Arq Neuropsiquiatr 1994, 52(4): 493-500

EXPRESSION OF HLA-DR IN PHERIPHERAL NERVE OF AMYOTROPHIC LATERAL SCLEROSIS

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SUMMARY - To investigate the possibility of local antigen presentation within the peripheral nerve in amyotrophic lateral sclerosis (ALS), cryostat sections of 83 peripheral nerve biopsies were stained for the demonstration of HLA-DR using a monoclonal antibody. Forty samples showed increased expression of HLA-DR in endoneurium. The phenotypic characteristics of the HLA-DR positive cells are chiefly Schwann cells, using S-100 protein as a marker. We did not detect any co-expression between HLA-DR and NF (axons) and HLA-DR and myelin marker. We also detected co-expression between HLA-DR and NGFr in a majority of HLA-DR positive cells. Inflammatory cells were infrequent, being detected only in 11 cases, predominantly around epineurial blood vessels. Motor and sensory nerve biopsies performed simultaneously showed higher expression of HLA-DR in motor nerves in 2 out of 4 patients. The significance of these findings is not clear. The presence of endoneurial cells expressing HLA-DR suggests that an autoimmune mechanism may be involved in ALS having Schwann as the main target.

KEY WORDS: amyotrophic lateral sclerosis, peripheral nerve, histocompatibity antigen.

Expressão de HLA-DR em nervo periférico de esclerose lateral amiotrófica

RESUMO - Para investigar a possibilidade de comprometimento auto imune no nervo periférico de pacientes com esclerose lateral amiotrófica (ELA) 83 biópsias do nervo periférico de 79 pacientes (51 sexo masculino, 28 sexo feminino) com média de idade de 62 anos (variação de 19 a 82) foram congeladas e coradas para demonstração de HLA-DR usando anticorpo monoclonal. Quarenta amostras (48%) mostraram expressão nitidamente aumentada de HLA-DR na região endoneural. Usando proteína S-100 como marcador, demonstramos que HLA-DR se expressava principalmente nas células de Schwann. Não encontramos co-expressão com HLA-DR usando anticorpos antineurofilamento ou antimielina, mas detectamos co-expressão de HLA-DR e anti-receptor de fator de crescimento nervoso na maioria das células HLA-DR positivas. Células inflamatórias foram encontradas ocasionalmente, sendo detectadas em somente 11 casos, predominantemente ao redor de vasos sanguíneos epineurais. Biopsias de nervo sensitivo e motor feitas simultaneamente mostraram maior expressão de HLA-DR em nervos motores de 2 dos 4 pacientes. A significância desses achados ainda não é clara. A presença de células endoneurais expressando HLA-DR sugere que mecanismos auto imunes podem estar envolvidos na ELA tendo a célula de Schwann como um dos principais alvos.

PALAVRAS-CHAVE: esclerose lateral amiotrófica, nervo periférico, antígeno de histocompatibilidade.

Amyotrophic lateral sclerosis (ALS) is a degenerative disease of the lower and upper motor neurons, leading to progressive weakness and death. The precise mechanism of degeneration is yet unknown[16]. In rcent years, studies have suggested autoimmunity as a cause of ALS[2,14]. Evidence for

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this hypothesis is based on enhanced expression of histocompatibility complex molecules (MHC) in spinal cord of ALS patients[7,8,19]. The MHC molecules are a family of proteins encoded by a complex of genes, located in humans on chromosome 6. The gene products of the MHC are divided into class I and class II molecules comprising a set of cell-surface glycoproteins. In man, class I MHC glycoproteins are encoded by 3 separate genetic loci, called HLA-A, HLA-B and HLA-C. MHC class II are encoded by the HLA-D locus, and are subdivided into DP, DQ and DR class I molecules have a wide tissue distribution and are expressed on the surface of target cells. They are essential for the recognition of foreign antigen by cytotoxic T cells. Class II molecules have a more limited tissue distribution. They are located at the surface of certain cells and are essential for the presentation and recognition of foreign antigen by helper T cells. Only cells expressing HLA-DR are able to induce T cells to respond, and this limited expression efficiently controls T cell activation[20]. In pathological conditions, especially associated with inflammation, HLA-DR molecules have been found on cells that do not express the protein normally[18]. Studying four cases of ALS, Lampson et al.[8] detected increased expression of class I and class II MHC in the affected areas of spinal cord. Troost et al.[19] showed an increase in inflammatory cells and MHC products in the corticospinal tracts and anterior horns in 8 ALS cases.

