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Analysis of the IgG subclass distribution and inflammatory infiltrates in patients with anti-Hu–associated paraneoplastic encephalomyelitis

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Article abstract—Using immunohistochemistry, we studied the IgG subclass distribution of the anti-Hu antibody in serum, nervous system, and tumor of patients with anti-Hu–associated paraneoplastic encephalomyelitis/sensory neuronopathy (PEM/PSN). The nervous system was also examined for deposits of complement and the distribution and type of inflammatory cells. IgG1 and IgG3 were the predominant isotypes of the anti-Hu IgG in serum, nervous system, and tumor. A few patients also had anti-Hu IgG2, but this isotype was not consistently present in all the regions of the nervous system studied. There was no correlation between neurologic symptoms and specific anti-Hu isotype, nor was there evidence that different anti-Hu isotypes recognized specific brain regions. Although IgG1 and IgG3 can activate complement, only weak complement reactivity was found, and that only in a few areas of the nervous system. This finding, in addition to the absence of natural killer (NK) cells, suggested that complement-mediated toxicity and antibody-dependent cell cytotoxicity mediated by NK cells are not pathogenic in PEM/PSN. Inflammatory infiltrates included CD19+ (B cells) and CD4+ (helper/inducer) cells in the perivascular spaces, and lymphocytes bearing CD8+CD11b− markers (cytotoxic T cells) in the interstitial spaces. Infiltrates of EBM11+ (monocyte/macrophage) cells were identified in the perivascular spaces (macrophage phenotype) and in those interstitial regions (microglial phenotype) with severe pathologic changes. The ability of the IgG1 and IgG3 isotypes to bind Fc receptors may have played a role in the recruitment of these monocyte/macrophage cells. We conclude that anti-Hu–associated PEM/PSN is a complex immune disorder in which both cell-mediated and humoral (probably non-CMT and non-ADCC) cytotoxic mechanisms appear to be involved in its pathogenesis.

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Paraneoplastic encephalomyelitis (PEM) and paraneoplastic sensory neuronopathy (PSN) are rare syndromes usually associated with small-cell lung cancer (SCLC).[1,2] Most patients with PEM/PSN and SCLC develop an immune response against a 35 to 40 kD neuronal protein (Hu antigen) expressed both in all neurons and tumor tissue.[3,4] This immune response is characterized by a high titer of anti-Hu IgG antibodies in serum and CSF and by inflammatory infiltrates of T and B cells in the nervous system and the tumor.[5,6] A family of genes encoding RNA-binding proteins (HuD, HuC, Hel N-1) that are specifically identified by the anti-Hu antibody have recently been cloned (Manley, unpublished data).[7,8] The restricted expression of the Hu proteins in neurons and their homology to the *Drosophila* protein Elav (embryonic lethal abnormal visual system) suggest that the Hu proteins play a role in the development and maintenance of the nervous system.[7]

In patients with PEM/PSN there is a correlation, albeit imprecise, among the neurologic symptoms, regions of major tissue damage, and the quantitative distribution of deposits of anti-Hu IgG.[9] In addition, Hu-specific infiltrating lymphocytes have been identified in the nervous system and tumor.[7] In a single case of anti-Hu–associated PEM/PSN in which only a few areas of the nervous system were examined, IgG was identified at the periphery of some neurons, but no complement or natural killer (NK) cells (involved in antibody-dependent cell-mediated cytotoxicity; ADCC) were found.[10] These findings suggested that anti-Hu IgG may play a role in the pathogenesis of the disease, but the exact mechanism is unproved.

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Mouse monoclonal antibodies directed against specific human IgG heavy-chain isotypes allow identification of the IgG subclass distribution in autoimmune diseases and correlation of the IgG subclass with the pathogenesis of these disorders. Of particular interest is the complement-activating potential of IgG1 and IgG3 and the capacity of these subclasses to bind Fc receptors on mononuclear cells.[11,12] Previous reports showed that various antinuclear antibodies, including anti-DNA, RNP, Sm, and SS-B, belonged mainly to the IgG1 and, to a lesser degree, IgG3 subclasses.[13-18] Furthermore, anti-glomerular basement membrane antibodies, whose pathogenicity has been well established by transfer to animals, were also shown[19] to be predominantly IgG1.

We hypothesized that if the anti-Hu antibodies were restricted to IgG1 or to IgG1 and IgG3 subclasses, this would suggest a pathogenic role of anti-Hu in PEM/PSN, either by complement-mediated cytotoxicity (CMT) or by ADCC. Additionally, if the inflammatory infiltrates were predominantly CD8 cytotoxic cells, this would indicate a role of cell-mediated toxicity in the pathogenesis of the disorder. Therefore, in the present study, we undertook to examine (1) the IgG subclass distribution of the anti-Hu antibody in the serum, nervous system, and tumor of patients with anti-Hu–associated PEM/PSN, (2) the presence of deposits of complement in the nervous system, and (3) the type of inflammatory infiltrates in the nervous system.

