basement reflectors, often terminated on the landward side by an escarpment, have been observed along several rifted continental margins and are thought to be at or near the transition from continental to oceanic crust[12]. Paralleling the Andenes and Explora escarpments at a distance of <300 km towards the south is the 130-km-long Vestfjella chain of nunataks, which experienced a regime of NW–SE tension during the early to middle Jurassic[13–15]. A 1–2-km-thick section of mostly tholeiitic basalts was laid down in subaerial conditions. The volcanic pile is cut by many dykes of predominantly NE–SW strike, and by normal faults with no significant component of strike-slip. Also, basement depths landward of the escarpments (Fig. 2) require a thin crust from isostatic considerations. Thus, the evidence available favours an interpretation of the Andenes–Explora escarpments as structures of a rifted margin or possibly a plate boundary with oblique spreading rather than pure strike-slip. A sequence of events beginning with a large initial shear and subsequent overprint by rifting cannot be ruled out, but is considered less likely in view of the structural history of adjacent land areas, as well as the structural simplicity of the Andenes and Explora escarpments.

A major event in the evolution of the Weddell Embayment was mid-Jurassic crustal extension between the East Antarctic craton and the Pacific facing arc[16], forming major north–south grabens below the Ronne and Filchner ice shelves[5,17]. A Gondwana plate margin represented by the linear Andenes–Explora escarpments and their western continuation cuts across the early extensional tectonic trend (Fig. 4), indicating a post-mid-Jurassic change in the regional stress field.

A post-rift-phase Filchner microplate[1,3] probably did not exist. The available multi-channel seismic data[4,7] (Fig. 1) demonstrate that the sediments below the continental slope and shelf between 15 and 40° W are characterized by a total absence of fold and fault structures induced by post-rift basement tectonics. Also, preliminary results of a seismic survey by *Polarstern*[18] along portions of a traverse from the East Antarctic craton to the Antarctic Peninsula, outside the Filchner and Ronne ice shelves, show the upper 1 s (two-way travel time) of sediments to be undisturbed except in an area within 150 km of the peninsula. Thus, the locus of any post-rift relative motion between the Antarctic Peninsula[19,20] and the East Antarctic craton is certainly west of 40° W and probably near the peninsula itself.

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# Capacity of purified Lyt-2$^+$ T cells to mount primary proliferative and cytotoxic responses to Ia$^-$ tumour cells

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**Allogeneic gene products of the major histocompatibility complex, the *HLA* complex in man and the *H–2* complex in mice, induce T lymphocytes to exert powerful mixed lymphocyte reactions (MLR) and cell-mediated lympholysis (CML). In mice, the subset of T cells carrying the L3T4 surface antigen but lacking the Lyt-2 antigen responds predominantly to H–2 class II (Ia) differences whereas the L3T4$^-$ Lyt-2$^+$ subset reacts to class I (K/D) differences[1,2]. For primary responses the stimulus for MLR and CML appears to be controlled by Ia$^+$ cells of the macrophage/dendritic cell lineages, for both L3T4$^+$ and Lyt-2$^+$ cells[3–6]. The finding that Ia$^+$ cells are required for responses involving Lyt-2$^+$ cells has been taken to imply that triggering of these cells is controlled by Ia-restricted L3T4$^+$ cells[7,8]. Lyt-2$^+$ cells have thus come to be regarded as crippled cells which are heavily dependent on 'help' from other T cells[9–11]. This well-entrenched view is challenged by evidence presented here that purified Lyt-2$^+$ cells can give high primary responses to certain Ia$^-$ tumour cells *in vitro*.**

Recent studies from this laboratory showed that highly purified Lyt-2$^+$ cells were able to mount high primary MLR and CML responses to class I but not class II H-2 differences *in*

