possible that such tumours are directly immunogenic for Lyt-2+ cells in vivo, involvement of L3T4+ cells responding to 'processed' tumour H-2 antigens being unnecessary for tumour rejection. In this respect, we now have preliminary evidence that the subcutaneous growth of P815 tumour cells in irradiated B6 mice can be prevented by mixing the injected tumour cells with unprimed purified B6 Lyt-2+ cells (unpublished data).

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Note added in proof: We have recently found that one T cell tumour (EL4) does stimulate high primary MLR by allogenic Lyt-2+ cells.

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Fig. 2 Micrographs showing the location of MBP-specific transcripts in cervical spinal cords of virus-infected animals at 2 (a,b), 3 (c,d), 4 (e,f) and 8 (g,h) weeks post-inoculation. Bright-field views of histological stained sections are shown (a,c,e,g), together with corresponding dark-field cryostat sections after in situ hybridization (b,d,f,h). The number of grains observed in dark field is directly proportional to the amount of hybridization with the MBP probe and thus the number of MBP-specific transcripts. Note some hybridization of the MBP-specific probe in white matter at 2 weeks post-inoculation, with a decrease in labelling in the lesion containing inflammatory cells (delineated by arrowheads). In contrast, at 3 and 4 weeks, intense hybridization is seen in regions surrounding the lesions (delineated by arrowheads) which are devoid of label. Increased hybridization extends into the normal-looking white matter: in f, the left ventral column has a lesion (under arrowheads) but increased label is also seen in the right ventral column. The dorsal right column also has a small lesion (arrow) surrounded by increased label. At 8 weeks (g,h), there is still a slight increase in white matter labelling without the differential distribution (G, grey matter of substantia gelatinosa) observed in the 3–4-week samples. x200.

Methods. For these in situ hybridization experiments, samples from the spinal cord were cryoprotected in 15% sucrose (RNase-free) before freezing in liquid nitrogen. Cryostat sections (10 μm thick) were cut and hybridized as described in detail previously. Briefly, the sections were treated with 0.2 M HCl for 20 min, washed in phosphate-buffered saline, incubated with 1 μg ml⁻¹ proteinase K (Boehringer Mannheim) for 15 min at 37°C, and then with the hybridization mixture without the probe for 2 h. The hybridization mixture was as follows: 50% formamide, 2 x SSC, 1 x Denhardt’s solution, salmon sperm DNA (Sigma) at 450 μg ml⁻¹, Escherichia coli transfer RNA (Sigma) at 500 μg ml⁻¹, 200 μg ml⁻¹ poly(A) (Sigma), 40 nM oligonucleotide d(pT)₈ (Collaborative Research, Inc.). The probe, at a concentration of 100 μg ml⁻¹ and specific activity of 1 x 10⁶ d.p.m. ml⁻¹, together with the salmon sperm DNA and E. coli tRNA, was denatured for 3 min at 100°C, quenched on ice for 3 min and mixed with the other constituents of the hybridization mixture with 40 mM dithiothreitol. The mixture was incubated for 15 min at 50°C, denatured again and incubated at 50°C for another 15 min. Each section was then incubated at 40°C with 10 μl of the hybridization mixture in a sealed chamber overnight (about 16 h), washed in 0.1 x SSC at 55°C for 3 h, treated with 75% and 95% ethanol with 300 mM ammonium acetate and air-dried. The slides were dipped in a Kodak NTB-2 emulsion with 300 mM ammonium acetate and stored at 4°C for 3 days before being developed using standard procedures. The sections were counterstained with cresyl violet before mounting in Permount.
The clinical and pathological features of the disease have been described elsewhere. Briefly, 5–7 days after infection, the mice developed signs of acute disease characterized by apathy, hunched posture, ruffled fur and tremor. Some mice exhibited paraparesis spontaneously whereas others showed more subtle motor deficits only during testing. During this acute episode, 70% of the mice died, but the remainder recovered from the disease. Signs of motor deficits were less obvious after 4 weeks. Histological examination of the brain and spinal cord at 7 days post-inoculation revealed scattered infiltrations of inflammatory cells. C3H mice, however, showed few perivascular infiltrates. After 14–21 days, numerous focal lesions exhibited vacuolation and fragmentation of the myelin sheaths. Naked axons, necrotic cells and areas of demyelination were evident around the lesions. Mild perivascular infiltration with mononuclear inflammatory cells was also seen in these plaques of demyelination. Such demyelinating lesions were present in almost every section of the spinal cords examined and were situated in the central, lateral or posterior columns. Immunocytochemistry revealed a significant increase in MBP in these lesions, the remaining stain being associated with myelin debris. After 4 weeks, the demyelinated foci were more prominent and most of the degenerated myelin had been phagocytosed (Fig. 1a). A few partially demyelinated axons with abnormally thin myelin sheath were scattered in the lesion at this time. Ten weeks after infection, remyelination was prominent and most axons in the previously demyelinated areas were surrounded by thinner than normal myelin sheaths (Fig. 2e). Immunostaining showed that MBP had reappeared in these areas and was associated with the newly formed myelin sheath. Invasion of the CNS by Schwann cells to remyelinate bare axons was not detected.