Methods. *Sera, tissues, and antibodies.* Serum was obtained from nine patients with anti-Hu–associated PEM/PSN. The presence of the anti-Hu antibody was established by immunohistochemistry and Western blot analysis. In three patients, serum was obtained within 2 months of death; serum from the other patients was obtained at various points during their illness. Serum from neurologically normal individuals was used as control.

The entire brain was available from four patients at autopsy and kept frozen at −70 °C. Only sections of tissue that contained deposits of anti-Hu IgG[9] were used for the present studies. Frozen blocks of spinal cord tissue were available from one patient and dorsal root ganglion (DRG) from another. SCLC tissue was available from three patients, two with anti-Hu–associated PEM/PSN and one without the anti-Hu antibody.

Brain tissue obtained at autopsy from neurologically normal individuals was used as control tissue and substrate for the immunohistochemical studies involving patients' sera. Normal mediastinal lymph nodes were obtained from autopsy and biopsy studies.

Anti-Hu IgG subclass distribution was determined by using mouse monoclonal antibodies directed against human IgG isotypes (table 1). Since both serum and normal human tissues (used as controls) contain a mixture of all types of IgG, the specific reactivity, and the absence of cross-reactivities, of the mouse monoclonal antibodies were pretested by a dot-blot assay in which immobilized human IgG isotypes (IgG1, IgG2, IgG3, IgG4) from myeloma (Chemicon, Temecula, CA), were reacted with the panel of mouse monoclonal antibodies against the human subclasses of IgG.

The presence and distribution of complement and the immunohistochemical analysis of the inflammatory infiltrates were established using the panel of mouse monoclonal antibodies shown in table 1. Immunoreactivity with these antibodies was evaluated as weak (+), moderate (++), or intense (+++).

Table 1. Panel of antibodies

Antibody	Dilution	Marker	Source
Anti-human pan-IgG	1:500	pan-IgG	Boehringer-Mannheim
IgG1	1:500	IgG1	FisherBiotech
IgG2	1:500	IgG2	FisherBiotech
IgG3	1:500	IgG3	FisherBiotech
IgG4	1:500	IgG4	FisherBiotech
T3 (CD3)	1:60	pan-T	Dako
Leu-12 (CD19)	1:10	B cell	Becton Dickinson
Leu-3a (CD4)	1:10	Helper/inducer	Becton Dickinson
Leu-2b (CD8)	1:10	Cytotoxic/suppressor	Becton Dickinson
Leu-19 (CD56)	1:10	NK cells	Becton Dickinson
Leu-15 (CD11b)	1:10	NK cells, T suppressor	Becton Dickinson
C3, HAV 3-4	1:10	Complement C3	Dako
C5b-9, aE11	1:10	Complement C5b-9	Dako
EBM11	1:1,000	Macrophage/ microglia	Dako

Immunohistochemical analysis of the anti-Hu IgG subclass distribution in serum. To determine the anti-Hu IgG subclass distribution in the serum of patients with PEM/PSN, sera were diluted 1:500 (approximately 22 μg of total IgG/dl) and reacted with sections of normal cerebral cortex. The isotype of anti-Hu IgG bound to neurons was then studied with a panel of mouse monoclonal antibodies against human IgG isotypes (table 1).

Seven-μm-thick frozen tissue sections of brain obtained at autopsy of neurologically normal individuals were fixed for 10 minutes in cold acetone (4 °C) and sequentially incubated with 0.3% hydrogen peroxide to avoid endogenous peroxidase activity and 10% normal horse serum (Vector, Burlingame, CA) to prevent nonspecific binding of the secondary antibody. Sections were then incubated with the indicated amounts of the patient's serum for 2 hours at room temperature (RT), washed, reacted with the panel of mouse monoclonal anti-human IgG isotypes (2 μg/ml) for 1 hour at RT and, after washing, incubated with biotinylated horse anti-mouse IgG antibody (Vector) diluted 1:1,000 for 1 hour at RT. Bound horse anti-mouse IgG was visualized by incubation with avidin-biotin-peroxidase complex (Vectastain ABC complex, Vector) and the substrate developed with 0.05 diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO).

Two types of controls were included: (1) sections sequentially incubated with serum from normal individuals (instead of the patient's serum) and the panel of mouse monoclonal anti-human IgG isotypes served as control for the absence of the anti-Hu IgG; and (2) sections sequentially incubated with the patient's serum and normal mouse IgG (instead of mouse monoclonal anti-human IgG) served as negative control for the assay.

