



the Bank Statement

News from

The UK Multiple Sclerosis Tissue Bank

ISSUE 3

A newsletter for you

In this issue, find out about...

...two aims...

(1) a facility

MS Matters = MS Society's magazine with distribution of 55,000

In 7 years...

...over 4,000 Information Packs sent out

and

...over 2,500 people registered as donors

we will keep on raising awareness of the importance of tissue to research

Welcome to the third issue of our newsletter. Whether you are already a registered donor; or, in the process of deciding whether it is right for you; or you are the relative or friend of someone who has donated tissue, we hope that our **Bank Statement** will keep **all of you** informed with what is going on here at the Tissue Bank. In this issue we are pleased to report on:

- how well the Tissue Bank is meeting its two overall aims
- 6 research projects supported by the Tissue Bank
- the Tissue Bank's updated website
- our **new** emergency donor line number – **07 659 132 045**
- the things we would like you to tell us

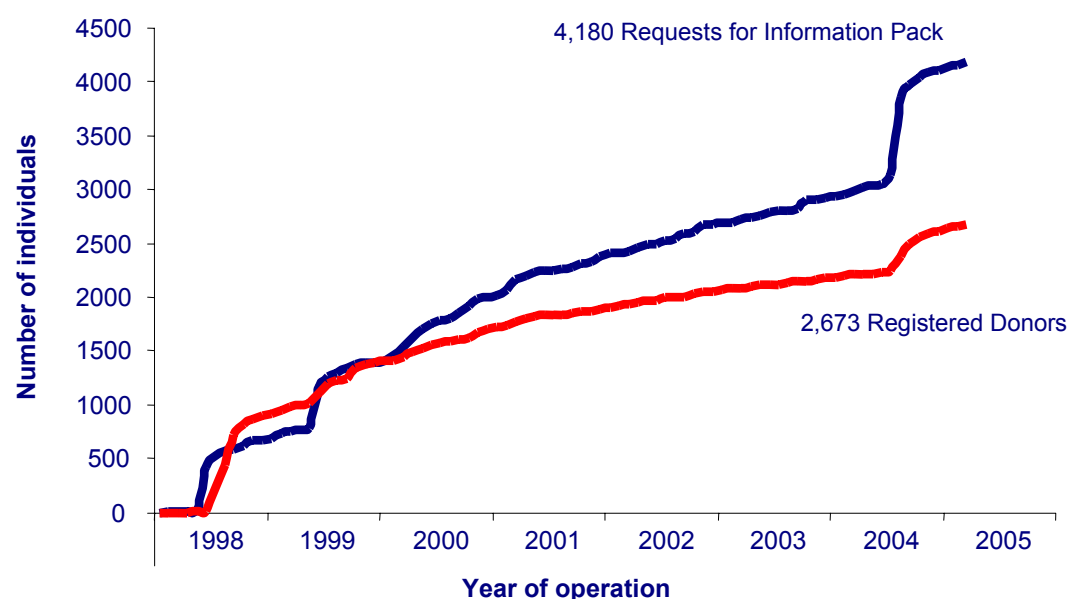
The Tissue Bank acts as two things:

- a facility for all those people (those with MS and those without the condition) who want to donate their brain and spinal cord to research; and,
- a resource for those scientists able to use samples of tissue in their research on the cause and treatment of MS.

We now describe how well we have been addressing these aims over the last 7 years that the Tissue Bank has been operating from the Charing Cross Campus of Imperial College London under the directorship of Professor Richard Reynolds:

-a facility for those wanting to donate tissue to research

The most effective way of raising the profile of the Tissue Bank and of recruiting individuals on to its prospective donor scheme remains to be one of publishing articles in MS Matters.



For example, the July/August 2004 issue carried an interview of Professor Reynolds and Dr Vora. This article alone resulted in **987** requests for more information and **336** individuals subsequently registering on the donor scheme.

we need even more people to register, so we will keep on promoting the work of the Tissue Bank

the Tissue Bank Team occupy a privileged position in being able to fulfil last wishes

80% of tissue samples preserved within 48hours of death...
...over 50% within 24hours