We next examined the cryostat sections of the spinal cord of animals at different stages of the disease after hybridization with the MBP-specific probe and autoradiography (Fig. 2). Since the sections were also stained with cresyl violet, we compared the distribution of the grains (corresponding to the amount of hybridization with the MBP cDNA probe) with the distribution of the lesions. In spinal cord of uninoculated mice, more grains were seen in the white matter than in the grey matter, as expected from dot-blot hybridization studies of 1-month-old mice (Table 1). A CDNA oligo- and cDNA probe specific for a structural phosphoprotein gene of vesicular stomatitis virus showed no significant hybridization to grey or white matter in either normal or infected animals. The MBP-specific probe, however, showed a slight increase in labelling of the white matter was detected at 2 weeks (Fig. 2b), and fewer grains were seen in areas of white matter inflammation (Fig. 2a, b). At 3–4 weeks post-inoculation (Fig. 2c–f), the intensity of labelling had increased dramatically in some areas of the white matter. When the distribution of the grains in dark field was compared with the size and location of the lesions as seen after cresyl violet staining (Fig. 2a, c, e, g), it became clear that the foci with inflammation and tissue degeneration were devoid of labelling. However, a striking increase in the number of grains was present at the edge of the lesion as well as in the surrounding, normal-appearing white matter of the entire column (Fig. 2d) and even in the opposite column (Fig. 2f). Animals examined 8 weeks after inoculation had slightly higher levels of labelling in the white matter than had the normals (Table 1), but there were no clearly demarcated unlabelled areas such as those seen during the peak of demyelination (Fig. 2g, h).

The evidence presented here suggests that, during a demyelinating process caused by a virus in rodents, an increase in transcription of the gene coding for MBP, a major myelin protein, occurs early when demyelination is still progressing. Similarly, in CNS demyelinating lesions caused by cuprizone in rats, MBP can be detected by immunocytochemistry in oligodendrocytes before remyelination. However, as MBP staining is also seen in macrophages containing myelin debris, it is difficult to distinguish between 'old' MBP and newly synthesized MBP in these lesions. In contrast, in situ hybridization with a MBP-specific probe has revealed an approximate 10-fold increase in the number of MBP transcripts in a widespread area surrounding the demyelinating lesions 3–4 weeks after inoculation (Table 1). In normal rodents only basal levels of MBP-specific RNA are expressed at this stage in development. Thus, in situ hybridization may be the method of choice for detecting the effects of a demyelinating process on myelin protein gene expression. At 2 months post-inoculation, the remyelinated areas showed only a slight increase in MBP mRNA in a diffuse area including the lesion. This suggests that myelin-forming cells have repopulated the demyelinated area and are completing the synthesis of MBP mRNA at that time. It also implies that, in the adult rodent CNS, a cell of the glial lineage may be capable of dividing and probably of migrating into the lesion to repair myelin. Transplantation studies have recently shown that myelinating cells can migrate significant distances in the CNS. Earlier electron microscopic and autoradiographic studies have shown that the cells associated with remyelination after a viral disease are newly generated oligodendroglia. Moreover, recent studies on newborn rat optic nerve have demonstrated the existence of a progenitor cell which, after a number of mitoses, can differentiate into an oligodendrocyte. In the adult CNS, similar progenitor cells have been identified in the optic nerve and in the rat cerebral cortex. Thus, it is now necessary to improve the resolution of our in situ hybridization method and combine this approach with the use of cell-specific markers in order to identify precisely the cell type responsible for remyelination in the CNS.

To our surprise, the increase in MBP gene transcription seemed to radiate from the edge of the plaque, far away into the surrounding normal white matter. This suggests that, in this virus-induced demyelinating disease in mice, a factor may be produced in the lesion which could diffuse into the normal white matter and trigger oligodendrocytes and/or their progenitor cells to participate in myelin repair. One possibility is that such a factor is secreted by inflammatory cells in the lesion, since it has been shown that spleen cells in mice produce factors which promote proliferation and maturation of astrocytes and oligodendrocytes in vitro. Moreover, interleukin-2 was recently shown to enhance MBP expression in cultured oligodendrocytes.

