Humoral and cellular immunity studies in patients with Vogt-Koyanagi-Harada syndrome and pars planitis

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We performed a comprehensive immunologic assessment of 17 patients with Vogt-Koyanagi-Harada syndrome (VKH) and nine with pars planitis (PP). Several variations in humoral and cellular immunity were found when the patients were compared with normal controls. Elevated serum IgD levels, positive results from various autoimmune profile assays, and deviations from normal levels of viral antibody titers were noted in both groups of patients. However, only some VKH patients had elevated β2-microglobulin, total complement level and E-active and EA rosette counts. HLA haplotype typing revealed no distinct correlation between patients when compared with a normal population.

Key words: immunoglobulin D, β2-microglobulin, total complement, C-reactive protein, rheumatoid factor, antigangliosides, virus antibody, rosettes, lymphocyte stimulation, histocompatibility antigens

The importance of immunologic mechanisms in the etiology of human uveitis has been supported by experimental models illustrating the alteration of the immune response in experimental allergic uveitis. Humoral immune reactions to uveal antigen in sympathic ophthalmia have been studied in humans.1-9 More recently, in vivo and in vitro cellular immunity tests have been used10-21 to investigate the agent responsible for the development of human uveitis.

The present study tested humoral and cellular immunity to assess the immunologic status of patients with Vogt-Koyanagi-Harada syndrome (VKH) and pars planitis (PP). Serum protein levels, autoimmune profiles, viral antibody titers, lymphocyte subpopulations, lymphocyte transformation, and histocompatibility antigens (HLAs) were investigated.

Various abnormalities in both humoral and cellular immune responses were found in our studied patients when compared with normal controls that were matched for race, age, and sex. Autoimmune mechanisms may be significantly involved in the pathogenesis of these diseases, as evidenced by the presence of autoimmune antibodies and the high titers of viral antibodies that may act as triggers of autoimmune mechanisms.

Materials and methods

Two peripheral blood samples were drawn from patients with VKH (17 cases) and PP (nine cases) at
The blood was drawn into heparinized tubes and with VKH and PP
Comparison of serum immunoglobulin levels (mean ± S.D.) in patients
Table I.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>IgG (mg/dl)</th>
<th>IgA (mg/dl)</th>
<th>IgM (mg/dl)</th>
<th>IgD (mg/dl)</th>
<th>IgE (IU/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VKH (17)</td>
<td>1070 ± 290</td>
<td>255 ± 93</td>
<td>109 ± 49</td>
<td>6.1 ± 1.5*</td>
<td>129 ± 168</td>
</tr>
<tr>
<td>PP (9)</td>
<td>1015 ± 222</td>
<td>160 ± 69</td>
<td>121 ± 32</td>
<td>6.1 ± 1.4*</td>
<td>216 ± 220</td>
</tr>
<tr>
<td>Controls (20)</td>
<td>1180 ± 271</td>
<td>197 ± 46</td>
<td>108 ± 42</td>
<td>3.3 ± 0.5</td>
<td>236 ± 140</td>
</tr>
</tbody>
</table>

*Significant at p < 0.01.

the Uveitis Clinic and from 20 normal healthy donors at the Blood Bank, University of Illinois Hospital. The serum sample was obtained from clotted (at 4°C) blood and used for quantitation of serum proteins and determination of antibodies. The blood was drawn into heparinized tubes and used for lymphocyte tests, including HLA typing.

Serum levels (mg/dl) of IgG, IgM, IgD, α2-macroglobulin (α2M), and α1-antitrypsin (α1AT) were determined by radial immunodiffusion (RID). IgE (International Unit) and β2-microglobulin (β2m; μg/ml) were measured by radioimmunoassay. Total serum complement (CH50) levels were measured by the complement fixation method of Kent and Fife after serum was separated from clotted blood at 4°C. C-reactive protein (CRP) was determined by the capillary tube method.

Rheumatoid factor (RF) was determined by a latex agglutination test; assay for antinuclear antibody (ANA), antidualle strand DNA, (AdDNA), smooth muscle antibody (SMA), and antithyroid antibody (ATA) were done by indirect immunofluorescent technique with tissue substrates. Antiganglioside antibody (AGs) was determined by a passive hemagglutination test as described by Yokoyama et al. Antibody titers to the following viruses were determined by complement fixation methods: mumps, cytomegalovirus (CMV), herpes simplex virus (HSV), adenovirus, Mycoplasma, lymphogranuloma venereum (LGV), varicella zoster (VZ), and measles. Antibody titers to Epstein Barr virus (EBV) were determined by immunofluorescent techniques.

