MB, Bloom BR. The effects of macrophage depletion on the clinical and pathologic expression of experimental allergic encephalomyelitis. **J Immunol** 1981;**126(2)**:614-620.
48 Bruck W, Friede RL. Anti-macrophage CR3 antibody blocks myelin phagocytosis by macrophages *in vitro*. **Acta Neuropathol** 1990;**80**:415-418.
49 Cabilly S,Gallily R. Non-immunological recognition and killing of xenogeneic cells by macrophages. I. Repertoire of recognition. **Immunology** 1981;**44(2)**:347-355.
50 Cadelli DS, Bandtlow CE, Schwab M. Oligodendrocytes and myelin-associated inhibitors of neurite growth: their involvement in the lack of CNS regeneration. **Exp Neurol** 1992;**115**:189-192.
51 Cammermeyer J. Morphologic distinctions between oligodendrocytes and microglial cells in the rabbit cerebral cortex. **Am J Anat** 1966;**118**:227-248.
52 Carson MJ, Reilly CR, Sutcliffe G, Lo D. Mature microglia resemble immature antigen-presenting cells. **Glia** 1998;**22**:72-85.
53 Castano A, Lawson LJ, Fearn S, Perry VH. Activation and proliferation of murine microglia are insensitive to glucocorticoids in Wallerian degeneration. **Eur J Neurosci** 1996;**8(3)**:581-588.

54 Castillo M, Quencer RM, Green BA, Montalvo BM. Syringomyelia as a consequence of compressive extramedullary lesions: postoperative clinical and radiological manifestations. **AJR** 1988;**150(2)**:391-396.
55 Chakrabortty S, Tamaki N, Ehara K, Ide C. Experimental syringomyelia in the rabbit: an ultrastructural study of the spinal cord tissue. **Neurosurgery** 1994;**35(6)**:1112-1120.
56 Chakrabortty S, Tamaki N, Ehara K, Takahashi A, Ide C. Experimental syringomyelia: late ultrastructural changes of spinal cord tissue and magnetic resonance imaging evaluation. **Surg Neurol** 1997;**48(3)**:246-254.
57 Chambers TJ. Multinucleated giant cells. **J Pathol** 1978;**126**:125-148.
58 Chao CC, Hu.S, Molitor TW, Shaskan EG, Peterson PK. Activated microglia mediate neuronal cell injury via a nitric oxide mechanism. **J Immunol** 1992;**149(8)**:2736-2741.
59 Chiari H. Uber Veranderungen des Kleinhirns, des Pons und der Medulla Oblongata in Folge von congenitaler Hydrocephalie des Grosshirns. **Denkschr Akad Wiss Wien** 1895;**63**:71-116.
60 Cho KH, Iwasaki Y, Imamura H, Hida K, Abe H. Experimental model of posttraumatic syringomyelia: the role of adhesive arachnoiditis in syrinx formation. **J Neurosurg** 1994;**80(1)**:133-139.
61 Choi BH. Hematogenous cells in the central nervous system of developing human embryos and fetuses. **J Comp Neurol** 1981;**196**:683-694.
62 Cleland J. Contribution to the study of spina bifida, encephalocoele, and anencephalus. **J Anat Physiol** 1883;**17**:257-291.
63 Cohen WA, Young W, DeCrescito V, Horii S, Kricheff II. Posttraumatic syrinx formation: experimental study. **AJNR** 1985;**6(5)**:823-827.
64 Colton CA. Microglial oxyradical production: causes and consequences. **Neuropathol Appl Neurobiol** 1994;**20(2)**:208-209.
65 Conway LW. Hydrodynamic studies in syringomyelia. *J.Neurosurg.* 1967;**27(6)**:501-514.
66 Cotran RS, Kumar V, Robbins SL, Schoen FJ. Inflammation and repair. In: Cotran RS, Kumar V, Robbins SL, Schoen FJ, eds. Pathologic basis of disease.Philadelphia:W.B. Saunders, 1994:51-92.
67 Damoiseaux JGMC, Dopp EA, Calame W, Chao D, MacPherson GG. Rat macrophage lysosomal membrane antigen recognised by monoclonal antibody ED1. **Immunology** 1994;**83**:140-147.
68 Dannenberg AM, Ando M, Shima K, Tsuda T. Macrophage turnover and activation in tuberculous granulomata. In: van Furth R, ed. Mononuclear phagocytes in immunity, infection and pathology. Oxford:Blackwell Scientific Publications, 1975:959-980.
69 Dannenberg AM. Pathogenesis of tuberculosis. In: Fishmann AP, ed. Pulmonary disease and disorders. New York:McGraw-Hill, 1980:1264-1281.
70 David S, Bouchard C, Tsatas O, Giftochristos N. Macrophages can modify the nonpermissive nature of the adult mammalian central nervous system. **Neuron** 1990;**5**:463-469.
71 Davies P, Bonney RJ. Secretory products of mononuclear phagocytes: A brief review. **J Reticuloendothel Soc** 1979;**26**:37-47.

72 Davison C, Keschner M. Myelitic and myelopathic lesions VI. Cases with marked circulatory interference and a picture of syringomyelia. **Arch Neurol Psychiatry** 1933;**30**:1074-1085.
73 de Groot CJA, Huppes W, Sminia T, Kraal G, Dijkstra CD. Determination of the origin and nature of brain macrophages and microglial cells in mouse central nervous system, using non-radioactive in situ hybridization and immunoperoxidase techniques. **Glia** 1992;**6**:301-309.
74 de Lotbiniere ACJ. Historical considerations. In: Anson JA, Benzel EC, Award IA, eds. Syringomyelia and the Chiari Malformations.USA:AANS Publications Committee, 1997:1-26.
75 De Simone R, Giampaolo A, Giometto B, and et al. The costimulatory molecule B7 is expressed on human microglia in culture and in multiple sclerosis acute lesions. **J Neuropathol Exp Neurol** 1995;**54**:175-187.
76 de Groot CJ, Huppes W, Sminia T, Kraal G, Dijkstra CD. Determination of the origin and nature of brain macrophages and microglial cells in mouse central nervous system, using non-radioactive in situ hybridization and immunoperoxidase techniques. **Glia** 1992;**6(4)**:301-309.
77 Del Cerro M, Monjan AA. Unequivocal demonstration of the hematogenous origin of brain macrophages in a stab wound by a double-label technique. **Neuroscience** 1979;**4** :1399-1404.
78 Dickson DW and Lee SC. Microglia. In: Davis RL, Robertson DM, eds. Textbook of Neuropathology, 3rd ed. Baltimore: Williams & Wilkins,1995:165-205.
79 Diesselhoff-den Dulk MMC, Crofton RW, van Furth R. Origin and kinetics of Kupffer cells during an acute inflammatory response. **Immunology** 1979;**37**:7-14.
80 Dijkstra CD, Dopp EA, Joling P, Kraal G. The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognised by monoclonal antibodies ED1, ED2 and ED3. **Immunology** 1985;**54**:589-599.
81 Dijkstra CD, Dopp EA, Huitinga I, Damoiseaux JGMC. Macrophages in experimental autoimmune diseases in the rat: a review. **Current Eye Research** 1992;**11(Suppl)**:75-79.
82 Dijkstra CD, de Groot CJA, Huitinga I. The role of macrophages in demyelination. **J Neuroimmunol** 1992;**40**:183-188.
83 Dijkstra CD, Dopp EA, van den Berg TK, Damoiseaux JGMC. Monoclonal antibodies against rat macrophages. **Journal of Immunological Methods** 1994;**174**:21-23.
84 Dijkstra CD. Personal communication, 1999.
85 Drake C. Syringomyelia, London:WB Saunders, 1973.
86 du Boulay G, Shah SH, Currie JC, Logue V. The mechanism of hydromyelia in Chiari type 1 malformations. **Br J Radiol** 1974;**47(561)**:579-587.
87 Dunning HS, Furth J. Studies on the relation between microglia, histiocytes and monocytes. **Am.J.Pathol.** 1935;**XI(6)**:895-913.
88 Dusart I, Schwab ME. Secondary cell death and the inflammatory reaction after dorsal hemisection of the rat spinal cord. **Eur J Neurosci** 1994;**6(5)**:712-724.

89 Elkabes S, Bloom EM ,DiCicco EM, Black IB. Brain microglia/macrophages express neurotrophins that selectively regulate microglial proliferation and function. **J Neurosci** 1996;**16(8)**:2508-2521.
90 Fantone JC, Ward PA. Role of oxygen-derived free radicals and metabolites in leucocyte-dependent inflammatory reactions. **AJP** 1982;**107**:397-418.
91 Faulhauer K, Loew K. The surgical treatment of syringomyelia. Long-term results. **Acta Neurochir Wien** 1978;**44(3-4)**:215-222.
92 Fedoroff S. Development of microglia. In: Kettenmann H, Ransom BR, eds. Neuroglia. New York:Oxford University Press, 1995:162-181.
93 Fehlings MG, Tator CH. A review of models of acute experimental spinal cord injury. In: Ilschner S, ed. Spinal Cord Dysfunction: Assessment .Oxford:Oxford University Press, 1988:3-33.
94 Feigin I, Ogata J, Budzilovich G. Syringomyelia: the role of edema in its pathogenesis. **J Neuropathol Exp Neurol** 1971;**30(2)**:216-232.
95 Ferrer I, Bernet E, Soriano E, Del Rio T, Fonseca M. Naturally occurring cell death in the cerebral cortex of the rat and removal of dead cells by transitory phagocytes. **Neuroscience** 1990;**39(2)**:451-458.
96 Feuerstein GZ, Liu T, Barone FC. Cytokines, inflammation, and brain injury: role of tumour necrosis factor-alpha. **Cerebrovasc Brain Metab Rev** 1994;**6**:341.
97 Fischer HG, Eder C, Hadding U, Heinemann U. Cytokine-dependent K+ channel profile of microglia at immunologically defined functional states. **Neuroscience** 1995;**64(1)**:183-191.
98 Flaris NA, Densmore TL, Molleston MC, Hickey WF. Characterization of microglia and macrophages in the central nervous system of rats: definition of the differential expression of molecules using differential expression of molecules using standard and novel monoclonal antibodies in normal CNS and in four models of parenchymal reaction. **Glia** 1993;7:34-40.
99 Ford CE, Hamerton JL, Barnes DWH, Loutit JF. Cytological identification of radiation chimeras. **Nature (London)** 1956;**113**:510-511.
100 Foster JB, Hudgson P. Traditional concepts of syringomyelia. In: Barnett HJM, Foster JB, Hudgson P, eds. Syringomyelia. London:W.B. Saunders, 1973:11-15.
101 Foster JB, Hudgson P. The clinical features of communicating syringomyelia. In: Barnett HJM, Foster JB, Hudgson P, eds. Syringomyelia. London:W.B. Saunders, 1973:16-29.
102 Foster JB, Hudgson P. Historical introduction. In: Barnett HJM, Foster JB, Hudgson P, eds. Syringomyelia, London:W.B. Saunders, 1973:3-10.
103 Foster JB, Hudgson P. The pathogenesis of communicating syringomyelia. In: Syringomyelia, edited by H. J. M. Barnett, J. B. Foster, and P. Hudgson, eds. Syringomyelia, London:W.B. Saunders, 1973:104-123.
104 Foster JB, Hudgson P. The pathology of communicating syringomyelia. In: Barnett HJM, Foster JB, Hudgson P, eds. Syringomyelia, London:W.B. Saunders, 1973:79-103.
105 Franzen R, Schoenen, J, Leprince P, Joosten E, Moonen G, Martin D. Effects of macrophage transplanation in the injured adult rat spinal cord: A combined immunocytochemical and biochemical study. **J Neurosci Res** 1998;**51**:316-327.

106 Frei K, Siepl C, Groscurth P, Bodmer S, Schwerdel C, Fontana A. Antigen presentation and tumor cytotoxicity by interferon-gamma-treated microglial cells. **Eur J Immunol** 1987;**17**:1271-1278.
107 Frei K, Leist TP, Meaget A, Gallo P, Leppert D, Zinkernagel RM, Fontana A. Production of B-cell stimulating factor-2 and interferon in the central nervous system during viral meningitis and encephalitis. **J Expl Med** 1988;**168**:449-453.
108 Frei K, Fredrickson S, Fontana A, Link H. Interleukin-6 is elevated in plasma in multiple sclerosis. **J Neuroimmunol** 1991;**31**:147-153.
109 Fujita S, Kitamura T. Origin of brain macrophages and the nature of the so-called microglia. **Acta Neuropathol Suppl Berl** 1975;**6(suppl)**:291-296.
110 Galluci M, Bozzao A, Splendiani A. Clinically manifest or silent primary and secondary syringomyelia: the magnetic resonance findings. **Radiol Med Torino** 1990;**79**:290-296.
111 Garcia JH, Kamijyo Y. Cerebral infarction: Evolution of histopathological changes after occlusion of a middle cerebral artery in primates. **J Neuropathol Exp Neurol** 1974;**33**:408-421.
112 Gardner WJ. Hydrodynamic mechanism of syringomyelia: its relationship to myelocele. **J Neurol Neurosurg Psychiat** 1965;**28**:247-259.
113 Gardner WJ. Embryologic origin of spinal malformations. **Acta Radiol Diagn Stockh** 1966;**5**:1013-1023.
114 Gardner WJ. Myelocele: rupture of the neural tube? **Clin Neurosurg** 1968;**15**:57-79.
115 Gardner WJ. *The dysraphic states*, Amsterdam:Excerpta Medica, 1973.
116 Gardner WJ. Letter: Danger of pantopaque myelography in syringomyelia. **J Neurosurg** 1974;**41(6)**:785.
117 Gardner WJ, McMurray FG. ""Non-communicating" syringomyelia: a non-existent entity. **Surg Neurol** 1976;**6(4)**:251-256.
118 Gardner WJ. Syringomyelia [letter]. **Surg Neurol** 1977;**7(6)**:370.
119 Gardner WJ, Bell HS, Poolos PN, Dohn DF, Steinberg M. Terminal ventriculostomy for syringomyelia. **J Neurosurg** 1977;**46(5)**:609-617.
120 Gardner WJ. Klippel-Feil syndrome, iniencephalus, anencephalus, hindbrain hernia and mirror movements: overdistention of the neural tube. **Childs Brain** 1979;**5(4)**:361-379.
121 Gehrmann J, Kreutzberg GW. Characterisation of two new monoclonal antibodies directed against rat microglia. **J Comp Neurol** 1991;**313**:409-430.
122 Gehrmann J, Banati RB, Kreutzberg GW. Microglia in the immune surveillance of the brain: human microglia constitutively express HLA-DR molecules. **J Neuroimmunol** 1993;**48(2)**:189-198.
123 Gehrmann J, Gold R, Linington C, Lannes-Vieira J, Wekerle H, Kreutzberg GW. Microglial involvement in experimental autoimmune inflammation of the central and peripheral nervous system. **Glia** 1993;**7**:50-59.
124 Gehrmann J, Kreutzberg GW. Experimental models to study microglial in vivo. The activated microglia: an early response element in the injured CNS? **Neuropathol Appl Neurobiol** 1994;**20(2)**:180-182.

