Effects of Herpes Virus Carrier Status on Peripheral T Lymphocyte Subsets


We studied the effects of herpes virus carrier status on peripheral blood T lymphocyte subsets in 334 healthy individuals. IgG-class antibodies against cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), and varicella-zoster virus (VZV) were used as markers for the carrier status of those viruses. CMV carrier status was associated with significant increases in the numbers of some T cell subsets, whereas the carrier status of EBV, HSV, and VZV had no significant effects. The 159 CMV-seropositive individuals had higher numbers of HNK1+ T cells than did the 175 CMV-seronegative individuals [mean (SD), 292 (196)/μL v 164 (89)/μL, respectively], including the CD4+ HNK1+ T cells [38 (48)/μL v 9 (13)/μL, respectively] and the CD8+ HNK1+ T cells [166 (146)/μL v 73 (54)/μL, respectively]. Morphological and cytochemical studies showed that the expression of HNK1 by the CD4+ and CD8+ T cells was associated with the occurrence of azurophilic cytoplasmatic granules and a loss of nonspecific esterase activity. The numbers of CD4+ HNK1+ and CD8+ HNK1+ T cells increased proportionally to the levels of the IgG-class CMV antibody titers. We suggest that the increased numbers of CD4+ HNK1+ and CD8+ HNK1+ granular T cells in CMV carriers reflect the persistent interaction between CMV and the immune system of its hosts.

AN IMPORTANT characteristic of the herpes group viruses is their ability to persist in the tissues of their hosts for many years after the initial infection. Consequently, the prevalence of antibodies against those viruses increases with age. Acute infection with cytomegalovirus (CMV) or Epstein-Barr virus (EBV) influences significantly the numbers of peripheral T lymphocytes, particularly those expressing the CD8 marker. Such increases in CD8+ T cells have also been reported in recipients of renal and bone marrow allografts. Active CMV infection is associated with a persistent increase of CD8+ T cells that also express the HNK1 antigen in recipients of cardiac and bone marrow allografts. The HNK1 marker was originally described as occurring on large granular lymphocytes with natural killer (NK) function.

The number of T cells in the peripheral blood decreases with increasing age, but the subsets of T cells that coexpress CD4 or CD8 and HNK1 increase in number. That situation may result from the increased numbers of viruses that the elderly carry as compared with younger individuals. We investigated whether the herpes virus carrier status of healthy individuals influences significantly their peripheral blood T cell subsets, particularly those coexpressing the HNK1 marker.

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MATERIALS AND METHODS

Healthy volunteer blood donors. Three hundred thirty-four healthy individuals (153 males and 181 females) participated in the study. Their age distribution is shown in Table I. Of those 334 individuals, 131 were blood donors of the Dutch Red Cross Blood Transfusion Service, 171 were hospital personnel (University Hospital, Leiden, The Netherlands) and 32 were relatives or friends of the hospital staff.

Isolation and fractionation of mononuclear cells. Heparinized venous blood samples were drawn between 8 and 11 AM to exclude the influence of circadian variations on T cell subsets. The mononuclear cells were isolated using Ficoll-Isopaque density-gradient centrifugation and enriched for T lymphocytes using 2-aminoethylisothiouroniumbromide hydrobromide (AET) pretreated sheep RBCs (SRBCs). The proportion of cells that formed rosettes with the AET-pretreated SRBCs was counted among 200 mononuclear cells of each sample using phase-contrast microscopy. The SRBC-rosetting cells are referred to as T cells in the following sections. After the SRBCs were lysed the T cell-enriched fractions were washed twice in phosphate-buffered saline containing 1% bovine serum albumin (PBS/BSA) and resuspended in RPMI 1640 medium containing 10% fetal calf serum (RPMI/FCS) until further use.

Prior to cell sorting, the T cells were incubated with the following mixtures of monoclonal antibodies (MCAs) that were conjugated either with fluorescein isothiocyanate (FITC) or phycoerythrin (PE): anti-Leu 3a/PE (CD4) + anti-Leu 7/FITC (anti-HNK1) and anti-Leu 2a/PE (CD8) + anti-Leu 7/FITC for 30 minutes at 4°C. After being washed once in PBS/BSA, the T cells were resuspended in RPMI/FCS and fractionated into CD4+ HNK1− and CD4+ HNK1+ or CD8+ HNK1− and CD8+ HNK1+ subsets using a FACS (FACS IV, Becton Dickinson, Mountain View, CA) equipped with an argon-ion laser tuned at 488 nm with an intensity of 300 mW. Fluorescent signals for each fluorochrome were detected selectively using a 530-nm band pass filter for green (FITC) emission and a 575-nm band pass filter for red-orange (PE) emission. Cells other than living T cells were electronically eliminated on the basis of forward and right-angle light scatter. The sorted cells were collected in plastic tubes prefixed with RPMI/FCS.