Immunohistochemical analysis of the anti-Hu IgG subclass distribution in tissues. Sections of nervous system and tumor from patients with anti-Hu–associated PEM/PSN were used in this assay. Analysis of the subclass anti-Hu IgG deposits (previously demonstrated in these tissues[9]) was done by sequential incubation of the slides as described above, omitting the step of incubation with the patient's serum. Sections of brain obtained at autopsy from neurologically normal individuals and SCLC from a patient without paraneoplastic symptoms served as tissue controls.

Immunohistochemical analysis of the presence of complement, and inflammatory infiltrates in the nervous system. Tissue sections were fixed in 4% buffered formalin and sequentially incubated with 0.3% hydrogen peroxide, 10% normal horse serum, and the mouse anti-human C3 (or C5b-9) fraction of the complement for 2 hours at RT. After washing, sections were incubated with biotinylated horse anti-mouse IgG and the reaction developed as described above.

Analysis of the presence of cells bearing the phenotype CD3 (T3, pan-T cell), CD4 (helper/inducer), CD8 (suppressor/cytotoxic), CD11b (NK cell, T suppressor), CD19 (B cell), EBM11 (monocyte/macrophage), and CD56 (NK cell) was done, using a similar immunohistochemical assay, with the primary antibodies and concentrations indicated in table 1.

Sections of brain from neurologically normal individuals and normal lymph node were used as controls.

Results. *Analysis of the anti-Hu IgG subclass distribution in serum.* After the serum of all nine patients was incubated with sections of normal cerebral cortex, the anti-Hu IgG bound to neurons reacted strongly with mouse anti-human IgG1 (table 2 and figure 1). In addition, one patient's anti-Hu (91/124) had IgG2 and IgG3 reactivities, and another patient's anti-Hu (91/257) had IgG2 reactivity. To detect the presence of minor species of anti-Hu antibodies, the patients' sera were concentrated such that 440 μg of total IgG per dl was reacted with cerebral cortex. At this concentration, the bound anti-Hu IgG from of all nine sera had strong IgG1 reactivity. In addition, the anti-Hu of five sera (56%) had IgG2 reactivity, and the anti-Hu of four sera (44%) had IgG3 reactivity. In two sera (92/008 and 89/350), the anti-Hu had IgG2 but not IgG3 reactivity; conversely, in one serum (92/032), the anti-Hu had IgG3 but not IgG2 reactivity. Anti-Hu of the IgG4 subclass was not detected in any of the nine sera.

Sections of cerebral cortex sequentially incubated with serum from normal individuals and mouse anti-human IgG isotypes demonstrated IgG (all four isotypes) reactivity in blood vessels but not in neurons; this reactivity corresponded to the endogenous IgG contained in cerebral vessels. No reactivity was observed in the sections sequentially incubated with the patients' sera and normal mouse IgG (instead of the mouse anti-human IgG isotypes).

Analysis of the anti-Hu IgG subclass distribution in the nervous system. The CNS from four patients with anti-Hu–associated PEM/PSN was examined for the IgG subclass distribution of the anti-Hu antibody (table 3). Two patients (89/042 and 89/37) had limbic encephalitis as their main clinical syndrome. The other two had cerebellar degeneration in addition to a motor neuron syndrome (89/350) and brainstem encephalitis (89/044). All the CNS regions studied from the four patients showed strong immunoreactivity with anti-IgG1 (figure 2) and pan-IgG antibodies. In addition, weak reactiv

Table 2. Analysis of the anti-Hu IgG subclass distribution in serum

Patient no.	IgG1	IgG2	IgG3	IgG4	pan-IgG
92/032	+++	—	—	—	*
92/019	+++	—	—	—	*
92/022	+++	—	—	—	*
92/008	+++	—	—	—	*
91/124	+++	+	+	—	+++
89/350	+++	—	—	—	*
91/257	+++	++	—	—	+++
89/044	+++	—	—	—	+++
88/182	+++	—	—	—	*

* Not done.
\+ Weak.
++ Moderate.
+++ Strong reactivity.

