Human monoclonal anti-MAG antibody and anti-Leu 7 recognise shared antigenic determinants in peripheral nerve and spinal cord

GUIDO STOLL, GÜNTHER SCHWENDEMANN, KURT HEININGER,
ANDREAS J STECK,* KLAUS V TOYKA

*From the Departments of Neurology, University of Duesseldorf, West Germany, and Lausanne, Switzerland**

SUMMARY Anti-Leu 7 monoclonal antibody (MAB), a marker of natural killer cells, and a human MAB to myelin-associated glycoprotein (MAG) from a patient with a demyelinating neuropathy specifically stained Schmidt-Lanterman incisures, paranodal and periaxonal regions in peripheral nerve myelin by immunocytochemistry on thin plastic sections, while compact myelin was labelled in paraffin-embedded material. Preabsorption studies indicated that the antigen recognised was a MAG epitope shared by MAG and Leu 7. In spinal cord both MABS bound to oligodendrocytes and a subclass of anterior horn cells. Our findings support the hypothesis that shared antigens between the nervous and the immune systems do exist *in situ*, which may be important in the pathogenesis of demyelinating neuropathies with monoclonal gammopathies.

A monoclonal antibody (MAB) to Leu 7, a marker of natural killer cells, and a rabbit polyclonal antibody to myelin-associated glycoprotein (MAG) recognise shared antigenic components of peripheral nerve myelin *in vitro*.[1] MAG is the putative antigenic molecule in a human demyelinating neuropathy which is associated with an IgM kappa gammopathy.[2 3] In contrast, in a recent immunocytochemical study the staining pattern of anti-Leu 7 MAB was not found to be suggestive of MAG being the antigen.[4] Here we report on an identical staining pattern of anti-Leu 7 MAB and a human IgM kappa MAB to MAG in peripheral nerve *in situ* thus supporting the concept that the antigen recognised by anti-Leu 7 MAB shares antigenic epitopes with MAG.

Materials and methods

Antisera The properties of the human IgM kappa MAB to MAG derived from a patient with a demyelinating neuropathy and a "benign" IgM gammopathy have been described in detail previously.[3 5] Briefly, it reacts with

MAG from human, monkey, calf, rabbit, and guinea pig but not with MAG from rat and mouse. The mouse IgM MAB to Leu 7 (Becton-Dickinson) is directed against a membrane antigen from the cultured T-cell line HSB-2.[6 7] A mouse IgG MAB to bovine P_0 was kindly provided by Dr Linington, Würzburg.

Immunocytochemistry
Sciatic nerves of marmoset monkeys and of mice were quickly dissected, fixed by immersion in 2·5% glutaraldehyde for 4 hours without osmication amd embedded in Spurr's low viscosity resin using ethanol and propylene oxide as dehydrating agents. In addition, sciatic nerves were fixed in 4% paraformaldehyde for 4 hours and embedded in paraffin. Spinal cords of monkeys and mice were fixed by perfusion for 30 minutes and postfixed 2 hours in 4% paraformaldehyde with 0·5% glutaraldehyde prior to embedding in paraffin.

