Immunologic Analysis of Cerebrospinal Fluid Lymphocytes in Vogt-Koyanagi-Harada Disease

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In this study, we focused upon the immunologic aspects of Vogt-Koyanagi-Harada disease (VKH) by comparing the cytotoxic activity of peripheral blood leukocytes (PBL) to that of cerebrospinal fluid leukocytes (CSFL) against the human melanoma cell line (P-36) and the human cervical carcinoma cell line (HeLa-S3). The PBL from patients with VKH showed significant cytotoxic activity against P-36 \((P < 0.01)\), but did not show cytotoxic activity against HeLa-S3. The CSFL showed significantly weaker cytotoxic activity against P-36 compared to that of PBL \((P < 0.02)\). We also analyzed the cell membrane surface markers applying monoclonal antibodies on PBL and CSFL. The percentage of OKT8\(^+\) (CD8: T cytotoxic/suppressor lymphocytes) cells was significantly lower in CSFL than in PBL \((P < 0.05)\). There was a tendency toward a higher percentage of HLA-DR\(^+\) cells (B lymphocytes, monocytes, macrophages, and activated T lymphocytes) and a higher ratio of OKT4\(^+\)/8\(^+\) cells (CD4/CD8: T helper/inducer lymphocytes / T cytotoxic/suppressor lymphocytes) in CSFL from patients with VKH than in their PBL \((P < 0.1)\). Invest Ophthalmol Vis Sci 31:1210-1216, 1990

Vogt-Koyanagi-Harada disease (VKH) is characterized by bilateral uveitis, meningitis, alopecia, perceptive deafness, and vitiligo. VKH is believed to be a systemic disorder affecting various organs containing melanocytes.\(^1\) It is presumed that cellular immune responses to self (or modified-self) systemic melanocytes play a key role in determining the immunopathology of this disease,\(^2\) as they do in other autoimmune diseases such as Hashimoto's disease.

Meningitis or pleocytosis in the cerebrospinal fluid (CSF) almost always occurs during the acute phase of the disease;\(^1\) however, the implication of this pleocytosis is not known. Many immunologic studies of peripheral blood leukocytes (PBL) in patients with VKH have been performed.\(^2\)-\(^7\) But, our knowledge of the cellular immune response of CSF leukocytes (CSFL) in patients with VKH is more limited.\(^8\)-\(^11\) The precise role of the immune response in the pathogenesis of VKH within the central nervous system (CNS) remains in question.

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We identified and compared the leukocyte subpopulations in PBL and CSFL, and compared their cytotoxicity against the human melanoma cell line (P-36), in patients with VKH; our goal in this was to observe how systemic events are manifested in CSFL and the degree to which localized processes are isolated from, or reflected in, the systemic circulation in this disease. We also compared the cell membrane surface markers on CSFL with those of PBL.

Materials and Methods

Subjects

From 1986 through 1988, nine patients (cases 1–9) with VKH, including three men and six women, from Matsumoto and its surroundings were studied. The patients were 18–62 yr of age, with a mean of 34.1 yr. They were admitted to the Department of Ophthalmology, Shinshu University School of Medicine with complaints of blurred vision, central scotoma, metamorphopsia, photophobia, severe headache, pain in the deep orbit, and slight fever. The diagnosis was confirmed on the basis of the typical clinical symptoms and findings: bilateral panuveitis with exudative retinal detachment, associated with polyopia, dysacusia, alopecia, vitiligo, cerebrospinal fluid pleocytosis, and meningeal signs. CSF was obtained by spinal tap from the patients during acute phases of their illnesses. A PBL specimen also was obtained from each patient on the same day the spinal tap was performed. All CSF and peripheral blood (PB) samples except in case 5 were taken before ste-
Isolation of PBL and CSFL

The PBL were isolated from heparinized venous blood by Ficoll-Conray density centrifugation as described in detail elsewhere. The CSFL were centrifuged at 250 g for 5 min and placed on ice. They were washed three times with Hanks' medium and then resuspended in RPMI 1640 (GIBCO, Grand Island NY) medium supplemented with 10% fetal calf serum (FCS; GIBCO) and kanamycin sulfate (400 μg/ml).