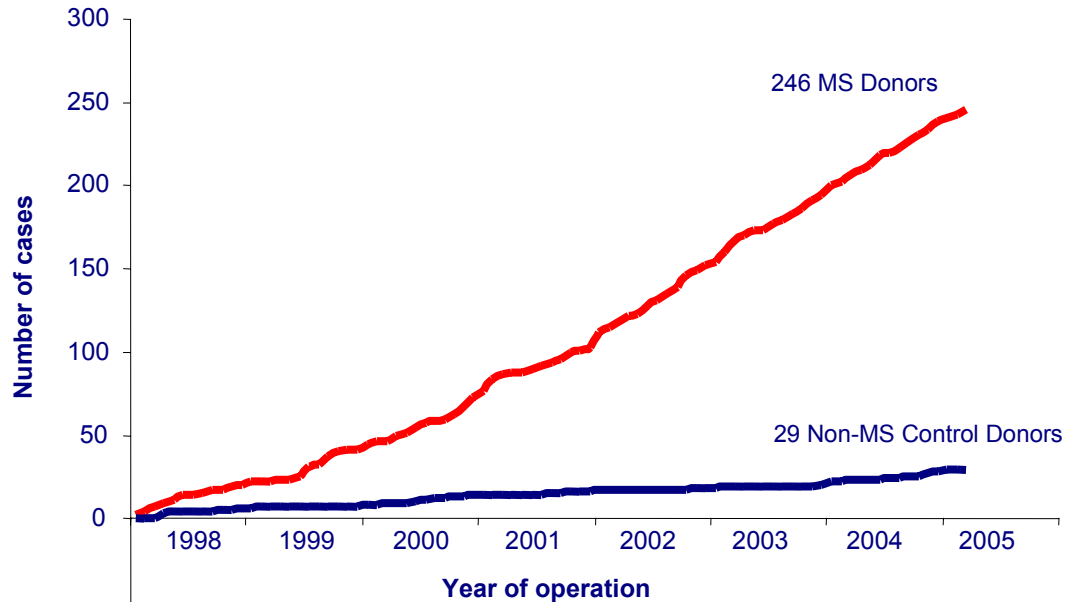
(2) a resource

89 projects supported over 7years

we value your feedback on all aspects of our work, our contact details are on the back cover

This brings the total number of Information Packs supplied since we began operations in 1998 to **4,180**. The blue line in the graph above shows how the number of Packs distributed has grown over the last seven years. The graph also shows the accumulation of people who have registered on the donor scheme over this time - the total number who have registered since 1998 is **2,673**.

The graph below shows the rate at which we have accrued tissue from **246** donors that had MS (red line) and **29** subjects without the condition (blue line) over the period 1998 to March 2005. With each and every one of these cases we are reminded of the privileged position that we occupy in being able to carry out the last wishes of the people registered with our donor scheme and the strength and determination of the donor's families in allowing us to carry out those wishes.



Our procedure for the rapid retrieval of donated brain and spinal cord was effective in procuring these tissues from 84% of the MS donors and 74% of the control donors within 48hours of death. In 63% of the MS cases and 55% of the control cases, the tissues were preserved within 24 hours of death. This demonstrates the willingness of a range of professionals (general practitioners, nurses, pathologists, coroner's officers, funeral directors etc) to help us to fulfil the last wishes of generous individuals registered on the donor scheme.

-a resource for those scientists able to use tissue in their research on MS

The Tissue Bank continues to act as an essential resource for research on multiple sclerosis. The number of projects that are being supported has risen steadily until at the end of June 2005, the Tissue Bank had provided 89 separate research projects with samples of tissue.

Year of operation	Total number of research projects supplied with tissue
1998	8
1999	16
2000	28
2001	31
2002	39
2003	59
2004	84
2005 (to June)	89

Tissue from 170 (69%) of the 246 donors that had MS and 22 (76%) of the 29 donors that did not have the condition has already been used in research.

a way of looking at how scientists find answers

the availability of tissue means giving MS researchers access to the crime scene

1st Project

each mitochondrion measures 1/4000 of a millimetre in length and many hundreds can be found in a single nerve cell

top row:
blue = myelin

bottom row:
brown = activity of mitochondria

MS lesion – scene of a crime?

Scientists studying MS lesions are a little like crime scene investigators examining scenes of crime: both identify and piece together fragments of relevant evidence in an effort to come up with a sequence of events that culminated in injury. For MS researchers, part of solving the crime is to understand (1) the way in which myelin and axons (an extension of a nerve cell along which impulses travel) are damaged; and, (2) how exactly the brain repairs itself. The ultimate aim of all research is to develop therapies that will halt the first and promote the second.