125 George R, Griffin JW. Delayed macrophage responses and myelin clearance during Wallerian degeneration in the central nervous system: the dorsal radiculotomy model. **Exp Neurol** 1994;**129(2)**:225-236.
126 Gerlach W. Ein Fall von kongenitaler Syringomyelie mit intramedullarer Teratombildung. **Dtsche Z Nervenheilkd** 1894;**5**:271-301.
127 Ginsburg I, Mitrani S, Ne'eman N, Lahav M. Granulomata in streptococcal inflammation: mechanisms of localization transport and degradation of streptococci in inflammatory sites. In: van Furth R, ed. Mononuclear phagocytes in immunity, infection and pathology, Oxford:Blackwell Scientific Publications, 1975:981-1014.
128 Giordana MT, Attanasio A, Cavalla P, Migheli A, Vigliani MC, Schiffer D. Reactive cell proliferation and microglia following injury to the rat brain. **Neuropathol Appl Neurobiol** 1994;**20(2)**:163-174.
129 Giulian D, Baker TJ. Characterization of ameboid microglia isolated from developing mammalian brain. **J Neurosci** 1986;**6(8)**:2163-2178.
130 Giulian D. Amoeboid microglia as effectors of inflammation in the central nervous system. **J Neurosci Res** 1987;**18**:155-171.
131 Giulian D, Chen J, Ingeman JE, George JK, Noponen M. The role of mononuclear phagocytes in wound healing after traumatic injury to adult mammalian brain. **J Neurosci** 1989;**9(12)**:4416-4429.
132 Giulian D, Robertson C. Inhibition of mononuclear phagocytes reduces ischemic injury in the spinal cord. **Ann Neurol** 1990;**27**:33-42.
133 Giulian D, Vaca K, Corpuz M. Brain glia release factors with opposing actions upon neuronal survival. **J Neurosci** 1993;**13(1)**:29-37.
134 Giulian D, Haverkamp LJ, Li J, Karshin WL, Yu J, Tom D, Li X, Kirkpatrick JB. Senile plaques stimulate microglia to relaease a neurotoxin found in Alzheimer brain. **Neurochem Int** 1995;**27(1)**:119-137.
135 Giulian D, Corpuz M, Richmond B, Wendt E, Hall ER. Activated microglia are the principal glial source of thromboxane in the central nervous system. **Neurochem Int** 1996;**29(1)**:65-76.
136 Gordon S, Cohn ZA. The macrophage. **Int Rev Cytol** 1973;**36**:171-214.
137 Gordon S. Biology of the macrophage. **J Cell Sci** 1986;**4(Suppl)**:267-286.
138 Graeber MB, Streit WJ, Kreutzberg GW. Axotomy of the rat facial nerve leads to increased CR3 complement receptor expression by activated microglial cells. **J Neurosci Res** 1988;**21(1)**:18-24.
139 Graeber MB, Tetzlaff W, Streit WJ, Kreutzberg GW. Microglial cells but not astrocytes undergo mitosis following rat facial nerve axotomy. **Neurosci.Lett.** 1988;**85**:317-321.
140 Graeber MB, Streit WJ, Kreutzberg GW. Formation of microglia-derived brain macrophages is blocked by adriamycin. **Acta Neuropathol Berl** 1989;**78(4)**:348-358.
141 Graeber MB, Streit WJ, Kreutzberg GW. Identity of ED2-positive perivascular cells in rat brain. **J Neurosci Res** 1989;**22(1)**:103-106.
142 Graeber MB, Streit WJ. Perivascular microglia defined [letter; comment]. **Trends Neurosci** 1990;**13(9)**:366.

143 Graeber MB, Streit WJ, Kreutzberg GW. The third glial cell type, the microglia: cellular markers of activation in situ. **Acta Histochem.Suppl.** 1990;**38**:157-160.
144 Graeber MB, Streit WJ, Kiefer R, Schoen SW, Kreutzberg GW. New expression of myelomonocytic antigens by microglia and perivascular cells following lethal motor neuron injury. **J Neuroimmunol** 1990;**27(2-3)**:121-132.
145 Graeber MB. Development of the microglia literature. **Neuropathol Appl Neurobiol** 1994;**20(2)**:215-216.
146 Graeber MB, Mehraein P. Microglial rod cells. **Neuropathol Appl Neurobiol** 1994;**20(2)**:178-180.
147 Grau V, Herbst B, van-der Meide PH, Steiniger B. Activation of microglial and endothelial cells in the rat brain after treatment with interferon-gamma in vivo. **Glia** 1997:**19(3)**:181-189.
148 Guizar-Sahagun G, Grijalva I, Madrazo I, Franco-Bourland R, Salgado H, Ibarra A, Oliva H, Zepeda A. Development of Post-traumatic Cysts in the Spinal Cord of Rats Subjected to Severe Spinal Cord Contusion. **Surg Neurol** 1994;**41**:238-240.
149 Gull W. Case of progressive atrophy of the muscles of the hands: enlargement of the ventricle of the cord in the cervical region, with atrophy of the gray matter (hydromyelus). **Guys Hosp.Report (3rd series)** 1862;**8**:244-250.
150 Hall EJ. Acute effects of total-body irradiation. In: Radiobiology for the Radiologist, Philadelphia:J.B.Lippincott Company, 1994:311-322.
151 P. Hall, J. Godersky, Muller J, R. Campbell R, Kalsbeck J. A study of experimental syringomyelia by scanning electron microscopy. **Neurosurgery** 1977;**1(1)**:41-47.
152 Hall P, Turner M, Aichinger S, Bendick P, Campbell R. Experimental syringomyelia: the relationship between intraventricular and intrasyrinx pressures. **J.Neurosurg.** 1980;**52(6)**:812-817.
153 Hall PV, Muller J, Campbell RL. Experimental hydrosyringomyelia, ischemic myelopathy, and syringomyelia. **J.Neurosurg.** 1975;**43(4)**:464-470.
154 Hanieh A, Simpson DA. Cavitation of the Spinal Cord in Association with Spina Bifida. **Z Kinderchir** 1980;**31(4)**:321-326.
155 Harding B, Copp AJ. Malformations. In: Graham DI, Lantos PL, eds .Greenfield's neuropathology, London:Arnold, 1997:486-490.
156 Harel A, Fainaru M, Hernandez M, Schwartz M. Optic nerve regeneration in adult fish and apolipoprotein A-I. **J.Neurochem.** 1989;**52**:1218-1228.
157 Hartung H-P, Jung S, Stoll G, Zielasek J, Schmidt B, Archelos JJ, Toyka KV. Inflammator mediators in demyelinating disorders of the CNs and PNS. **Neuroimmunol.** 1992;19:177-189.
158 Hartung HP, Archelos JJ, Zielasek R, Gold M, Koltzenburg K-H, Reiners K, Tokya V. Circulating adhesion molecules and inflammatory mediators in demyelination: a review. **Neurology** 1995;**45(Suppl(6))**:S22.
159 Hassin GB. A contribution to the histopathology and histogenesis of syringomyelia. **Arch.Neurol.Psychiatry** 1920;**3**:130-147, 1920.
160 Hayes GM, Woodroofe MN, Cuzner ML. Microglia express MHC class II in normal and demyelinating human white matter. **Ann.N.Y.Acad.Sci.** 1988;**540**:501-503.
161 He BP, Tay SS, Leong SK. Do microglial cells have a neuroprotective function? **J.Hirnforsch.** 1997;**38(3)**:309-315.

162 Heese K, Hock C, Otten U. Inflammatory Signals induce neurotrophin expression in human microglial cells. **J.Neurochem.** 1998;**70**:699-707.
163 Hennenberg R, Koch M. Zur Pathogenese der Syringomyelie und uber Hamatomyelie bei Syringomyelie. **Monatsschr.Psychiatr.Neurol.** 1923;**54**:117-140.
164 Herscowitz HB, Holden HT, Bellanti JA, Ghaffar A. Manual of macrophage methodology, New York:Dekker, 1981.
165 Heumann R, Linndholm D, Bandtlow C, Meyer M, Radeke KJ, Miko TP, Shooter E, Theonen H. Differential regulation of mRNA encoding nerve growth factor and its receptor in sciatic nerve furing development, degeneration, and regeneration: role of macrophages. **Proc.Natl.Acad.Sci.U.S.A.** 1987;**84**:8735-8739.
166 Heyes MP, Nowak TSJ. Delayed increases in regional brain quinolinic acid follow transient ischaemia in the gerbil. **J.Cereb.Blood Flow Metab.** 1990;**10**:660-667.
167 Heyes MP, Achim CL, Wiley CA, Major EO, Saito K, Markey S. Human microglia convert L-tryptophan into the neurotoxin quinolinic acid. **Biochem.J.** 1996;**320**:595-597.
168 Hickey WF, Kimura H. Perivascular microglial cells of the CNS are bone marroe-derived and present antigen in vivo. **Science** 1986;**239**:290-292.
169 Hickey WF. Migration of hematogenous cells through the blood-brain barrier and the initiation of CNS inflammation. **Brain Pathol.** 1991;**1(2)**:97-105.
170 Hickey WF, Vass K, Lassmann H. Bone marrow-derived elements in the central nervous system: An immunohistochemical and ultrastructural survey of rat chimeras. **J.Neuropathol.Exp.Neurol.** 1992;**51(3)**:246-256.
171 Hickey WF. Personal communication, 1999.
172 Hinokuma K, Ohama E, Oyanagi K, Kakita A, Kawai K, Ikuta F. Syringomyelia. A neuropathological study of 18 autopsy cases. **Acta Pathol.Jpn.** 1992;**42**:25-34.
173 Hirsch S, Gordon S. Surface antigens as markers of mouse macrophage differentiation. **Int.Rev.Exp.Pathol.** 1983;**25**:51-75.
174 Hirsch S, Gordon S. The use and limitation of monoclonal antibodies against mononuclear phagocytes. **Immunobiol.** 1982;**161**:298-307.
175 Ho MK,Springer TA. Mac-2, a novel 32,000 M mouse macrophage subpopulation-specific antigen defined by monoclonal antibodies. **J.Immunol.** 1982;**128**:1221-1228.
176 Hormigo A, Lobo-Antunes J, Bravo-Marques J. Syringomyelia secondary to compression of the cervical spinal cord by an extramedullary lymphoma. **Neurosurgery** 1990;**27**:834-836.
177 Hu S, Chao CC, Khana KV, Gekker G, Peterson PK, Molitor TW. Cytokine and free radical production by porcine microglia. **Clinical Immunology and Immunopathology** 1996;**78(1)**:-93.
178 Huitinga I, van Rooijen N, de Groot CJA, Uitdehaag BMJ, Dijkstra CD. Suppression of experimental allergic encephalomyelitis in Lewis rars after elimination of macrophages. **J.Exp.Med.** 1990;**172**:1025-1033.
179 Huitinga I, Ruuls SR, Jung S, van Rooijen N, Hartung HP, Dijkstra CD. Macrophages in T cell line-mediated, demyelinating, and chronic relapsing experimental autoimmune encephalomyelitis in Lewis rats. **Clin.Exp.Immunol.** 1995;**100(2)**:344-351.

180 Hume DA, Gordon S. The mononuclear phagocyte system of the mouse defined by immunohistochemical localisation of antigen F4/80: identification of resident macrophages in renal and cortical interstitium and the juxtaglomerular complex. **J.Exp.Med.** 1983;**157**:1704-1709.

181 Hume DA, Gordon S. The mononuclear phagocyte system of the mouse defined by immunohistochemical localisation of antigen F4/80. In: Mononuclear phagocytes. Characteristics, physiology and function. Boston:Martnus Nijhoff, 1985:9-17.

182 Imamoto K, Leblond CP. Presence of labeled monocytes, macrophages and microglia in a stab wound of the brain following an injection of bone marrow cells labeled with 3H-uridine into rats. **J.Comp.Neurol.** 1977;**174(2)**:255-279.

183 Imamoto K, Fujiwara R, Nagai T, Maeda T. Distribution and fate of macrophagic amoeboid cells in the rat brain. **Arch.Histol.Jap.** 1982;**45(5)**:505-518.

184 Innocenti GM, Clarke S, Koppel H. Transitory macrophages in the white matter of the developing visual cortex. II. Development and relations with axonal pathways. **Dev.Brain Res** 1983;**11**:56-66.

185 Inoue K, Nakajima K, Morimoto T, Kikuchi Y, Koizumi S, Illes P, Kohsaka S. ATP stimulation of Ca2+ -dependent plasminogen release from cultured microglia. **Br.J.Pharmacol.** 1998;**123(7)**:1304-1310.

186 Issekutz TB, Issekutz AC, Movat HZ. The *in vivo* quantification and kinetics of monocyte migration into acute inflammatory tissue. **Am.J.Pathol.** 1981;**103**:47-55.

187 Ivy OG, Killackey HP. Transient populations of glial cells in developing rat telencephalon revealed by horseradish peroxidase. **Brain Res.** 1978;**158**:213-218.