For the enumeration of lymphocyte subpopulations, lymphocytes were separated from the heparinized blood by density gradient centrifugation. Monocytes were removed by mixing with silica gel solution. The cell count was adjusted to 3.0 x 10^9/ml in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (PBS-BSA) for the rosette test.

E rosette assays for T lymphocyte counts were done with sheep red blood cells (SRBCs) that were resuspended in PBS-BSA to provide a 0.5% suspension. An equal volume (0.1 ml) of lymphocyte and SRBC suspension were mixed. One tube of the mixture was used for the determination of the percentage of “active” T lymphocytes. The other tube was used for “total” T lymphocyte count. Total T lymphocytes were also determined by a rosette test with neuraminidase-treated SRBC.

EA and EAC rosette assays were carried out for B lymphocyte counts. Ox red blood cells (ORBCs) were used for sensitization with a predetermined subagglutinating dose of rabbit anti-ORBC IgG serum. The lymphocyte suspension was mixed with an equal volume (0.1 ml) of the sensitized ORBC suspension, and the rosette-forming cells (RFC) were counted. The EAC rosette tests were also done in the same manner as the EA assay. However, in this case SRBCs were sensitized with an appropriate dilution of rabbit anti-SRBC IgM serum, and the sensitized cells were further mixed with a predetermined dose of human serum complement. The percentage of RFC was also designated as B lymphocyte count.

For lymphocyte stimulation tests, lymphocytes were isolated in the same manner as described, except that sterile conditions were used for all steps in this test. The washed lymphocytes were resuspended in RPMI-1640 medium with 100 µg/ml penicillin, 100 µg/ml streptomycin, and 10% fetal calf serum; the cell count was adjusted to 1.5 x 10^6 cells/ml. The microtiter plate assay was performed with phytohemagglutinin (PHA) and pokeweed mitogen (PWM) in dilutions of 1:100 and 1:1000. All cultures were tested in triplicate. The cultured cells were labeled at 72 hr with 1 µCi/ml tritiated thymidine. After 3 more hours of incubation, the lymphocytes were collected on filter paper, and radioactivity was measured in a liquid scintillation counter. The degree of lymphocyte transformation was measured as counts per minute of tritiated thymidine incorporated into the DNA of the proliferating cells. The maximum responses of the patients' lymphocyte to each PHA and PWM were compared to those of the control. Stimulation index (SI) was the mean counts per minute taken from triplicates of each mitogen stimulation divided by the mean counts.
Table II. Results of $\alpha_1$AT, $\alpha_2$M, $\beta_2$m, CH$_{50}$ levels (mean ± S.D.) in patients with VKH and PP

<table>
<thead>
<tr>
<th>Subjects</th>
<th>$\alpha_1$AT (mg/dl)</th>
<th>$\alpha_2$M (mg/dl)</th>
<th>$\beta_2$m ($\mu$g/ml)</th>
<th>CH$_{50}$ (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VKH (17)</td>
<td>260.1 ± 53.7</td>
<td>290.2 ± 66.6</td>
<td>5.4 ± 7.1*</td>
<td>229 ± 75*</td>
</tr>
<tr>
<td>PP (9)</td>
<td>287.4 ± 108.1</td>
<td>296.5 ± 108.1</td>
<td>2.1 ± 1.0</td>
<td>176 ± 47</td>
</tr>
<tr>
<td>Controls (20)</td>
<td>267.8 ± 59.0</td>
<td>267.8 ± 59.0</td>
<td>1.5 ± 0.6</td>
<td>183 ± 45</td>
</tr>
</tbody>
</table>

*Significant at p < 0.05.

Table III. Serum levels of CRP and autoantibodies in patients with VKH and PP

<table>
<thead>
<tr>
<th>Subjects</th>
<th>CRP</th>
<th>RF</th>
<th>ACgr</th>
<th>ANA</th>
<th>AdDNA</th>
<th>ATA</th>
<th>SMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>VKH (17)</td>
<td>3 (18)*</td>
<td>4 (24)</td>
<td>12 (71)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PP (9)</td>
<td>1 (11)</td>
<td>1 (11)</td>
<td>4 (44)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Controls (20)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate percent positive.