Analysis of T cell subsets. The T cell subsets are described using the CD nomenclature of the Third International Workshop and Conference on Human Leukocyte Differentiation Antigens (Oxford, England, September 21–26, 1986), except for the HNK1+ and NKH1+ subsets, for which no Workshop nomenclature is
HERPES VIRUS CARRIER STATUS AND T CELL SUBSETS

Table 1. Prevalence of Herpes Virus Antibodies

<table>
<thead>
<tr>
<th>Age Category (yr)</th>
<th>Prevalence of Virus Antibodies*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CMV</td>
</tr>
<tr>
<td>5-20</td>
<td>38</td>
</tr>
<tr>
<td>21-30</td>
<td>42</td>
</tr>
<tr>
<td>31-40</td>
<td>52</td>
</tr>
<tr>
<td>41-50</td>
<td>56</td>
</tr>
<tr>
<td>51-60</td>
<td>42</td>
</tr>
<tr>
<td>61-64</td>
<td>67</td>
</tr>
</tbody>
</table>

Abbreviations: HSV, herpes simplex virus; VZV, varicella-zoster virus.

*Percentage of seropositive individuals available. Therefore, we refer to those subsets using the nomenclature given by original investigators.15,16

The following MCAgs were used: anti-Leu 4 (CD3); anti-Leu 3a (a CD4 MCA of the IgG, subclass); FK16 (a CD8 MCA of the IgG subclass); and anti-Leu 7 (an anti-HNK1 MCA of the IgM class); anti-Leu 19 (an anti-HNK1 MCA of the IgG subclass); and anti-HLA-DR (an anti-class II backbone MCA of the IgG, subclass). The coexpression of HNK1 by CD4+, CD8+, and NKHI anti-Leu 19 (an anti-NKHI MCA of the IgG, subclass); and FK18 (a CD8 MCA of the IgG3 subclass) was studied using two-color immunofluorescence. For those purposes, the HNK1 marker was identified using anti-Leu 7 conjugated with biotin (B), and the T cells were incubated with the following mixtures: anti-Leu 3a + anti-Leu 7/B; FK16 + anti-Leu 7/B; anti-Leu 19 + anti-Leu 7/B; and anti-HLA-DR + anti-Leu 7/B. In the second step of the single-color immunofluorescence analyses, goat-anti-mouse Ig (GAM) conjugated with FITC was used. In the second step of the two-color immunofluorescence analyses, the appropriate mixtures of streptavidin conjugated with PE and class-specific and subclass-specific GAM antibodies conjugated with FITC were added to the T cells. All incubations were for 30 minutes at 4 °C. The anti-Leu MCA and the streptavidin were purchased from Becton Dickinson; FK16 was purchased from the Netherlands Institute of Public Health and Environmental Hygiene (Bilthoven, The Netherlands), and all GAM antibodies were purchased from Nordic Immunological Laboratories (Tilburg, The Netherlands).

Flow cytometry was performed using forward and right-angle light scatter on the FACS IV. For single-color immunofluorescence, histograms were recorded for 10,000 T cells per sample and for two-color immunofluorescence, 5,000 HNK1+ T cells were analyzed per sample.

The absolute numbers of the different T cell subsets were calculated from the percentages obtained by flow cytometry and from the simultaneously obtained absolute T cell counts. The latter were calculated from the percentages of SRBC-rosetting cells among the mononuclear cells and the absolute numbers of mononuclear cells as determined with a Hemalog D cell counter (Technicon, Tarrytown, NY).

Morphological studies. The sorted T cells were washed once with PBS/BSA, and cytocentrifuge preparations were made. The slides were stained with May-Grünwald Giemsa (MGG) and for acid phosphatase

antigens of herpesvirus were detected using an

enzyme-linked immunosorbent assay (ELISA). Antibody titers of ≥40 in the IgG assay and of ≥200 in the IgM assay were considered positive.