To our knowledge, no studies of MHC in nerve have been reported in ALS. Here we have studied the expression and celular localization of HLA-DR in peripheral nerve of patients with ALS.

MATERIALS AND METHODS

We studied 83 peripheral nerve biopsies from 79 patients (51 men, 28 women) median age 62 years (range 19 to 82) with ALS followed at the Columbia Presbyterian Medical Center. The diagnosis of ALS was made by clinical and laboratory investigations. All patients fulfilled the criteria for ALS[21]. None of the patients had other causes of peripheral neuropathies, or antibodies against gangliosides. Sural nerves were obtained in the majority of the patients. Four patients were submitted, simultaneously, to motor (gracilis motor nerve) and sensory (medial cutaneous nerve) nerve biopsies. Control sural nerves were obtained from 5 patients with other disorders (inclusion body myositis, polymyositis, Leigh's disease, episodic ataxia and Parkinson's disease), but with no known neuropathy.

Antibodies. The primary antibodies employed in the present study were as follows: a monoclonal anti-human HLA-DR antibody (Dakopatts, Santa Barbara, CA), directed against the common framework of MHC class II antigens; a rabbit antiserum to the 200 KD subunit of human neurofilament protein (Sigma Chemical Company), a marker of axons; a rabitt antiserum to S-100 protein (Sigma), a Schwann cell marker; a monoclonal antibody to human nerve growth factor receptor (anti-NGFr) (Boehringer-Manheim); a monoclonal antibody to Ulex europaeus agglutinin 1 (UEA-1), biotinylated (Dakopatts), detecting endothelial cells[10]; a monoclonal human anti-IgM (our source - Armstrong), that recognizes myelin associated glycoprotein, a marker for myelin sheath; a monoclonal antibody to leucocyte common antigen (LCA) (Dakopatts), a putative CD45 antibody which reacts with all four glycoproteins of the LCA family (MW 180, 190, 205 and 220 KD), being a marker for lymphocytes, macrophages and granulocytes[4]; a monoclonal anti-human T cell antibody, CD3, (Dakopatts), a pan T cell marker; a monoclonal anti-human B cell antibody, CD20, (Dakopatts).

As secondary antibodies, we used: fluorescein-conjugated, affinity-purified goat antibodies to mouse (Amersham); biotinylated sheep anti-mouse Ig (Amersham); biotinylated goat anti-human IgM (Sigma); Texas-red streptavidin (Amersham); Texas-red conjugated, affinity-purified donkey antibodies to rabbit IgG (Amersham).

Immunohistochemical methods. Nerves were rapidly frozen by immersion in isopentane cooled nitrogen for immunohistochemistry. Cross and longitudinal serial sections (10 micrometers) were cut, mounted on 0.1% poly-D-lysine coated glass slides and allowed to air-dry for a few minutes at room temperature. Dilutions of antibodies and washing steps of sections were performed using phosphate buffered saline (PBS) - sodium phosphate, 0.01 M, and 0.9% saline, at pH 7.4. The sections were placed in PBS for 10 minutes, fixed in cold acetone for 10 minutes and rinsed twice in PBS prior to staining. Sections were exposed for 10 minutes by 10% normal goat serum to block nonspecific binding sites. The sections were incubated at room temperature with consecutively, monoclonal anti-HLA-DR antibody (1:20) during 2 hours, and FITC - conjugated goat anti-mouse antibody (1:20) for 30 minutes. In between each step, the sections were thoroughly washed for 15 min in

Table 1. Doublelabel immunostaining with HLA-DR and a second antigen. All the antibodies were incubated in sequence. The working dilution of each antibody is represented.