188 Jackson DI, Barclay AN. The extra segments of sequence in rat leucocyte common antigen (L-CA) are derived by alternative splicing of only three exons and show extensive O-linked glycosylation. **Immunogenetics** 1989;**29(5)**:281-287.

189 Jacobson LO, Simmons EL, Marks EK, Eldredge JH. Recovery from radiation injury. **Science** 1951;**113**:510-511.

190 James AE Jr, Flor WJ, Novak GR, Strecker EP, Burns B. **J Neurosurg** 1978;**48(6)**: 970-4.

191 Jordan FL, Thomas WE. Brain macrophages: questions of origin and interrelationship. **Brain Res.** 1988;**472(2)**:165-178.

192 Juba A. Untersuchungen uber die Entwicklung der Hortegaschen Microglia des Menschen. **Arch.Psychiat.Nervenkr.** 1934;**101**:577-592.

193 Jun CD, Hoon-Ryu Um JY, Kim TY, Kim JM, Kang SS, Kim HM, Chung HT. Involvement of protein kinase C in the inhibition of nitric oxide production from murine microglial cells by glucocorticoid. **Biochem.Biophys.Res.Commun.** 1994;**199(2)**:633-638.

194 Kampinga J, Kroese FGM, Pol GH, Opstelten D, Seijen HG, Boot JHA, Roser B, Nieuwenhuis P. RT7-defined alloantigens in rats are part of the leukcocyte common antigen family. **Scand.J.Immunol.** 1990;**31**:699-710.

195 Kaur C, Ling EA, Wong WC. Scanning electron microscopy of transitory subependymal cysts in the developing midbrain of postnatal rats. **Arch.Histol.Cytol.** 1989;**52**:311-317.

196 Kerns JM, Hinsman EJ. Neuroglial response to sciatic neurectomy. I. Light microscopy and autoradiography. **J.Comp.Neurol** 1973;**151(3)**:237-254.

197 Kim YS, Kim SU. Oligodendroglial death induced by oxygen radicals and its protection by catalase. **J.Neurosci.** 1991;**29**:100-106.
198 Kitamura T, Hattori H, Fujita S. Autoradiographic studies in histogenesis of brain macrophages in the mouse. **J.Neuropathol.Exp.Neurol.** 1972;**31**:502-518.
199 Kitamura T. The origin of brain macrophages- Some considerations on the microglia theory of Del Rio-Hortega. **Acta.Path.Jap.** 1973;**23(1)**:11-26.
200 Kitamura T, Miyake T, Fujita S. Genesis of resting microglia in the gray matter of mouse hippocampus. **J.Comp.Neurol.** 1984;**226**:421-433.
201 Konat GW, Wiggins RC. Effect of reactive oxygen species on myelin membrane proteins. **J.Neurochem.** 1985;**45**:1113-1118.
202 Konigsmark BW, Sidman RL. Origin of brain macrophages in the mouse. **J.Neuropathol.Exp.Neurol.** 1963;**22**:643-676.
203 Koshinaga M, Whittemore SR. The temporal and spatial activation of microglia in fiber tracts undergoing anterograde and retrograde degeneration following spinal cord lesion. **J.Neurotrauma.** 1995;**12(2)**:209-222.
204 Kreutzberg GW. Microglia, the first line of defence in brain pathologies. **Arzneimittelforschung.** 1995;**45(3A)**:357-360.
205 Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. **Trends.Neurosci.** 1996;**19(8)**:312-318.
206 Kreutzberg GW. Introduction. **Neuropathol.Appl.Neurobiol.** 1990;**20**:176. (Abstract)
207 Kurz H,Christ B. Embryonic CNS macrophages and microglia do not stem from circulating, but from extravascular precursors. **Glia** 1998;**22**:98-102.
208 Lassmann H, Wisniewski HM. Chronic relapsing experimental allergic encephalomyelitis: Clinicopathological comparison with multiple sclerosis. **Arch.Neurol.** 1979;**36**:490-498.
209 Lassmann H, Zimprich F, Rossler K, Vass K. Inflammation in the nervous system. Basic mechanisms and immunological concepts. **Rev.Neurol.Paris.** 1991;**147(12)**:763-781.
210 Lassmann H, Zimprich F, Vass K, Hickey WF. Microglial cells are a component of the perivascular glia limitans. **J.Neurosci.Res.** 1991;**28(2)**:236-243.
211 Lassmann H, Schmied M, Vass K, Hickey WF. Bone marrow derived elements and resident microglia in brain inflammation. **Glia** 1993;**7(1)**:19-24.
212 Lassmann H, Hickey WF. Radiation bone marrow chimeras as a tool to study microglia turnover in normal brain and inflammation. **Clin.Neuropathol.** 1993;**12(5)**:284-285.
213 Lassmann H, Rinner W, Hickey WF. Differential role of hematogenous macrophages, resident microglia and astrocytes in antigen presentation and tissue damage during autoimmune encephalomyelitis. **Neuropathol.Appl.Neurobiol.** 1994;**20(2)**:195-196.
214 Lawson LJ, Perry VH, Dri P, Gordon S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. **Neuroscience 1990;39(1)**:151-170.
215 Lawson LJ, Perry VH, Gordon S. Turnover of resident microglia in the normal adult mouse brain. **Neuroscience** 1992;**48(2)**:405-415.

216 Lawson LJ, Frost L, Risbridger J, Fearn S, Perry VH. Quantification of the mononuclear phagocyte response to Wallerian degeneration of the optic nerve. **J.Neurocytol.** 1994;**23(12)**:729-744.
217 Lazarov-Spiegler O, Solomon AS, Zeev-Brann AB, Hirschberg DL, Lavie V, Schwartz M. Transplanatation of activated macrophages overcomes central nervous system regrowth failure. **FASEB J.** 1996;**10**:1296-1302.
218 Lazarov-Spiegler O, Solomon AS, Schwartz M. Peripheral nerve-stimulated macrophages simulate a peripheral nerve-like regenerative response in rat transected optic nerve. **Glia** 1998;**24**:329-337.
219 Lazarov-Spiegler O, Rapalino O, Agranov G, Schwartz M. Restricted inflammatory reaction in the CNS: a key impediment to axonal regeneration? **Molecular Medicine Today** 1998;**4**:337-342.
220 Lee CS, Outteridge PM. The identification and ultrastructure of macrophages from the mammary gland of the ewe. **Aust.J.Exp.Biol.Med.Sci.** 1976;**54(1)**:43-55.
221 Lees GJ. The possible contribution of microglia and macrophages to delayed neuronal death after ischemia. **J.Neurol.Sci.** 1993;**114(2)**:119-122.
222 Levy R, Rosenblatt S, Russell E. Percutaneous drainage and serial magnetic resonance imaging in the diagnosis of symptomatic posttraumatic syringomyelia: case report and review of the literature. **Neurosurgery** 1991;**29**:429-433.
223 Levy WJ, Mason L, Hahn JF. Chiari malformation presenting in adults: a surgical experience in 127 cases. **Neurosurgery** 1993;**12(4)**:377-390.
224 Lewis PD. The fate of the subependymal cells in the adult rat brain, with a note on the origin of microglia. **Brain** 1968;**91**:721-738.
225 Leyden E. Uber Hydromyelus and Syringomyelie. **Arch.Path.Anat.Physiol.** 1968;**68**:1-20.
226 Li YB, Kaur C, Ling EA. Labeling of amoeboid microglial cells and intraventricular macrophages in fetal rats following a maternal injection of a fluorescent dye. **Neurosci.Res.** 1997;**28(2)**:119-125.
227 Liber AF, Lisa JR. Rosenthal fibers in non-neoplastic syringomyelia: a note on the pathogenesis of syringomyelia. **J.Nerv.Ment.Dis.** 1937;**86**:549-559.
228 Lichtenstein BW. Cervical syringomyelia and syringomyelia-like states associated with Arnold-Chiari deformity and platybasia. **Arch.Neurol.Psychiat.** 1943;**49**:194.
229 Lindholm D, Castren E, Kiefer R, Zafra F, Thoenen H. Transforming growth factor-ß1 in the rat brain: Increase after injury and inhibition of astrocyte proliferation. **J.Cell Biol.** 1993;**117**:395-400.
230 Lindsley DL, Odell TT, Tausche FG. Implantation of functional erythropoietic elements following total-body irradiation. **Proc.Soc.Exp.Biol.N.Y.** 1955;**90**:512-515.
231 Ling EA, Tan CK. Amoeboid microglial cells in the corpus callosum of neonatal rats. **Arch.Histol.Jpn.** 1974;**36**:265-280.
232 Ling EA. Light and electron microscopic demonstration of some lysosomal enzymes in the amoeboid microglia in neonatal rat brain. **J.Anat.** 1977;**123(3)**:637-648.

233 Ling EA. Transformation of monocytes into amoeboid microglia in the corpus callosum of postnatal rats, as shown by labelling monocytes by carbon particles. **J.Anat.** 1979;**128(4)**:847-858.
234 Ling EA. Evidence for a haematogenous origin of some of the macrophages appearing in the spinal cord of the rat after dorsal rhizotomy. **J.Anat.** 1979;**128(1)**:143-154.
235 Ling EA, Penny D, Leblond CP. Use of carbon labeling to demonstrate the role of blood monocytes as precursors of the 'amoeboid cells' present in the corpus callosum of postnatal rats. **J.Comp.Neurol.** 1980;**193**:631-657.
236 Ling EA, Kaur C, Wong WC. Light and electron microscopic demonstration of non-specific esterase in amoeboid microglial cells in the corpus callosum in postnatal rats: a cytochemical link to monocytes. **J.Anat.** 1982;**135**:385-394.
237 Ling EA, Wong WC. The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. **Glia** 1993;**7(1)**:9-18.
238 Ling EA. Monocytic origin of ramified microglia in the corpus callosum in postnatal rat. **Neuropathol.Appl.Neurobiol.** 1994;**20(2)**:182-183.
239 Linington C, Bradl M, Lassmann H, Brunner C, Vass K. Augmentation of demyelination in rat acute experimental allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrovyte glycoprotein. **Am.J.Pathol.** 1988;**130**:443-454.
240 Linnik MD, Zobrist RH, Hatfield MD. Evidence supporting a role for programmed cell death in focal cerebral ischaemia in rats. **Stroke** 1993;**24**:2002-2009.
241 Logue V, Edwards MR. Syringomyelia and its surgical treatment--an analysis of 75 patients. **J.Neurol.Neurosurg.Psychiatry** 1981;**44(4)**:273-284.
242 Lorenz E, Uphoff DE, Reid TR, Shelton E. Modification of irradiation injury in mice and guinea pigs by bone marrow injections. **J.Nat.Cancer Inst.** 1951;**12**:197-201.
243 Lorenz E, Congdon C, Uphoff D. Modification of acute irradiation injury in mice and guinea-pigs by bone marrow injections. **Radiology** 1952;**58**:863-877.
244 Lubaroff DM, Hunt HD, Rasmussen GT. Evidence from capping experiments for independence of the RT& alloantigen and the leucocyte common antigen in the rat. **J.Immunogenetics** 1985;**12**:239-245.
245 Lubin AF. Adhesive spinal arachnoiditis as a cause of intramedullary cavitation: comparison with syringomyelia. **Arch.Neurol.Psychiatry** 1940;**44**:409-420.
246 Lumsden CE. The study of tissue culture of tumours of the nervous system. In: Russell DS, Rubenstein LJ, eds. Tumours of the Nervous System. London:Edward Arnold, 1971.
247 Mackay RP, Favill J. Syringomyelia and intramedullary tumour of the spinal cord. **Arch.Neurol.Psychiatry** 1935;**33**:1255-1278.
248 Madsen JR, Scott RM. Chiari malformations, syringomyelia, and intramedullary spinal cord tumors. **Curr.Opin.Neurol.Neurosurg.** 1993;**6(4)**:559-563.
249 Madsen PW, Yezierski RP, Holets VR. Syringomyelia: clinical observations and experimental studies. **J.Neurotrauma.** 1994;**11(3)**:241-254, 1994.
250 Mallat M, Houlgatte R, Brachet P, Prochiantz A. Lipopolysaccharide-stimulated rat brain macrophages release NGF in vitro. **Developmental Biology** 1989;**133**:309-311.

251 Mariani C, Cislaghi MG, Barbieri S, Filizzolo F, Palma F Di, Farina E, D'Aliberti G, Scarlato G. The natural history and results of surgery in 50 cases of syringomyelia. **J.Neurol.** 1991;**238**:433-438.
252 Marie P. Lectures on diseases of the spinal cord. London:New Sydenham Society, 1895..
253 Martiney JA, Rajan AJ, Charles PC, Cerami A, Ulrich PC, Macphail S, Tracey KJ, Brosnan CF. Prevention and treatment of experimental autoimmune encephalomyelitis by CNI-1493, a macrophage-deactivating agent. **J.Immunol.** 1998;**160**:5588-5595.
254 Marty S, Dusart I, Peschanski M. Glial changes following an excitotoxic lesion in the CNS--I. Microglia/macrophages. **Neuroscience** 1991;**45(3)**:529-539.
255 Mathew RC, Gupta SK, Katayama I, Curtis J, Turk JL. Macrophage specific antigen is expressed by resting microglia in the CNS but not by Langerhans cells in the skin. **J.Pathol.** 1983;**141**:435-440.
256 Matsumoto Y, Hara N, Tanaka R, Fujiwara M. Immunohistochemical analysis of the rat central nervous system during experimental allergic encephalomyelitis, with special refernce to Ia-positive cells with dendritic morphology. **J.Immunol.** 1986;**136(10)**:3668-3676.
257 Matsumoto Y, Fujiwara M. Absence of donor-type major histocompatibility complex class I antigen-bearing microglia in the rat central nervous system of radiation bone marrow chimeras. **J.Neuroimmunol.** 1987;**17**:71-82.
258 G. Mayrhofer. Personal communication 1999.
259 McIlroy WJ, Richardson JC. Syringomyelia: a clinical review of 75 cases. **Can.Med.Assoc.J.** 1965;**93(14)**:731-734.
260 McKeever PE, Balentine JD. Macrophage migration through the brain parenchyma to the perivascular space following particle ingestiion. **Am.J.Pathol.** 1978;**93**:153-164.
261 McLaurin RL, Bailey OT, Schurr PH. Myeolomalacia and multiple cavitations of spinal cord secondary to adhesive arachnoiditis: an experimental study. **Arch.Pathol.** 1954;**57**:138-146.
262 McVeigh JF. Experimental cord crushes: with special reference to the mechanical factors involved and subsequent changes in the areas of the cord affected. **Arch.Surg.** 1923;**7**:573-600.
263 Merchant RE, Low FN. Scanning electron microscopy of the subarachnoid space in the dog; evidence for non-hematogenous origin of subarachnoid macrophages. **Am.J.Anat.** 1979;**156**:183-206.
264 Merrill JE, Ignarro LJ, Sherman MP, Melinek J, Lane TE. Microglial cell cytotoxicity of oligodendrocytes is mediated through nitric oxide. **J.Immunol.** 1993;**151(4)**:2132-2141.
265 Milhorat TH, Adler DE, Heger IM, Miller JI, Hollenberg-Sher JR. Histopathology of experimental hematomyelia. **J.Neurosurg.** 1991;**75**:911-915.
266 Milhorat TH, Johnson WD, Miller JI, Bergland RM, Hollenberg-Sher J. Surgical treatment of syringomyelia based on magnetic resonance imaging criteria. **Neurosurgery** 1992;**31(2)**:231-245.