The “crime scene” for MS researchers is complicated for a number of reasons:

- It may be that more than one culprit is responsible for the injury – we will learn of two possible ways in which axons may be damaged (**project 1 and 2**).
- The culprit(s) causing damage to axons may be different to those that are injuring myelin - we learn about antibodies that may attack myelin (**project 3**).
- Changes will occur as a scene of destruction transforms into one of repair - we learn about one change that is vital to the function of axons (**project 4**).
- Molecules and cells designed to be of benefit may actually hamper repair – we learn how clotting and scarring may hinder remyelination (**projects 5 and 6**).

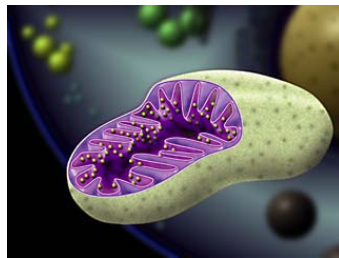
The 6 projects described below were made possible only by the generosity and forethought of the people who have donated their tissues to research on MS.

Is working harder bad for the axon?

Dr Philip Nichols

MS Research Group, University of Newcastle upon Tyne

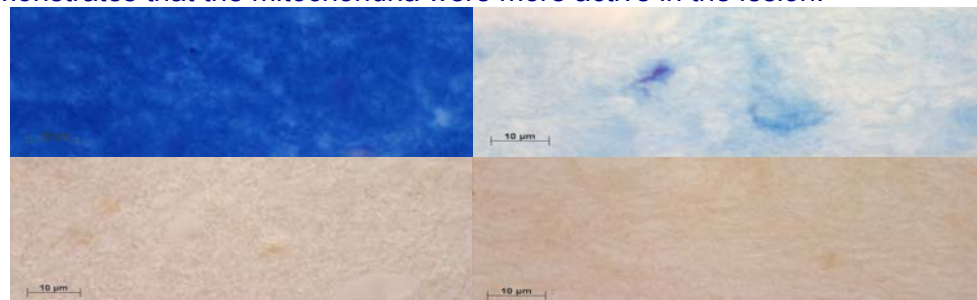
An examination of MS lesions reveals two casualties – myelin and axons. What is less clear is how these injuries occur. Over the last two years, Dr Nichols’ group has been trying to find out how axons are damaged in MS lesions; and what role special structures within the nerve cell - the “mitochondria” play in this damage.



The mitochondria, one shown here in a cartoon, provide energy to the cell and so are referred to as the cell’s “batteries”. In nerve cells, mitochondria provide the energy that is needed to drive nerve impulses along the axon. The group asked “do the ‘batteries’ in axons within lesions have to work harder?” In order to answer this they examined brain tissue from MS donors that contained a lesion and an adjacent sample that appeared “normal”.

They first confirmed the presence of a lesion by treating a very thin slice of the tissue with a dye that stains myelin blue. They then cut another slice and used a special dye with which they could “see” how hard the mitochondria were working.

The picture in the top left panel (below) shows normal tissue with normal amounts of myelin (dark blue); a lesion in which the myelin has been destroyed is shown top right (lack of blue). The brown stain in the bottom panels shows the activity of mitochondria – the darker staining of the tissue containing the lesion (bottom right) demonstrates that the mitochondria were more active in the lesion.



This is perfectly understandable, as an axon devoid of the insulating layer of myelin will need more energy to conduct nerve impulses than a fully insulated, myelinated axon. But, is it possible that the very system by which the axon is trying to cope with the lack of myelin is actually making it more vulnerable to

next step...

...stopping a fatal side effect of mitochondrial over activity

2nd Project

macrophage = "big eater" (literally)

chemokines = chemicals causing kinesis (movement)

lesions expand like ripples produced when a stone is thrown into a still pond

Left: large lesion blue = myelin

Right: 3 high power pictures of different areas of the lesion brown = MCP-1

periplaque = area surround lesion

Edge of lesion:
-large amounts of MCP-1&IP-10
-large numbers of macrophages
-large amounts of toxins for axons

damage and eventual death? In order to answer this, Dr Nichols' group is now trying to find out whether an unfortunate side effect of the increased activity of the mitochondria could contribute to axons being damaged within lesions. Understanding the exact mechanism of this could provide a target for treatments aimed at stopping damage to axons and this would in turn bring us one step closer to limiting or even preventing the disability caused by MS.