			HLA-DR (1:20)			
			FITC anti-mouse (1:20)			
Arm (1:50)	Ulex (1:20)	S100 (1:50)	NGFr (1:20)	NF (1:200)	Tcell (1:20)	Bcell (1:20)
bio IgM (1:100)	TR stre (1:200)	TR anti rabbit (1:20)	TR anti mouse (1:20)	TR anti rabbit (1:20)	bio anti mouse (1:100)	bio anti mouse (1:100)
TR stre (1:200)					TR stre (1:200)	TR stre (1:200)

2 changes of PBS. After a final wash in PBS, the sections were mounted with a synthetic mountant (Biomeda Corporation, Foster City, CA) containing 2% DABCO (Sigma) to inhibit inactivation of fluorescence during prolonged exposure of the sections to ultraviolet light. For detection of inflammatory cells, the sections were immunostained with the anti-LCA antibody (1:20) using a similar method.

The nerve specimens with increased expression of HLA-DR were evaluated by double immunofluorescence staining for HLA-DR and for various cell markers. All the primary antibodies were incubated for 2 hours at room temperature, except for T and B cell antibodies, which were applied overnight at 4 degrees C. All immunostaining was performed on successive sectioned 10-micrometers tissue sections and the antibodies were incubated separately in sequence. The optimal dilution of each antibody was determined by titration.

The double labeling of HLA-DR and the marker for either T cells or B cells was performed only when the immunostaing with LCA showed the presence of inflammatory cells. A schematic description of the double labeling is shown in Table 1.

As controls the primary antibody was ommited or was replaced by either normal mouse serum or rabitt serum. In double labeling studies, only one primary antibody was applied. Positive and negative controls were used for each antibody.

The sections were examined and photographed with an epifluorescence microscope (Zeiss) equipped with filters to detect fluorescein and Texas red signals separately.

RESULTS

In 43 nerves (52%) HLA-DR expression was the same found in normal controls. Forty nerves (48%) showed markedly increased HLA-DR expression within the endoneurium. The immunostaining had a spotted aspect, generally focal and linear, with an irregular distribution pattern. The expression varied from slight to increased among different fascicles. In some areas within the fascicles, many and intense foci of increased HLA-DR expression were seen, whereas in other areas little or no staining was detectable.

Specimens with increased expression of HLA-DR were stained with double label immunofluorescence to identify immunoreactive cells to both antisera. The immunostaining clearly showed no colocalization of HLA-DR with either axons (neurofilament antibody) or myelin (antimyelin antibody). In some areas, a strongly immunoreactive HLA-DR was detected in between bundles of fine neurofilament-positive axons with no detectable myelin as judged by phase optics. Combined staining for endothelial cells and HLA-DR showed that most, but not all vessels expressed HLA-DR. Capillaries were most frequently positive and large vessels were usually negative.