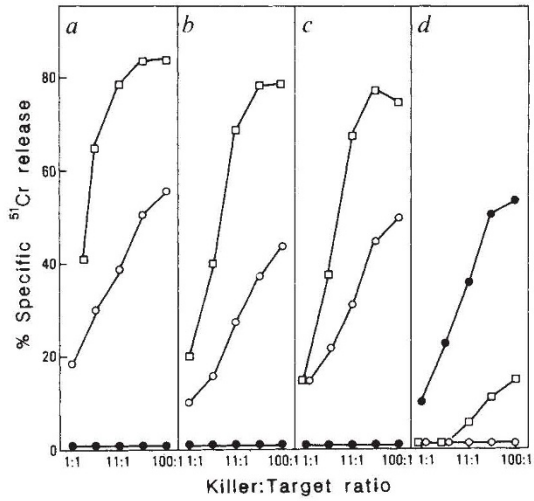


**Fig. 1** Cytolytic activity of purified B6 (H-2$^b$) Lyt-2$^+$ cells cultured for 4 days *in vitro* with P815 (H-2$^d$) tumour cells. Doses of $2\times10^6$ responder cells were cultured with lightly irradiated (1,500 rad) DBA/2 (H-2$^d$) spleen cells or with $0.5\times10^6$ heavily irradiated (20,000 rad) P815 tumour cells in a volume of 2 ml in 24-well plates; as controls for specificity of lysis, DBA/2 T (J11d-treated LN) cells were cultured with B10 (H-2$^b$) spleen cells. In experiment *c*, the purified Lyt-2$^+$ cells were pretreated with anti-Ia$^b$ antibody plus C′ before culture. After 4 days the cells pooled from several wells were counted and then cultured in varying numbers for 3 h at 37 °C with fixed numbers ($10^4$) of $^{51}$Cr-labelled target cells, that is, P815 cells (□), concanavalin A-stimulated B10.D2 (H-2$^d$) (○) or B10 (H-2$^b$) (●) spleen cells; target cells were labelled with 300 μCi $^{51}$Cr per $4\times10^6$ cells at 37 °C for 1 h and then washed thoroughly. The per cent $^{51}$Cr release from target cells was measured by standard techniques, taking release of isotope from detergent-treated cells as 100% release. Each data point represents the mean per cent specific $^{51}$Cr release of triplicate cultures; the data in *a*, *b*, and in *c*, *d* were obtained in two different experiments.

**Table 1** Primary MLR of B6 T cells and purified B6 Lyt-2$^+$ cells stimulated with H-2-incompatible spleen cells or P815 tumour cells

| Stimulators | No. of stimulators ($\times 10^5$) | $^3$H-TdR incorporation (c.p.m. $\times 10^3$) with responders ($2 \times 10^5$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | B6 T ($H$-$2^b$) | | | B6 Lyt-2$^+$ ($H$-$2^b$) | | | No responders | | |
| | | Day 3 | Day 4 | Day 5 | Day 3 | Day 4 | Day 5 | Day 3 | Day 4 | Day 5 |
| B6 spleen | 0.2 | 0.3 | 0.4 | 1.3 | 0.1 | 0.1 | 0.1 | — | — | — |
| ($H$-$2^b$) | 1 | 0.6 | 1.1 | 7.6 | 0.1 | 0.1 | 0.1 | — | — | — |
| | 5 | 2.3 | 6.7 | 17.8 | 0.3 | 0.7 | 0.7 | 0.2 | 0.3 | 0.5 |
| DBA/2 spleen | 0.2 | 15.9 | 34.1 | 51.8 | 18.8 | 44.5 | 25.5 | — | — | — |
| ($H$-$2^d$) | 1 | 86.7 | 171.4 | 44.6 | 82.4 | 188.3 | 16.1 | — | — | — |
| | 5 | 144.2 | 275.0 | 58.9 | 132.6 | 263.0 | 44.9 | 0.4 | 0.5 | 0.5 |
| P815 | 0.2 | 28.0 | 62.7 | 14.4 | 38.8 | 66.7 | 11.5 | 0.4 | 0.5 | 0.2 |
| ($H$-$2^d$) | 1 | 8.2 | 16.9 | 16.3 | 26.8 | 49.4 | 30.6 | 0.4 | 0.3 | 0.2 |
| | 5 | 4.3 | 3.6 | 3.8 | 3.3 | 3.6 | 4.4 | 2.3 | 2.7 | 2.1 |