IgG-class antibodies to herpes simplex virus (HSV), to varicella-zoster virus (VZV), and to the capsid antigen of EBV were detected using indirect immunofluorescence. Human embryonic lung fibroblasts were infected with an HSV-2 strain or a VZV strain. PHR, cells were cultured for the production of EBV capsid antigen. Smears of the infected antigen-bearing cells and the noninfected control cells were made on slides, which were then fixed by acetone and methanol. Before testing, nonspecific fluorescent factors were removed from the test sera by absorption. Dilutions were made in veronal-buffered saline containing 10% fresh guinea pig serum. After incubation and washing, the slides were treated with sheep anti-human IgG conjugated with FITC (prepared by Dr J. Nagel, National Institute of Public Health and Environmental Hygiene).

Table 2. Influence of Age and Gender on T Cell Subsets

<table>
<thead>
<tr>
<th>T Cell Subset</th>
<th>Age Categories (yr)</th>
<th>Correlation Coefficient*</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-30 (n = 150)</td>
<td>31-60 (n = 150)</td>
<td>51-64 (n = 34)</td>
</tr>
<tr>
<td>CD3+</td>
<td>1,378</td>
<td>1,339</td>
<td>1,133</td>
</tr>
<tr>
<td>CD4+</td>
<td>845</td>
<td>845</td>
<td>777</td>
</tr>
<tr>
<td>CD8+</td>
<td>465</td>
<td>437</td>
<td>369</td>
</tr>
<tr>
<td>HNK1+</td>
<td>167</td>
<td>195</td>
<td>242</td>
</tr>
<tr>
<td>CD4+ HNK1+</td>
<td>7</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>CD4+ HNK1-</td>
<td>816</td>
<td>814</td>
<td>709</td>
</tr>
<tr>
<td>CD8+ HNK1+</td>
<td>81</td>
<td>97</td>
<td>117</td>
</tr>
<tr>
<td>CD8+ HNK1-</td>
<td>363</td>
<td>328</td>
<td>246</td>
</tr>
</tbody>
</table>

*Correlation coefficient: r = +0.11 or < −0.11 differed significantly from 0 at the P < .05 level.
†Median number of T cells per microliter.
‡P = .002 (Wilcoxon’s test).
§P = .01 (Wilcoxon’s test).
¶Median (5th and 95th percentiles) number of T cells per microliter.
Influence of age and gender on T cell subsets. Because age and gender influence some lymphocyte subpopulations significantly, particularly HNK1+12,15 we examined the effects of those parameters on the T cell subset counts to correct for their possible confounding effects in a multivariate statistical model. The significant influences of age and gender on T cell subsets as analyzed using univariate statistics are shown in Table 2. With increasing age, the CD3+ and CD8+ T cells decreased in number, whereas the HNK1+ T cells increased in number. As a result, the CD4+HNK1+ and CD8+HNK1+ T cells increased, whereas the CD8+HNK1− T cells decreased in number. Males had significantly higher numbers of HNK1+ and CD8+HNK1+ T cells than did females. Consequently, we decided to analyze the influence of the herpes virus carrier status on T cell subsets using multivariate statistical models that also controlled for age and gender. In those analyses, the above-described effects of age and gender on T cell subsets and their significance were confirmed (data not shown).

Influence of herpes virus carrier status on T cell subsets. The effects of CMV and EBV carrier status on T cell subsets as analyzed using univariate statistics are shown in Table 3; those of HSV and VZV are shown in Table 4. The CD3+, CD4+, CD8+, and especially the HNK1+ T cell subsets were all significantly increased in number in the CMV-seropositive group as compared with the CMV-seronegative group. Analysis of the coexpression of HNK1 by the CD4+ and CD8+ T cells revealed that the