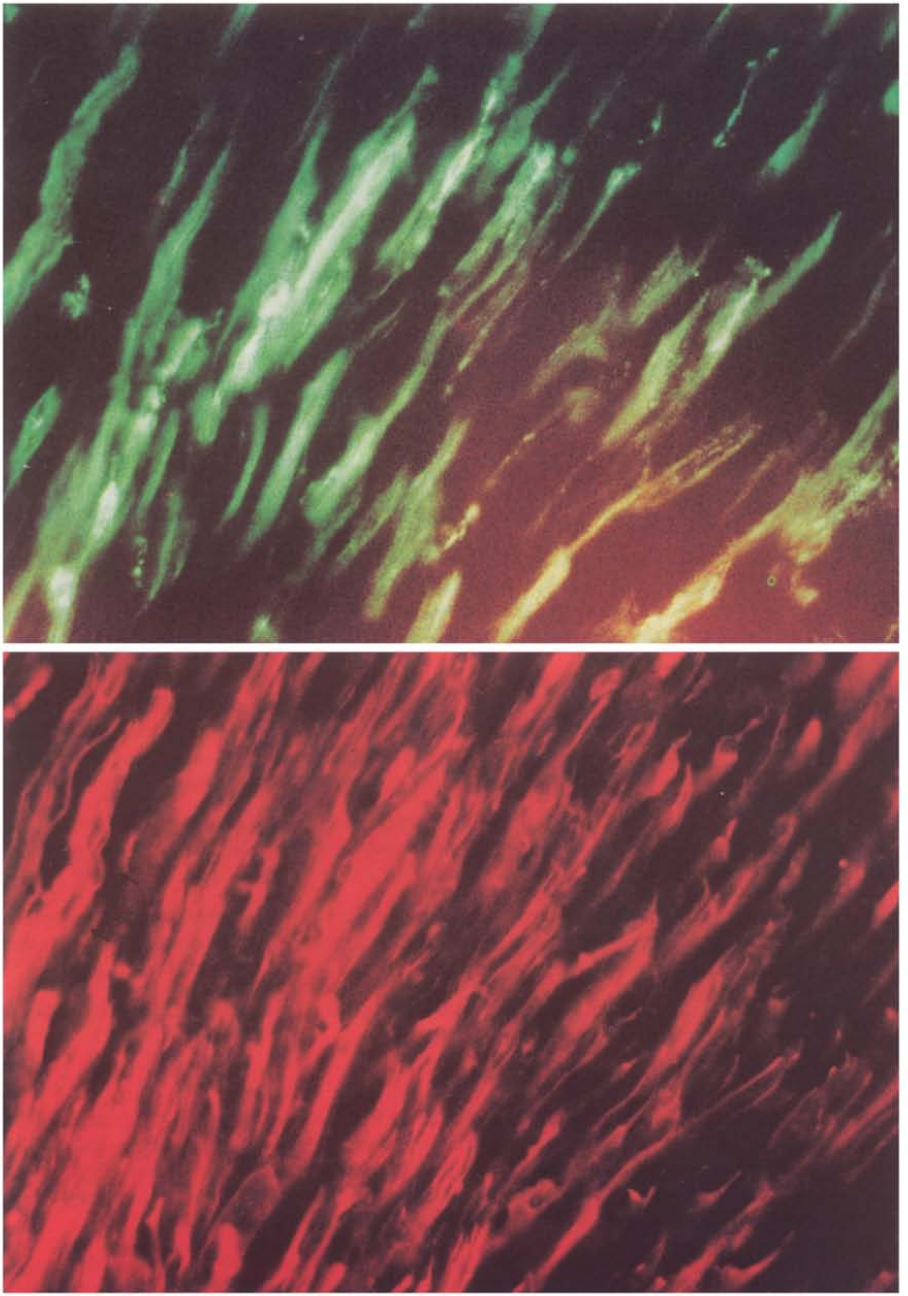

Fig 1. Sural nerve of ALS case doubly immunostained for HLA-DR (green) and anti-S100 (red). Notice many, but not all, positive S100 cells are also positive for HLA-DR.