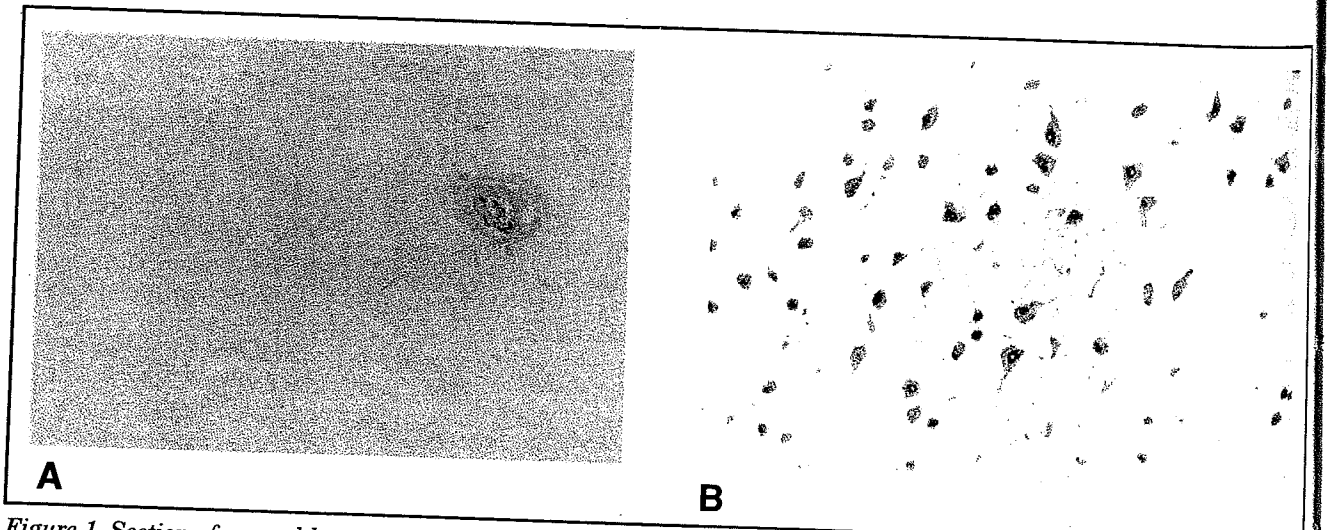


Figure 1. Section of normal human brain incubated with (A) serum from a normal individual and (B) anti-Hu serum; both panels have been reacted with mouse anti-human IgG1. In panel A, IgG1 reactivity is observed only in blood vessels and perivascular areas. The reactivity observed in panel B demonstrates that anti-Hu IgG is predominantly IgG1. Sections not counterstained.

Table 3. Analysis of the anti-Hu IgG subclass distribution in the nervous system

Patient no.	IgG1	IgG2	IgG3	IgG4	pan-IgG	Mouse IgG1
89/350						
Cervical spinal cord	+++	+	+	—	+++	—
Thoracic spinal cord	+++	+	—	—	+++	—
Medulla	+++	—	—	—	+++	—
Amygdala	+++	—	—	—	+++	—
89/377						
Medulla	+++	++	+	—	*	*
Hippocampus	+++	—	—	—	+++	—
Amygdala	++	+	+	—	+++	—
Dentate	+++	+	+	—	+++	—
89/044						
Medulla	+++	—	+	—	+++	—
Hippocampus	+++	+	—	—	+++	—
89/042						
Dorsal root ganglion	+	—	—	—	+	—
Medulla	+++	+	—	—	+++	—
Hippocampus	+++	+	—	—	+++	—

* Not done.

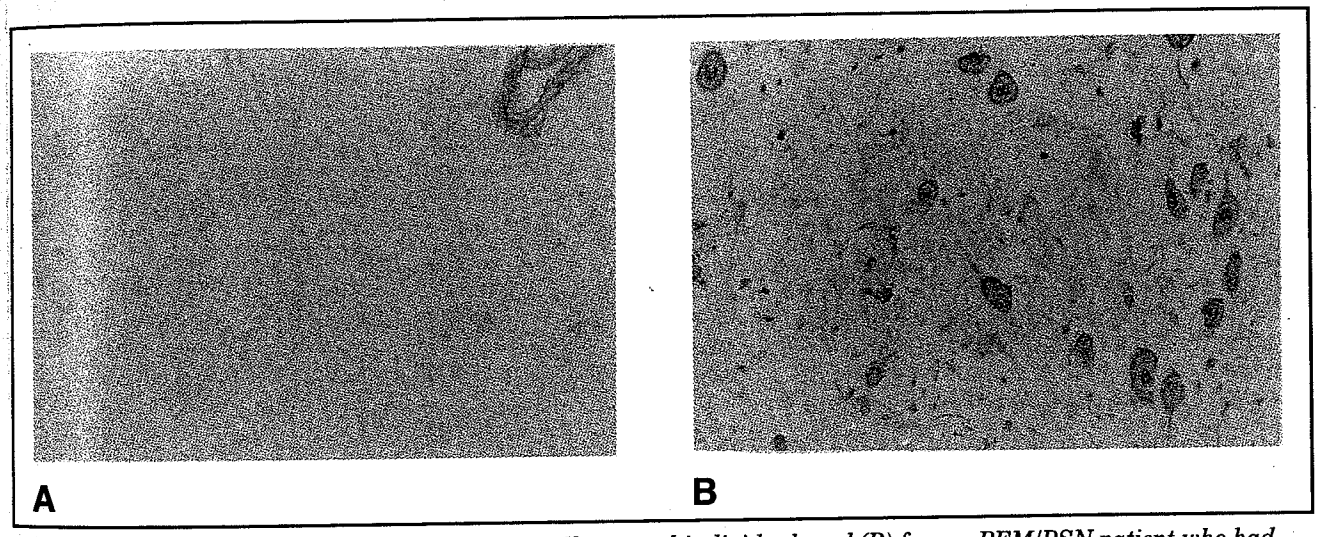


Figure 2. Section of medulla from (A) a neurologically normal individual, and (B) from a PEM/PSN patient who had deposits of anti-Hu IgG in the nervous system; both sections have been reacted with mouse anti-human IgG1. The neuronal deposits of anti-Hu IgG are predominantly IgG1.