<table>
<thead>
<tr>
<th>T Cell Subset</th>
<th>CMV Seropositive* (n = 175)</th>
<th>CMV Seronegative* (n = 159)</th>
<th>EBV Seropositive* (n = 283)</th>
<th>EBV Seronegative* (n = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+</td>
<td>1,382 (783, 2,681)</td>
<td>1,265 (523, 2,291)</td>
<td>1,319 (647, 2,421)</td>
<td>1,436 (533, 2,524)</td>
</tr>
<tr>
<td>CD4+</td>
<td>877 (457, 1,840)</td>
<td>814 (354, 1,541)</td>
<td>826 (391, 1,636)</td>
<td>888 (372, 1,690)</td>
</tr>
<tr>
<td>CD8+</td>
<td>470 (231, 1,096)</td>
<td>415 (171, 776)</td>
<td>430 (210, 865)</td>
<td>454 (169, 1,026)</td>
</tr>
<tr>
<td>HNK1+</td>
<td>237 (83, 629)</td>
<td>149 (56, 342)</td>
<td>194 (63, 528)</td>
<td>167 (64, 436)</td>
</tr>
<tr>
<td>CD4+HNK1+</td>
<td>23 (&lt;5, 150)</td>
<td>7 (&lt;5, 31)</td>
<td>11 (&lt;5, 107)</td>
<td>5 (&lt;5, 77)</td>
</tr>
<tr>
<td>CD4+HNK1−</td>
<td>846 (452, 1,814)</td>
<td>794 (351, 1,525)</td>
<td>801 (382, 1,619)</td>
<td>870 (367, 1,680)</td>
</tr>
<tr>
<td>CD8+HNK1+</td>
<td>136 (21, 332)</td>
<td>66 (12, 186)</td>
<td>95 (15, 285)</td>
<td>66 (9, 335)</td>
</tr>
<tr>
<td>CD8+HNK1−</td>
<td>331 (123, 750)</td>
<td>342 (124, 710)</td>
<td>327 (128, 686)</td>
<td>404 (114, 808)</td>
</tr>
</tbody>
</table>

Table 4. HSV and VZV Carrier Status and T Cell Subsets

<table>
<thead>
<tr>
<th>T Cell Subset</th>
<th>HSV Seropositive* (n = 203)</th>
<th>HSV Seronegative* (n = 131)</th>
<th>VZV Seropositive* (n = 326)</th>
<th>VZV Seronegative† (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+</td>
<td>1,297 (619, 2,508)</td>
<td>1,363 (615, 2,421)</td>
<td>1,328 (635, 2,425)</td>
<td>1,446 (544, 3,323)</td>
</tr>
<tr>
<td>CD4+</td>
<td>817 (386, 1,660)</td>
<td>848 (402, 1,577)</td>
<td>836 (390, 1,647)</td>
<td>882 (346, 1,555)</td>
</tr>
<tr>
<td>CD8+</td>
<td>415 (185, 912)</td>
<td>464 (183, 885)</td>
<td>433 (190, 893)</td>
<td>512 (186, 1,379)</td>
</tr>
<tr>
<td>HNK1+</td>
<td>198 (69, 540)</td>
<td>171 (62, 464)</td>
<td>193 (65, 523)</td>
<td>151 (64, 460)</td>
</tr>
<tr>
<td>CD4+HNK1+</td>
<td>11 (&lt;5, 111)</td>
<td>8 (&lt;5, 71)</td>
<td>11 (&lt;5, 104)</td>
<td>13 (&lt;5, 66)</td>
</tr>
<tr>
<td>CD4+HNK1−</td>
<td>794 (372, 1,654)</td>
<td>828 (399, 1,568)</td>
<td>812 (385, 1,620)</td>
<td>837 (342, 1,540)</td>
</tr>
<tr>
<td>CD8+HNK1+</td>
<td>97 (21, 293)</td>
<td>80 (8, 282)</td>
<td>89 (15, 285)</td>
<td>68 (12, 149)</td>
</tr>
<tr>
<td>CD8+HNK1−</td>
<td>307 (113, 674)</td>
<td>373 (139, 738)</td>
<td>333 (125, 710)</td>
<td>448 (150, 1,296)</td>
</tr>
</tbody>
</table>

P values were determined using standard regression analyses that controlled for age, gender, and carrier status of CMV, EBV, HSV, and VZV (described in the Materials and Methods section). None of the P values reached the .05 significance level.

*Median (5th and 95th percentiles) number of T cells per microliter.
†Median (minimum and maximum) number of T cells per microliter.
very few CD4+ HNK1+ T cells and the larger subset of CD8+ HNK1+ T cells were clearly and significantly increased in the CMV-seropositive group as compared with the CMV-seronegative group. There was also a slight increase in CD4+ HNK1− T cells in the CMV-seropositive group as compared with the CMV-seronegative group that was of borderline significance (P = .03). The effects of EBV, HSV, and VZV carrier status on T cell subsets was not significant except for an EBV-associated increase in CD4+ HNK1+ T cells that was of borderline significance (P = .048).