Chemicals that draw "big eaters" to a site of injury

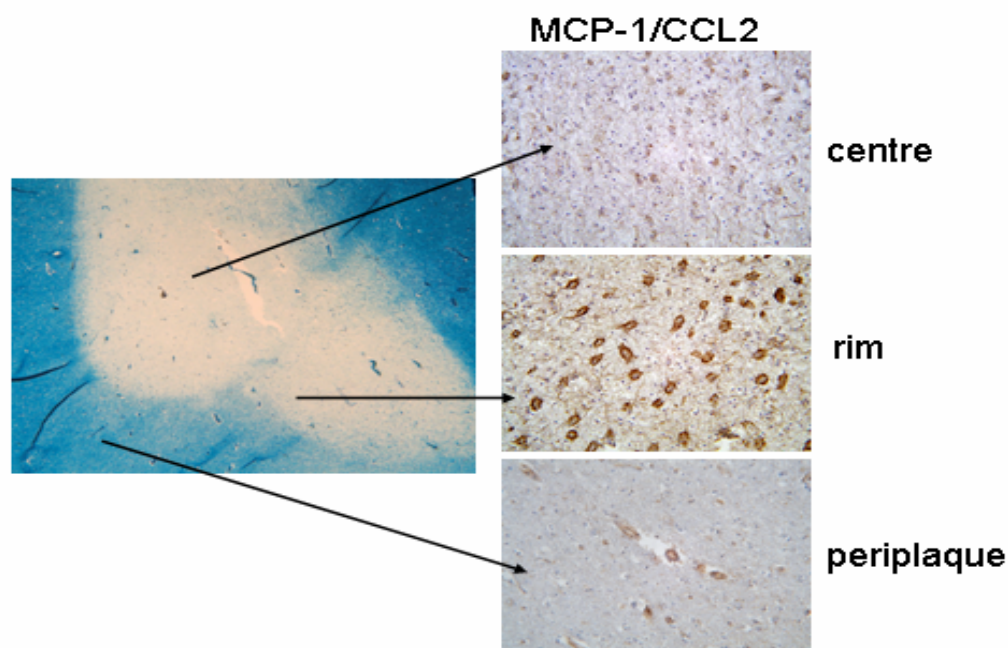
Professor Yoh Matsumoto

Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan

Professor Matsumoto's group is following another lead to explain the injury to axons. The amount of axonal damage within a lesion has been linked to the number of a particular type of cell called a macrophage present at the "crime scene". Macrophages release toxic chemicals that kill bacteria and tumour cells, and they then engulf and digest the resulting debris. Since these activities could be directed to injuring axons, it is vital to control this "powerful" cell within the brain. Professor Matsumoto has gone to the very beginning of the chain of events and asked: "What initially draws macrophages to a site where axons are or about to be injured?"

Chemicals that cause cells to move towards the site of release are called chemokines, and the group found two types in MS lesions: monocyte chemoattractant-1 (MCP-1) and interferon-gamma inducible protein-10 (IP-10). It is known that MS lesions grow by expanding outward from a central starting point. This means that in a large active lesion, the ongoing destruction will be occurring on the lesion edge. High concentrations of MCP-1 and IP-10 were found on the rim of the lesions, and low levels in the inactive, lesion centre and in the surrounding "normal" tissue.

These pictures from Professor Matsumoto's research show on the left a thin slice of tissue treated with the blue myelin dye – demonstrating the presence of a large lesion. The next slice was treated with a stain that picks up the chemokine MCP-1. The three high power views on the right, show low amounts of MCP-10 at the centre and outside the lesion and large amounts on the lesion rim (small brown dots). Similar pictures were seen when slices were stained for IP-10.



Large numbers of macrophages were also found in the rim, and these had on their surface receptors that would capture MCP-10 and IP-10. Importantly these macrophages had produced a toxic enzyme (matrix metalloproteinase) that is capable of damaging axons.

...another way in which axons may be injured

3rd Project

oligoclonal bands detected in CSF are due to antibodies

B cells
= B lymphocytes
= type of white blood cell

lymph nodes, located at strategic sites around the body...

...they house a collection of cells that are required for the body to mount an immune response

sustained – because oligoclonal bands can be detected throughout the life of a person living with MS

Blue = nuclei of cells
= mostly plasma cells
Green = antibody

The plasma cells are in a sea of antibody that is leaking into the surrounding tissue

-stop B cell follicles
-stop antibodies
-stop demyelination?