Etching of plastic semithin sections was done by incubation in a 1:4 dilution of a saturated sodium ethoxide stock solution in ethanol for 15 min. In addition, some sections were treated with a 5% H_2O_2 solution for 2 minutes. Paraffin sections were deparaffinised with xylene for 10 min. All sections were then stained by the avidin-biotin-complex technique.[8] Following incubation with 5% bovine serum albumin for 30 min and rinsing in PBS, the primary antibody was added (human-anti MAG MAB, mouse anti-Leu 7 MAB, diluted 1:100 in PBS, or mouse anti P_0 MAB 1:500) for 16 hours at 4°C. After rinsing in PBS, biotinylated antihuman IgM, anti-mouse IgM or IgG (Vektor) was added at a 1:50 dilution for 30 min. Sections were then incubated with avidin-biotinylated-peroxidase-
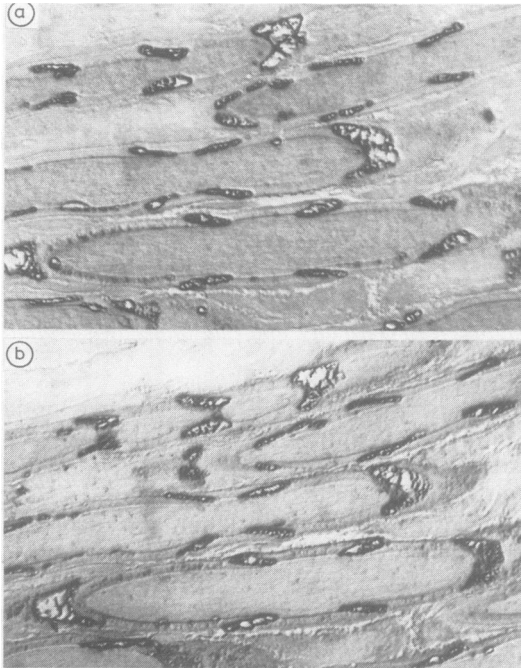
Fig 1 *(a) normal monkey sciatic nerve incubated with human IgM MAB to MAG 1:100. The dark reaction product occurs at Schmidt-Lanterman incisures, paranodal regions, and, more faintly, at periaxonal sites. (b) adjacent 1 μm section to (a). Incubation was done with anti-Leu 7 MAB, 1:100. The corresponding regions to (a) are stained indicating cross-reactivity with epitopes in the myelin sheath; (× 660).*

complex (Vektor) 1:50 after another wash in PBS. Following a rinse in Tris-HCl buffer (0·05 m, pH 7·6) the peroxidase reaction was performed by incubation with diaminobenzidine (10 mg/50 ml with 0·004% H_2O_2 diluted in Tris-HCl buffer) for 10 min. Sections were dehydrated, embedded in Eukitt and examined by Nomarski optics.

For controls mouse anti-Leu 7 MAB and human anti-MAG MAB were preabsorbed with 1 μg of purified MAG in 1 μl antiserum final dilution for 12 h at 4°C before incubating the sections. MAG was isolated from bovine brain by the method of Quarles and Pasnak.[9] In further control sections primary antibody was replaced by PBS.

Results

In paraffin sections of monkey sciatic nerves treated with human MAB to MAG compact myelin was intensively stained (data not shown). In plastic sections staining was limited to Schmidt-Lanterman incisures and paranodal regions of myelin (fig 1a). After prolonged etching with sodium ethoxide up to one hour, additional staining occurred at the periaxonal region but not in compacted myelin. In poorly

fixed material with multiple vesicular artefacts in the myelin sheaths staining occurred also in compact regions. Further pretreatment with H_2O_2 did not change staining pattern. In contrast, a mouse MAB to P_0 stained compact myelin but not Schmidt-Lanterman incisures and paranodal regions in both paraffin and plastic sections. When anti-Leu 7 MAB was used as the primary antibody the staining pattern was indistinguishable from that with human anti-MAG MAB in all paraffin or plastic sections (fig 1b). Replacement of the primary antibody by PBS or preabsorption of antisera with purified bovine MAG abolished all immunoreactivity. In contrast to monkey nerve, all sections from mouse sciatic nerve were negative even at high concentrations of the primary antibody.

In the monkey spinal cord anti-Leu 7 MAB and the human anti-MAG MAB stained oligodendrocytes, a proportion of anterior horn cells, (fig 2) and only some myelin sheaths at the periaxonal region. No specific staining was seen in mouse spinal cord material.

