Reactive microglia in patients with senile dementia of the Alzheimer type are positive for the histocompatibility glycoprotein HLA-DR

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(Received 13 March 1987; Revised version received and accepted 1 April 1987)

Key words: Immune system; Alzheimer's disease; HLA-DR; Immunohistochemistry; Phagocytosis; Glial fibrillary acidic protein (GFAP); Cholinergic system

HLA-DR is a class II cell surface glycoprotein of the human histocompatibility complex usually expressed on the surface of cells that are simultaneously presenting foreign antigen to T-lymphocytes. Using immunohistochemical procedures with two specific monoclonal antibodies to HLA-DR, HLA-DR-positive reactive microglia were found in gray matter throughout the cortex of postmortem brains of patients with senile dementia of the Alzheimer type (SDAT) and were particularly concentrated in the areas of senile plaque formation. Double immunostaining with antibodies to glial fibrillary acidic protein (GFAP) showed that the HLA-DR-positive cells were different from the reactive astrocytes although the occasional positively staining giant astrocyte was also seen. Small numbers of resting microglia were HLA-DR-positive in white matter of both normal and SDAT brains. The SDAT cases also had reduced cortical choline acetyltransferase (ChAT) levels. In the 11 brains studied, the number of hippocampal HLA-DR-positive cells was positively correlated with the numbers of plaques and negatively correlated with average cortical ChAT.

HLA-DR is a class II cell surface glycoprotein of the human major histocompatibility complex. Class II or Ia (immunity associated) glycoproteins are associated with the immune response and are derived from genes on the short arm of chromosome 6. They are expressed on most B-lymphocytes, some T-lymphocytes, some macrophages, thymus epithelial cells, and selected cells of various body tissues [2]. Their classic function on the cell surface is to signal T-helper cells that foreign antigen is being simultaneously presented. The expression of Ia or HLA-DR by a cell does not necessarily indicate that this process is taking place; many reports exist of these surface proteins being detected in apparently non-pathological tissue. For example, in brain, HLA-DR has been reported on microglia in normal white matter and occasionally on astrocytes [3, 9]. Ia Expression has also been induced in glial cells in cul-
ture by lymphokine stimulation [11]. We confirm here the existence of HLA-DR on small numbers of resting microglia in normal white matter. We also report the presence of large numbers of intensely HLA-DR-positive phagocytosing macrophages (reactive microglia), particularly concentrated in the gray matter, in many areas of brains from patients with senile dementia of the Alzheimer type (SDAT). Double immunostaining for HLA-DR and glial fibrillary acidic protein (GFAP) shows that these antigens exist on separate cell populations. Thus, the reactive microglia are different from the reactive astrocytes which provide scar tissue following neuronal death in Alzheimer's disease and other neuropathological processes.

Six SDAT brains and 5 from non-neurological cases were obtained from 2–12 h after death. Seven small cortical samples were dissected from the left hemisphere for determination of choline acetyltransferase (ChAT) by previously reported methods [5]. The brains were then fixed for 2–3 days in 4% paraformaldehyde and prepared for immunohistochemistry by techniques which yield satisfactory immunohistochemical results on human tissue [6, 8]. Brains were soaked in a 15% sucrose solution in 0.1 M phosphate-buffered saline (PBS) for at least 2 weeks before sectioning on a freezing microtome at 30 μm thickness. Sections were rinsed for several hours with PBS containing 0.3% Triton X-100 before being stained immunohistochemically as free-floating sections. They were incubated with a primary antibody solution followed by appropriate Vectastain and ABC second antibody solutions at concentrations recommended by the supplier (Vector Labs.). They were then rinsed and incubated in a staining mixture containing 0.01% 3,3'-diaminobenzidine (DAB, Sigma), 0.6% nickel ammonium sulfate (Fisher), 0.05% imidazole and 0.0003% H2O2 [8]. A dark purple reaction product formed. For double immunostaining, the steps were repeated commencing at the primary antibody step, again utilizing an appropriate Vectastain and ABC second antibody system. The final staining step, however, utilized the standard mixture, consisting of 0.5% DAB and 0.008% H2O2 in 0.05 M Tris buffer, which gives a brown reaction product [6]. Sections were washed, mounted on glass slides, dehydrated with graded alcohols and, in some cases, counterstained with