So it seems that as lesions expand, more and more macrophages are drawn to the active edge by MCP-1 and IP-10; once there, the cells attack the axon, and perhaps the myelin and then engulf and digest fragments of the resulting debris. The important role played by chemokines in guiding macrophages to the edge of a lesion, provides a target for therapies, since stopping the action of chemokines would stop macrophages from reaching the crime scene and injuring axons.

A source of antibodies in the brain of patients with MS

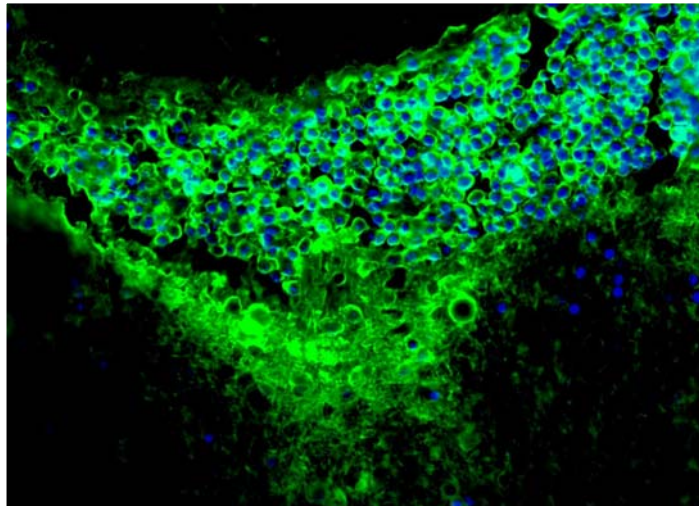
Dr Francesca Aloisi

Istituto Superiore di Sanità, Rome, Italy

Another “usual suspect” that should be looked at very carefully whenever the immune system is involved in injury is the antibody. Circumstantial evidence suggesting a role for antibodies in causing damage in MS comes from their presence in the fluid that bathes the brain and spinal cord (cerebrospinal fluid, CSF). It is these antibodies that are detected in the sample of CSF drawn from a lumbar puncture, and that help the diagnosis of MS. Dr Aloisi’s research is aimed at finding out how these antibodies are made within the brains of MS patients.

Antibodies are produced by plasma cells; plasma cells develop from B cells. B cells can be found in lymph nodes and in the blood. In the nodes, the B cells are organised into “small sacs” called follicles that are extremely efficient at producing antibodies. Dr Aloisi asked whether these “antibody producing factories” were responsible for making antibodies in the brains of people with MS.

The study, initiated in Rome and continued in the Tissue Bank laboratories by Dr Roberta Magliozzi, used special staining techniques to detect cells and molecules that are unique to B cell follicles. Follicles, containing multiplying B cells, plasma cells, and other cells and chemical messengers necessary for the production of antibodies were found within the brains of patients that had had the secondary progressive form of MS. All this evidence taken together suggests that B cell follicles may indeed be responsible for the sustained production of antibodies within the brains of MS patients. Follicles were not found in tissue from patients that had had other forms of MS or in tissue from patients that did not have MS.



In this picture of a B cell follicle, the fluorescent blue dye marks the nuclei of all cells; but most of these are plasma cells. The green stain is picking out the antibodies. The mass of green stain shows that large amounts of antibody are being produced by plasma cells in this follicle. The antibody is leaking into the surrounding brain tissue.

When B cell follicles were present, they were only found in the membranes (meninges) that encase the brain; they were never seen within demyelinated lesions. However, single B- and plasma cells were present within some lesions, which raises the question: “what is the relationship between the presence of antibody producing B cell follicles in the meninges and the presence of B/plasma cells in lesions, and how is all that related to the degree of demyelination?” Dr Aloisi’s group is now working to answer these important questions because if these antibodies prove to have a role in the destruction of myelin, then developing strategies that would inhibit the formation B cell follicles may be beneficial to patients with secondary progressive MS.

