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Phenotypic study of $CD4^+$ and $CD8^+$ lymphocyte subsets in relation to cytomegalovirus carrier status and its correlate with pokeweed mitogen-induced B lymphocyte differentiation

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SUMMARY

A characteristic of active cytomegalovirus (CMV) infection is its suppressive effect on *in vitro* assays of immune function. The expression of CD11b by the $CD4^+$ and $CD8^+$ lymphocytes allows the identification of subsets with distinct regulatory functions of pokeweed mitogen (PWM) induced B cell differentiation. In order to relate that result with our previous observations that CMV carriers have significantly increased numbers of $CD4^+$, $HNK1^+$ and $CD8^+$, $HNK1^+$ lymphocytes in their peripheral blood compared with non-carriers, we performed a three-colour flow cytometric analysis of the co-expression of CD11b and HNK1 by the $CD4^+$and $CD8^+$ lumphocytes obtined from 27 CMV carriers and 42 non-carriers. The differences between CMV carriers and non-carriers were significant for the $CD4^+$, $HNK1^+$ lymphocytes (median [5th and 95th percentiles], 59 [18 and 123 *versus* 24/7 and 73 per mm^3, respectively; $P < 0{\cdot}001$) and $CD8^+$, $HNK1^+$ lymphocytes (105/28 and 259] *versus* 52 [23 and 139] per mm^3; $P < 0{\cdot}001$), but not for the $CD4^+$, $CD11b^+$ lymphocytes (59 [18 and 135] *versus* 52 [17 and 104] per mm^3) and the $CD8^+$, $CD11b^+$ lumphocytes (85 [34 and 293] *versus* 82 [21 and 248] per mm^3). The $CD4^+$, $HNK1^+$ and $CD8^+$, $HNK1^+$ lymphocytes that were increased in CMV carriers compared with non-carriers included mostly $CD11b^-$, but also $CD11b^+$ lymphocytes. After sorting $CD4^+$ and $CD8^+$ lymphocytes for four CMV carriers into $HNK1^+$ and $HNK1^-$ fractions, we analysed their regulatory functions on PWM-driven B cell differentiation. Helper function to PWM-driven B cell differentiation was exclusively associated with the $CD4^+$, $HNK1^-$ lymphocytes; the $CD4^+$, $HNK1^+$ generally did not show helper or suppressor activity in this assay. Both $CD8^+$, $HNK1^+$ and $CD8^+$, $HNK1^-$ lymphocytes showed suppressor activity. Thus, the HNK1 marker does not constitute a phenotypical correlate for suppressor cells of PWM-driven B-cell differentiation.

Keywords cytomegalovirus lymphocyte subpopulations regulation immunoglobulin synthesis flow cytometry

INTRODUCTION

Several active viral infections are accompanied by transient increases in the numbers of peripheral blood $CD8^+$ lymphocytes (Reinherz *et al.*, 1980; De Waele, Thielemans & Van Camp, 1981; Carney, Iacoviello & Hirsch, 1983). These $CD8^+$ lymphocytes contribute to the immunosuppressive effects associated with such infections (Carney, Iacoviello & Hirsch, 1983; Verdonck & De Gast, 1984; Cauda *et al.*, 1987). The $CD8^+$ lymphocytes that increase in number in recipients of allogeneic heart (Maher *et al.*, 1985) and bone marrow (Würsch *et al.*, 1985) transplants with active cytomegalovirus (CMV) infection also express the HNK1 antigen. Healthy CMV carriers maintain increased numbers of $CD4^+$, $HNK1^+$ and $CD8^+$, $HNK1^+$ lymphocytes compared with non-carriers (Gratama *et al.*, 1987a), whereas the carrier status of Epstein–Barr virus, herpes simplex virus or varicella zoster virus is not associated with such alterations in lymphocyte phenotypes (Gratama *et al.*, 1987b).