The relation between the expression of the HNK1 and NKH1 markers was studied on the T cells of 90 individuals (Table 5). The number of HNK1+ T cells lacking the NKH1 marker were significantly higher in 42 CMV-seropositive individuals (P = .002), whereas the numbers of T cells expressing both HNK1 and NKH1 were similar. The expression of HLA-DR was studied on the T cells of 32 individuals (12 CMV seropositive and 20 CMV seronegative). A median of only 2% (range 1% to 12%) of their T cells reacted with the anti-HLA-DR MCAs above background level. The seven individuals who had ≥4% of their T cells stained with anti-HLA-DR were all CMV seronegative. Almost no HNK1+ T cells reacted with anti-HLA-DR (range 1% to 3%).

We then studied the interactions between the levels of the IgG-class CMV antibody titers and the numbers of HNK1+, CD4+ HNK1+, and CD8+ HNK1+ T cells. The numbers of CD8+ HNK1+ T cells are plotted against the IgG-class CMV antibody titers in Fig 1. We analyzed those correlations in the 159 CMV-seropositive individuals with multivariate statistics that controlled for age, gender, IgG-class CMV antibody titers, and the presence or absence of IgM-class CMV antibodies. The analyses confirmed the significance of those correlations (HNK1+ T cells, P = .01; CD4+ HNK1+ T cells, P = .003, and CD8+ HNK1+ T cells, P = .003). The influence of IgM-class CMV antibodies on those parameters was not significant.

**Morphological studies of sorted T cell subsets.** We examined the morphological and cytochemical characteristics of the CD4+ and CD8+ T cells of three CMV-seropositive individuals in relation to the HNK1 expression by those cells (Table 6). They were selected because of their relatively high proportions of T cells expressing HNK1. Staining of the sorted T cell subsets with MGG revealed that the majority of CD4+ HNK1+ and CD8+ HNK1+ T cells had azuropholic granules scattered through moderate to large amounts of cytoplasm. The CD4+ HNK1− T cells had scarce amounts of cytoplasm and were generally agranular. Also, the majority of CD8+ HNK− T cells had small amounts of cytoplasm without granules, but in 20% to 26% of these cells more cytoplasm was present containing several azuropholic granules.

After staining was done for nonspecific esterase activity, a dotlike staining pattern was observed in a sizable proportion of CD4+ HNK1− and CD8+ HNK1− T cells, whereas only a minority of CD4+ HNK1+ and CD8+ HNK1+ T

<table>
<thead>
<tr>
<th>T Cell Subset</th>
<th>CMV Seropositive* (n = 42)</th>
<th>CMV Seronegative* (n = 48)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNK1+</td>
<td>222 (65, 591)</td>
<td>137 (37, 421)</td>
<td>.004</td>
</tr>
<tr>
<td>NKH1+</td>
<td>127 (36, 382)</td>
<td>103 (28, 331)</td>
<td>NS‡</td>
</tr>
<tr>
<td>HNK1+ NKH1+</td>
<td>54 (11, 222)</td>
<td>56 (11, 173)</td>
<td>NS‡</td>
</tr>
<tr>
<td>HNK1− NKH1+</td>
<td>132 (21, 385)</td>
<td>60 (16, 278)</td>
<td>.002</td>
</tr>
</tbody>
</table>

*Median (5th and 95th percentiles) number of T cells per microliter.
†P values were determined using standard regression analyses that controlled for age, gender, and CMV carrier status (described in the Materials and Methods section).
‡NS, P value > .05.

Fig 1. Interaction between the numbers of peripheral blood CD6+ HNK1+ T cells and the levels of IgG-class cytomegalovirus (CMV) antibody titers in 334 healthy individuals. Each point in the figure represents one individual. A logarithmic scale has been used for the vertical axis to compress the figure.
Table 6. Morphological Characteristics of CD4+ and CD8+ T Cells in Relation to HNK1 Expression

<table>
<thead>
<tr>
<th>T Cell Subset</th>
<th>CD4+ HNK1+</th>
<th>CD4+ HNK1-</th>
<th>CD8+ HNK1+</th>
<th>CD8+ HNK1-</th>
</tr>
</thead>
<tbody>
<tr>
<td>May-Grünwald Giemsa</td>
<td>73, 78, 74</td>
<td>13, 11, 11</td>
<td>2, 1, 7</td>
<td>9, 89, 83</td>
</tr>
<tr>
<td>Cytoplasmatic Granules*</td>
<td>2, 5, 3</td>
<td>ND, 51, 54</td>
<td>ND, 6, 3</td>
<td>ND, 20, 26</td>
</tr>
<tr>
<td>Dotlike Staining</td>
<td>Granular Staining</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