Discussion

Our immunocytochemical results support and extend recent findings that natural killer cells and nervous tissue share antigenic determinants.[1 4]
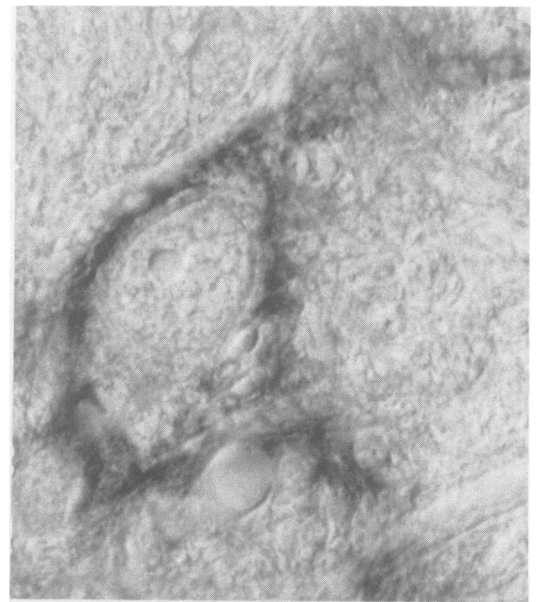
Fig 2 *Anterior horn cell of monkey spinal cord incubated with human MAB to MAG, 1:5000. The surface of the perikaryon and of the cell processes are intensively stained. (× 1150)*

Anti-Leu 7 MAB stains peripheral nerve myelin with the same pattern as does a human MAB to MAG. The staining of paranodal and periaxonal regions and of Schmidt-Lanterman incisures in semithin plastic sections is identical to that of previous reports on immunocytochemical localisation of MAG utilising polyclonal antisera raised in animals.[10][11] Our finding that preabsorption with purified MAG abolishes all immunoreactivity indicates that the antigen recognised by anti-Leu 7 MAB is most likely a MAG epitope. These results are in accordance with immunoblot experiments showing that anti-Leu 7 MAB binds specifically to purified MAG.[1][12] Furthermore, the species restriction known to be displayed by the human anti-MAG MAB *in vitro* (by immunoblot analysis)[5][13] and *in situ* (this study) also applies to anti-Leu 7 MAB since we could not find any immunoreactivity in mouse peripheral nerve myelin.

The diffuse staining pattern of compact myelin[14] has recently been attributed to the identification of gangliosides cross-reacting with human MAG MABs.[13] Our observations that in plastic sections only parts of the myelin sheats were labelled suggest that the epitope in compact myelin, possibly a ganglioside, may be more vulnerable during the fixation, embedding, and etching procedures. Similarly, this may also apply to the staining pattern with the mouse MAB to Leu 7. Labelling of compact myelin in both paraffin and plastic sections by a mouse MAB to P_0 indicates that, in principal, compact myelin can be stained even in plastic sections. In the CNS, we confirm the surprising finding[4] that anterior horn cells can be stained, in which no MAG like antigen has hitherto been demonstrated. The fact that only a proportion of these cells show positive staining, leads us to speculate that anterior horn cells may subdivide in groups with different antigenic markers similar to findings in oligodendrocytes.[15] Whether the antigen on anterior horn cells recognised by anti-Leu 7 and the human MAG MAB is a MAG epitope or is a cross-reacting epitope of a myelin ganglioside remains to be shown. A recent study on "benign" human monoclonal paraproteins from patients with polyneuropathy demonstrated binding to a certain group of neurons of the rodent brain including pyramidal cells.[16] Although the specific binding to MAG was not stated, these MABS may indeed resemble the human MAB used in this study.

A cross reactivity between cells of the immune system and the myelin sheaths of a natural human MAB may be relevant to the pathogenesis of the polyneuropathy associated with monoclonal gammopathies. Natural killer cells are known to play an important role in homeostasis of the antibody response.[17] If the initial sensitising event leading to autoantibody formation occurs at the level of the natural killer cells, a self-sustained formation of these antibodies may ensue and ultimately attack the shared MAG antigen in peripheral nerve.[12]

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