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Fig. 1. A: SDAT hippocampus stained for HLA-DR showing many clusters of reactive microglia. B: adjacent section stained for plaques and tangles by the Bielschowsky silver method. Note the correspondence between HLA-DR-positive microglia clusters and senile plaques. Arrows in A and B point to corresponding areas. C: HLA-DR-positive staining in SDAT hippocampus, counterstained with Neutral red to reveal neurons and glia. D: HLA-DR-positive staining of a plaque in SDAT cingulate gyrus counterstained with Congo red. A collection of HLA-DR-positive cells surrounds the Congo red-positive amyloid core. E: HLA-DR-positive giant astrocyte in SDAT hippocampus. F: double immunostained SDAT cingulate gyrus. The initial antibody was anti-HLA-DR producing a purple stain followed by anti-GFAP antibody, producing a brown stain. Notice the purple staining cells showing typical morphology of reactive microglia while the brown staining cells show the typical morphology of reactive astrocytes. G, H: HLA-DR staining of hippocampal gray (G) and white (H) matter in control brain. Neutral red counterstaining. Notice in H the occasional small cell showing the typical morphology of a resting microglia. I: a section of SDAT hippocampus stained with anti-leu 4 mouse monoclonal antibody. No positive reaction is seen. Bars = 250 μm in A and B; 50 μm in C and E–I; and 20 μm in D.
standard dyes before coverslipping with Entellan (Merck). Double immunostained sections gave a deep purple color with the first primary antibody and a brown color with the second. Control staining was done by substituting for the primary antibody normal mouse or rabbit serum, monoclonal culture medium without cells, or a mouse monoclonal antibody indifferent to brain tissue (e.g. anti-leu 4, Becton Dickinson).

Two HLA-DR monoclonal antibodies were used: one from the HB-104 American Cell Type Culture line diluted 1:5000 (gift of Dr. F. Takei) and the other from a commercial supplier (Becton Dickinson) diluted 1:50. GFAP antibody (Dakopatts) was used at a dilution of 1:1000. Bielschowsky's silver, Congo red, or eosin staining was done by standard histochemical procedures on mounted sections [1].

Fig. 1A shows a typical lower power field of HLA-DR staining of SDAT brain tissue by the HB-104 antibody. Many swollen, reactive microglia intensely positive for HLA-DR are found throughout the gray matter. Fig. 1B shows an adjacent section stained by Bielschowsky's silver stain, displaying the typical tangles and plaques of Alzheimer's disease. Comparison of Fig. 1A and B illustrates the tendency of these reactive microglia to congregate in areas of plaques. Fig. 1C shows a high power field of some reactive microglia close to degenerating neurons which are revealed by Neutral red counterstaining. Fig. 1D is from a SDAT cingulate gyrus. It shows HLA-DR-positive cells surrounding the site of a neuritic plaque. Congo red counterstaining reveals the presence of amyloid in the core. In this case it is not possible to distinguish between tissue microglia and endothelial cells which also may show a positive HLA-DR reaction. Occasional positively reacting giant astrocytes are also seen (Fig. 1E).

Fig. 1F illustrates that the abnormal HLA-DR-positive phagocytosing cells in SDAT disease are different from the reactive astrocytes that replace neurons in degenerated brain tissue. The section is from a SDAT cingulate gyrus stained initially with the HLA-DR antibody (purple), followed by the GFAP antibody (brown). It can be seen that the brown colored reactive astrocytes have a different morphology and location than the purple HLA-DR-positive reactive microglia.

Fig. 1G and H show typical HLA-DR staining of hippocampal tissues from non-neurological cases. In gray matter (G), HLA-DR-positive glia are seldom seen. In white matter (H), a few cells having the morphology of resting microglia are usually seen. Again, Neutral red shows the presence of normal neurons and glia. Many areas of normal brains show no HLA-DR-positive cells in gray or white matter.