Target Cells

In the assay for cytotoxicity, the following cell lines were used: human melanoma cell line (P-36; SK-MEL-28) originally established at the Memorial Sloan-Kettering Cancer Center (New York, NY); the human cervical carcinoma cell line (HeLa-S3); and K562 cell line. All of the cell lines were maintained in RPMI 1640 medium supplemented with 10% FCS at 37°C in an atmosphere with 5% CO2 and 100% humidity.

Assay for Cytotoxicity against P-36 and HeLa-S3

Cytotoxicity against P-36 and HeLa-S3 was assayed in cases 1 through 5. The details of the assay method have been described elsewhere. In brief, 5 x 10^6 target cells in 0.5 ml RPMI 1640 medium supplemented with 10% FCS were incubated with 50 μCi ^51Cr (sodium chromate, spec. act. 445.39 mCi/mg; NEN Products, MA), for 45 min at 37°C with occasional gentle shaking. For cytotoxicity assay of PBL, 5 x 10^4 labeled target cells in 0.2 ml were placed in 96-well round-bottom microtiter plates (Nunclon, Roskilde, Denmark) at an effector/target (E/T) ratio of 10:1 and 20:1. After centrifugation for 2 min, the cells were incubated for 4 hr at 37°C in a 5% CO2 incubator. The supernatants were harvested, and the radioactivity was determined by the trypan blue dye exclusion method. The PBL from 6 age- and sex-matched controls were analyzed in a similar manner.

Assay for Natural Killer (NK) Cell Activity

NK cell activity of PBL was assayed in all cases except in case 5. NK cell activity was determined in a standard 4-hr ^51Cr-specific release assay by using K562 target cells as described previously. Briefly, effector cells in complete medium were added to triplicate cultures of 1 x 10^4 ^51Cr-labeled K562 cells in 0.2-ml vol in V-bottomed microtiter plates (Nunclon) at an E/T ratio of 10:1 and 20:1. After centrifugation at 25 g for 8 min, the cells were incubated for 4 hr at 37°C in a 5% CO2 incubator. The supernatants were harvested, and the radioactivity was determined with the use of a gamma counter. The cytotoxicity was calculated by the above same formula.

The PBL from 50 age- and sex-matched controls were analyzed in a similar manner. NK cell activity of CSFL was not assayed because of limited cell numbers.

Distribution of the Cell Population

The distribution of leukocyte subsets in PB and CSF from five patients (case 1 and cases 6-9) was examined by cytofluorometric analysis with fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies. We used monoclonal antibodies to the surface antigens of human leukocytes using T11 (CD2: lymphocytes that form rosettes with sheep red blood cells); OKT3 (CD3: pan T lymphocytes); OKT4 (CD4: T helper/inducer lymphocytes); OKT8 (CD8: T cytotoxic-suppressor lymphocytes); B1 (CD20: B lymphocytes); and HLA-DR (B lymphocytes, monocytes, macrophages and activated T lymphocytes) to determine the distribution of leukocytes in PB and CSF from patients with VKH.

The distribution of Leu 11a + (CD16: NK cells) cells in PB was examined in all cases except in cases 6 through 8, but was not examined in CSF except in case 9.

The PBL from 50 age- and sex-matched controls were analyzed in a similar manner.

T11 and B1 monoclonal antibodies were purchased from Coulter Immunology (Hialeah, FL). OKT3, OKT4, and OKT8 monoclonal antibodies were purchased from Ortho Diagnostic Systems (Raritan, NJ). HLA-DR and Leu 11a monoclonal antibodies were purchased from Becton-Dickinson (Sunnyvale, CA).
Table 1. Clinical and CSF findings