T cells and T-cell subsets were purified from pooled lymph nodes (LN) of adult B6 mice as described elsewhere[2]. To purify T cells, LN cells were pretreated at 37 °C for 1 h *in vitro* with an anti-B-cell monoclonal antibody (J11d) plus guinea pig serum as a source of complement (C'); the surviving cells (>98% Thy 1$^+$) were then passed through Ficoll gradients to remove dead cells. To prepare Lyt-2$^+$ cells, LN cells were first pretreated at 37 °C *in vitro* with a mixture of J11d and anti-L3T4 (GK1.5) antibodies plus C'. The surviving cells were then washed and allowed to adhere to anti-Lyt-2-coated dishes for 1 h at 4 °C. After gently washing non-adherent cells from the dishes, the adherent cells were eluted by vigorous pipetting. The adherent cells were >99% Lyt-2$^+$ by FACS analysis and contained no detectable L3T4$^+$ cells[2]. For MLR, doses of $2 \times 10^5$ B6 T or Lyt-2$^+$ responder cells were cultured in flat-bottom microtitre plates with varying numbers of lightly irradiated (1,500 rad) normal B6 or DBA/2 spleen cells or with heavily irradiated (20,000 rad) *in vitro*-passaged P815 tumour cells in a final volume of 200 μl. Cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum and standard additives[2]; IL-2 was not added to the cultures. Cultures were pulsed with 1 μCi tritiated thymidine ($^3$H-TdR) 18 h before collection. The data show the mean levels of radioactivity in triplicate cultures. s.d., omitted for simplicity, were generally within 10–20% of the mean. Pretreatment of P815 cells with mitomycin C rather than irradiation led to comparable MLR with B6 Lyt-2$^+$ cells.

*vitro*[2]; other workers have reported similar findings[12]. MLR to class I differences were not reduced by removing T cells from the stimulator population or by adding anti-L3T4 monoclonal antibody to the cultures[2,12]; addition of anti-Ia monoclonal antibody caused only minimal inhibition of the response. Although these findings suggested that the response of Lyt-2$^+$ cells could not be attributed to minor contamination of the cultures with L3T4$^+$ cells, removal of Ia$^+$ cells from the stimulator population (spleen cells) virtually abolished the response[2,12]. At face value the simplest explanation of this finding is that the response of Lyt-2$^+$ cells to alloreactive class I molecules requires co-recognition of class II (Ia) molecules. The problem with this notion is that, unless one invokes cryptic participation of L3T4$^+$ cells, it is difficult to envisage how Ia molecules might control Lyt-2$^+$ cell induction. In addition, one must account for the fact that certain Ia$^+$ cells—small B lymphocytes—are non-stimulatory for unprimed Lyt-2$^+$ cells[6]. An alternative possibility is that Ia$^+$ cells with stimulatory function display a putative 'second signal' required by Lyt-2$^+$ cells[12,13], with the joint expression of Ia molecules and the second signal being largely coincidental. This raises the question of whether some Ia$^-$ cells might be stimulatory for Lyt-2$^+$ cells. Rammensee *et al.*[14] have shown that Ia$^-$ Lyt-2$^+$ T cells have the capacity to 'veto' the induction of allogeneic Lyt-2$^+$ cytolytic precursors, the veto function of T cells being attributed to the failure of these cells to express a requisite second signal. Since spleen cell suspensions depleted of Ia$^+$ cells consist largely of T cells, the poor antigen-presenting cell function of Ia$^-$ spleen cells thus does not preclude the possibility that a spectrum of cell types might act as antigen-presenting cells for Lyt-2$^+$ cells, the notable exception being T cells. To assess this idea, we arbitrarily tested the antigen-presenting function of Ia$^-$ tumour cells.