4th Project

...giving the axon the look of a string of sausages

1mm length of axon will have about 20 nodes of Ranvier

...neurofascin is one of many adhesion molecules that stick myelin to axons

green = neurofascin
red = myelin
blue = cell nucleus

from
2 short bands
to
1 long band
to
3 short bands
to
2 short bands
again

in order to promote repair,
we must find out exactly what happens during repair

5th Project

just as a builder will remove the scaffolding once he/she has carried out the repairs ...so fibrin must be removed after the healing process

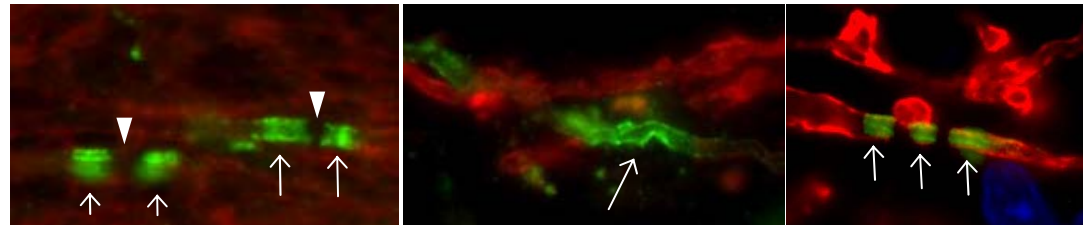
Nodes of Ranvier during demyelination and remyelination

Dr Owain Howell

Dept. of Cellular & Molecular Neuroscience, Imperial College London

If one looks carefully along the length of a myelinated axon, one finds that the myelin is not present as one uniform coating, but that there are minute gaps (called nodes of Ranvier) at which the axon is “naked”. This structure, consisting of stretches of insulation (myelinated axon) interrupted by un-insulated gaps (the nodes), allows the “electrical” nerve impulses, to jump from one node to the next in a process called saltatory conduction. The formation and maintenance of the nodes is therefore vital to the ability of the axon to rapidly conduct messages to and from the rest of the body and within the brain itself.

Working in collaboration with Professor Brophy at the University of Edinburgh, Dr Howell has been studying the edges of the node - the very point at which myelination stops. Here, the myelin sheets bind tightly to the axon using “sticky proteins” called adhesion molecules; Dr Howell has been looking at the presence of one such molecule called neurofascin in MS brain tissue in order to see how its presence changes during the transition from normal tissue to demyelinated lesion and from demyelinated lesion to remyelinated lesion. In the three pictures below,



very thin slices of tissue were treated with a green fluorescent dye that stains neurofascin and a red dye that picks-up myelin. The first picture shows the “normal” situation. There are two axons lying next to each, running across the picture with their envelope of myelin stained red. The arrow heads show the position of two nodes. The nodes are flanked on both sides by the green staining neurofascin. The middle panel shows an area in which there is on-going demyelination. The neurofascin is no longer present as a pair of discrete bands, but has now spread along the axon - arrow. The last panel shows an area that is remyelinating; here the neurofascin has again become concentrated into discrete bands. It is thought that with time the middle band will be lost so that a “normal” situation similar to the one shown in first panel is re-established.

The effective conduction of nerve impulses along an axon is dependent upon the presence of nodes of Ranvier; this study shows what happens to these vital structures during demyelination and remyelination. The study again demonstrates the complexity of repair and highlights just one structure that needs to be rectified in a large re-construction in order for normal function to be restored after an injury.

Fibrin clots – good guys and bad guys?

Dr Djordje Gveric

Institute of Neurology, University College London

Fibrinogen is a protein that circulates in the blood and that can be thought of as a member of a “rapid response team”. When a blood vessel wall is torn at a site of injury anywhere in the body, fibrinogen escapes from the blood and enters the surrounding tissue. Here single molecules of fibrinogen stick to one another and form a mesh called fibrin. Fibrin seals the tear and stops any further leakage from the blood vessel. It also attracts cells and molecules to the site where they play a role in the healing process. An essential step at the end of this procedure is the removal of the fibrin; this is achieved by an enzyme called tissue plasminogen activator (tPA) that dissolves away the fibrin mesh.