Fig. 1I is an example of many sections of SDAT cortex and hippocampus that were stained with control materials; all were negative. Similar, but weaker staining to that shown in Fig. 1A–H was obtained with the commercial HLA-DR monoclonal antibody.

Table 1 is a semiquantitative assessment of the extent of HLA-DR-positive staining in the hippocampus, comparing 6 SDAT and 5 non-neurological cases. The hippocampus was chosen because of its almost universal involvement in Alzheimer's disease. As can be seen from the table, in every case of SDAT, there was extensive HLA-DR-positive reactive microglia in the gray matter of hippocampal tissue; there was very little staining in the aged-matched non-neurological cases. The cortical ChAT
TABLE I
FREQUENCY OF HLA-DR-POSITIVE REACTIVE MICROGLIA AND SENILE PLAQUES IN SDAT AND CONTROL HIPPOCAMPUS AND CORTICAL ChAT

Average values for ChAT in nmol/100 mg protein·h in 7 samples (temporal tip, midtemporal gyrus, pre-central gyrus, postcentral gyrus, Broca’s area, occipital gyrus and frontal pole) of each brain were used to calculate these group means; in these 11 cases the average cortical ChAT levels correlated negatively both with numbers of plaques ($r = -0.76$) and the numbers of HLA-DR-positive cells ($r = -0.82$) counted in 5 high power fields of the hippocampus. Each high power field was 33 mm$^2$ and the section 30 μm thick. The correlation between plaques and HLA-DR-positive cells was positive ($r = 0.78$).

<table>
<thead>
<tr>
<th>Type</th>
<th>$n$</th>
<th>Age</th>
<th>No. of plaques</th>
<th>No. of HLA-DR-positive cells</th>
<th>Average cortical ChAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDAT</td>
<td>6</td>
<td>78 ± 3.0</td>
<td>102.7 ± 68.6</td>
<td>118.3 ± 40.0</td>
<td>0.27 ± 0.17</td>
</tr>
<tr>
<td>Controls</td>
<td>5</td>
<td>73 ± 8.7</td>
<td>8.2 ± 15.7</td>
<td>10.8 ± 7.1</td>
<td>0.77 ± 0.17</td>
</tr>
</tbody>
</table>

$P$ for group comparison: n.s. <0.02 <0.001 <0.001

levels showed the expected lower values in the SDAT cases. There was a strong correlation between the cortical ChAT deficit and the numbers of plaques and HLA-DR-positive cells.

These data illustrate two principles: the first is that active phagocytosis of dying neurons is taking place in SDAT cortex; the second is that there is an association of these phagocytosing cells with the class II glycoprotein, HLA-DR. The macrophages have the classical morphology of the reactive microglia originally described by del Rio Hortega and later by Penfield [7]. It is not known, however, whether these cells are stimulated from the resting state by some pathological process as suggested by these authors, or are derived at the time the pathology starts from peripheral monocytes, as suggested by more recent pulse thymidine labeling experiments [10], or some combination of the two. In any event, HLA-DR seems to be an excellent marker of these cells, at least in SDAT.

We have also observed such HLA-DR-positive reactive microglia in affected regions in Parkinson’s disease and Huntington’s chorea. Cells were observed phagocytosing substantia nigra pars compacta cells and neostriatal cells, respectively, in these two conditions [4]. On the basis of these results, we suggest that staining for HLA-DR may be a useful method to detect active pathological processes occurring in human brain at the time of death.

This research was supported by grants from the Medical Research Council of Canada and the Alzheimer’s Association of B.C.. We acknowledge the excellent technical assistance of Joane Suzuki. We are grateful for the generous gift of HLA-DR antibody from the HB-104 cell line from Dr. F. Takei of the University of B.C.


8 Tago, H., McGeer, P.L., Bruce, G. and Hersh, L.B., Distribution of cholineacetyltransferase-containing neurons of the hypothalamus, Brain Res., in press.