The capability to suppress immunological functions *in vitro*, such as pokeweed mitogen (PWM) induced B lymphocyte differentiation, antigen- or mitogen-induced T lymphocyte

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proliferation or interleukin 2 (IL-2) production, is a characteristic of CD8+, CD11b+ lymphocytes in healthy individuals (Landay, Gartland & Clement, 1983; Clement, Grossi & Gartland, 1984; Abo *et al.*, 1987) and of CD8+, CD11b+ and CD8+, HNK1+ lymphocytes in marrow-graft recipients (Gebel, Kaizer & Landay, 1987; Leroy *et al.*, 1988). These two subsets consist of granular lymphocytes and may overlap (Clement, Grossi & Gartland, 1984). However, suppressor cells of PWM-induced B lymphocyte differentiation have also been identified among the CD8+, CD11b- lymphocytes (Takeuchi *et al.*, 1988). Small subsets of granular CD4+ lymphocytes expressing CD11b or HNK1 or both have also been identified. CD4+ granular lymphocytes have been detected in reactive tonsils (Velardi *et al.*, 1986b) and increased numbers of granular CD4+ lymphocytes in the blood of patients with various types of cancer (Velardi *et al.*, 1985; Velardi, Clement & Grossi, 1985). These granular CD4+ lymphocytes neither help nor suppress PWM-driven B lymphocyte differentiation; they also differ from typical CD4+ helper lymphocytes in their low ability to produce IL-2 after mitogenic stimulation (Morishita *et al.*, 1986).

In order to relate these functional studies to our previous work on the influence of CMV carrier status on CD4+ and CD8+ lymphocytes, we investigated the expression of HNK1 and CD11b by peripheral blood CD4+ and CD8+ lymphocytes of healthy donors in relation to their CMV carrier status using three-colour immunofluorescence and flow cytometry. This analysis was coupled with a study of whether the HNK1 marker allowed the identification of different regulatory subsets among the CD4+ and CD8+ lymphocytes with respect to PWM-induced B cell differentiation.

MATERIALS AND METHODS

Healthy volunteer blood donors

Sixty-nine healthy individuals (40 men and 29 women) participated in the study. Their ages ranged from 18 to 51 years. Of these 69 individuals, 16 were blood donors of the Dutch Red Cross Blood Transfusion Service and 53 were hospital personnel.

Assessment of CMV carrier status

We took the presence of IgG-class antibodies in the sera of the blood donors as a marker for their CMV carrier status. We determined IgG-class antibodies against CMV late antigen with an indirect ELISA (Sundqvist & Wahren, 1981) and IgM-class antibodies against that antigen with a direct ELISA (Sundqvist, 1982).

Isolation and fractionation of mononuclear cells

Mononuclear cells were isolated from heparinized venous blood samples using Ficoll-Isopaque density gradient centrifugation and washed twice in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA). For studies involving absolute lymphocyte counts, those samples were drawn between 8 and 11 AM in order to exclude the influence of circadian variations on lymphocyte subsets (Ritchie *et al.*, 1983).

For the PWM-induced B cell differentiation assays, the mononuclear cells were separated into T cell and non-T cell enriched fractions using 2-aminoethylisothioureumbromide hydrobromide pre-treated sheep red blood cells (Gratama *et al.*, 1983). All non-T cell fractions contained < 5% T cells. We used RPMI-1640 containing 20% fetal calf serum (FCS) that had been absorbed with sheep erythrocytes during all fractionation procedures.

Before sorting, the cells were incubated with one of the following mixtures of fluorescein isothiocyanate (FITC)- or phycoerythrin (PE) conjugated monoclonal antibodies: anti-Leu3a/PE (CD4) + anti-Leu7/FITC (HNK1), or anti-Leu2a/PE (CD8) + anti-Leu7/FITC, for 30 min at 4°C. After washing once, the cells were resuspended and fractionated into CD4+HNK1+ and CD4+HNK1- subsets, or CD8+ HNK1+ and CD8+ HNK1- subsets using a fluorescence-activated cell sorter (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 orange-red (PE) emission. If necessary, correction for the spectral overlap of these two wavelengths was performed by using the dual compensation network of the FACS IV. Living lymphocytes were electronically selected on the basis of their FLS and PLS characteristics. During the sorting procedures, the cell rate was kept below 2500 cells/sample in order to obtain optimal purity of the sorted cell fractions, which was >90% as determined by microscopic re-analysis.