In multiple sclerosis lesions, fibrinogen escapes from the blood into brain tissue across a broken down blood-brain barrier. Dr Gveric has been looking to see

two fluorescent dyes were used to treat this slice of tissue:
red = axon
green = fibrin

the two dyes together give...
yellow = fibrin on axon

Inhibiting the inhibitor will allow fibrin to be dissolved

6th Project

this is as true for a scratch on the arm as it is for MS lesions in the central nervous system

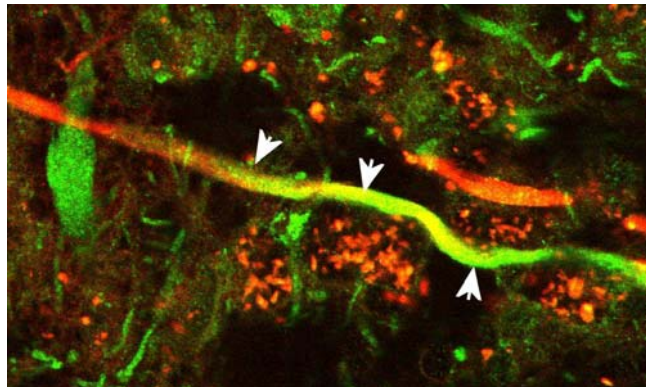
astrocyte = "star-like" cell (literally)

left – astrocytes in a lesion (identified by the black dots)

right – astrocytes growing in a dish

every time the Tissue Bank receives a donation from a non-MS donor, a member of the group travels from Exeter to London, collects a tissue sample and processes it back in Exeter

whether the failure of successful repair (remyelination) of MS lesions could be due to fibrin not being cleared away from the site. One of the ways in which the body controls the activity of tPA is with an enzyme inhibitor called plasminogen activator inhibitor-1 (PAI-1) which binds to tPA and inactivates it. When the group examined active MS lesions they found an increase in the activity of PAI-1, a resulting decrease in the activity of tPA, and an accumulation of fibrin around blood vessels and on axons. In this photograph, fibrin is stained green and axons red - there is one running across the photograph. The arrowheads point to areas where fibrin has been laid down on the axon resulting in a yellow colour. The extended presence of fibrin would inhibit the repair process and may actually favour damage to axons by components of the immune system. Since the action of tPA is inhibited by PAI-1, hindering the action of PAI-1 would release tPA to dissolve the fibrin. Dr Gveric's group is now trying to develop a strategy that will block the action of PAI-1 and so aid recovery and reduce further damage.



Dr Gveric's group is now trying to develop a strategy that will block the action of PAI-1 and so aid recovery and reduce further damage.

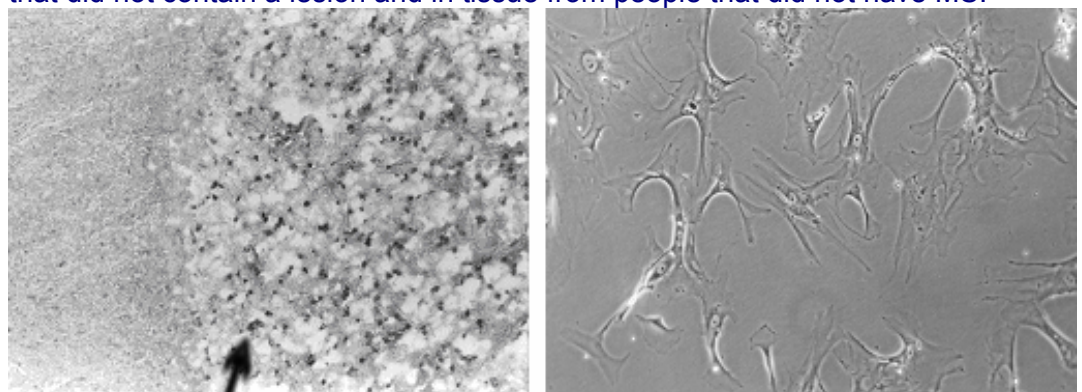
Could preventing scarring help repair?

Dr Nick Gutowski

Peninsula Medical School, Exeter

A part of the normal response of the body to damage is to form a scar; the characteristic grey, firm MS lesion in the white matter is actually a scar that has grown as the myelin and/or axons have been destroyed. These scars mainly consist of a meshwork of fibres that are the outgrowth of a cell resident in the normal CNS – the astrocyte. Could this normal response of astrocyte scarring actually be hampering remyelination?

Dr Gutowski's group has been trying to find out what makes a normal resting astrocyte turn into cell that lays down a fibrous scar. The group first made "identikits" that would allow astrocytes at different stages along this process to be distinguished according to the proteins that were present on the surface of each cell. Using the identikits, the group were able to distinguish "scar" astrocytes that were present in MS lesions from resting astrocytes that were in MS brain tissue that did not contain a lesion and in tissue from people that did not have MS.