ity with anti-IgG2 was detected in some regions of the four patients but was not consistently present in all the regions studied. Reactivity with anti-IgG3 was detected in three patients but was present only in the cervical spinal cord of patient 89/350; medulla, amygdala, and dentate nucleus of patient 89/377; and the olivary nucleus of patient 89/044. Regardless of the subtype of IgG studied, the IgG reactivity predominated in the nuclei of the neurons and to a lesser degree in the cytoplasm. Intense reinforcement of IgG reactivity on the neuronal cell surface was frequently observed (figure 2B).

No tissue section from any of the four patients showed intracellular deposits of IgG4; very weak IgG4 reactivity was observed in vessels. Spinal cord and brain from neurologically normal individuals were negative for the presence of intracellular deposits of any of the four IgG isotypes.

Analysis of the anti-Hu IgG subclass distribution in tumors. The SCLC tissue from two patients with anti-Hu–associated PEM/PSN were examined for the IgG subclass distribution of the anti-Hu antibody (table 4). Tumor tissue from patient 89/044 showed strong immunoreactivity with anti-human IgG1 antibody and weak reactivity with anti-IgG2 and anti-IgG3 antibodies. The immunostaining with all three mouse monoclonal antibodies was both cytoplasmic and nuclear. There was no reac-

Table 4. Analysis of the anti-Hu IgG subclass distribution in tumors

Patient no.	IgG1	IgG2	IgG3	IgG4	pan-IgG	Mouse IgG1
89/044	+++	+	+	—	+++	—
88/182	+++*	+	+	—	+++	—
Control	+†	—	—	—	+†	—

* Cell surface reactivity only.
† Nonspecific reactivity of the interstitium.

Table 5. Analysis of the inflammatory infiltrates in the nervous system

Patient no.		B	T4	T8	Macrophage/ Monocyte	Complement C3	Complement C5b-9	T8 sup
89/350								
Cervical spinal cord	pv	++	++	+	—			NS
	it	+	+	++	+	+	—	NS
Thoracic spinal cord	pv	+++	+++	+	+			NS
	it	+	+++ (nod)	++ (nod)	++	NS	NS	NS
Lumbar spinal cord	pv	+++	+++	+	+			—
	it	+	+++	++	+++ (nod)	—	+	+
Medulla	pv	++	++	+	—			NS
	it	+	+	++ (nod)	+++	+	—	NS
89/044								
Medulla	pv	+++	+++	+	+			+
	it	+	++ (nod)	+++ (nod)	++ (nod)	+	NS	—
89/042								
Hippocampus	pv	+	+	—	—			NS
	it	—	+	+	+	++	NS	NS
DRG	pv	—	—	—	—			—
	it	+	++ (nod)	++ (nod)	+	—	NS	+
89/377								
Medulla	pv	+	+	—	+			+
	it	+	+ (nod)	+ (nod)	+	—	—	—
Amygdala	pv	+	++	+	+			+
	it	+	+	+++ (nod)	+	—	NS	—
Dentate	pv	++	++	—	—			NS
	it	+	+	++ (nod)	+	+	—	NS

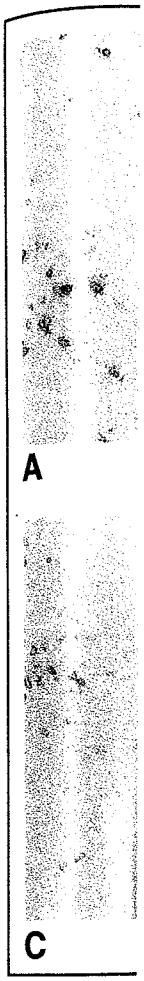
DRG Dorsal root ganglia.
pv Perivascular.
it Interstitial.
nod Nodule.
NS Not studied.

tivity with anti-IgG4 antibody.

The tumor tissue of patient 88/182 reacted strongly with anti-human IgG1 antibody. The reactivity was almost entirely confined to the cell surface. There was weak intracellular reactivity with anti-human IgG2 and IgG3 antibodies. Tumor cells from SCLC of a patient without the anti-Hu antibody did not immunoreact with all the anti-human IgG subclass antibodies, but there was reactivity with anti-human IgG1 and pan–anti-human IgG in the interstitium of the tumor tissue (table 4).