Analysis of lymphocyte subsets

For study of the expression of CD11b and HNK1 by the CD4+ and CD8+ lymphocytes, mononuclear cells were incubated with mixtures of anti-Leu15/PE (CD11b), biotinylated anti-Leu7, and anti-Leu3a/FITC or anti-Leu 2a/FITC. After washing once in PBS/BSA, streptavidin conjugated with allophycocyanin (APC) was added to the cells . All monoclonal antibodies and the streptavidin were purchased from Becton Dickinson and were used in saturating concentrations. After the final washing step, flow cytometry was performed on the RELACS-2 flow cytometer. Details of that instrumentation have been described by Trask *et al.* (1985). Briefly, an argon–ion laser (400 mW, 488 nm) was used for excitation of FITC and PE, and a R6G dye laser (model 375, Spectra Physics, Mountain View, CA) (400 mW, 600 nm) was used for excitation of APC. Fluorescent signals for each fluorochrome were detected selectively using a 530 nm band pass filter for green emission (FITC), a 575 nm band pass filter for orange-red emission (PE), and a 630 nm long pass filter for deep red (APC) emission. FLS, PLS and green, orange-red and deep red fluorescence characteristics of 25 000 cells/sample were measured and stored in list mode for subsequent analyses. The green and orange-red fluorescence signals were corrected for spectral overlap using the electronic compensation network of the RELACS-2.

In the first step of our analyses, cells other than living lymphocytes were gated out by setting threshold triggers on the FLS and PLS parameters. Histograms of green fluorescence were then generated, from which the percentages of CD4+ and CD8+ lymphocytes were computed. In the second step, the CD4+ and CD8+ lymphocytes were selected by setting threshold triggers on the FLS, PLS and green fluorescence parameters. Correlated data on orange-red and deep red fluorescence were then collected into 64 × 64 matrices and displayed as contour plots; these were divided into quadrants to represent

unstained cells, cells stained with both PE and APC, and cells stained with either PE or APC. The absolute numbers of the different lymphocyte subset were calculated from the percentages of lymphocyte subsets obtained by flow cytometry and from the simultaneously obtained absolute lymphocyte counts. The latter were determined using a Hemalog D cell counter (Technicon, Tarrytown, NY).

PWM-induced B-cell differentiation assay

For the assessment of T cell helper function on PWM-induced B cell differentiation, 20 000 (sorted) irradiated (15 Grey) T cells were cultured in RPMI-1640 medium containing 15% pooled human serum with 20 000 non-T cells and 10 μg/ml PWM (GIBCO, Grand Island, NY) per well in round-bottomed microtitre plates (De Gast & Platt-Mills, 1979; Verdonck & De Gast, 1984). T cell suppressor function was studied by culturing 10 000 (sorted) T cells with 20 000 irradiated T cells, 20 000 non-T cells and PWM. Per cell combination cultures of 24 wells were set up. Control cultures included non-T cells, and non-T cells with labelled and sham-sorted T cells (with or without irradiated T cells, depending on the type of assay). B cell differentiation was evaluated after 6 days by counting the proportion of cytoplasmatic Ig-positive (cIg^+) cells among 500 cells in cytocentrifuge preparations that had been fixed in ethanol/glacial acetic acid (19/1 v/v) and stained using FITC-conjugated goat $F(ab')_2$ anti-human immunoglobulin (Nordic Immunological Laboratories, Tilburg, The Netherlands).

At the end of each culture, the number of cIg^+ cells was expressed as a proportion of the number of non-T cells that had been set up for each culture. The results obtained with the sorted T cell subsets were related to those obtained with labelled and sham-sorted T cells. Helper activity was considered to be present if the result of a sorted T cell subset exceeded that of the sham-sorted control by > 50%. Suppressor activity was considered to be present if the result of a sorted T cell subset was < 50% of that of the sham-sorted control.

In one of the four experiments (donor 4), the number of immunoglobulin-secreting cells was also determined using an ELISA-spot assay (Logtenberg *et al.*, 1985). The results of this experiment showed a positive correlation with those based on enumeration of cIg^+ cells (Table 1).

Statistical analysis

Inspection of the distribution curves of the lymphocyte subset counts and related variables revealed that many of them were non-Gaussian. Therefore, non-parametric statistics were used for univariate analyses of the effects of age, gender and CMV carrier status on those parameters. In accordance with previous reports (Abo *et al.*, 1983; Gratama *et al.*, 1987b), age and gender influenced significantly some of the lymphocyte subset counts in the present study. Therefore, the effects of CMV carrier status on lymphocyte subset counts and related variables was further studied, using multivariate analyses, prior to which the data were transformed to their natural logarithms or square roots in order to reduce skewness. For those multivariate analyses, standard linear regression procedures were used that controlled for age, gender and CMV carrier status and the results of those analyses are presented. Only P values < 0.05 were considered significant.