Although certain Ia$^-$ tumours, such as the P815 mastocytoma, are known to elicit primary T-cell responses *in vitro*[13,15], it is unclear whether these responses require help from the L3T4$^+$ T-cell subset. Table 1 compares the capacity of B6 (H-2$^b$) T cells and >99% purified B6 Lyt-2$^+$ cells to mount primary MLR to DBA/2 (H-2$^d$) spleen stimulators (1,500 rad) or P815 (H-2$^d$) tumour stimulators (20,000 rad) in the absence of added interleukin-2 (IL-2); the P815 tumour, of DBA/2 origin, completely lacks Ia molecules as assessed by fluorescence-activated cell sorter (FACS) analysis, but is strongly positive for class I molecules (data not shown). B6 T and B6 Lyt-2$^+$ cells both gave high responses to DBA/2 spleen. Peak responses occurred on day 4 and the responses decreased progressively as the dose of stimulators was lowered. With P815 stimulators, large doses of tumour cells ($5 \times 10^5$ per culture) elicited virtually no MLR. Low tumour doses ($10^4$–$10^5$), by contrast, elicited highly significant MLR. Responsiveness to the P815 tumour seemed to be restricted to Lyt-2$^+$ cells because: (1) responses were appreciably higher with B6 Lyt-2$^+$ cells than with unseparated B6 T cells (Table 1), (2) there was no response to the tumour using purified B6 L3T4$^+$ cells (Table 2, expts *a*, *b*) (implying that the tumour cells remained Ia$^-$ in culture), and (3) in marked contrast to anti-Lyt-2 monoclonal antibody, adding anti-L3T4 to the cultures failed to inhibit the response of B6 Lyt-2$^+$ cells to the tumour (Table 2, expt *a*; the response to the bm1 and bm12 mutants controlled for the specificity of the blocking effects of anti-Lyt-2 and anti-L3T4 monoclonal antibodies, see Table 2 legend). Heat-killed P815 cells and P815 cells exposed to ultraviolet light were completely non-stimulatory, even when reconstituted with recombinant IL-1 (data not shown).

The response of B6 Lyt-2$^+$ cells to the P815 tumour appeared to be specific for H-2 antigens, as the tumour failed to stimulate H-2-identical DBA/2 Lyt-2$^+$ cells (Table 2, expt *b*) but did stimulate Lyt-2$^+$ cells from another H-2$^b$ strain, C3H.SW (Table 2, expt *a*). Further evidence for antigen specificity was obtained by studying the capacity of the tumour to elicit CML activity. In all three experiments performed, two of which are illustrated in Fig. 1, culturing B6 Lyt-2$^+$ cells for 4 days with irradiated P815 cells in the absence of added IL-2 led to high levels of lysis against $^{51}$Cr-labelled P815 cells. With concanavalin A-stimulated spleen blast cells as targets, lysis was high on B10.D2 (H-2$^d$) targets but absent on B10 (H-2$^b$) targets, implying that lysis was directed to H-2$^d$ determinants rather than to tumour-specific antigens. The specific lytic activity of B6 Lyt-2$^+$ cells cultured with P815 stimulators was only slightly lower than with cells cultured with DBA/2 spleen stimulators (compare Fig. 1*a* and *b*). Pretreating the B6 Lyt-2$^+$ cells with anti-I-A$^b$ antibody plus complement (C') to remove any residual Ia$^+$ cells before culture failed to impair CML activity (Fig. 1*c*).

The above results indicate that Ia$^-$ P815 tumour cells are able to stimulate purified allogeneic Lyt-2$^+$ cells to proliferate extensively and differentiate into H-2-specific (presumably class I-

**Table 2** MLR of T-cell subsets to P815 (H-$2^d$) and L929 (H-$2^k$) tumour cells: failure of anti-L3T4 antibody to inhibit MLR of Lyt-$2^+$ cells