Analysis of the inflammatory infiltrates and the presence of complement. To examine the inflammatory infiltrates and the presence of complement, we selected those areas of the nervous system of four patients with anti-Hu–associated PEM/PSN that in previous studies had a high content of anti-Hu IgG and severe pathologic changes.[9] These changes predominantly involved the gray matter of multiple regions of the nervous system and included neuronal degeneration, gliosis, and inflammatory infiltrates. In some instances, neurons or Purkinje cells were found closely surrounded by T lymphocytes.[6,9]

Table 5 shows the immunohistochemical analysis of the inflammatory infiltrates in these patients. Widespread perivascular and interstitial infiltrates of T (CD3+) cells were observed. In addition, the perivascular infiltrates were composed of B (CD19+)



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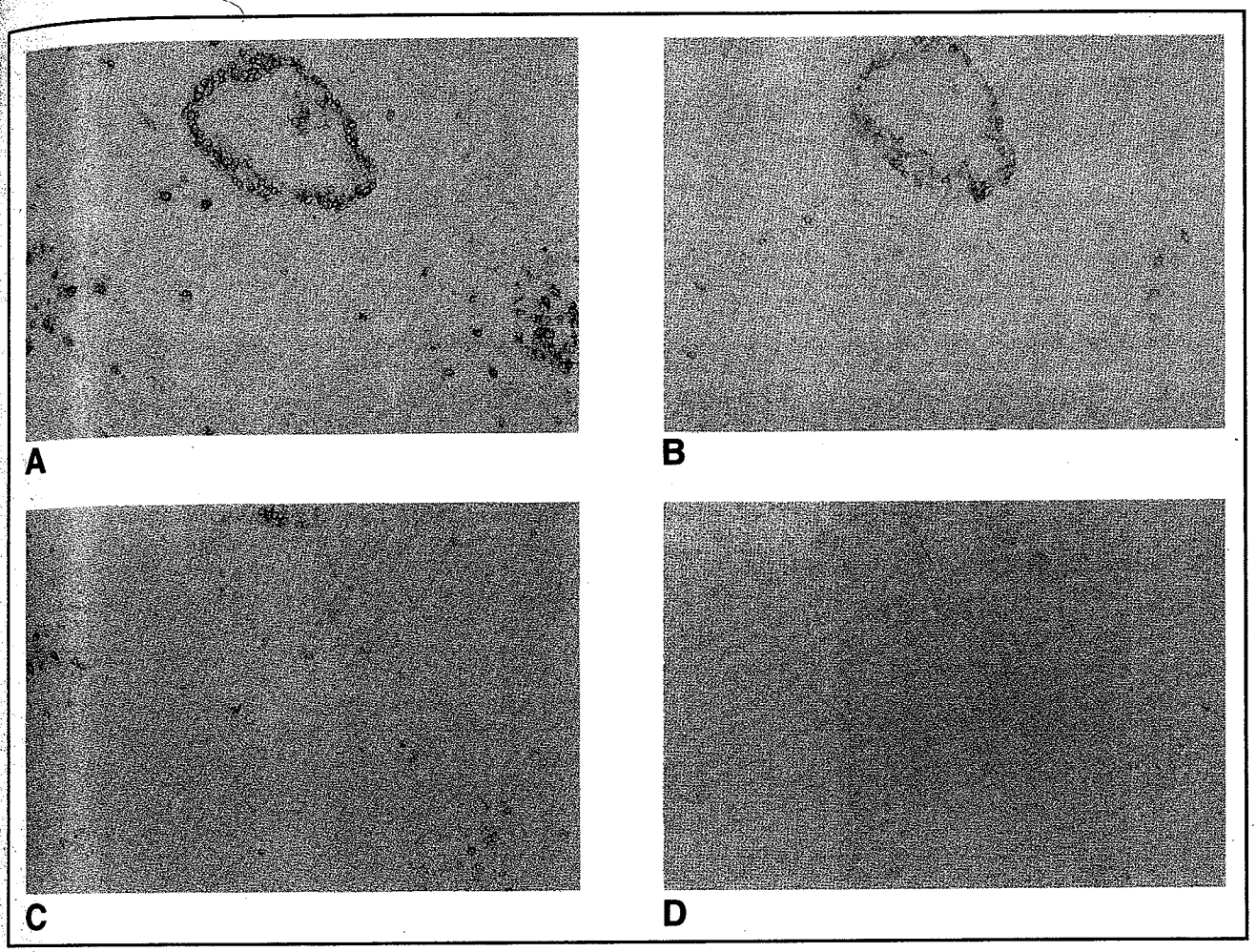