| Case | Age (yr) | Sex | Iritis | DE | RD | Sun | Del | Alo | Vit | Men | Deaf | Ophthalmic findings | General findings | Pressure (mm H2O) | Cells (per mm³) | IgG (mg/dl) | Protein (mg/dl) | Sugar (mg/dl) | Days |
|------|---------|-----|--------|-----|----|-----|-----|-----|-----|-----|------|--------------------|---------------|----------------|--------------|-------------|--------------|--------------|------------|-----|
| 1    | 32      | M   | ++     | +   | ++ | +   | +   | +   | +   | +   | +    |                   |               | 70            | 12.7         | 138         | 44           | 23          |        |
| 2    | 6       | F   | +      | +   | +  | +   | +   | +   | +   | +   | +    |                   |               | 70            | 3.9          | 31          | 63           | 27          |        |
| 3    | 48      | F   | +      | +   | +  | +   | +   | +   | +   | +   | +    |                   |               | 210           | 58           | 8.9         | 52           | 57          | 12         |
| 4    | 18      | F   | +      | +   | +  | +   | +   | +   | +   | +   | +    |                   |               | 80            | 33           | 5.3         | 40           | 56          | 18         |
| 5    | 18      | F   | +      | +   | +  | +   | +   | +   | +   | +   | +    |                   |               | 170           | 20           | 2.3         | 45           | 68          | 39         |
| 6    | 29      | M   | +      | +   | +  | +   | +   | +   | +   | +   | +    |                   |               | NT            | 241          | 23.1        | 188          | 45          | 17         |
| 7    | 40      | F   | +      | +   | +  | +   | +   | +   | +   | +   | +    |                   |               | 130           | 24           | 1.1         | 7            | 57          | 43         |
| 8    | 24      | M   | +      | +   | +  | +   | +   | +   | +   | +   | +    |                   |               | 210           | 13           | 2.3         | 19           | 64          | 7          |
| 9    | 36      | F   | +      | +   | +  | +   | +   | +   | +   | +   | +    |                   |               | 105           | 70           | 6.3         | 41           | 74          | 23         |
| Mean | 34.1    |     |        |     |    |     |     |     |     |     |      |                   |               | 156           | 132          | 7.3         | 62           | 59          | 23         |

Normal value

Mean 70–180

<table>
<thead>
<tr>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5</td>
</tr>
<tr>
<td>1–3</td>
</tr>
<tr>
<td>10–40</td>
</tr>
<tr>
<td>50–75</td>
</tr>
</tbody>
</table>

DE, optic disc edema; RD, retinal detachment; Sun, sunset glow fundus; NT, not tested.

HLA phenotypes: case 1: A24, Bw52, B44, DR2, DRw6, Dw1; case 2: A2, A24, Bw61, B51, Cw3, DR4, Dw3; case 3: A24, Bw54, B60, Cw1, DR2, DR4, DQw3; case 4: A24, A30, Bw54, Cw1, DR2, DQ, Dw3; case 5: A24, B13, Cw1, Cw3, DR4, Dw3; case 6: A24, Bw54, Cw1, DR4, Dw3; case 7: A11, A24, Bw55, Cw1, Cw3, DR4, Dw3; case 8: A26, A31, Bw56, Cw1, Cw4, DR4, Dw3; case 9: A24, Bw55, Cw1, DR4, DQw1, Dw3.

Statistical Methods

Statistical analysis was performed with the paired nonpaired t-test.

Informed consent was obtained after the nature of the procedure had been fully explained.

Results

Clinical and Laboratory Findings (Table 1)

At an acute phase, the range of pressure in the CSF of the eight cases (case 6 was not tested) was 80–210 mm H₂O, with a mean of 156 mm H₂O. The mean of the CSF cell counts in nine cases was 132 per mm³. In the CSF cytogram, the average percentage of lymphocytes was 97.3%. Protein content in the CSF was elevated in five of the nine cases, and was decreased in one of the nine cases, whereas IgG levels were increased in six of the nine cases. Sugar content in the CSF was decreased in two of the nine cases.

Cytotoxic Activity of PBL and CSFL against P-36 and HeLa-S3 (Table 2)

The PBL from patients with VKH showed significantly stronger cytotoxic activity against P-36 than the CSFL cell counts in nine cases was 132 per mm³. In the CSF cytogram, the average percentage of lymphocytes was 97.3%. Protein content in the CSF was elevated in five of the nine cases, and was decreased in one of the nine cases, whereas IgG levels were increased in six of the nine cases. Sugar content in the CSF was decreased in two of the nine cases.

Table 2. Cytotoxic activity of PBL and CSFL against P-36 and HeLa-S3 from patients with VKH disease (% specific ⁵¹Cr release*)

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Case</th>
<th>P-36</th>
<th>HeLa-S3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10:1</td>
<td>50:1</td>
</tr>
<tr>
<td>Patients' PBL†</td>
<td>1</td>
<td>23.3 ± 0.9</td>
<td>63.8 ± 5.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25.0 ± 0.4</td>
<td>61.6 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>27.2 ± 0.5</td>
<td>43.7 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>35.8 ± 4.2</td>
<td>60.8 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>8.1 ± 1.6</td>
<td>17.0 ± 2.1</td>
</tr>
<tr>
<td>Control PBL (n = 6)</td>
<td></td>
<td>8.2 ± 2.2</td>
<td>8.5 ± 1.0</td>
</tr>
<tr>
<td>Patients' CSFL‡‡</td>
<td>1</td>
<td>5.4 ± 0.3</td>
<td>7.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.3 ± 0.5</td>
<td>12.1 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10.8 ± 1.7</td>
<td>21.7 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.1 ± 2.0</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.4 ± 0.2</td>
<td>NT</td>
</tr>
</tbody>
</table>