The panel on the left shows the edge of an MS lesion; scarring astrocytes in the lesion have been marked with the identikit (black dots in right half of picture). Finding an accurate way to recognise scar astrocytes was essential to be able to answer the next question: "what signals make a resting astrocyte change into a scarring astrocyte?" Astrocytes were isolated from tissue donated by people that did not have MS, and grown in plastic dishes as shown in the photograph in the right panel above. These resting astrocytes can then be used to test the ability of chemical messengers to convert them into a scarring astrocyte as recognised by

stopping scarring
helping
remyelination

News from the Tissue Bank

The website also has
details of:

--members of our
management board,

--projects that we have
supplied with tissue,

--where we have given
talks on our work

...the new
emergency number

...the new Tissue
Bank Donor Card

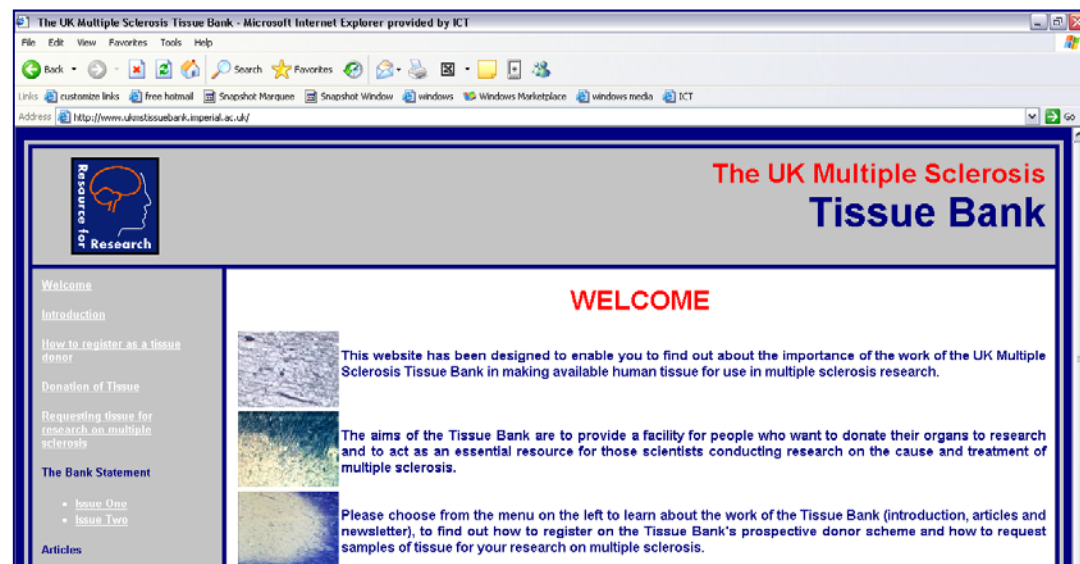
And finally...

The Tissue Bank is
most grateful to
BiogenIdec Limited,
Seroxo Limited and
Schering Health Care
Limited for
generously meeting
the cost of printing
and posting the Bank
Statement

the identikit. It is hoped that this work will lead to developing ways of stopping scarring; and this combined with therapies that encourage remyelination may provide a means of allowing demyelinated axons to regain their function.

Tissue Bank Website: ukmstissuebank.imperial.ac.uk

We have recently updated the Tissue Bank's website so that you now have access to the two previous issues of the Bank Statement and all articles on the Tissue Bank that have appeared in MS Matters.



Emergency Donor Line Number: **07 659 132 045**

In January 2005, we had to change the number of our 24-hour Emergency Donor Line. This is the number that people should use if they need an immediate response from the Tissue Bank; for example, to inform us of the death of someone registered on our donor scheme. In the New Year we sent all registered donors a new Donor Card, like this...



...if you are a registered donor and do not have a card like this, please let us know. Please also update the number on all your paperwork and let all relevant people know of the change. We are very sorry for the inconvenience that this is going to cause.

- **please let us know...**if you do not wish to receive another newsletter
- **please let us know...**of any changes that we need to make to our copy of your consent forms, (eg change of address, next-of-kin or general practitioner)

Telephone:-----020 8846 7324

Email:-----ukmstissuebank@imperial.ac.uk

Post:-----The UK Multiple Sclerosis Tissue Bank
Division of Neuroscience and Mental Health
Imperial College London
Charing Cross Campus
Fulham Palace Road
London W6 8RF

the Bank Statement was written by Abhi Vora (Manager of the Tissue Bank)