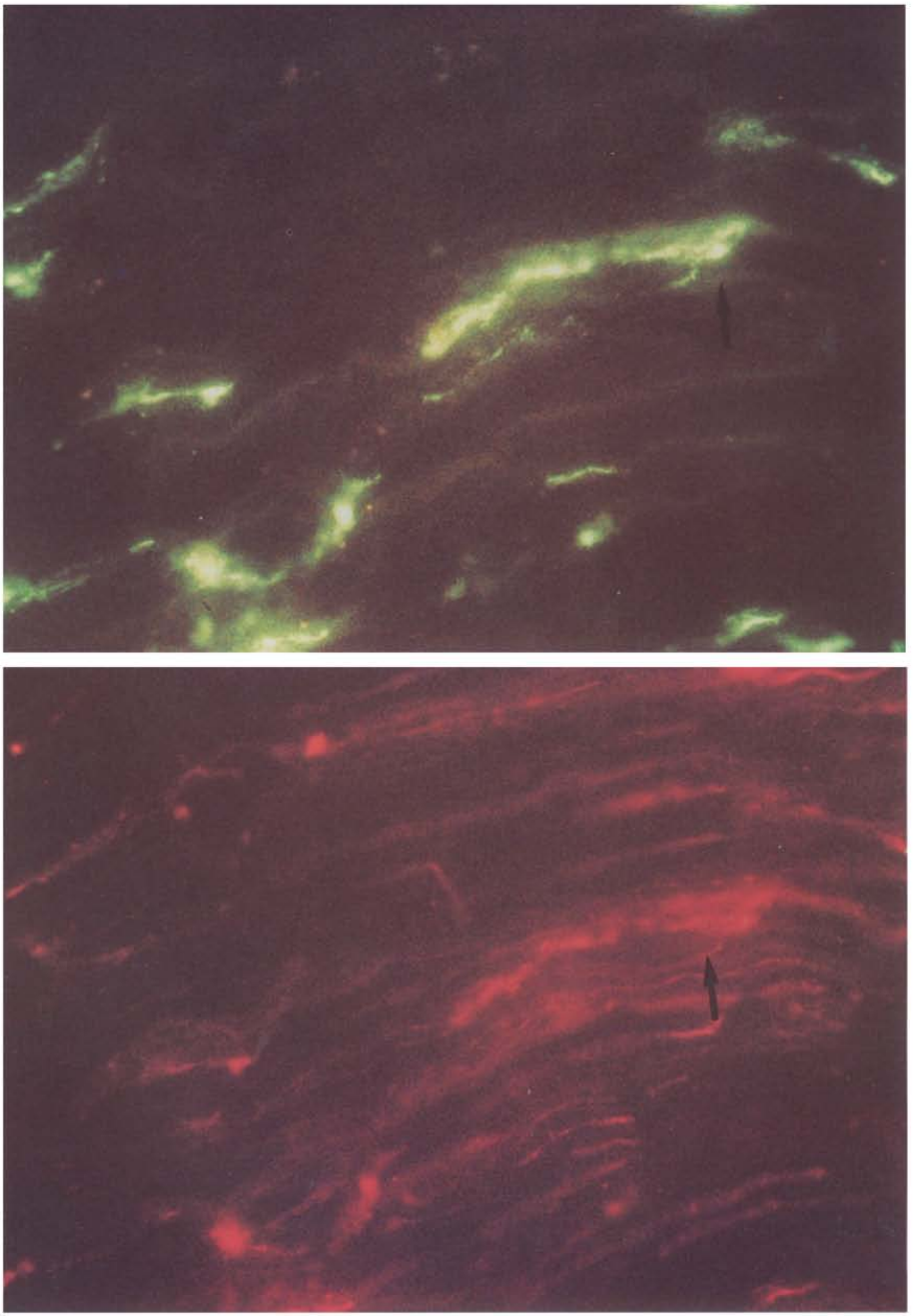

Fig 2. Sural nerve of ALS case doubly immunostained for HLA-DR (green) and NGFr (red). Notice the areas that are expressing HLA-DR also have NGFr expression.