* Percent specific ⁵¹Cr release is expressed as mean ± SD.
† Cytotoxic activity of PBL from patients with VKH against P-36 was significantly higher than that of control PBL (P < 0.01 at 10:1 E/T ratio and P < 0.001 at 50:1 E/T ratio by nonpaired t-tests). Significance of cytotoxic activity of PBL from patients with VKH against P-36 was P < 0.01 at both 10:1 and 50:1 E/T ratios by paired t-tests, when compared with cytotoxic activity against HeLa-S3.
‡ Significance of cytotoxic activity of CSFL from patients with VKH against P-36 was P < 0.01 at 10:1 E/T ratio by paired t-tests, when compared with cytotoxic activity of PBL. There was a tendency toward a weaker cytotoxic activity of CSFL from patients with VKH against P-36 than that of PBL at 50:1 E/T ratios.
§ Duplicate.
¶ One well.
NT: not tested.
against HeLa-S3 ($P < 0.01$ at both 10:1 and 50:1 E/T ratios). HeLa-S3 cells were used as a negative control, as in a previous study.7 Case 5, whose PBL showed low cytotoxicity against P-36, suffered from severe iritis with fibrin. Her iritis recurred over 6 months. This case is discussed later.

On the other hand, CSFL from patients with VKH showed cytotoxic activity against P-36 that was significantly weaker than that of PBL ($P < 0.02$ at 10:1 E/T ratio). CSFL from case 3, which showed weak cytotoxic activity against P-36, was obtained after only 12 days from the onset of the disease at a much earlier stage.

PBL from six controls showed no cytotoxic activity against P-36 and HeLa-S3.

**NK Cell Activity (Fig. 1)**

NK cell activity of PBL from cases 1 through 4 with VKH was 6.9, 18.7, 8.1, and 19.3% cytotoxicity at 10:1 E/T ratio (controls: 8.9–29.5%). NK cell activity of PBL from eight cases (case 5 was not tested) with VKH was 9.7, 34.5, 11.4, 39.0, 67.0, 23.0, 65.0, and 44.0% cytotoxicity, respectively, at 20:1 E/T ratio (controls: 17.1–48.5%). There was no significant NK cell activity difference between patients and normal controls.

**Distribution of Leukocytes (Fig. 2)**

The percentages of leukocyte subsets were analyzed as follows. The values for T11+ (CD2), OKT3+ (CD3), OKT4+ (CD4), OKT8+ (CD8), B1+ (CD20), and HLA-DR+ cells in PBL and CSFL were, respectively, 79.0 ± 7.7, 66.4 ± 13.0, 41.0 ± 8.8, 32.8 ± 3.1, 5.8 ± 1.9, 10.2 ± 4.9, and 79.8 ± 15.5, 70.2 ± 14.7, 44.6 ± 9.8, 22.4 ± 5.2, 6.6 ± 2.7, and 18.6 ± 11.1%. In PBL, the distribution of leukocytes was in the range of healthy controls. The percentage of OKT8+ (CD8) cells was significantly lower in CSFL than in PBL ($P < 0.05$). There was a tendency toward a higher percentage of HLA-DR+ cells and a higher ratio of OKT4/8+ cells in CSFL than in their PBL ($P < 0.01$) (Fig. 3).

The percentages of Leu 11a+ (CD16) cells in PBL from all cases except in cases 6 through 8 were 4.1, 14.8, 9.0, 20.2, 5.2, and 17.0% (controls: 3.9–23.5%) respectively. There was no significant difference between the patients and controls. The percentage of Leu 11a+ (CD16) cells in CSFL from case 9 was 1%.

**Discussion**

The CSF represents a dynamic and accessible source of information about events occurring within the CNS. The investigation of human immune responses in anatomically compartmentalized sites such as the CNS offers a unique opportunity to learn about both the immune function outside of peripheral circulation and the pathogenesis of diseases that involve such compartments. Patients with VKH often have suffered from mild cases of meningitis.1 It is very important, therefore, to clarify the immunopathologic mechanism of CSFL for better understanding of the meningitis associated with VKH.