Figure 3. Consecutive sections of amygdala from a patient with anti-Hu–associated PEM/PSN incubated with a panel of T-cell markers (A, pan–T-cell marker). Cells bearing the helper/inducer phenotype (CD4) predominate in perivascular areas (B). Cells with the suppressor/cytotoxic phenotype (CD8) predominate in the interstitial spaces (C). The virtual absence of CD11b+ (T-suppressor) cells in the interstitial spaces (D) suggests that the majority of CD8+ cells (C) have a cytotoxic phenotype.

and monocyte/macrophage (EBM11+) cells. B and T cells constituted more than 85% of the perivascular infiltrates, B cells generally predominating over T cells. The majority (>90%) of the perivascular T (CD3+) cells were CD4+ (helper/inducer). Only a minority of the interstitial infiltrating cells were B cells (CD19+). CD8+CD11b− (cytotoxic) cells predominated in the interstitial infiltrates and constituted more than 70% of the lymphocytic nodules (figure 3). However, in a few areas, the lymphocytic nodules were composed largely of CD4+ cells. Conspicuous infiltrates of cells bearing the monocyte/macrophage lineage marker EBM11 were identified in the perivascular and interstitial spaces. The interstitial infiltrates of EBM11+ cells predominated in the areas of major tissue damage. In these areas, the EBM11+ cells were microglia-like; in contrast, the perivascular EBM11+ cells had a macrophage-like phenotype.

Although there were severe inflammatory infiltrates and deposits of anti-Hu IgG in multiple areas of the nervous system in each patient, C3 immunoreactivity (deposits of complement) was restricted to some vessel walls and a few interstitial areas. In these areas, C3 reactivity was usually weak and diffusely involved the cytoplasm and nuclei of the neurons; C3 reactivity was prominent in the hippocampus and olivary nucleus of one patient (89/042) who had had a cardiac arrest and hypoxic encephalopathy after resuscitation. In regions with severe pathologic changes, weak C3 reactivity was also observed in the cytoplasm of glial cells. In the DRG, there was strong C3 reactivity in the interstitial space, but no immunostaining was observed in the remaining neurons or in areas of inflammation. C5b-9 reactivity was observed in vascular areas and in a few neurons of the spinal cord of a patient (89/350) with cerebellar degeneration and a motor neuron syndrome, and in the olivary nucleus of the patient (89/042) with hypoxic changes.

Antibodies against the CD56 (NK cell) marker gave weak diffuse background staining in all the tissue sections obtained from either patients or normal controls. In the DRG there was stronger reac-

tivity with the capsular cells surrounding the neurons, but no increased reactivity over background was detected in any of the inflammatory cells or neuronophagic nodules present in brain and DRG.

Sections of brain obtained from neurologically normal individuals did not show reactivity with any of the antibodies against the following markers: CD3, CD4, CD8, CD19, and CD11b. A few perivascular cells had EBM11+ reactivity (macrophage-microglia), and C3 and C5b-9 reactivities were frequently observed in vascular vessels.

Cells bearing CD3, CD4, CD8, CD19, CD11b, CD56, or EBM11 markers were identified in sections of normal mediastinal lymph node, which was used as positive tissue control (data not shown).

Discussion. Using a double antibody assay with mouse monoclonal antibodies, we have demonstrated that IgG1 is the predominant isotype of anti-Hu IgG in the serum, brain, and tumor of patients with PEM/PSN. Since the anti-Hu antibody is polyclonal in origin, it is not surprising to find anti-Hu of other IgG subtypes. However, in the serum, the predominance of the IgG1 species is clearly in excess of the normal distribution of IgG isotypes mentioned above. A 20-fold increase in serum concentration led to only a slight increase in immunoreactivity with monoclonal anti-IgG2 and anti-IgG3 antibodies.

In the four patients from whom brain tissue was available, the quantitative distribution of anti-Hu IgG in the nervous system and tumor was known from a previous study.[9] In these patients, IgG1 was the predominant isotype despite the different concentration of total anti-Hu IgG found in each region. This was also true irrespective of the presenting signs and symptoms of the four patients, which included limbic encephalopathy, sensory neuropathy, motor weakness, ataxia, orthostatic hypotension, and seizures. The finding that a minor IgG isotype was present in regions of the brain but not detected in the same patient's serum (for example, 89/044 and 89/350) can be explained by the concentration of the antibody subtype in the presence of its antigen.

There was no correlation between the presence of specific IgG subtypes and the neurologic symptoms. For example, IgG2 was present as a minor anti-Hu species in the hippocampus of patient 89/042 with limbic encephalopathy, but it was absent in the hippocampus of patient 89/377 who had a similar clinical syndrome. It is therefore unlikely that the minor isotype species of anti-Hu antibody play a significant role in the pathogenesis of paraneoplastic symptoms. Furthermore, we did not find a correlation between the presence of a particular isotype of anti-Hu IgG and the region of the CNS studied. Therefore, there is no evidence to suggest that anti-Hu of different isotypes recognizes different epitopes limited to specific brain regions.