Table IV. Positive antibody titers against viruses in sera of patients with VKH and PP

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mumps</th>
<th>CMV</th>
<th>HSV</th>
<th>Adenovirus</th>
<th>Mycoplasma</th>
<th>LGV</th>
<th>VZ</th>
<th>EBV</th>
<th>Measles</th>
</tr>
</thead>
<tbody>
<tr>
<td>VKH (17)</td>
<td>2/15* (13.3)</td>
<td>4/16</td>
<td>2/16</td>
<td>0/16</td>
<td>0/16</td>
<td>4/16</td>
<td>0/16</td>
<td>1/15</td>
<td>2/15</td>
</tr>
<tr>
<td>PP (9)</td>
<td>2/7 (25.0)</td>
<td>0/6</td>
<td>0/6</td>
<td>1/8</td>
<td>0/8</td>
<td>2/8</td>
<td>1/8</td>
<td>1/7</td>
<td>1/7</td>
</tr>
</tbody>
</table>

*Antibody titers equal or greater than 1:8 for mumps, 1:80 for EBV, and 1:64 for other viruses suggest number of serum samples positive per number tested.

Results

Except for IgD, all immunoglobulin levels in both patient groups were within normal limits when the results were compared with those of the control group (Table I). Serum levels of $\alpha_1$AT and $\alpha_2$M were also found to be normal in comparison with the control values (Table II). A significant elevation of $\beta_2$m and CH$_{50}$ levels were found in the VKH group, whereas those of the PP group were within normal values. Positive CRP was found in 18% of VKH and 11% of PP patients. In autoantibody test RF was present in 24% of VKH and 44% of PP patients. AGgs's were detected in 71% of VKH and 44% of PP patients. There were no ANAs, AdDNAs, ATAs, and SMAs detected in either test group (Table III). All control sera were negative for all the above-mentioned antibody tests.

Positive viral titers were found in the VKH group for mumps, CMV, HSV, LGV, measles, and EBV. Positive titers were demonstrated for mumps, adenovirus, LGV, VZ, measles, and EBV for PP patients (Table IV).

As shown in Table V active T lymphocyte counts of the VKH group were found to be significantly higher than those of the PP and control groups. However, the total T lymphocyte counts were normal in both patient groups as compared with the controls. The EA rosette results showed significantly elevated values in the VKH group. The results of the EAC rosette count did not reveal a statistical difference among groups.

Lymphocytes were stimulated with PHA and PWM mitogens at two different dilutions. No significant difference in stimulation...
Table V. Comparison of percentage of T and B lymphocyte subpopulations in patients with VKH and PP

<table>
<thead>
<tr>
<th>Subjects</th>
<th>T lymphocytes</th>
<th>B lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active</td>
<td>Total</td>
</tr>
<tr>
<td>VKH (17)</td>
<td>32.8 ± 9.1*</td>
<td>56.2 ± 7.3</td>
</tr>
<tr>
<td>PP (9)</td>
<td>28.7 ± 8.6</td>
<td>57.0 ± 7.4</td>
</tr>
<tr>
<td>Controls</td>
<td>24.1 ± 8.1</td>
<td>50.8 ± 10.2</td>
</tr>
</tbody>
</table>

*Significant vs. controls at p < 0.001.
1 Number in parentheses indicate total T lymphocyte counts with neuraminidase-treated SRBC.

Table VI. Results of lymphocyte stimulation tests expressed with SI of patients with VKH and PP

<table>
<thead>
<tr>
<th>Subjects</th>
<th>PHA (1:100)</th>
<th>PHA (1:1000)</th>
<th>PWM (1:100)</th>
<th>PWM (1:1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VKH (17)</td>
<td>99.3 ± 62.6</td>
<td>93.7 ± 52.9</td>
<td>26.9 ± 20.5</td>
<td>37.9 ± 30.0</td>
</tr>
<tr>
<td>PP (9)</td>
<td>140.2 ± 91.0</td>
<td>113.8 ± 66.6</td>
<td>37.4 ± 22.2</td>
<td>35.9 ± 19.4</td>
</tr>
<tr>
<td>Controls</td>
<td>96.1 ± 43.2</td>
<td>88.0 ± 38.1</td>
<td>24.7 ± 17.5</td>
<td>43.9 ± 24.7</td>
</tr>
</tbody>
</table>

values between the disease and control group was found (Table VI).

HLA antigens for 14 VKH patients tested are listed in Table VII. In the A locus, A1, A2, A3, A9, A28, Aw30, and Aw31 were frequently found, whereas the most frequent B locus antigens were B7, B8, B12, and Bw35. However, no significant association of the HLA antigens with the disease was found.