267 Milhorat TH, Nobandegani F, Miller JI, C. Rao C. Noncommunicating syringomyelia following occlusion of central canal in rats. Experimental model and histological findings. **J.Neurosurg.** 1993;**78(2)**:274-279.
268 Milhorat TH, Johnson RW, Milhorat RH, Jr Capocelli AL, Pevsner PH. Clinicopathological correlations in syringomyelia using axial magnetic resonance imaging. **Neurosurgery** 1995;**37(2)**:206-213.
269 Milhorat TH, Jr Capocelli AL, Anzil AP, Kotzen RM, Milhorat RH. Pathological basis of spinal cord cavitation in syringomyelia: analysis of 105 autopsy cases. **J.Neurosurg.** 1995;**82(5)**:802-812.
270 Milligan CE, Levitt P, Cunningham TJ. Brain macrophages and microglia respond differently to lesions of the developing and adult visual system. **J.Comp.Neurol.** 1991;**314(1)**:136-146.
271 Minghetti L, Levi G. Induction of prostanoid biosynthesis by bacterial lipopolysaccharide and isoproterenol in rat microglia cultures. **J.Neurochem.** 1995;**65**:2690-2698.
272 Minghetti L, Levi G. Microglia as effector cells in brain damage and repair: focus on prostanoids and nitric oxide. **Prog.Neurobiol.** 1998;**54(1)**:99-125.
273 Miwa T, Furukawa S, Nanjima K, Furukawa Y, Kohsaka S. Lipopolysaccharide enhances synthesis of brain-derived neurotrophic factor in cultured rat microglia. **J.Neurosci.Res.** 1997;**50**:1023-1029.
274 Miyake R, Schelper RL. Axoplasmic transport of adriamycin blocks true microglial proliferation induced by hypoglossal axotomy. **J.Neuropathol.Exp.Neurol.** 1987;**46**:350.
275 Miyake T, Tsuchihashi Y, Kitamura T, Fujita S. Immunohistochemical studies of blood monocytes infiltrating into the neonatal rat brain. **Acta Neuropathol.Berl** 1984;**62(4)**:291-297.
276 Montero-Menei CN, Sindji L, Pouplard-Barthelaix A, Jehan F, Denechaud L, Darcy F. Lipopolysaccharide intracerebral administration induces minimal inflammatory reaction in rat brain. **Brain Res.** 1994;**653**:101-111.
277 Moore S, Thanos S. The concept of microglia in relation to central nervous system disease and regeneration. **Prog.Neurobiol.** 1996;**48(4-5)**:441-460.
278 Mori S, Leblond CP. Identification of microglia in light and electron microscopy. **J.Comp.Neurol.** 1969;**135(1)**:57-80.
279 Morioka T, Kalehua AN, Streit WJ. The microglial reaction in the rat dorsal hippocampus following transient forebrain ischemia. **J.Cereb.Blood Flow Metab.** 1991;**11(6)**:966-973.
280 Morioka T, Streit W. Expression of immunomolecules on microglial cells following neonatal sciatic nerve axotomy. **J.Neuroimmunol.** 1991;**35**:21-30.
281 Morioka T, Kalehua AN, Streit WJ. Characterization of microglial reaction after middle cerebral artery occlusion in rat brain. **J.Comp.Neurol.** 1993;**327(1)**:123-132.
282 Muller HW, Gebicke-Harter PJ, Hangen DH, Shooter EM. A specific 37,000-dalton protein that accumulates in regenerating but not in nonregenerating mammalian nerves. **Science** 1985;**228**:499-501.

283 Murabe Y, Sano Y. Morphological studies on neuroglia. I. Electron microscopic identification of silver-impregnated glial cells. **Cell Tissue Res**. 1981;**216**:557-568.
284 Murabe Y, Ibata Y, Sano Y. Morphological studies on neuroglia. IV. Proliferative response of non-neuronal elements in the hippocampus of the rat to kainic acid-induced lesions. **Cell Tissue Res.** 1982;**222(1)**:223-226.
285 Murabe Y, Sano Y. Morphological studies on neuroglia. VI. Postnatal development of microglial cells. **Cell Tissue Res.** 1982;**225**:469-485.
286 Murabe Y, Sano Y. Morphological studies on neuroglia. VII. Distribution of "brain macrophages" in brains of neonatal and adult rats, as determined by means of immunohistochemistry. **Cell Tissue Res.** 1983;**229**:85-95.
287 Myrianthopoulos NC. Epidemiology of central nervous system malformations. **Handbook of Clinical Neurology** 1987;**6**:46-69.
288 Nagahiro S, Matsukado Y, Kuratsu J, Saito Y, Takamura S. Syringomyelia and syringobulbia associated with an ependymoma of the cauda equina involving the conus medullaris: case report. **Neurosurgery** 1986;**18(3)**:357-360.
289 Nakajima K, Kohsaka S. Functional roles of microglia in the brain. **Neurosci.Res.** 1993;**17(3)**:187-203.
290 Nathan CF. Secretory products of macrophages. **J.Clin.Invest.** 1987;**79**:319-326.
291 Netsky MG. Syringomyelia: A clinicopathologic study. **Arch.Neurol.Psychiat.** 1953;**70**:741-777.
292 Nogues MA. Syringomyelia and syringobulbia. **Handbook of Clinical Neurology** 1987;**6**:1-17.
293 Normann SJ, Noga SJ. Population kinetic study on the origin of guinea pig monocyte heterogeneity. **Cell.Immunol.** 1986;**101**:534-547.
294 D. W. C. Northfield. The surgery of the central nervous system, 1973. 529 pages.
295 Oakes WJ. Chiari malformations, hydromyelia, syringomyelia. In: Wilkins RH, Rengchary SS, eds. Neurosurgery. New York:McGraw-Hill, 1996:3593-3616.
296 Oehmichen M, Torvik A. The origin of reactive cells in retrograde and wallerian degeneration. **Cell Tissue Res.** 1976;**173**:343-348.
297 Oehmichen M, Wietholter H, Greaves MF. Immunological analysis of human microglia: Lack of monocytic and lymphoid membrane differentiation antigens. **J.Neuropathol.Exp.Neurol.** 1979;**38**:99-103.
298 Oemichen M. Mononuclear phagocytes in the central nervous system. Origin, mode of distribution, and function of progressive microglia, perivascular cells of intracerebral vessels, free subarachnoidal cells, and epiplexus cells. **Schriftenr.Neurol.** 1978;**21**:1-167.
299 Ogawa N, Tanaka K, Kondo Y, Asanuma M, Mizukawa K, Mori A. The preventative effect of cyclosporin A, an immunosuppressant, on the late onset reduction of muscarinic acetylcholine receptors in gerbil hippocampus after transient forebrain ischaemia. **Neurosci.Lett.** 1993;**152**:173-176.
300 Okada S, Nakagawa Y, Hirakawa K. Syringomyelia extending to the basal ganglia. Case report [see comments]. **J.Neurosurg.** 1989;**71(4)**:616-617.

301 Oldfield EH, Muraszko K, Shawker TH, Patronas NJ. Pathophysiology of syringomyelia associated with Chiari I malformation of the cerebellar tonsils. Implications for diagnosis and treatment [see comments]. **J.Neurosurg.** 1994;**80(1)**:3-15.
302 Ollivier d'Angers CP. Traite de la Moelle Epiniere et de ses Maladies, Paris:Chez Crevot, 1827.
303 Padovani R, Cavallo M, Gaist G. Surgical treatment of syringomyelia: favorable results with syringosubarachnoid shunting. **Surg.Neurol.** 1989;**32(3)**:173-180.
304 Panegyres PK, Hughes J. The neuroprotective effects of the recombinant interleukin-1 receptor antagonist rhIL-1ra after excitotoxic stimulation with kainic acid and its relationship to the amyloid precursor protein gene. **J.Neurol Sci.** 1998;**154(2)**:123-132.
305 Pender MP. Demyelination and neurological signs in experimental allergic encephalomyelitis. **J.Neuroimmunol.** 1987;**15**:11-24.
306 Perry VH, Hume DA, Gordon S. Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. **Neuroscience** 1985;**15(2)**:313-326.
307 Perry VH, Gordon S. Macrophages and microglia in the nervous system. **Trends.Neurosci.** 1988;**11(6)**:273-277.
308 Perry VH, Gordon S. Macrophages and the nervous system. **International Review of Cytology** 1991;**125**:203-244.
309 Perry VH, Andersson PB. The inflammatory response in the CNS. **Neuropathol.Appl.Neurobiol.** 1992;**18(5)**:454-459.
310 Perry VH, Brown MC, Andersson P. Macrophage responses to central and peripheral nerve injury. **Adv.Neurol.** 1993;**59(309)**:314.
311 Perry VH, Andersson PB, Gordon S. Macrophages and inflammation in the central nervous system. **Trends.Neurosci.** 1993;**16(7)**:268-273.
312 Perry VH. Modulation of microglia phenotype. **Neuropathol.Appl.Neurobiol.** 1994;**20(2)**:177.
313 Perry VH, Lawson LJ, Reid DM. Biology of the mononuclear phagocyte system of the central nervous system and HIV infection [see comments]. **J.Leukoc.Biol.** 1994;**56(3)**:399-406.
314 Perry VH, Bell MD, Brown HC, Matyszak MK. Inflammation in the nervous system. **Curr.Opin.Neurobiol.** 1995;**5**:636-641.
315 Perry VH, Tsao JW, Fearn S, Brown MC. Radiation-induced reductions in macrophage recruitment have only slight effects on myelin degeration in sectioned peripheral nerves of mice. **Eur.J.N eurosci.** 1995;7:271-280.
316 Pillay PK, Awad IA, Hahn JF. Gardner's hydrodynamic theory of syringomyelia revisited. Cleve.**Clin.J.Med** 1992;**59**:373-380.
317 Polman CH, Dijkstra CD, Sminia T, Koetsier JC. Immunohistological analysis of macrophages in the central nervous system of Lewis rats with acute experimental allergic encephalomyelitis. **J.Neuroimmunol.** 1986;**11(3)**:215-222.

318 Popovich, PG, Guan Z, Wei P, Huitinga I, van Rooijen N, Stokes BT. Depletion of hematogenous macrophages promotes partial hindlimb recovery and neuroanatomical repair after experimental spinal cord injury. **Exp.Neurol.** 1999;**158**:351-365.

319 Poser CM. The Relationship between Syringomyelia and Neoplasm. Springfield, Illinois:Charles C Thomas, 1956.

320 Prewitt CM, Niesman IR, Kane CJ, Houle JD. Activated macrophage/microglial cells can promote the regeneration of sensory axons into the injured spinal cord. **Exp.Neurol.** 1997;**148(2)**:433-443.

321 Puca A, Cioni B, Colosimo C. Spinal neurenteric cyst in association with syringomyelia: case report. **Surg Neurol** 1992;**37**:202-227.

322 Quencer RM, Gammal T. el, Cohen G. Syringomyelia associated with intradural extramedullary masses of the spinal canal. **AJNR.Am.J.Neuroradiol.** 1986;**7(1)**:143-148.

323 Rabchevsky AG, Streit J. Grafting of cultured microglial cells into the lesioned spinal cord of adult rats enhances neurite growth. **J.Neurosci.Res.** 1997;**47**:34-48.

324 Rabinovitch M, De Stefano MJ. Interactions of red cells with phagocytes of the wax-mouth (Galleria mellonella, L.) and mouse. **Exp.Cell.Res.** 1970;**59(2)**:272-282.

325 Raine CS, Snyder DH, Valsamis MP, Stone SH. Chronic experimental allergic encephalomyelitis in inbred guinea pigs: An ultrastructural study. **Lab.Invest.** 1974;**31**:369-380.

326 Raine CS. Biology of disease, Analysis of autoimmune demyelination: Its impact upon Multiple Sclerosis. **Lab.Invest.** 1984;**50**:608-635.

327 Ranson PA, Thomas WE. Pinocytosis as a select marker of ramified microglia in vivo and in vitro. **J.Histochem.Cytochem.** 1991;**39(6)**:853-858.

328 Rapalino O, Lazarov-Spiegler O, Agranov E, Velan GJ, Yoles E, Fraidakis M, Solomon A, Gepstein R, Katz A, Belkin A, Hadani M, Schwartz M. Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats. **Nat.Med.** 1988;**4**:814-821.

329 Rascher K, Booz KH, Donauer E, Nacimiento AC. Structural alterations in the spinal cord during progressive communicating syringomyelia. An experimental study in the cat. **Acta Neuropathol.Berl.** 1987;**72(3)**:248-255.

330 Rascher K, Donauer E. Experimental models of syringomyelia--personal observations and a brief look at earlier reports. **Acta Neurochir.Wien.** 1993;**123(3-4)**:166-169.