| Stimulators | No. of stimulators ($\times 10^5$) | No. of responders ($\times 10^5$) | Antibody added to cultures | $^3$H-TdR incorporation (c.p.m. $\times 10^3$) with responders: B6 Lyt-$2^+$ (*bbbb*) | C3H.SW Lyt-$2^+$ (*bbbb*) | B6 L3T4$^+$ (*bbbb*) | No responders |
|---|---|---|---|---|---|---|---|
| Expt *a* | | | | | | | |
| B6 spl (*bbbb*)* | 5 | 1 | — | 0.3 | 0.8 | 3.1 | — |
| | 5 | 2 | — | 0.8 | 1.2 | 6.3 | — |
| bml spl (*bmlbbb*) | 5 | 1 | — | 74.5 | 57.4 | 3.8 | — |
| | 5 | 1 | Anti-L3T4 | 74.5 | 51.0 | — | — |
| | 5 | 1 | Anti-Lyt-2 | 2.7 | 2.7 | — | — |
| | 5 | 2 | — | 178.3 | 82.0 | — | — |
| bm12 spl (*bbm12bb*) | 5 | 1 | — | 2.2 | 1.9 | 22.9 | — |
| | 5 | 1 | Anti-L3T4 | — | — | 2.1 | — |
| | 5 | 1 | Anti-Lyt-2 | — | — | 30.3 | — |
| | 5 | 2 | — | — | — | 81.5 | — |
| P815 (*dddd*) | 0.5 | 2 | — | 25.4 | 18.2 | 0.3 | 0.2 |
| | 0.5 | 2 | Anti-L3T4 | 21.9 | 17.6 | — | — |
| | 0.5 | 2 | Anti-Lyt-2 | 0.8 | 0.6 | — | — |
| | | | | B6 Lyt-$2^+$ (*bbbb*) | DBA/2 Lyt-$2^+$ (*dddd*) | B6 L3T4$^+$ (*bbbb*) | No responders |
| Expt *b* | | | | | | | |
| B6 spl (*bbbb*) | 5 | 2 | — | 1.1 | 29.0 | 4.2 | 0.1 |
| DBA/2 spl (*dddd*) | 5 | 2 | — | 104.1 | 0.4 | 210.2 | 0.1 |
| P815 (*dddd*) | 0.25 | 2 | — | 32.3 | 0.5 | 1.1 | 0.1 |
| | 0.5 | 2 | — | 40.9 | 1.2 | 0.8 | 0.1 |
| | 1 | 2 | — | 43.0 | 1.4 | 3.1 | 0.2 |
| | | | | B6 Lyt-$2^+$ (*bbbb*) | CBA/Ca Lyt-$2^+$ (*kkkk*) | No responders | |
| Expt *c*† | | | | | | | |
| B6 spl (*bbbb*) | 5 | 1 | — | 0.2 | 20.7 | 0.1 | |
| CBA/Ca spl (*kkkk*) | 5 | 1 | — | 55.5 | 0.7 | 0.3 | |
| bml spl (*bm1bbb*) | 0.2 | 1 | — | 2.2 | 0.8 | 0.3 | |
| | 5 | 1 | — | 70.5 | 31.1 | 0.1 | |
| | 5 | 1 | Anti-L3T4 | 62.0 | — | — | |
| | 5 | 1 | Anti-Lyt-2 | 3.2 | — | — | |
| L929 (*kkkk*) | 0.04 | 1 | — | 5.5 | 0.6 | 0.5 | |
| | 0.2 | 1 | — | 16.7 | 1.1 | 1.1 | |
| | 0.2 | 1 | Anti-L3T4 | 24.8 | — | — | |
| | 0.2 | 1 | Anti-Lyt-2 | 2.2 | — | — | |
| | 1 | 1 | — | 22.4 | 1.7 | 1.7 | |
| | 5 | 1 | — | 0.6 | 0.4 | 0.4 | |