*Percentage of cells in three CMV seropositive individuals. Results obtained in those individuals are presented in the same order through.

cells did so (Table 6). A granular staining pattern for nonspecific esterase activity was apparent in <10% of all four sorted T cell subsets. Staining for ANAE and ANBE activities yielded similar results (data not shown). Cytochemical acid phosphatase localization revealed that >95% of all four sorted T cells subsets were positive.

The ultrastructural characteristics of the CD4+ and CD8+ T cells of two CMV-seropositive individuals were studied in relation to the expression of HNK1 by those cells (Figs 2 and 3). The CD4+ HNK1+ and CD8+ HNK1+ T cells generally had an extended cytoplasm as compared with the CD4+ HNK1- and CD8+ HNK1- T cells, and they often had an irregularly shaped nucleus. The cytoplasm of the HNK1+ T cells contained heterogeneous electron-dense bodies, which were somewhat more numerous in the CD8+ HNK1+ T cells than in the CD4+ HNK1+ T cells.

The Golgi apparatus of those cells was moderately developed.

DISCUSSION

CMV carrier status of healthy individuals is associated with significant increases in number of some peripheral blood T cell subsets, particularly those expressing the HNK1 marker. Those T cells also express CD4 or CD8 but not NK1 and have the morphological, cytochemical, and ultrastructural characteristics of granular lymphocytes.

The interaction between CMV carrier status and T cell subsets is independent of age and gender. We noted similar changes in T cell subsets with increasing age, as reported by other investigators, ie, decreases in CD3+, CD4+, CD8+, and CD8+ HNK1- T cell numbers and increases in HNK1+, CD4+ HNK1+, and CD8+ HNK1+ T cell numbers.11,12,15,21 Our observation that males have higher numbers of HNK1+ T cells is in accordance with a previous report.13

The significance of the effects of herpes virus carrier status on T cell subsets appears to be limited to CMV, since those effects are not significant for EBV and HSV. One should be cautious in assessing the significance of the effect of the VZV carrier status on T cell subsets because of the very low number of VZV-seronegative individuals in our study group. The positive correlations between the levels of IgG-class CMV antibody titers and the numbers of HNK1+, CD4+ HNK1+, and CD8+ HNK1+ T cells may indicate that the magnitude of the CMV-specific immune
response relates to the HNK1 expression by peripheral T cells.

The positive correlation between age and HNK1 expression by peripheral blood T cells suggest that antigenic stimuli other than CMV may also be involved. A marked and significant increase in CD8+ HNK1+ lymphocytes has been reported in patients with the acquired immunodeficiency syndrome (AIDS), the AIDS-related complex of clinical signs, and also in patients treated with clotting factor concentrates for their congenital coagulation defects. Those increases in CD8+ HNK1+ lymphocytes may have been due to infection with the human immunodeficiency virus, but also to chronic exposure to CMV or other antigenic stimuli present in semen or clotting factor concentrates. Increased numbers of CD4+ HNK1+ and CD8+ HNK1+ lymphocytes have been reported in long-term survivors after allogeneic kidney, heart, or bone marrow transplants (BMTs) and correlated with CMV infection. The results are all consistent with the hypothesis that the increased numbers of HNK1+ T cells represent an immune response to chronic antigenic stimulation.

Interestingly, the occurrence of increased proportions of peripheral blood HNK1+ granular lymphocytes coexpressing CD4 or CD8 has been reported in patients with B cell malignancies. However, the majority of the CD4+ lymphocytes in two studies also express the CD11b antigen in contrast to the situation in healthy individuals. In this context, the reported tissue distribution of CD4+ HNK1+ and CD8+ HNK1+ lymphocytes may be particularly illuminating. Most HNK1+ lymphocytes in the germlin centers of immunologically activated lymph nodes are also CD4+. The CD8+ HNK1+ lymphocytes are rare in tissues, although they are predominant among the very few HNK1+ T cells in the bone marrow and must be considered as a predominantly bloodborne subset. The increased numbers of HNK1+ lymphocytes coexpressing T cell markers in the blood of some patients with B cell malignancies, which represent a significant proportion of the cells in germinal centers, suggest that HNK1+ T cells have a regulatory role in B cell differentiation and expansion.