331 Reid DM, Perry VH, Andersson PB, Gordon S. Mitosis and apoptosis of microglia in vivo induced by an anti-CR3 antibody which crosses the blood-brain barrier. **Neuroscience** 1993;**56(3)**:529-533.

332 Reisert I, Wildemann G, Grab D, Pilgrim CH. The glial reaction in the course of axon regeneration: A stereological study of the rat hypoglossal nycleus. **J.Comp.Neurol.** 1984;**229**:121-128.

333 Relton JK, Rothwell NJ. Interleukin-1 receptor antagonist inhibits ischaemic and excitotoxic neuronal damage in the rat. **Brain Res Bull** 1992;**29(2)**:243-246.

334 Relton JK, Martin D, Russel D. Effects of peripheral IL-1RA treatment after focal cerebral ischaemia in the rat. **Soc.Neurosci.** 1993;**19**:673-673. (Abstract)
335 Rice-Edwards JM. A pathological study of syringomyelia. **J.Neurol.Neurosurg.Psychiatry** 1977;**40**:198.
336 Richardson A, Neuhaus J, Fedoroff S. Microglia progenitor cells and development of microglia. **Neuropathol.Appl.Neurobiol.** 1994;**20(2)**:183-185.
337 Rinner WA, Bauer J, Schmidts M, Lassmann H, Hickey WF. Resident microglia and hematogenous macrophages as phagocytes in adoptively transferred experimental autoimmune encephalomyelitis: an investigation using rat radiation bone marrow chimeras. **Glia** 1995;**14(4)**:257-266.
338 Rio-Hortega P del. Microglia. In: Penfield W, ed. Cytology and Cellular Pathology of the Nervous System. New York:Paul B. Hoeber, 1932:481-534.
339 Risau W, Wolburg H. Develpoment of the blood brain barrier. **Trends.Neurosci.** 1990;**13**:174-178.
340 Roessmann U, Friede RL. Entry of labelled monocytic cells into the central nervous system. **Acta Neuropathologica** 1968;**10**:359-362.
341 Ropper AH, Martin JB. Diseases of the spinal cord. In: Wilson JD, Braunwald KJ, Isselbaxher KJ, Petersdorf RG, Martin JB, Fauci AS, Root RK, eds. Harrison's principles of internal medicine. New York:McGraw-Hill, Inc., 1991:2081-2088.
342 Ruan RS, Leong SK, Yeoh KH. Glial reaction after facial nerve compression in the facial canal of the albino rat. **Acta Otolaryngol.Stockh.** 1994;**114(3)**:271-277.
343 Rydberg E. Cerebral injury in newborn children consequent on birth trauma, with an enquiry into the normal and pathological anatomy of the neuroglia. **Acta Pathol.Microbiol.Scand.Suppl.** 1932;**10**:1-247.
344 Santha K, Juba A. Weitere Untersuchungen uber die Entwicklung der Hortegaschen Mikroglia. **Arch.Psychiat.Nervenkr.** 1933;**98**:598-613.
345 Sawada M, Suzumura A, Marunouchi T. Down regulation of CD4 expression in cultured microglia by immunosuppressants and lipopolysaccharide. **Biochem.Biophys.Res.Comm.** 1992;**189**:869-876.
346 Schelper RL, Adrian EK Jr. Monocytes become macrophages; they do not become microglia: a light and electron microscopic autoradiographic study using 125-iododeoxyuridine. **J.Neuropathol.Exp.Neurol.** 1986;**45(1)**:1-19.
347 Schlesinger EB, Antunes JL, Michelsen WJ, Louis KM. Hydromyelia: clinical presentation and comparison of modalities of treatment. **Neurosurgery** 1981;**9(4)**:356-365.
348 H. Schlesinger. Die Syringomyelie, Leipzig/Vienna:Franz Deuticke, 1902.
349 Schnell L, Schwab ME. Axonal elongation in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors. **Nature (London)** 1990;**343**:269-272.
350 Schnitzer J, Scherer J. Microglial cell responses in the rabbit retina following transection of the optic nerve. **J.Comp.Neurol.** 1990;**302**:779-791.
351 Schwartz M, Lazarov-Spiegler O, Rapalino O, Agranov I, Velan G, Hadani M. Potential repair of rat spinal cord injuries using stimulated homologous macrophages. **Neurosurgery** 1999;**44(5)**:1041-1045.
352 Scolding NJ, Compston AS. Oligodendrocyte-macrophage interaction in vitro triggered by specific antibodies. **Immunology** 1991;**72**:127-132.

353 Sedgwick JD, Schwender S, Gregersen R, Dorries R, Meulen V. ter. Resident macrophages (ramified microglia) of the adult brown Norway rat central nervous system are constitutively major histocompatibility complex class II positive. **J.Exp.Med.** 1993;**177(4)**:1145-1152.

354 Sedgwick JD, Ford AL, Foulcher E, Airriess R. Central nervous system microglial cell activation and proliferation direct interaction with tissue-infiltrating T cell blasts. **J.Immunol.** 1998;**160**:5320-5330.

355 Selmaj K, Bradbury K, Chapman J. Multiple sclerosis: effects of activated T-lymphocyte-derived products on organ cultures of nervous tissue. **J.Neuroimmunol.** 1988;**18(3)**:255-268.

356 Sengpiel B, Preis E, Krieglstein J, Prehn JH. NMDA-induced superoxide production and neurotoxicity in cultured rat hippocampal neurons: role of mitochondria. **Eur.J.Neurosci.** 1998;**10(5)**:1903-1910.

357 Sgouros S, Williams B. A critical appraisal of drainage in syringomyelia. **J.Neurosurg.** 1995;**82(1)**:1-10.

358 Shrikant P, Benveniste EN. The central vernous system as an immunocompetent organ. Role of glial cells in antigen presentation. **J.Immunol.** 1996;**157**:1819-1822.

359 Sjostrand J. Neuroglial proliferation in the hypoglossal nucleus after nerve injury. **Exp.Neurol.** 1971;**30**:178-189.

360 Skoff RP, Vaughn JE. An autoradiographic study of cellular proliferation in degenerating rat optic nerves. **J.Comp.Neurol.** 1971;**141**:131-156.

361 Sloff JL, Kernohan JW, MacCarthy CS. Primary intra-medullary tumours of the spinal cord and filum terminale. Philadelphia:W.B.Saunders Company Ltd, 1964.

362 Slonecker CE. The cellular composition of an acute inflammtory exudate in rats. **J.Reticuloendothel.Soc.** 1971;**10**:269-282.

363 Small J. Difficult labour and communicating syringomyelia [letter]. **Lancet** 1977;**2(8038)**:609.

364 Small JA, Sheridan PH. Research priorities for syringomyelia: a National Institute of Neurological Disorders and Stroke workshop summary. **Neurology** 1996;**46(2)**:577-582.

365 Smith ME. Phagocytosis of myelin by microglia in vitro. **J.Neurosci.Res.** 1993;**35(5)**:480-487.

366 Smith SJ, Greenberg M, Vogelzang RL, Greenberg B, Neiman HL, Greenberg I. Diagnosis of syringomyelia. **IMJ.Ill.Med.J.** 1985;**167(2)**:128-130.

367 Solomon RA, Stein BM. Unusual spinal cord enlargement related to intramedullary hemangioblastoma. **J.Neurosurg.** 1988;**68**:550-553.

368 Sorokin S, Hoyt RF Jr, Blunt DG, McNelly NA. Macrophage development:II. Early ontogeny of macrophage populations in brain, liver, and lungs of rat embryos as revealed by a lectin marker. **Anat.Rec.** 1992;**232**:527-550.

369 Spector WG, Coote E. Differentially labelled blood cells in the reaction to paraffin oil. **J.Pathol.Bacteriol.** 1965;**90**:589-598.

370 Springer TA. Monoclonal antibody analysis of complex biological systems. Combination of cell hybridization and immunoadsorbents in a novel cascade procedure and its application to the macrophage cell surface. **J.Biol.Chem.** 1981;**256**:3833-3839.

371 Stenwig AE. The origin of brain macrophages in traumatic lesions, wallerian degenration, and retrograde degeneration. **J.Neuropath.Exp.Neurol.** 1972;**31**:696-704.
372 Stoll G, Muller H. Macrophages in the peripheral nervous system and astroglia in the central nervous system of rat commonly express apolipoprotein-E during development but differ in their response to injury. **Neurosci.Lett.** 1986;**72**:233-238.
373 Stoll G, Griffin JW, Li CY, Trapp BD. Wallerian degeneration in the peripheral nervous system: participation of both Schwann cells and macrophages in myelin degradation. **J.Neurocytol.** 1989;**18**:671-683.
374 Stoodley MA, Jones NR, Brown CJ. Evidence for rapid fluid flow from the subarachnoid space into the spinal cord central canal in the rat. **Brain Res.** 1996;**707(2)**:155-164.
375 Stoodley MA. Pathophysiology of syringomyelia. Thesis 1996.
376 Stoodley MA, Jones NR. Syringomyelia. In: Clark CR, ed. The cervical spine. Philadelphia:Lippincott-Raven Publishers, 1998:565-583.
377 Streit WJ, Graeber MB, Kreutzberg GW. Functional plasticity of microglia: a review. **Glia** 1988;**1(5)**:301-307.
378 Streit WJ, Kreutzberg GW. Response of endogenous glial cells to motor neuron degeneration induced by toxic ricin. **J.Comp.Neurol.** 1988;**268(2)**:248-263.
379 Streit WJ, Graeber MB, Kreutzberg GW. Expression of Ia antigen on perivascular and microglial cells after sublethal and lethal motor neuron injury. **Exp.Neurol.** 1989;**105**:115-126.
380 Streit WJ, Graeber MB. Heterogeneity of microglial and perivascular cell populations: insights gained from the facial nucleus paradigm. **Glia** 1993;**7(1)**:68-74.
381 Sulica A, Gherman M, Medesan C, Sjoquist J, Ghetie V. IgG-binding sites on macrophage cell membrane. I. Identification of two distinct Fc receptors on mouse peritoneal macrophages. **Eur.J.Immunol.** 1979;**9(12)**:979-984.
382 Sutton W, Weiss L. Transformation of monocytes in tissue culture into macrophages, epitheloid cells and multinucleate giant cells. **J.Cell.Biol.** 1966;**28**:303-332.
383 Suzumura A, Marunouchi T, Yamamoto H. Morphological transformation of microglia in vitro. **Brain Res.** 1991;**545**:301-306.
384 Svensson M, Aldskogius H. Infusion of cytosine-arabinoside into cerebrospinal fluid of the rat brain inhibits the microglial cell proliferation after hypoglossal nerve injury. **Glia** 1993;7:286-298.
385 Svensson M, Eriksson NP, Aldskogius H. Evidence for activation of astrocytes via reactive microglial cells following hypoglossal nerve transection. **J.Neurosci.Res.** 19993;**35**:373-381.
386 Takeuchi A, Isobe KI, Miyaishi O, Sawada M, Fan ZH, Nakashima I, Kiuchi K. Microglial NO induces delayed neuronal death following acute injury in the striatum. **Eur.J.Neurosci.** 1998;**10(5)**:1613-1620.
387 Tamaki K, Lubin AJ. Pathogenesis of syringomyelia: case illustrating the process of cavity formation from embryonic cell rests. **Arch.Neurol.Psychiatry** 1938;**40**:748-761.

388 Tator CH, Meguro K, Rowed DW. Favorable results with syringosubarachnoid shunts for treatment of syringomyelia. **J.Neurosurg**. 1982;**56(4)**:517-523.
389 Tauber ES, Langworthy OR. A study of syringomyelia and the formation of cavities in the spinal cord. **J.Nerv.Ment.Dis** 1935;**81**:245-264.
390 Taylor J, Greenfield JG, Martin JP. Two cases of syringomyelia and syringobulbia oberved clinically over many years and examined pathologically. **Brain** 1922;**45**:323-356.
391 Terae S, Miyasaka K, Abe S, Abe H, Tashiro K. Increased pulsatile movement of the hindbrain in syringomyelia associated with the Chiari malformation: cine-MRI with presaturation bolus tracking. **Neuroradiology**. 1994;**36(2)**:125-129.
392 Thanos S, Kacza J, Seeger J, Mey J. Old dyes for new scopes: the phagocytosis-dependent long-term fluorescence labelling of microglial cells in vivo. **TINS** 1994;**17(5)**:177-182.
393 Thery C, Chamak B, Mallat M. Free radical killing of neurons. **Eur.J.Neurosci**. 1991;**3**:1155-1164.
394 Thomas WE. Characterization of the dynamic nature of microglial cells. **Brain Res.Bull.** 1990;**25**:351.
395 Thomas WE. Brain macrophages: evaluation of microglia and their functions. **Brain Res.Brain Res.Rev.** 1992;**17(1)**:61-74.
396 Thyberg J, Stenseth K. Endocytosis of native and cationized horseradish peroxidase by cultured mouse peritoneal macrophages. Variations in cell surface binding and intracellular traffic and effects of colchicine. **Eur.J.Cell Biol**. 1981;**25(2)**:308-318.
397 Toku K, Tanaka J, Yano H, Desaki J, Zhang B, Yang L, Ishihara K, Sakanaka M, Maeda N. Microglial cells prevent nitric oxide-induced neuronal apoptosis in vitro. **J.Neurosci.Res**. 1998;**53(4)**:415-425.
398 Torvik A. The relationship between microglia and brain macrophages. **Acta Neuropathol**. 1975;**VI**:297-300.
399 Trachtenberg MC. Glial endocytosis of protein in the traumatized brain. **J.Neurosci.Res.** 1983;**9**:413-423.
400 Tran EH, Hoekstra K, van Rooijen N. , Dijkstra CD, and Owens T. Immune invasion of the central nervous system parenchyma and experimental allergic encephalomyelitis, but not leukocyte extravasation from blood, are prevented in macrophage-depleted mice. **J.Immunol**. 1998;**161 (7)** :3767-3775.
401 Tseng CY, Ling EA, and Wong WC. Scanning electron microscopy of amoeboid microglial cells in the transient cavum septum pellucidum in pre- and postnatal rats. **J.Anat.** 1983,**136**:251-263.
402 Tsuchihashi Y, Kitamura T, and Fujita S. Immunofluorescence studies of the monocytes in the injured rat brain. **Acta Neuropathol.Berl**. 1981;**53**:213-219.
403 Ulvestad E, Williams K, Matre R, Nyland H, Olivier A, and Antel J. Fc receptors for IgG on cultured human microglia mediate cytotoxicity and phagocytosis of antibody-coated targets. **J.Neuropathol.Exp.Neurol** 1994;**53 (1)**:27-36.
404 Valentino KL and Jones EG. Morphological and immunocytochemical identification of macrophages in the developing corpus callosum. **Anat.Embryol.** 1981;**163**:157-172.
405 van Bekkum DW. Present status of bone marrow transplantation following whole body irradiation. **Oncologia** 1965;**20** (Suppl):60-72.