Discussion

The results of the present study showed elevated IgD levels in the patient groups when the results were compared with those of the normal group; other immunoglobulin levels were within normal limits. Our results of serum IgD levels of normal healthy subjects were in agreement with previously published reports. Rowe and Fahey reported a medium level of 3.0 mg/dl with an RID method, and Bachman with a single-diffusion technique reported a level of 3.1 ml/dl. The exact role of IgD is not fully understood, but the elevated values may be indicative of the stage of the disease. In particular, during relapses the present results seem to indicate some integral involvement.

Gangliosides are a class of glycosphingolipids. Yokoyama et al. initially demonstrated that gangliosides have antigenic properties and can elicit an antibody response in the host. AGgs was detected in the serum of patients with neuropsychiatric diseases, including multiple sclerosis, amyotrophic lateral sclerosis, kuru, and schizophrenia. We postulated that the presence of AGgs in VKH and PP patients was due to the
magnitude of the role of the autoimmune phenomena in their diseased state. Our study showed this antibody to be present in 71% of the VKH and 44% of the PP patients but not in any of the normal controls. Shimizu and Inaba and Aoyama reported that the serum samples from patients with neuro-Behçet's disease contained antibody against myeline basic protein (demyelinating antibody) and galactocerebroside. However, it is doubtful that this antibody causes the disease. It may, however, represent a response to damaged neural tissue as seen in systemic slow viral infections as opposed to localized ocular disease.

The presence of CRP and RF in some of the VKH and PP patients represent the immunologic spectrum. CRP is an acute-phase protein that is found in acute inflammatory states. On the other hand, RF is predominant in the serum of patients with chronic recurrent and systemic inflammatory disease. The appearance of acute- and chronic-phase proteins may suggest a variable multiple immunologic involvement in VKH and PP patients. Because the other autoimmune profile tests were negative, a systemic autoimmune phenomenon was ruled out. Although the etiology of uveitis remains unknown, viruses, bacteria, and fungi have been implicated. It might also be possible to assume that the inflammation is caused by a slow virus infection of chronic duration that appears with the syndrome without prior clinical symptoms.

Initially we were impressed with the history of early, severe measles infections in some of the PP patients. Antibody titers in sera of most of the patients were within normal limits; a few VKH and PP patients showed higher than normal titers to some viruses, e.g., mumps, CMV, HSV, and LGV. Through the observation of individual cases, high antibody titers against CMV and HSV were predominantly found in VKH patients. It might be assumed that these patients were hyperreactive to the two viruses in contrast to PP patients who were reactive. Because all specimens were handled in an identical manner, this difference suggests a difference in the age and socioeconomic class of the two groups of patients. The PP patients were younger and from a higher socioeconomic class than the VKH patients. Weber et al. reported that a few uveitis patients specifically were hyperreactive for adenovirus, measles, mumps, and parainfluenza 1 and 3 but had no reaction to HSV and CMV.

SRBC RFC are regarded largely as representing T lymphocytes, which play an active role in cell-mediated immunity. In this study the VKH group revealed a significant elevation of active T lymphocyte counts, whereas the total counts were within normal limits. An increase in active T lymphocyte counts was reported to be associated with lymphoma and Hodgkin's disease, and the results may suggest a clinical linkage between VKH and lymphoproliferative disorders.

B lymphocytes were enumerated by EA and EAC rosette formation via the Fc and complement receptors present in the surface membrane of the cells. The elevated EA rosette values we found in our VKH patient group might be attributed to an increase in the percentage of T lymphocytes possessing receptors for the Fc portion of IgG called suppressor T lymphocytes. This finding supports the theory that the process of autoimmunity is based on an immunologic imbalance of the regulatory mechanism of T lymphocytes.

Mitogenic transformation of lymphocytes with PHA and PWM was normal in our patients with VKH and PP when the SI was compared with that of the control group. However, Hammer has demonstrated transformation in response to uveal pigments in patients with sympathetic ophthalmitis and VKH. Wong et al. also reported enhanced lymphocytic transformation in the presence of homologous uveal-retinal extract. In their study patients with the higher degree of transformation manifested the least clinical evidence of active disease. Marak reported increased blastogenic transformation to normal ocular antigen. A specific cell-mediated immune response of the patients to antigens with or without antibody-mediated immune responses could certainly be considered as factors in this disease.

The HLA antigens for the A and B loci did
not reveal any significant patterns in association with the diseases evaluated in this study. The incidence of these antigens closely parallels that obtained by Mittal\textsuperscript{34} in healthy black and white subjects from the Chicago area.

REFERENCES