Lyt-$2^+$ cells were prepared as for Table 1. An analogous procedure was used to prepare L3T4$^+$ cells; that is, pretreatment of LN cells with J11d + anti-Lyt-2 (3.168) antibodies + C′ followed by positive panning on dishes coated with anti-L3T4. The P815 mastocytoma and the L929-transformed fibroblast line were both totally I-A-negative by FACS analysis but were strongly positive for expression of class I molecules. As for P815, the L929 cells were passaged *in vitro* without feeder cells; the cells were exposed to 20,000 rad before use as stimulators. The amount of antibody added to the cultures was 2 μl of undiluted ascites fluid for anti-Lyt-2 and 2 μl of 1:10 diluted ascites fluid for anti-L3T4. As controls for the specificity of inhibition by anti-L3T4 and anti-Lyt-2, these antibodies were added to cultures in which MLR were directed solely to a class I H-2 difference (B6 Lyt-$2^+$ cells responding to the H-2K-different B6.C-H-$2^{bm1}$ (bm1) mutant) or to a class II H-2 difference (B6 L3T4$^+$ cells responding to the I-A-different B6.C-H-$2^{bm12}$ (bm12) mutant); as reported elsewhere[2] (confirmed in the table), anti-L3T4 selectively inhibits anti-class II MLR whereas anti-Lyt-2 selectively inhibits anti-class I MLR. MLR (mean of triplicate cultures) were measured on day 4 for each experiment shown.

* Alleles of *H-2* subregions, that is, *H-2K, I-A, I-E, H-2D.*

† Responder T cells in this experiment were pretreated with anti-I-$A^b$ + C′ before culture.

specific) cytotoxic cells in the absence of exogenous IL-2 (although IL-2 production by a subset of Lyt-$2^+$ T 'helper' cells[6] cannot be excluded). Similar findings were observed with the L929 (H-$2^k$) Ia$^-$ transformed fibroblast line (tested only for MLR) (Table 2, expt *c*). In the case of non-neoplastic cells, we have obtained preliminary evidence that Thy 1$^-$ Ia$^-$ cells from normal bone marrow can stimulate primary MLR by allogeneic Lyt-$2^+$ cells (unpublished data). Thus, the capacity to stimulate Lyt-$2^+$ cells is apparently not a property unique to Ia$^-$ tumour cells.

In contrast to these findings with Thy 1$^-$ cells, studies with three Thy 1$^+$ Lyt-2$^-$ T-cell tumours have shown that, like normal T cells, these tumours cannot stimulate unprimed allogeneic Lyt-$2^+$ cells in the absence of added IL-2 (unpublished data of the authors); the reason for this is unclear. Interestingly, the ability of T cells to mediate veto function is largely restricted to activated Lyt-$2^+$ T-killer cells and is abolished by irradiation[14]. Hence, the poor stimulatory function of typical small T cells (for example, anti-Ia + C′-treated spleen) and Lyt-2$^-$ T tumours is unlikely to reflect a veto effect. Moreover, we have seen no evidence of suppression when Lyt-$2^+$ responders are exposed to a mixture of spleen stimulators supplemented with irradiated small T cells or T tumours. The most likely explanation for the poor stimulatory function of (non-cytotoxic) T cells is that these cells simply lack some requisite second signal required by Lyt-$2^+$ cells.

Although the nature of the putative second signal provided by Thy 1$^-$ Ia$^-$ H-2-different tumour cells is unknown, it is

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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# Increased levels of myelin basic protein transcripts gene in virus-induced demyelination

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**In multiple sclerosis, a demyelinating disease of young adults, there is a paucity of myelin repair in the central nervous system (CNS) which is necessary for the restoration of fast saltatory conduction in axons[1,2]. Consequently, this relapsing disease often causes marked disability. In similar diseases of small rodents, however, remyelination can be quite extensive, as in the demyelinating disease caused by the A59 strain of mouse hepatitis virus (MHV-A59)[3,4], a coronavirus of mice. To investigate when and where oligodendrocytes are first triggered to repair CNS myelin in such disease, we have used a complementary DNA probe specific for one major myelin protein gene, myelin basic protein (MBP), which hybridizes with the four forms of MBP messenger RNA in rodents[5]. Using Northern blot and *in situ* hybridization techniques, we previously found that MBP mRNA is first detected at about 5 days after birth, peaks at 18 days and progressively decreases to 25% of the peak levels in the adult[5-7]. We now report that in spinal cord sections of adult animals with active demyelination and inflammatory cells, *in situ* hybridization reveals a dramatic increase in probe binding to MBP-specific mRNA at 2–3 weeks after virus inoculation and before remyelination can be detected by morphological methods. This increase of MBP-specific mRNA is found at the edge of the demyelinating area and extends into surrounding areas of normal-appearing white matter. Thus, *in situ* hybridization with myelin-specific probes appears to be a useful method for detecting the timing, intensity and location of myelin protein gene reactivation preceding remyelination. This method could be used to elucidate whether such a reactivation occurs in multiple sclerosis brain tissue. Our results suggest that in mice, glial cells react to a demyelinating process with widespread MBP mRNA synthesis which may be triggered by a diffusible factor released in the demyelinated areas.**