406 van Dam AM, Bauer J, Man-A-Hing WKH, Marquette C, Tilders FJH, and Berkenbosch F. Appearance of inducible nitric oxide synthase in the rat central nervous system after rabies infection and during experimental allergic excephalomyelitis but not after administration of endotoxin. **J.Neurosci.Res.** 1994;**40**:251-260.

407 van Furth R and Cohn ZA. The origin and kinetics of mononuclear phagocytes. **J.Exp.Med.** 1968;**128**:415-435.

408 van Furth R, Cohn ZA, Hirsch JG, Humphry JH, Spector WG, and Langevoort HL. The mononuclear phagocyte system: a new classification of macrophages, monocytes and their precursor. **Bull WHO** 1972;**46**:845-852.

409 van Furth R, Langevoort HL, and Schaberg A. Mononuclear phagocytes in human pathology- proposal for an approach to improve classification. In: van Furth R, ed. Mononuclear phagocytes in immunity, infection, and pathology. Oxford:Blackwell Scientific Publications, 1975:1-15.

410 van Furth R. Cells of the mononuclear phagocyte system: nomemclature in terms of sites and conditions. In: van Furth R, ed. Mononuclear phagocytes. Functional aspects. Boston:Martnis Nijhoff 1980:1-30.

411 van Furth R, van der Meer JWM, van Oud Alblas Blusse A, and Sluiter W. Development of mononuclear phagocytes. In: Mizuno D, Cohn ZA, Takeya K, Ishida N, eds. Self-defense mechanisms. Role of macrophages. New York:Elsevier, 1982:25-41.

412 van Furth R, Nibbering PH, van Dissel JT, and Disselhoff-den Dulk MMC. The characterization, origin, and kinetics of skin macrophages during inflammation. **J.Invest.Dermatol.** 1985;**85**:398-402.

413 van Furth R. Development and distribution of mononuclear phagocytes. In: Gallin JI, Goldstein IM, Snyderman R, eds. Inflammation: Basic Principles and Clinical Correlates. New York:Raven Press, 1992:325-339.

414 van Furth R and Fedorko ME. Ultrastructure of mouse mononuclear phagocytes in bone marrow colonies grown in vitro. **Lab.Invest.** 1976;**34 (4)**:440-450.

415 Vass K, Lassmann H, Wekerle H, and Wisniewski HM. The distribuion of Ia antigen in the lesions of rat acute experomental allergic encephalomyelitis. **Acta.Neuropathol.(Berl)** 1986;**70 (2)**:149-160.

416 Vaugn JE and Peters A. A third neuroglial cell type. An electron microscopic study. **J.Comp.Neurol.** 1968;**133**:269-288.

417 Vaugn JE, Hinds PL, and Skoff RP. Electron microscopic studies of Wallerian degeneration in rat optic nerves. I. The multipotential glia. **J.Comp.Exp.** 1970;**140**:175-206.

418 Vedeler C, Ulvestad E, Grundt I, Conti G, Nyland H, Matre R, and Pleasure D. Fc receptor for IgG (FcR) on rat microglia. **J.Neuroimmunol**. 1994;**49 (1-2)**:19-24.

419 Volkman A and Gowans JL. The origin of macrophages from the bone marrow in the rat. **Br.J.Exp.Pathol.** 1965;**46**:50-61.

420 Volkman A and Gowans JL. The production of macrophages in the rat. **Br.J.Exp.Pathol.** 1965;**46**:50-61.

421 Volkman A. The origin and turnover of mononuclear cells in peritoneal exudates in rats. **J.Exp.Med.** 1966;**124**:241-254.

422 Vos O, Davids JAG, Weyzen WWH, and van Bekkum DW. Evidence for the cellular hypothesis in radiation protection by bone marrow cells. **Acta Physio.Pharmacol.Neerl.** 1956;**4**:482-486.
423 Wagner FC, van Gilder JC, and Dohrmann GJ. Pathological changes from acute to chronic in experimental spinal cord injury. **J.Neurosurg.** 1978;**48 (92)**:98.
424 Walz W, Ilschner S, Ohlemeyer C, Banati R, and Kettenmann H. Extracellular ATP activates a cation conductance and a K^+ conductance in cultured microglia from mouse brain. **J.Neurosci.** 1993;**13**:4403-4411.
425 Watanabe T, Yamamoto T, Abe Y, Saito N, Kumagai T, Kayama H. Differential activation of microglia after experimental spinal cord injury. **J. Neurotrauma** 1999;16(3):255-65.
426 Weil A. Textbook of Neuropathology, New York:Grune and Stratton, 1945.
427 Weir DM and Stewart J. Immunology, Edinburgh:Churchill Livingstone, 1993.
428 West RJ and Williams B. Radiographic studies of the ventricles in syringomyelia. **Neuroradiology.** 1980;**20 (1)**:5-16.
429 Whitelock OVS. The reticuloendothelial system. **Am.N.Y.Acad.Sci.** 1960;**88**:1-280.
430 Williams B. The distending force in the production of communicating syringomyelia. **Lancet** 1969;**2(7622)**:696.
431 Williams B and Weller RO. Syringomyelia produced by intramedullary fluid injection in dogs. **J.Neurol.Neurosurg.Psychiatry** 1973, **36 (3)**:467-477.
432 Williams B. Cerebrospinal fluid pressure changes in response to coughing. Brain 1976;**99 (2)**:331-346.
433 Williams B. A critical appraisal of posterior fossa surgery for communicating syringomyelia. **Brain** 1978;**101(2)**:223-250.
434 Williams B. On the pathogenesis of syringomyelia: a review. **J.R.Soc.Med.** 1980;**73(11)**:798-806.
435 Williams B, Bentley J. Experimental communicating syringomyelia in dogs after cisternal kaolin injection. Part 1. Morphology. **J.Neurol.Sci.** 1980;**48(1)**:93-107.
436 Williams B. Experimental communicating syringomyelia in dogs after cisternal kaolin injection. Part 2. Pressure studies. **J.Neurol.Sci.** 1980;**48(1)**:109-122.
437 Williams B. Chronic herniation of the hindbrain. **Ann.R.Coll.Surg.Engl.** 1981;**63(1)**:9-17.
438 Williams B. Post-traumatic syringomyelia. In: Frankel HL, ed. Handbook of Clinical Neurology. Wlsevier Science Publishers B.V., 1992:375-398.
439 Williams B. Syringobulbia: a surgical review. **Acta Neurochir.Wien.** 1993;**123(3-4)**:190-194.
440 Williams B. Pathogenesis of syringomyelia. **Acta Neurochir.Wien.** 1993;**123(3-4)**:159-165.
441 Williams B. Surgery for hindbrain related syringomyelia. **Adv.Tech.Stand.Neurosurg.** 1993;**20**:107-164.
442 Williams B. Management schemes for syringomyelia: Surgical indications and nonsurgical management. In: Anson JA, Benzel EC, Awad IA, eds. Syringomyelia and the Chiari malformations. USA:AANS Publications Committee, 1997:125-143.

443 Wisoff JH, Epstein F. Management of hydromyelia. **Neurosurgery** 1989;**25(4)**:562-571.
444 Wood GW, Gollahon KA, Tilzer SA, Vats T, Morantz RA. The failure of microglia in normal brain to exhibit mononuclear phagocyte markers. **J.Neuropathol.Exp.Neurol.** 1979;**38**:369-376.
445 Wood PL. Microglia as a unique cellular target in the treatment of stroke: potential neurotoxic mediators produced by activated microglia. **Neurol.Res.** 1995;**17(4)**:242-248.
446 Woodroofe MN, Hayes GH, Cuzner ML. Fc receptor density, MHC antigen expression and superoxide production are increased in interferon-gamma-treated microglia isolated from adult rat brain. **Immunology** 1989;**68(3)**:421-426.
447 Wyburn-Mason R. The vascular abnormalities and tumours of the spinal cord and its membranes, London:H. Kimpton, 1943.
448 Yamaya M, Fukushima T, Sekizawa K, Ohrui T, Sasaki H. Cytoplasmic motility reflects phagocytic activity in alveolar macrophages from dog lungs. **Respir.Physiol.** 1995;**101(2)**:199-205.
449 Yao J, Harvath L, Gilbert DL, Colton CA. Chemotaxis by a CNS macrophage, the microglia. **J.Neurosci.Res.** 1990;**27(1)**:36-42.
450 Young W, Kume-Kick J, Constantini S. Glucocorticoid therapy of spinal cord injury. **Ann.N.Y.Acad.Sci.** 1994;**743**:241-263.
451 Zager EL, Ojemann RG, Poletti CE. Acute presentations of syringomyelia. Report of three cases. **J.Neurosurg.** 1990;**72**:133-138.
452 Zajicek JP, Wing M, Scolding NJ, Compston DA. Interactions between oligodendrocytes and microglia. A major role for complement and tumour necrosis factor in oligodendrocyte adherence and killing. **Brain** 1992;**115(6)**:1611-1631.
453 Zhang SC, Fedoroff S. Neuron-microglia interactions in vitro. **Acta Neuropathol.Berl.** 1996;**91**:-385.
454 Zimmerman GA, Martin SB, Tracey KJ. The neuroimmunology of tumour necrosis factor-α. **Clin.Immunother.** 1994;**1**:67.

PRIZES

1. ***Justin Miller Memorial Prize 1999***

   - best paper delivered at the Annual Scientific Meeting

     (SA and NT), Wirrina Cove Paradise, South Australia.

2. ***NimmoResearch Prize 2001***

   - *best paper (full time category)*

   - *Royal Adelaide Hospital, South Australia*

SCIENTIFIC PRESENTATIONS

1. RACS Annual Scientific Meeting

   (South Australia and Northern Territory)

   Wirrina Cove Paradise,

   South Australia.

   August 7, 1999.

2. Neurosurgical Society of Australasia/

   Neural Injury Society of Australia

   Annual Scientific Meeting

   Hamilton Island,

   Queensland.

   October 9, 1999.

3. Spinal Society of Australasia

   Annual Scientific Meeting

   Fremantle,

   Western Australia.

   April 29, 2001.

Appendix

From: Graham Mayrhofer <gmayrhof@gina.science.adelaide.edu.au>
To: Gabriel Lee <glee@digitech.net.au>
Date: Friday, September 11, 1998 1:50 PM
Subject: Re: microglia/macrophages

At 05:44 PM 9/10/98 +0930, you wrote:
>Dear Dr Mayrhof,
>
>I spoke to you the other day on the phone regarding how one might be able to distinguish the macrophages from microglia by immunological means. Since then I have spoken with Professor Holt and quite a few other researchers.
>
>I became interested with the concept of using chimeric animals/rats which differ by only one allelle such that a group of cells can be identified with particular and specific antibodies. I just wonder if you have experience with such an experimental technique please?
>
>Thank you for your kind help.
>
>Kind regards
>Gabriel Lee

Dear Gabriel,
The answer is yes and we have one such congenic pair of rats. Perhaps we should meet and discuss your requirements, because we have just produced these animals and have not yet published on them.

Regards,

Graham Mayrhofer

Graham Mayrhofer
Department of Microbiology and Immunology
University of Adelaide
Adelaide, SA
Australia 5005

Phone: 61 8 303 5389
FAX: 61 8 303 4362
Email: gmayrhof@microb.adelaide.edu.au
WWW: http://www.microbiology.adelaide.edu.au/index.htm

/2/01

From: William F. Hickey <William.F.Hickey@Hitchcock.ORG>
To: glee@digitech.net.au <glee@digitech.net.au>
Date: Saturday, January 16, 1999 2:41 AM
Subject: Rat radiation chimeras

DEAR DR LEE,

THANK YOU FOR YOUR NOTE. I WILL PROVIDE ANSWERS TO THE EXTENT I CAN.

(1) what were the approximate weights of the rats you used? WE USE RATS OF APPROXIMATELY 150. GRAMS.

(2) what antibiotic regimen did you use (type/route/duration) etc? WE HAVE NEVER USED ANTIBIOTICS, NOR HAVE WE HAD TO. THE RATS DO VERY WELL ON THEIR OWN.

(3) what was the mortality rate following the irradiation process (with the cell transfers) at 2 months and when did these rats (if any) died?

OUR MORTALITY IS ZERO. THE RATS TOLERATE THIS WHOLE PROCEDURE VERY WELL. HOWEVER, THERE IS AN IMPORTANT POINT TO MAKE: USE THE MARROW FROM ONE ENTIRE RAT (BOTH FEMURS, BOTH TIBIAE AND BOTH HUMERI) TO MAKE THREE CHIMERAS. DON'T TRY TO "STRETCH" THE BONE MARROW INTO FIVE OR SIX RATS. IT JUST ISN'T ENOUGH. YOU NEED ONE DONOR FOR EACH THREE RECIPIENTS. ALSO, IT IS VERY IMPORTANT TO BE GOOD AT TAIL VEIN INJECTIONS. ALL THE MARROW MUST GO INTO THE TAIL VEIN.

WE USE IRRADIATION OF 1000. RADS GIVEN IN A SINGLE DOSE OVER ABOUT 5 MINUTES.