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Table 1 Quantitation of MBP mRNA levels in spinal cord sections of MHV-A59-infected animals using in situ hybridization

| Day post-inoculation | 0 | 7 | 14 | 20 | 28 | 60 |
|----------------------|---|---|----|----|----|----|
| No. of mice          | 4 | 1 | 3  | 4  | 2  | ++ |
| Degree of labelling   | + | + | ++ | +++| +++| +  |

The appearance of MBP mRNA was quantified by counting grains over the white matter using a scaled reticle and rates as follows: +, 2–3 times more grains over white than grey matter; ++, 3–6 times more grains over white than grey matter; +++, 10 times more grains over white than grey matter.
Chondroitin-sulphate-rich proteoglycan is an essential component of the matrix of cartilage since it enables the tissue to resist compression during load-bearing. Loss of proteoglycan, such as occurs in rheumatoid arthritis, osteoarthrosis and other joint diseases, results in severe impairment of the function of cartilage. IL-1 is the only purified cytokine known to cause cartilage to degrade its proteoglycan\(^4,5\), and to inhibit synthesis\(^2\).

Figure 1a shows the amount of proteoglycan (measured as percentage of total chondroitin sulphate) released from porcine articular cartilage during 6 days of culture in the presence of human recombinant TNFa or pure porcine IL-1. The TNFa caused up to 75% of the proteoglycan to be released, although it was less potent than the IL-1, which was significantly active at a 20-fold lower dose (0.5 PM). Figure 1b shows a similar experiment carried out on cartilage from bovine nasal septum which was cultured for a shorter period (48 h): again, the IL-1 was more potent. The time dependence of the release of proteoglycan from bovine cartilage caused by sub-maximal concentrations of the two agents revealed that their effects were additive. Figure 1c shows that 50 pM IL-1 or 290 pM TNFa caused a similar rate of release, and that this was approximately doubled when the agents were combined. Maximal stimulation of cartilage by IL-1 caused more rapid release of proteoglycan than did TNFa (Fig. 1d); results for two concentrations of each cytokine demonstrate that responses were maximal. Supranormal doses of these two agents in combination doubled the rate of release that was considerably faster than that due to TNFa alone, but was not significantly greater than that seen with IL-1 alone. The failure of TNFa to augment the maximal response to IL-1 may be because the limit of the chondrocytes’ ability to degrade their matrix in vitro was being approached.

The enzymatic mechanism by which the proteoglycan is degraded in cartilage is not understood. Normally, cartilage proteoglycans aggregate in a specific manner with hyaluronic acid, and it is thought that the large size of these aggregates causes them to be trapped in the matrix. Cartilage stimulated by IL-1 releases fragments of proteoglycan which, as judged by gel filtration, are smaller than normal proteoglycan monomers and are unable to aggregate with hyaluronic acid\(^3\). There is no evidence of degradation of their chondroitin sulphate chains. These changes suggest that degradation is by limited proteolysis of the protein core. The fragments of proteoglycan that were released by cartilage stimulated with TNFa behaved similarly on gel filtration to those generated by stimulation with IL-1 (Fig. 2). The bulk of the fragments generated by stimulation with either agent emerged from a Sepharose 2B column at a region between the elution positions of intact proteoglycan and proteoglycan acid, and it is thought that the large size of these aggregates causes them to be trapped in the matrix. Cartilage stimulated by IL-1 released fragments of proteoglycan which, as judged by gel filtration, are smaller than normal proteoglycan monomers and are unable to aggregate with hyaluronic acid\(^3\). There is no evidence of degradation of their chondroitin sulphate chains. These changes suggest that degradation is by limited proteolysis of the protein core. The fragments of proteoglycan that were released by cartilage stimulated with TNFa behaved similarly on gel filtration to those generated by stimulation with IL-1 (Fig. 2). The bulk of the fragments generated by stimulation with either agent emerged from a Sepharose 2B column at a region between the elution positions of intact proteoglycan and proteoglycan digested with papain (which consists largely of single-chain chondroitin sulphate peptides). Addition of hyaluronic acid to the proteoglycan fragments before chromatography caused little or no formation of aggregates. This suggested that the hyaluronate binding region was blocked or had been lost. When the proteoglycan fragments were chromatographed under dissociative conditions (4 M guanidine-HCl in the chromatographic buffer) the position of the main peak was unchanged. These experiments showed that chondrocytes activated by TNFa or IL-1 caused a similar limited proteolysis of the proteoglycans.

In order to study the effect of TNFa on the synthesis of proteoglycan, cartilage was stimulated for 48 h, and \(^{35}S\)SO\(_4\) was added to the culture medium for the last 6 h. In this procedure the isotope becomes incorporated into newly synthesized sulphated glycosaminoglycan (mainly chondroitin sulphate). At the end of the experiment the medium and cartilage were digested with papain, and glycosaminoglycan was precipitated from the digests with cetylpyridinium chloride. The amount of radioactivity present in the precipitates was a measure of chondroitin sulphate (and, by inference, proteoglycan) synthesis. In experiments made with porcine articular (Fig. 3a) or bovine nasal septal (Fig. 3b) cartilages, TNFa caused a marked sup-