Recently, information has been available indicating that NK cells may be involved in the pathogenesis of autoimmune disease.14,15 In the current study, PBL from patients with VKH showed significantly strong cytotoxic activity against P-36. The cell population producing specific cytotoxic activity against P-36 belongs mainly in the T cell category.7 There is a possibility, however, that those responding to P-36 are derived not only from cytotoxic T cells, but also from the NK cells. The NK cells may nonspecifically kill the P-36. However, our results revealed that there was no significant NK cell activity of PBL from patients with VKH. Thus, cytotoxic T lymphocytes that were antigen-specifically induced may play a more important role in pathogenesis of VKH than NK cells that mediate certain "nonspecific" cytotoxicity.

On the other hand, CSFL from patients with VKH showed cytotoxic activity against P-36 that was significantly weaker than that of PBL. Moreover, the sur-
Fig. 2. Leukocyte subsets in PB and CSF from patients with VKH disease. CD, cluster designation. Open triangles, case 1; open squares, case 6; circles, case 7; filled triangles, case 8; filled squares, case 9. The stippled area represents the range of normal controls (n = 50). The percentage of OKT8+ cells was significantly lower in CSFL than in PBL (P < 0.05). There was a tendency toward a higher percentage of HLA-DR+ cells in CSFL than in PBL (P < 0.1). NS, not significant.

Fig. 3. OKT4+/8+ ratio of PBL and CSFL from patients with VKH disease. The bar shows the mean ± SD. Open triangles, case 1; open squares, case 6; circles, case 7; filled triangles, case 8; filled squares, case 9. The stippled area represents the range of normal controls (n = 50). There was a tendency toward a higher ratio of OKT4+/8+ cells in CSFL than in PBL (P < 0.1).

Face markers of CSFL were different from those of PBL. Kato and co-workers have shown that the serum interferon-γ (IFN-γ) titer was significantly elevated, whereas no IFN-γ could be detected in the CSF of the patients with VKH in spite of severe meningeal inflammation. Furthermore, almost all patients' CSF show a moderate increase in the number of cells, predominantly lymphocytes, not polymorphonuclear leukocytes. From these viewpoints, the blood-brain barrier may not be damaged so severely as to permit the entrance of serum IFN-γ and polymorphonuclear leukocytes into the CSF in VKH.

Assuming that the CSF pleocytosis is of hematogenous origin, we may answer the essential questions of why CSFL showed weaker or negative cytotoxic activity against P-36 in vitro than did PBL, and why the differences have been observed in composition between cells isolated from the CSF and from the PB, as follows.

Monoclonal antibodies against various specific cell surface antigens distinguishing leukocyte cell types (eg, T cells, B cells, monocytes, NK cells) including human T cell subsets have been well established.
In some immunologically mediated diseases, such as rheumatoid arthritis and primary Sjögren’s syndrome, activated T cells have been seen to increase markedly at the site of inflammation. For example, in rheumatoid arthritis, significantly more activated T cells are present in the synovium than in the blood. Differences have been observed in the composition of T cell subsets between cells isolated from the CSF and from PB of patients with neurologic diseases. In mumps meningitis, the ratio of OKT4+/8+ cells in CSF has been observed to be significantly lower than that in PBL, showing that suppressor/cytotoxic T cells had selectively accumulated in CSF and were highly antigen-specific. In multiple sclerosis (MS), selective migration of T8+ (CD8) cells to CSF, migration of T8+ (CD8) cells at an earlier time (when peripheral blood T8+ (CD8) cells were normal), or differences in the life span of T4+ (CD4) and T8+ (CD8) cells within CSF, have been considered possible explanations. Differences in leukocyte subsets from CSFL and PBL have been found, even from patients with VKH. Some reports have shown that OKT4+ (CD4) and OKT11+ (CD2) cells were significantly higher, and that OKT8+ (CD8) and Bl+ (CD20) cells were significantly lower in CSFL than in PBL from patients with VKH. In our study, there were significantly fewer OKT8+ (CD8) cells in CSFL than in PBL. Therefore, the ratio of OKT4+/8+ cells in the CSFL from the patients with VKH generally may be observed to be higher than in their PBL. The increased percentage of OKT4+ (CD4) cells and the decreased percentage of OKT8+ (CD8) cells in CSF seemed to be the important factor responsible for the weak or negative cytotoxicity of CSFL against P-36. Although the lymphocytosis in CSF observed in VKH has a similarity to that of MS and viral meningitis, including mumps meningitis, meningeval symptoms in VKH are usually mild; Kernig’s sign is negative, and as a rule no other neurologic symptoms are associated. Because in VKH the uveal inflammation is more severe than the meningitis, P-36-specific cytotoxic T lymphocytes may be accumulated in PB, more precisely in the uvea. For this reason, very few P-36-specific cytotoxic T lymphocytes were found in CSF from the patients with VKH.