THERE IS ANOTHER VARIATION WHICH COULD ALSO HELP. IF IT IS NOT CRITICAL THAT YOU GIVE ONLY BONE MARROW CELLS, YOU CAN REMOVE A SPLEEN AND LYMPH NODES FROMT HE DONOR ALSO. IF YOU INFUSE THESE WHEN YOU INJECT THE BONE MARROW, YOU ARE GIVING THE RECIPIENT A "PREFORMED" IMMUNE SYSTEM. IT WILL NOT HAVE ANY PROBLEMS. OF COURSE, NOT KNOWING YOUR RESEARCH GOALS, I CANNOT SAY WHETHER THIS WOULD BE APPROPRIATE.

ALL OF THIS ASSUMES THAT YOUR ANIMAL COLONY IS RELATIVELY CLEAN. IF YOU HAVE DIRTY ANIMALS, THEN THE INFECTION RATE WILL NOT BE LOW.

IF YOUR RATS DIE BECAUSE OF THE IRRADIATION, THEY TYPICALLY DO SO BETWEEN 8 TO 10 DAYS FOLLOWING THE IRRADIATION ..... BUT, AS I'VE SAID, WE'VE NEVER HAD IT HAPPEN.

(4) did your rats lose considerable amounts of weight following the radiation?

NO, THE RATS DO VERY VERY WELL. NO DIARRHEA OR BLEEDING, AND TYRPICALLY NO INFECTIONS. HOWEVER, WE GIVE THE RATS THE BONE MARROW TRANSFER ABOUT 4 HOURS AFTER THEY HAVE BEEN IRRADIATED. WE DON'T WAIT DAYS.

I WISH YOU THE BEST OF LUCK. I HOPE THIS ADVICE HELPS. CHIMERAS ARE A VERY POWERFUL RESEARCH TOOL, AND THE RAT IS A VERY GOOD ANIMAL SYSTEM. IF YOU HAVE OTHER QUESTIONS, LET ME KNOW!

WITH MY REGARDS,

BILL HICKEY

From: William F. Hickey <William.F.Hickey@Hitchcock.ORG>
To: glee@digitech.net.au <glee@digitech.net.au>
Date: Thursday, January 21, 1999 9:06 AM
Subject: Re: Rat radiation chimeras

DEAR GABRIEL,

MY RESPONSES ARE IN UPPER CASE. I AM SORRY FOR THE DELAY..... BUT THERE ARE
OTHER ISSUES TO WHICH I MUST ATTEND (ALTHOUGH I PREFER RESEARCH).

I irradiate my rats at the same dose of 1000 rads at 95 rads/min. (NO PROBLEM WITH THIS). They started losing weight the same day (BAD SIGN...SOUNDS LIKE INFECTION OR RADIATION POISONING) for a total of 4 days before plateauing. As you said most have died at day 6-7. We used prophylactic gentamicin as suggested by the vet. HERE I HAVE FOUR THOUGHTS. FIRST, IF YOUR ANIMAL COLONY HARBORS PATHOGENS, AND EVERY RAT ENTERING BECOMES INFECTED (MYCOPLASMA
IS ONE OF THE ONES I HAVE IN MIND), THEN IRRADIATION WILL ELIMINATE THE IMMUNE
RESPONSE AND THE RATS DIE. SOME FUNGI AND PROTOZOAN INFECTIONS COULD DO THE
SAME. SECOND, IT COULD BE AS SIMPLE AS TRANSFERING TOO FEW STEM CELLS AND NO
IMMUNE CELLS (REMEMBER..... DOES YOUR RESEARCH OBJECTIVE REQUIRE THE USE OF
ONLY STEM CELLS?? IF IT DOESN'T, THROW IN A SPLEEN FOR THE RATS' HEALTH! THRE
ARE SOME SCIENTIC QUESTIONS WHICH WOULD PREVENT THIS, BUT NOT MANY.) THIRD,
THE CALIBRATION OF YOUR SPECIFIC IRRADIATOR MAY BE OFF .... IT MAY BE WAY, WAY
OFF. ASK THE GROUP THAT OVERSEES THE IRRADIATOR FOR CALIBRATION DATA. YOU
MAY BE LITERALLY FRYING YOUR RATS. FOURTH AND FINALLY IS SOMETHING I SUSPECT
MAY BE AT THE ROOT OF THE ISSUE. YOU ARE USING GENTEMICIN. THIS IS NEPHROTOXIC. ARE YOU KILLING YOUR RATS WITH UREMIA BECAUSE YOU ARE SEVERELY
DAMAGING THE KIDNEYS WITH WHAT MIGHT BE MASSIVE DOSES OF GENT?? YOU SHOULDN'T
NEED ANTIBIOTICS AT ALL IN A CLEAN COLONY AND WITH PROPER IRRADIATION AND
APPROPRIATE STEM CELL TRANSFER. IF YOU DO NEED THEM, THEN TETRACYCLINE IN THE
DRINKING WATER IS THE WAY I'D GO (CHAT WITH YOUR VET).

We used 1 x 10-7 cells for each rat. This meant that we used up to1 donor rat for 12 DA rats. I was therefore again surprised that you used a donor for 3 rats. We also injected the rats within 4 hours. We also know that the cells are immuno-histocompatible from skin grafts and previous cell transfers. After the injections, our rats were kept in cages of 3. YOUR TRANSFERED CELL NUMBER IS VERY MUCH TOO SMALL. (ALSO SEE ABOVE).

Could you tell me please if you have irradiated DA rats before? I USE THEM ROUTINELY. NO DIFFERENCE FROM OTHER STRAINS WITH REGARD TO SENSITIVITY TO
RADIATION, ETC. PLEASE FILE THIS NOTE AWAY FOR FUTURE REFERENCE RELATIVE TO
RATS IF YOU USE DA RATS. I HAVE A RAT CALLED THE DA(RP) RAT. ITS GENES ARE
DA EXCEPT FOR THE MHC CLASS TWO MOLECULES. THOSE ARE RT-1L (LIKE LEWIS).
THERE MHC CLASS ONE GENES ARE NOT LEWIS. SHOULD THIS TYPE OF RAT EVERY BECOME
INTERESTING TO YOU RELATIVE TO YOUR STUDIES (ESPECIALLY FOR MAKING VERY
INTERESTING CHIMERAS) LET ME KNOW.
Were they housed in separate cages after the injection of cells? NO - GROUPS
OF 3 WORKS BEST....THEY GET LONELY AND STRESSED IF HOUSED ALONEWere they reared
in special conditions prior? NO WAY!

I SINCERELY HOPE THIS INFORMATION GETS YOU OFF THE GROUND. THERE'S NO
QUESTION YOU'RE BATTLING TECHNICAL PROBLEMS. LUCKILY, THEY'RE CAN BE CORRECTED....UNLESS YOU HAVE AN ENDEMICALLY INFECTED ANIMAL FACILITY. IF
THAT'S THE CASE, FIND ANOTHER ONE ON CAMPUS OR NEARBY THAT IS CLEAN, AND MAKE
ARRANGEMENTS TO HOUSE THE CHIMERAS THERE WITHOUT EVER PASSING THROUGH THE DIRTY
COLONY.

ONCE AGAIN, BEST OF LUCK. LET ME KNOW IF YOU NEED MORE INFO.

BILL HICKEY

From: Emma <emma@serotec.demon.co.uk>
To: Gabriel Lee <glee@digitech.net.au>
Date: Friday, February 12, 1999 6:10 PM
Subject: RE: Re: Re: Re: RT7.2

Dear Dr Lee,

Hello again!

The clone originator for MCA473S is going to contact me on Monday. Apparently, he has not been in the lab this week which explains the delay in replying to me.

Serotec QCs most antibodies in flow cytometry. The information concerning the use of our antibodies in immunohistology is generally obtained from published references, from clone originators or sometimes from external testing where we send samples of our antibodies away for testing. At present, our labs are not set up for immunohistology, although in the near future we will be beginning an immunohistology project testing our antibodies "in house".

Although you have had little success with the tissue culture supernatant supplied by another company, I would be surprised if a dilution of 1/50 would work as we recommend using supernatants neat-1/5. Typically, supernatants have an antibody concentration of between 10 and 50 micrograms per ml (unless of course they are concentrated supernatants).

As soon as the clone originator for MCA473S contacts me on Monday, I will e-mail you the information I receive.

I hope this will be of assistance to you and that you have a good weekend.

Best regards,

Emma

From: Gabriel Lee
Sent: 11 February 1999 05:49
To: Emma
Subject: Re: Re: Re: RT7.2

Dear Emma

I was wondering if there was any chance that the Serotec laboratory could actually test the product of RT7,2 antibody on paraffin sections on a semi-urgent basis (ie in the next few days)? I suspect that this may be much faster than waiting for a reply from the clone originator to confirm the information on the data sheet.

will certainly test it as soon as I obtain the samples although this will certainly take up to the end of next week at the very earliest. However, should your lab be able to reproduce the results on paraffin sections, this would give me some confidence that the procedure really works.

Unfortunately, I am due to start on my animal work early next week and thus I would need to have a definite plan as to whether I proceed with paraffin sections or frozen sections by the end of next week.

In fact yesterday I got a small aliquot of the RT7.2 antibody from a neighbouring laboratory (BUT from a different product company) to test its efficacy on paraffin sections. Due to the small volume that was given, I made a 1/20 dilution (enough for 2 slides). Unfortunately even with a nickel DAB technique it does not appear to have worked at all. I did not see any staining at all wheras I expected to see perhaps patchy staining even at this low dilution. It is also a supernatant product and this has perhaps made me even more keen to demonstrate that the Serotec product does certainly work on these sections as stated on the data sheet. Despite a number of possible reasons for failure to work (including the dilution factor), the situation is rather worrying.

I look forward to a reply from the clone originator and hope that the Serotec laboratory will be able to assist in this regard as well.

Many thanks again.

Kind regards
Gabriel

-----Original Message-----
From: Emma <emma@serotec.demon.co.uk>
To: Gabriel Lee <glee@digitech.net.au>
Date: Tuesday, February 09, 1999 10:41 PM
Subject: RE: Re: Re: RT7.2

>Dear Dr. Lee,
>
>Serotec couriers all products to Australia by air (when I say shipping, I really mean despatching). The products will therefore take 2-3 days to get to our Australian distributor. I cannot say how long it will take for the products to reach you once they are in the hands of the distributor as I do not know which system is used to pass on the products. Perhaps you could contact the distributor to ask how long it will take once the products are in Australia.
>
>I hope this will resolve the situation and please don't worry about contacting me - if it wasn't for Serotec's mistake, you wouldn't have to keep e-mailing!

>
>Best regards,
>
>Emma.
>
>----------
>From: Gabriel Lee
>Sent: 09 February 1999 23:07
>To: Emma
>Subject: Re: Re: RT7.2
>
>Dear Emma
>
>Thank you for taking the trouble to contact the clone originator. I will be
>most interested in what you find out.
>
>Thank you also for sending the free vials of antibody. However, I am afraid
>shipping will imply another delay of at least 2-3 weeks. My testing of the
>antibody has already been delayed by at least 3 weeks and a further delay
>would not be helpful at all. In fact, I had spoken with the representative
>at Jomar Diagnostics toady to arrange an urgent courier service ie by air
if
>this was possible. This antibody is critical to my current research and I
>hope that Serotec would be able to kindly assist in this matter.
>
>My numerous e-mails must be wearing you down. I am extremely grateful for
>all the help that you have given over the phone as well as via electronic
>mail.
>
>I look forward to your reply with regards to both the information of
>staining paraffin sections as well as delivery of the antibody samples.
>
>Thank you kindly once again.
>
>
>Kind regards
>Gabriel Lee
>
>-----Original Message-----
>From: Emma <emma@serotec.demon.co.uk>
>To: Gabriel Lee <glee@digitech.net.au>
>Date: Tuesday, February 09, 1999 7:20 PM
>Subject: RE: Re: RT7.2
>
>
>>Dear Dr. Lee,
>>
>>Thank you for your e-mail regarding the free of charge vials of MCA473S.
>>
>>I have arranged for these to be shipped to our Australian distributor
>tomorrow so you should receive the vials shortly.

>>I will be contacting the clone originator of MCA473S today to discuss the
>suitability of MCA473S on paraffin sections. Unfortunately, I am unable to
>supply the details for the clone originator as this is confidential
>information, however, I will contact him today to see if there are any
>special conditions required for the technique.
>>
>>Once again, I would like to apologise for the problems you have
>experienced with Serotec on this occasion.
>>
>>Best regards,
>>
>>Emma Slater
>>Technical Services Advisor
>>
>>----------
>>From: Gabriel Lee
>>Sent: 09 February 1999 07:14
>>To: Emma
>>Subject: Re: RT7.2
>>
>>Dear Emma
>>
>>Thank you for your fax which I received yesterday.
>>
>>Indeed the events that have occurred have been rather unfortunate.
However,
>>I am grateful for Serotec's offer of 2 free vials of MCA 473S (RT7.2 rat
>>antibodies). I will inform Jomar Diagnostics today to state that my
>>department will take up your kind offer. The testing of the antibody will
>be
>>carried out as soon as possible and I am hopeful that the vials will be
>>delivered urgently.
>>
>>I look forward to hearing from you with regards to the use of this
>>particular antibody on paraffin sections or at least who the clone
>>originator. If this information cannot be provided due to confidentiality,
>>would you kindly assist in contacting the clone originator through Serotec
>>to confirm the information provided on your data sheets please?
>>
>>Thank you.
>>
>>
>>Kind regards
>>Gabriel Lee
>>
>>
>>
>>
>>-----Original Message-----
>>From: Emma <emma@serotec.demon.co.uk>
>>To: glee@camtech.net.au <glee@camtech.net.au>
2/01

>>Date: Thursday, February 04, 1999 3:46 AM
>>Subject: RT7.2
>>
>>
>>>Dear Dr. Lee,
>>>
>>>I apologise for the confusion surrounding your order of MCA473.
>>>
>>>It would seem as though you have received the ascites preparation
0.25ml)
>>which should be diluted approximately 1/500 for use on paraffin sections.
>>>
>>>I have searched through my files for information regarding the use of
>>MCA473 on paraffin sections and it appears as though the information was
>>obtained from testing by the clone originator of the antibody. As far as

>>am aware, the information has not been published.
>>>
>>>I am sorry I am unable to provide additional information at present. If

>>can be of any further assistance, please do not hesitate to contact me.
>>>
>>>Best regards,
>>>
>>>Emma Slater
>>>Technical Services Advisor
>>>
>>>
>>>
>>>
>>>
>>
>>
>>
>>
>>
>
>
>
>
>

From: Emma <emma@serotec.demon.co.uk>
To: Gabriel Lee <glee@digitech.net.au>
Date: Tuesday, February 16, 1999 10:53 PM
Subject: RE: Re: Re: Re: RT7.2

Dear Dr Lee,

Once again, I apologise for the delay in contacting you concerning the use of HIS41 on paraffin sections.