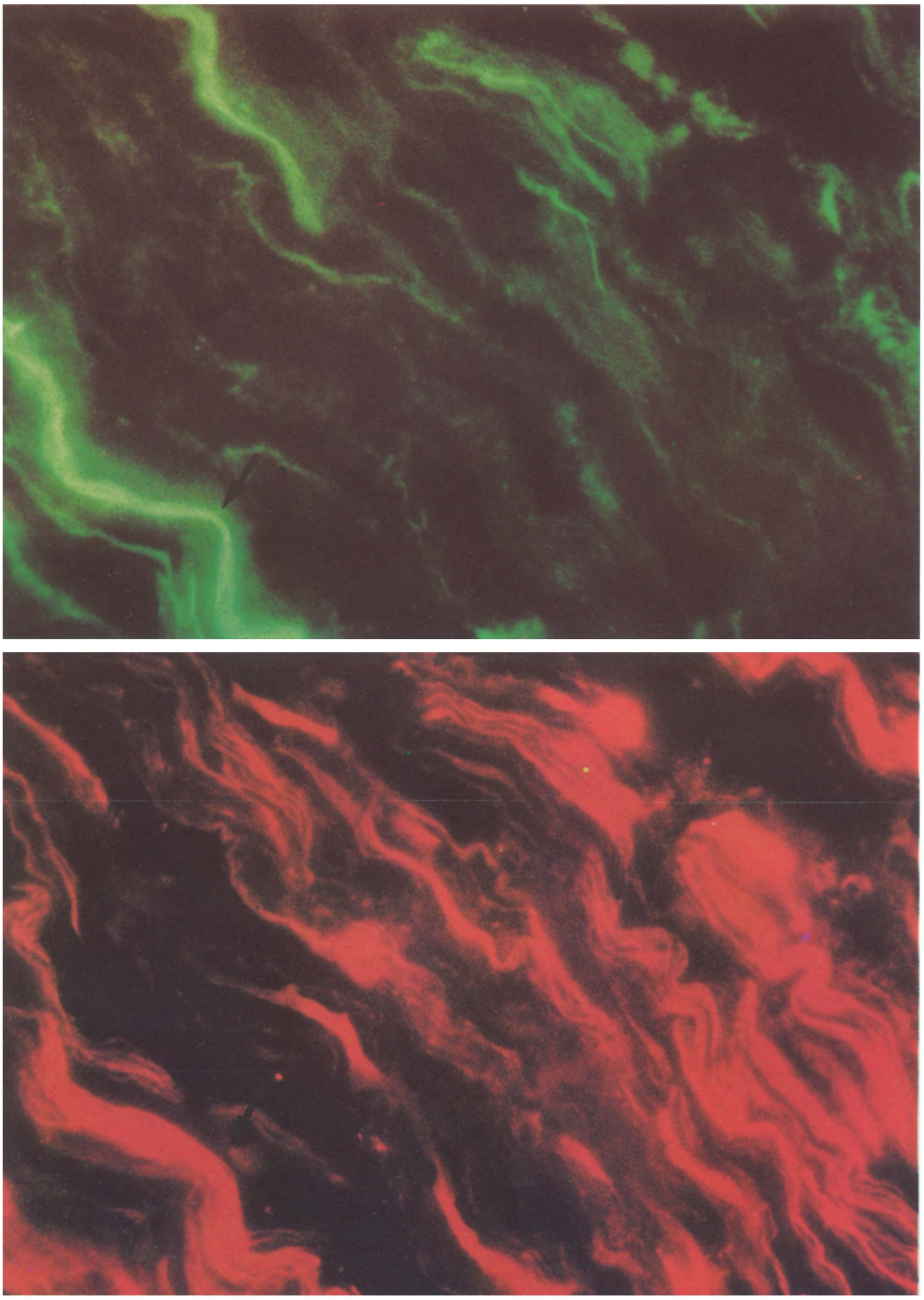

Fig 3. Sural nerve of ALS case doubly immunostained for S-100 and NGFr. Notice NGFr (green) positive cells in areas with S-100 (red) immunostaining.

Combined staining of HLA-DR and S-100 disclosed that at least 50% of all HLA-DR-positive cells were Schwann cells. In some specimens, nearly all of this protein was localized to Schwann cells (Fig 1).