Okubo and co-workers reported that OKla1+ cells (B lymphocytes, monocytes, macrophages, null cells, and activated T lymphocytes) were significantly lower in CSFL than in PBL from the patients with VKH, and that the surface markers on Con A-activated cells expressed remarkably less OKla1+ and Tac+ (CD25; T lymphocytes bearing the interleukin-2 receptors) in CSFL than in PBL. In contrast, our results revealed that there was a tendency toward more HLA-DR+ cells in CSFL than in PBL. Although all other CSF show no cytotoxicity against P-36, CSF from case 3, taken in the more acute phase of the illness, showed weak cytotoxicity against P-36. There may be kinetic differences in the percentage of the activated T cells and the cytotoxicity against P-36. It may also be possible to interpret the weak or negative cytotoxicity of CSFL against P-36 as the immune suppression mechanism operating in CSF. The PBL of case 5 showed no significant cytotoxic activity against P-36 target cells. It was revealed that the immunosuppression by HLA-DQ-mediated Leu 2a+ (CD8) suppressor T cells manifested the low cytotoxic responsiveness of the PBL to P-36 (manuscript in preparation).

It is well known that the recognition by T cells of antigens displayed or presented on the cell surface is generally restricted by the products of genes in the major histocompatibility complex (MHC). However, certain cell surface antigens, including melanocyte-specific antigen recognized by cytotoxic T lymphocytes, seem not to be subject to such rigid MHC restriction. It already has been found that murine cytotoxic T lymphocytes specific for syngeneic melanoma cells kill human as well as mouse melanoma cells. Sugira produced vitiligo in a monkey by intracutaneous inoculation of CSF from a patient with VKH (presumably caused by cytotoxicity of human cytotoxic T lymphocytes against monkey melanocytes). We have also shown that the PBL from patients with VKH showed a significant cytotoxic activity against P-36 human melanoma cells, and confirmed the specificity of the reaction by the cold target inhibition test and also by the use of HeLa-S3 human cervical carcinoma cells, against which no significant cytotoxicity was seen. These studies show that the recognition by cytotoxic T lymphocytes is not necessarily MHC-restricted. However, strictly MHC-restricted cytotoxic T lymphocytes also may exist even in VKH; yet there may be technical difficulties in preparing relevant target cells (sharing all possible determinants of MHC with cytotoxic T lymphocytes from patients with VKH) in order to study this possibility.

Regarding the affected organ specificity in VKH, there are several arguments about the differences among antigenic determinants in melanocytes. In an individual animal, ocular melanins can have different chemical structures from hair melanins. Furthermore, it has been suggested that surface antigenic changes occur during melanocyte differentiation. Therefore, it may be postulated that the human melanoma cells and meningeal melanocytes do not share common antigenic determinants or antigenically cross-reactive components on their cell surfaces. One might speculate from this that antigenic determi-
nants of uveal, meningeal, and epidermal melanocytes recognized by cytotoxic T lymphocytes may differ.

The current study leads to the conclusion that the activity of the cytotoxic T lymphocytes against P-36 is quite different in the PBL and CSFL from patients with VKH, and further, that the surface markers of CSFL also are different from those of PBL in patients with VKH. There clearly is a need to study the relationship between the function of CSFL and cell-surface markers in the future. Further characterization of the CSFL cellular response in VKH, not only with respect to functional activity and more refined cell-surface markers, but also with respect to the mechanisms of autoreactive-like immune responses in VKH, may result in a better understanding of the immunopathogenesis of the disease.

**Key words:** Vogt-Koyanagi-Harada disease, uveitis, cellular immunology, CSF, lymphocyte subsets.

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**References**