I can finally confirm that the recommended procedure for paraffin sections is as follows;

De-paraffinise 4% paraformaldehyde fixed paraffin sections in xylene solvent and hydrate through a graded series of graded alcohols. Wash twice in TBS.

Block endogenous peroxidase using 0.3% (w/v) hydrogen peroxide in methanol for approx. 30 minutes. Wash in TBS.

Block non-specific binding by incubating your sections in 10% normal serum for approx. 10 mins. The serum should be from the species in which the secondary antibody is raised. I do not know which secondary antibody you are using so cannot advise as to the species of the serum to use.

Incubate the sections with HIS41 for at least 1 hour at room temperature. Wash 3 times in TBS.

Add your enzyme-labeled secondary antibody at the recommended dilution (according to the manufacturers instructions) and wash in water.

Develop colour as normal.

I have been informed that microwave antigen retrieval may enhance staining. Should you wish to try this, the retrieval step should be performed following the endogenous peroxidase block.

I hope this information will be of assistance to you.

Best regards,

Emma.

From: Andy <andy@serotec.demon.co.uk>
To: glee@digitech.net.au <glee@digitech.net.au>
Date: Friday, February 19, 1999 6:12 PM
Subject: Re: HIS41

Dear Dr Lee,

I have been asked to review the details of the problems that you have experienced, in various ways, with our anti-rat RT7.2 antibody, product code MCA473.

Firstly I should add my apologies to those you have already received regarding the confusion about the format change from ascites to culture supernatant. The position with regard to activity of the antibody in paraffin-embedded material appears to be rather more complicated, and I should take the opportunity to review the position with you.

Our information regarding the applications of the antibody originates from our supplier. In this particular instance our originating supplier is not in fact the clone originator - although our confidentiality obligations do not permit us to identify our supplier, I believe that you have now actually been in touch with the clone originator yourself. In this respect you probably have an advantage over us, as we are not able to contact them directly.

I can confirm that our supplier originally indicated, and then confirmed, that the clone was active in paraffin embedded material with the assistance of microwave antigen retrieval. It would appear from a very recent communication (received after Emma forwarded you methodological details) that they cannot actually support this, which may reflect further information that they have received from the clone originator. Obviously I cannot speculate as to the source of their original information, but can only be frustrated by the confusion it has caused.

Your latest e-mail raised a number of technical points, to which I will respond as far as possible below:-

1. With the exception of titre, antibody format (i.e. ascites vs. TCS) should not have any affect upon activity of an antibody in staining paraffin sections.

2. Staining intensity in paraffin sections may often be less intense than with the same antibody used in frozen sections.

3. It could well be that the patchy weak staining you have seen reflects some fixation damage, which is likely to be additional to damage caused by the delay in processing the organs after euthanasia.

Given the support that we have received to date from our source I think it is fair to say that we are unlikely to receive specific answers regarding fixation technique. We do not have any data regarding expected expression of RT7.2 in heterozygotes, and I would not like to speculate about this without any knowledge of the genetic background of the animals you are using, or indeed without any knowledge about the genetic dominance of RT7 alleles.

Obviously I am unhappy with this situation, particularly regarding the unsupported claims that have been made by our supplier, and passed on in good faith by ourselves. My personal feeling from your results is that the antibody is probably weakly active in paraffin-embedded tissues, and that improved results may be obtained with even more sensitive detection systems (e.g. Streptavidin.ABC), although I am not in a position to be able to support this in any definite way.

I can offer a full credit in this case for your original purchase, but am aware from your correspondence that you may prefer to use this clone in frozen sections instead. If this is the case I would be pleased to supply you with 2 further vials free-of-charge. I would be grateful if you could let me know if you would like to have these so that I can make arrangements accordingly.

With best regards,

Yours sincerely

Andy

Andrew C. Lane PhD
Product Manager
Serotec Ltd
22 Bankside
Station Approach
Kidlington
Oxford
OX5 1JE
UK
Tel: +44 1865 852700
Fax: +44 1865 373899
E-mail: andy@serotec.demon.co.uk

From: F.G.M.KROESE <f.g.m.kroese@med.rug.nl>
To: Gabriel Lee <glee@digitech.net.au>
Date: Monday, February 22, 1999 9:16 PM
Subject: Re: RT7.2 rat antibody

Dear Gabriel Lee,

Thank you for your resending the e-mail. I am very sorry for you to hear the problems and frustrations related to the HIS41 antibody. Hope things are solbved now, and that HIS41 will fullfill your expectancies.

Regarding your questions:

Question #1
Protocol for iimunohistology:
1. Kill rat (does not matter how. We use now CO2 inhalation, but we also have used overdosis ether or i.p. injections of euthasate).

2. Remove spleen and keep on ice in some PBS

3. Snap freeze spleen on piece of filter paper in liquid freon, liquid nitrogen or on dry ice. (You may use tissue-tec).

4. Store tissue at -70 until use.

5. Make tissue sections (5-7 um)

6. Let them dry thorougly (e.g. 1 hour room temp, with cold haiblower).

7. Fix with acetone (best quality you can get; must be waterfree!), 10 minutes room temp.

8. Airdry, at least one hour (usually we do it overnight).

9. Wash slides one with PBS (room temp, 5 min)

10. Incubate with appropriately diluted antibody (if we do not know the dilution, we take undiluted supernatants or 1/200 diluted ascites, or 10 ugr/ml). Incubate for one hour at room temp. Do not let dry out; use a humidified chamber!

11. Wash three times with PBS, 3 x 10 min, room temp

12. Incubate with second step reagent (we use rabbit anti mouse

conjugated to peroxidase from DAKO). This reagent is diluted (1/50) n PBS contaning 1% normal rat serum, to prevent unspecific staining. ncubate for 30 minutes at room temp (humidified chamber)

13. Wash three times with PBS, 3 x 10 min, room temp.

14. Develop peroxidase, e.g. with diaminobenzidine as usual.

15 counterstain if needed with hematoxylin, dehydrate and mount sections in Depex.

I hope this will work.

Question #2:
You can store the tissues at -70 in small containers for many years.

Question #3:
Heterozygotes have enough RT7 to show up

Question #4
Staining pattern should be clear and easy for interpretation. The only problem you may encounter when you are dealing with macrophages and granulocytes is endogenous peroxidase.

Question #5
Just CO2

Question #6
Yes, no problems with culture supernantants.

I hope this will help to fix the problems. Please do not hesitate to contact me again, and I would like to hear whether it works or not.

Best regards,

Frans G.M. Kroese

Dr Frans G.M. Kroese
Dept. Histology and Cell Biology
University of Groningen,
Oostersingel 69/1
9713 EZ Groningen
The Netherlands
Phone: +31-50-3632522 or +31-50-3632534
/2/01

From: William F. Hickey <William.F.Hickey@Hitchcock.ORG>
To: glee@digitech.net.au <glee@digitech.net.au>
Date: Wednesday, March 31, 1999 11:47 PM
Subject: Re: Interpretation of ED1 macrophage marker staining pattern

---

Dear Gabriel.

The counting is difficult. I believe that ED1 stains BOTH activated microglial cells and infiltrating macrophages fromthe blood. The question of cross staining of neutrophils with ED1 is of little concern since in rat EAE there are virtually none of these in the CNS. The smaller cells you mention are probably microglial cells that are becoming activated (but remember some small percentage of your large "macrophages" started as microglia.

Hope all is going well with this project. Keep up the good work!

Bill Hickey

From: CD Dijkstra <CD.Dijkstra.Cell@med.vu.nl>
To: glee@digitech.net.au <glee@digitech.net.au>
Date: Tuesday, April 06, 1999 6:56 PM
Subject: re: ED1

Dear Dr. Lee,

As for the ED1:

The original observation was that ED1 does not stain granulocytes and indeed in tissues granulocytes are normally negative for ED1. However, in a personal communication by Jurgen Westermann I lerned that granulocytes in the peripheral blood are sometimes positive for ED1. I have never seen ED1 positive granulocytes in the CNS.

As for your second question: indeed (activated) microglia can be positive for ED1, we often observe that in EAE. I agree with you that this makes quantification of macrophages in the CNS difficult in your model. Morphology is in fact maybe the most reliable feature to discriminate between macrophages and microglia. Maybe you should also look in the papers of Jon Sedgwick for discrimination between macrophages and microglia by FACs analysis.

With kind regards,

Christine Dijkstra

---------- Original Message ----------
Dear Professor Dijkstra

Greetings from Australia.

I am a neuro-surgical researcher working on a post-graduate degree. I am using rat radiation chimeras to study the origin of CNS macrophages in my rat animal model.

I wonder if I could seek your kind advice on several aspects of the monoclonal antibody of ED1 which you originated. It would be very much appreciated.

(1) Does ED1 label neutrophils as well- the data sheet from Serotec suggests that it does stain neutrophils wheras your publication seem to suggest that it does not. In other words, can I be confident that in an ED1 positive inflammatory infiltrate, there are only macrophages which are

labelled/stained?

(2) I stain ED1 macrophages in the CNS (rat model). The staining intensity is usually very strong. However, there are smaller areas which stain more weakly- do you accept these as being macrophages as well? These variations in terms of staining intensity leads to difficulty in confident macrophage counting. I believe that activated microglia are also ED1 positive- would this be your experience as well?

I apologise for taking your time but know that there is probably no better person to seek advice on this matter than yourself.

Thank you kindly again.

Kind regards
Gabriel Lee (Dr)

---------- End of Original Message ----------

From: Hans Lassmann <hans.lassmann@univie.ac.at>
To: Gabriel Lee <glee@digitech.net.au>
Date: Tuesday, April 13, 1999 9:55 PM
Subject: Re: Interpretation of ED1 macrophage marker staining pattern

Dear Mr. Lee:

Thank you very much for your message dated March 31, 1999. Indeed ED1 stains both, macrophages as well as microglia. We have addressed this point in detail in a paper (Rinner et al, Glia 14:257-266; 1995) which by using bone marrow chimeras clearly shows that both, macrophages and microglia express this marker. In our hand ED1 does not stain neutrophils, at least in paraffin sections. For detailed cell identification we use rather thin paraffin sections for ED1 staining and counterstain them with hematoxylin. In this case it is easy to define the different cell types by cell and nuclear morphology. I hope this information is useful for you.

With best regards,
yours sincerely,

Prof. Dr. Hans Lassmann

At 23:30 31.03.1999 +0930, you wrote:
>Dear Professors Hickey and Lassmann
>
>
>Thank you for your previous advice on creating rat radiation chimeras. That aspect has gone rather smoothly and been quite successful. I am currently at the stage of looking at and analysing the results to assess the percentage of exogenous macrophages.
>
>I wonder if I could further trouble you by asking for your kind advice with regards to the use of the ED1 macrophage marker. My staining pattern within the central nervous tissue shows that the macrophages stain extremely strongly with Nickel DAB (in my model). In fact in a dense infiltrate, it proves quite difficult to count the number of macrophages. Furthermore, there are also much smaller smaller cells which stain positively (looking quite distinct). I presume that these are activated microglia- is that your experience? The data sheet from Serotec also suggests that neutrophils may stain weakly with ED1- how do you overcome this problem of trying to decide which cells are in fact macrophages when you perform a count.
>
>I am most grateful for your advice and experience. This has presented a real problem for me.

>
>Thank you once again and I apologise for the inconvenience.
>
>
>Kind regards
>Gabriel Lee
>
>
>Attachment Converted: "C:\MAIL\Interpre"
>

From: Graham Mayrhofer <graham.mayrhofer@adelaide.edu.au>
To: Gabriel Lee <glee@digitech.net.au>
Date: Wednesday, July 28, 1999 6:24 PM
Subject: Re: Quick question please

Dear Gabriel,
The problem that you face with this sort of protocol is
hat the second primary antibody can be bound by free valencies of the
secondary antibody used in the initial indirect labelling procedure -
e. by the donkey anti-mouse. You are attempting to block this by the
use of 1B5 antibody, but this will not be at a concentration that will
be effective. You should block by incubation with 50% normal mouse serum
and then perform the final incubation with the HIS/RT7.2 conjugate
diluted in 50% normal mouse serum. That way, you ensure that the donkey
anti-mouse is kept blocked throughout by normal mouse IgG.

Good luck,

Graham

> Gabriel Lee wrote:
>
> Dear Graham
>
> Just a quick question if you don't mind, please.
>
> I have managed to co-localise the RT7.2 and ED1 antigens on the same
> sections using immunoflurorescence as the last step of my project. It
> appears to have worked well. I initially sought advice from the
> Leukemia research lab at IMVS.
>
> However there remains some doubt in my mind as to whether the
> methodology can be open to criticism. The difficulty with the 2
> primary antibodies is that they are of the same isotype and are both
> mouse anti rat.
>
> Hence I ended using the following incubation sequence of antibodies:
>
> (1) primary ED1(monoclonal) as a first step
>
> (2) secondary donkey anti mouse antibody conjugated to Texas Red
>
> (3) anti-giardia 1B5 to flood the sections
>
> (4) finally the HIS/RT7.2 (monoclonal) which is directly conjugated to
> FITC
>

> I wonder Graham if you could kindly advise me as to whether you see
> any problems with this protocol. I know that there is a theoretical
> possibility that the Texas Red conjugated secondary may be dislodged
> to bind onto RT7.2 but thought this would be unlikely.
>
> Thank you again for your kind advice.
>
>
> Kind regards
> Gabriel