Among the four IgG subclasses, IgG1 and IgG3 can bind C1q, and thereby fix complement. These are also the only two subclasses of IgG for which there exist specific receptors on monocyte/macrophages.[11,12] Many autoantibodies are restricted to the IgG1 and IgG3 subclasses. These include anti-Sm, anti-U1-RNP, anti-glomerular basement membrane, and anti-acetylcholine receptor antibodies.[13] This predominance of IgG1 and IgG3 isotypes has led some authors to conclude[20,21] that complement fixation and ADCC may play a role in the pathogenesis of these autoimmune diseases. Autoantibodies of the IgG2 subclass are distinctly rare,[13] but IgG4 has been reported as the predominant subclass antibodies against thyroglobulin, microsomes, and factor VIII.[22,23] In some disorders, IgG4 antibodies appear to mask and protect epitopes from the binding of the pathogenic IgG1 antibodies.[21,22]

The predominance of anti-Hu IgG1 antibodies suggests that fixation of complement and ADCC may play a role in the immune response against the tumor and nervous system. However, Graus et al[10] did not identify NK cells (which are predominantly involved in ADCC) or deposits of complement in the nervous system of a patient with anti-Hu–associated PEM/PSN. In the present study, although NK cells were not identified, we did detect small amounts of complement in a few areas of the nervous system of all four patients. This finding supports a minor role for CMT or ADCC in the degeneration and loss of neurons. An alternative explanation would be that CMT has a role in damaging the neurons, but that the fractions C3 or C5b-9 of complement can be detected immunohistochemically for only a short time.

There is some preliminary evidence that the anti-Hu antibody is rapidly (1 hour) internalized by SCLC cell lines (Hormigo et al, unpublished data) and rat granule cells in tissue culture.[24] Greenlee and associates observed that the uptake of anti-Hu IgG by rat granule cells resulted in neuronal destruction; complement was not required, but its presence accelerated the neuronal damage.[24] These in vitro studies give support to the idea that internalization of anti-Hu IgG by the neurons and tumor cells may negatively affect the function of the cells and eventually lead to cell destruction.

The distribution of B and T cells in our patients is similar in many respects to that of other inflammatory disorders of the nervous system regardless of their etiology.[25,26] The finding of interstitial nodules with a predominance of either CD8+ or EBM11+ (macrophage/microglial) cells and, less frequently, CD4+ cells, may represent different stages of the inflammatory process in the same patient. Nodules with predominant EBM11+ cells were observed in areas of severe tissue damage and neuronal loss, suggesting a later stage of inflammation. The ability of the IgG1 isotype (which predominates in the anti-Hu deposits) to bind macrophage/monocyte cells could play a role in the recruitment of these cells.[11,12]

CD11b is an antigen present in NK cells and a subset of T lymphocytes involved in suppression; the presence of CD8+CD11b− antigens is indicative of a cytotoxic phenotype.[27] In the present

study, the absence of NK cells using another NK cell marker (CD56) and the virtual absence of CD11b+ cells in many interstitial CD8+ infiltrates indicate that the majority of these cells have a cytotoxic phenotype. This finding suggests that a cell-mediated cytotoxic mechanism is also involved in the pathogenesis of anti-Hu–associated PEM/PSN, and may explain the difficulties in creating an animal model of the disorder by passive transfer of anti-Hu IgG (Delattre and Posner, unpublished).[28] In preliminary experiments, animals immunized with the neuronal recombinant Hu protein develop antibodies with anti-Hu immunoreactivity but do not develop the disease (Fatallah, Smitt, unpublished data). Whether this is due to the recombinant Hu protein lacking putative "pathogenic epitopes" contained in the native Hu tumor antigen or that the animals do not develop a cell-mediated immune response is not known.

The present study suggests that anti-Hu–associated PEM/PSN is a complex immune disorder in which both humoral and cell-mediated mechanisms may play a role. ADCC (mediated by NK cells) and complement fixation by the predominant IgG1 isotype of anti-Hu are less likely to be involved in the pathogenesis of the disorder. Internalization of the anti-Hu IgG suggested by the IgG cell surface reactivity in neurons and the in vitro studies using tissue cultures may result in inhibition of a protein (Hu) which, by homology with other proteins (Elav), appears to have a crucial role in establishing and maintaining the neuronal phenotype. This process may then result in irreversible cell damage and neuronal death. In addition, the subtyping of the inflammatory infiltrates suggests a role of cell-mediated cytotoxicity in the pathogenesis of the disorder.

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