Our morphological, cytochemical, and ultrastructural studies of CD4+ HNK1+ and CD8+ HNK1+ T cells have revealed that those cells have a large cytoplasmic to nuclear ratio, moderate numbers of electron-dense cytoplasmic granules, and a moderately developed Golgi apparatus. More than 95% of those cells are acid phosphatase positive, but they are generally negative for nonspecific esterase activity. Thus, our CD4+ HNK1+ T cells closely
resemble the peripheral blood CD4+ granular lymphocytes described by other researchers,\(^1\) of which a significant proportion expresses the HNK1 marker. The CD4+ HNK1+ lymphocytes recovered from reactive tonsils show numerous electron-dense granules and abundant Golgi vesicles, suggesting that they are in a more active phase of granule formation than their peripheral blood counterparts.\(^1\)

Morphological studies of CD8+CD11b+ T cells, of which 70% to 80% express HNK1, have revealed a scattered staining pattern for nonspecific esterase activity\(^3\) and numerous electron-dense azurophilic granules and prominent Golgi apparatus.\(^4\) In contrast to those studies, our CD8+ HNK1+ T cells are generally negative for nonspecific esterase activity and lack the CD11b marker (data not shown). In addition, our ultrastructural studies suggest that our CD8+ HNK1+ T cells are in a less active phase of granule formation. The CD8+ HNK1+ lymphocytes that circulate in large numbers in the blood of long-term survivors after allogeneic BMT have only sparse granules or no granules at all.\(^5\)

Typically, T cells have a dotlike staining pattern for nonspecific esterase activity,\(^6\) which changes to a granular pattern during the early phases of in vitro cell activation, whereas blast cells have lost their nonspecific esterase activity.\(^7,8\) The possibility that the CD4+ HNK1+ and CD8+ HNK1+ T cells in our study may represent activated T cells is contradicted by the observations that they express neither HLA-DR antigens nor interleukin 2 (IL 2) receptors (data not shown). Similarly, the CD8+ HNK1+ T cells in long-term survivors of allogeneic BMT generally do not express HLA-DR antigens.\(^5\) The lack of nonspecific esterase activity in CD4+ HNK1+ and CD8+ HNK1+ T cells may indicate that those cells have returned to a resting state after a previous phase of activation, eg, while combattting CMV infection.

Functional studies of peripheral blood CD4+ granular lymphocytes and tonsillar CD4+ HNK1+ lymphocytes have shown neither helper nor suppressor activity in the pokeweed mitogen (PWM) driven-Ig synthesis assay.\(^3,4\) In contrast, the peripheral blood CD8+CD11b+(HNK1+) lymphocytes of healthy individuals\(^8\) and the CD8+(CD11b−) HNK1+ lymphocytes of long-term survivors of allogeneic BMT\(^9\) show suppressor function in that assay. HNK1+ lymphocytes that also express T cell markers have low cytotoxic activities against tumor cell lines used as targets in NK assays.\(^10,11\) However, lectin-dependent cellular cytotoxicity (LDCC) can be induced even against NK-insensitive target cell lines, in peripheral blood HNK1+ T cells\(^3\) and tonsillar CD4+ HNK1+ lymphocytes.\(^4\) Consequently, the suppressive effects of HNK1+ T cells on PWM-induced Ig synthesis may be mediated by PWM-dependent cellular cytotoxicity against the PWM-responsive lymphocytes.\(^4\)

Our observation that the HNK1+ T cells that increase in number in association with CMV carrier status do not express NKH1 suggests that they do not represent the subset of T cells that mediates non-HLA restricted cytotoxicity, which has the CD3+ NKH1+ phenotype.\(^12\) The suggestion that the HNK1+ T cells include in vivo primed antiviral cytotoxic T cells\(^13\) is of interest in view of our observation that those cells increase in number proportionally to the levels of IgG-class CMV antibody titers. The identification of such in vivo primed anti-CMV cytotoxic T cells and the elucidation of their activation mechanisms may yield a new therapeutic option for immunocompromised patients in whom CMV reactivations can often be life-threatening. In conclusion, our results illustrate the unique position of CMV among the herpes viruses in interacting with the immune systems of its carriers, as reflected by its persistent effects on peripheral blood T lymphocyte subsets.

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