A co-expression of HLA-DR and NGFr was also detected inside fascicles. In longitudinal and cross sections, NGFr immunostaining had an irregular distribution. Some areas of the fascicle disclosed a rare, dense, immunostaining pattern, and other areas had little or no staining (Fig 2). Double labelling of NGFr and S-100 revealed a co-expression of these two antibodies in the same areas, partial in some cases and almost total in others (Fig 3).

Antibody to LCA detected inflammatory cells in 11 specimes, predominantly around epineurial blood vessels. Anti pan T-cell monoclonal antibody revealed small numbers of T cells in 7 of these nerves. The monoclonal antibody for B cell revealed no B cells. There was no correlation in our samples between HLA-DR expression and the presence of inflammatory cells.

Two of the 4 cases that were submitted to motor and sensory nerve biopsies presented normal HLA-DR. One showed normal HLA-DR expression in the sensory nerve and incresead in the motor, and the other showed increased expression in both nerves, but more in the motor.

COMMENTS

We have demonstrated increased HLA-DR expression in almost half of 83 peripheral nerves of ALS patients.

In normal human nerves, immunoreactive HLA-DR is localized on endothelial and perivascular cells and in rare perineurial and endoneurial cells[12]. Schroder et al.[13] found strong expression of HLA-DR in peripheral nerve of different neuropathies, and its presence correlated with severity and activity of the neuropathy. Pollard et al.[11] found immune response associated HLA-DR in mononuclear and Schwann cells within the nerves of patients with chronic inflammatory demyelinating polyneuropathy, Guillain Barré syndrome, hereditary motor sensory neuropathy type I and demyelinating neuropathy associated with monoclonal IgM. It has been proposed that the magnitude of the imune response may be directly correlated to the cell surface levels of MHC molecules, both class I and class II[9].

The evidence that HLA-DR expression is increased in peripheral nerves of ALS may be relevant for pathogenetic mechanisms and raises speculation that this disease has some immunological basis.

In our samples we have demonstrated that about 50% of HLA-DR positive cells were Schwann cells, and we have not found, with double staining, any correlation between HLA-DR and neurofilament or myelin. A disorder in the functioning of Schwann cells in ALS has previously been suggested[5]; nonetheless the mechanism of this disorder remains to be explained, and could not, in our cases, be accounted for visible inflammatory destruction. Inflammatory cells in our samples were detected in the epineurial blood vessels of only 11 out of 83 nerves, and was not correlated with an increase of HLA-DR expression. These findings seem to confirm the hypothesis of Pollard[11] who suggested that class II molecules can be expressed at a certain stage of Schwann cell differentiation or dedifferentation. In this case, HLA-DR antigen appearance on Schwann cell may reflect not only an immunologically mediated phenomenon but also a dependence on Schwann cell interaction[12]. By other hand, it has been suggested however that the upregulation of MHC can be suficient per se to induce cell damage[1,6].

We have also demonstrated that most of the areas that overexpressed HLA-DR, and expressed S-100 also co-expressed NGFr. Animal experiments have shown that nerve transection is followed by expression of NGFr by Schwann cells of motor and sensory nerves distally to the site of the lesion[17]. Snider and Johnson[15] have demonstrated that denervated Schwann cells also express NGFr.

Based on our findings, we suggest that the expression of HLA-DR in Schwann cells could explain the mild reduction in the total number of myelinated fibers and the slight abnormalities of unmyelinated fibers found in sural nerves in ALS[3].

The key question is whether the presence of HLA-DR in peripheral nerve biopsies is a primary pathogenetic event or whether it is an epiphenomenon. It seems, nevertheless, that this finding is not a non-specific consequence of nerve degeneration since peripheral nerve from patients with various neuropathies, showing severe pathological changes, do not strongly and consistently express HLA-DR[11].

Further investigations studying motor nerves are required to establish the mechanisms of induction of HLA-DR in peripheral nerve from patients with ALS, since we have also demonstrated in two patients a higher expression of HLA-DR in motor nerves when compared to sensory nerves.

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