Earlier studies have shown that some MHV-A59 strains can cause chronic demyelination in mice and rats[4,8-15]. For instance, a high incidence of demyelination occurs in C3H and C57 black mice[3,4] after intracerebral inoculation of the prototype MHV-A59 strain[16]. The virus replicates preferentially in oligodendrocytes, destroying the cells in focal areas of the white matter during the first weeks of infection[3,4,8-15]. Viral antigen persists in the cytoplasm of oligodendrocytes within the demyelinating lesions for up to 4 weeks[3]. Untranslated viral genome may be present in other areas of the CNS and in other cell types[17].

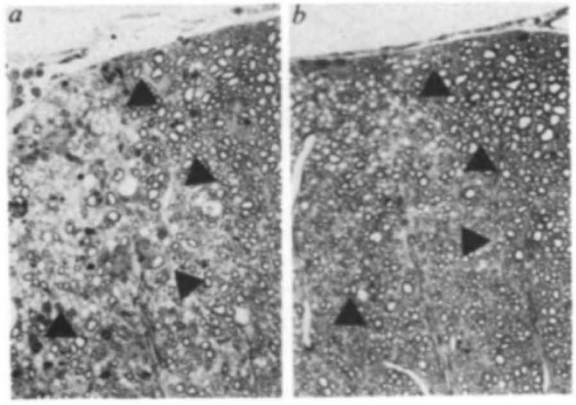


**Fig. 1** Micrographs showing demyelinated (*a*) and remyelinated (*b*) areas (delineated by arrowheads) in the anterior column of mice at 4(*a*) and 10(*b*) weeks post-inoculation with MHV-A59. Active demyelination is seen at 4 weeks while thinly remyelinated axons are seen at 10 weeks. Animals were perfused with 4% glutaraldehyde in Sorensen phosphate buffer, and slices of various levels of the spinal cord were embedded in Epon. 1-μm-thick sections were stained with toluidine blue. ×200.

In the present investigation, 4-week-old C57Bl/6J mice (obtained from Jackson Laboratories, Bar Harbor, Maine) were injected intracerebrally with 500–1,000 plaque-forming units of MHV-A59 (obtained from Dr L. S. Sturman, New York State Department of Health, Albany, New York). At 1, 2, 3, 4 and 8 weeks after injection, groups of three or four mice were perfused through the heart with periodate-lysine-formaldehyde[18], and the brains and spinal cords of the perfused animals were then dissected. Transversely cut slices of various regions of the brain and spinal cord were prepared by freezing and cryosectioning for *in situ* hybridization as described in Fig. 2 legend. As a probe, we used a small cDNA clone, NZ-112, which encodes amino acids 60–93 of mouse MBP[5]. By adding 20–25 $^{35}$S-labelled dATP residues to the 3′ ends of the gel-purified DNA fragments using terminal deoxynucleotidyl transferase, a specific activity of $1\text{–}2\times10^9$ d.p.m. per μg DNA was obtained[6,7]. Other CNS tissue slices were embedded in paraffin for histological examination and immunocytochemistry. For histology, paraffin sections were stained with Luxol fast blue and cresyl violet or haematoxylin/eosin. For immunocytochemistry, paraffin sections were incubated with dilutions of mouse or goat anti-MBP antibodies and stained by the peroxidase-antiperoxidase method[19]. In addition, two or three mice at each time point were perfused and processed as described in Fig. 1 legend in order to analyse the details of the demyelinating and remyelinating process in semi-thin plastic sections.