RESULTS

Expression of HNK1 and CD11b by CD4+ and CD8+ lymphocytes in relation to CMV carrier status

Twenty-seven of the 69 individuals had IgG-class CMV antibodies and non had IgM-class antibodies. The absolute numbers of the subsets of the $CD4^+$ and $CD8^+$ lymphocytes as defined by the HNK1 and CD11b markers in relation to CMV carrier status is set out in Tables 2 and 3.

The differences between CMV carriers and non-carriers were significant for the $CD4^+$, $HNK1^+$ lymphocytes (median [5th and 95th percentiles]: 59 [18 and 123] *versus* 24 [7 and 73] per mm^3, respectively; $P < 0.001$) and the $CD8^+$, $HNK1^+$ lymphocytes (105 [28 and 259] *versus* 52 [23 and 139] per mm^3, respectively; $P < 0.001$), but not for the $CD4^+$, $CD11b^+$ lymphocytes (59 [18 and 135] *versus* 52 [17 and 104] per mm^3, respectively) and the $CD8^+$, $CD11b^+$ lymphocytes (85/34 and 293 *versus* 82/21 and 248 per mm^3, respectively).

Among the $CD4^+$ and $CD8^+$ lymphocytes, both the larger $HNK1^+$, $CD11b^-$ and smaller $HNK1^+$, $CD11b^+$ subsets were significantly increased in CMV carriers as compared to non-carriers (Table 2). The differences between both groups were not significant for the $HNK1^-$, $CD11b^-$ and $HNK1^-$, $CD11b^+$ subsets. There was no preferential expansion of $HNK1^+$ lymphocytes among the large $CD11b^-$ or small $CD11b^+$ subsets. Consequently, the proportions of $CD11b^+$ cells among the $HNK1^+$ and $HNK1^-$ fractions of the $CD4^+$ and $CD8^+$ lymphocytes were similar in CMV carriers compared to non-carriers (Table 3). Relatively large fractions of the small $CD4^+$, $HNK1^+$ and $CD8^+$, $HNK1^+$ subsets expressed CD11b, whereas the proportions of the large $CD4^+$, $HNK1^-$ and $CD8^+$, $HNK1^-$ subsets expressing CD11b were relatively small. Particularly among the $CD4^+$, $HNK1^+$ and $CD8^+$, $HNK1^+$ lymphocytes, there were wide variations in CD11b expression which were not related to CMV carrier status.

Regulatory functions of CD4+ and CD8+ lymphocytes on PWM-induced B cell differentiation in relation to HNK1 expression

We selected four CMV-seropositive men on the basis of the relatively high HNK1 expression by their $CD4^+$ and $CD8^+$ lymphocytes, which allowed the sorting of enough lymphocytes of each fraction to perform the functional tests. After sorting of the $CD4^+$ and $CD8^+$ lymphocytes into $HNK1^+$ and $HNK1^-$ fractions, those subsets were tested for their helper and suppressor capacities on PWM-induced B cell differentiation (Table 1). The results of the sorted fractions were compared with those of labelled (either with anti-Leu3/PE + anti-Leu7/FITC or with anti-Leu2/PE + anti-Leu7/FITC) and sham-sorted lymphocytes. There was a large difference between donor 1 and the other three donors with respect to the proportion of cIg^+ lymphocytes in the control cultures with labelled and sham-sorted lymphocytes, irrespective of the type of assay (helper or suppressor function) or the MCA used for labelling.

The $CD4^+$, $HNK1^+$ enriched lymphocytes showed helper function in one of the four cases, whereas their $HNK1^-$ counterparts did so in three of the four cases. Donor 1 showed relatively high numbers of cIg^+ lymphocytes after co-culture with labelled and sham-sorted E-rosetting cells; therefore, our standard test conditions may not have been optimal to assess helper function among his $CD4^+$, $HNK1^-$ lymphocytes. The

Table 1. Regulatory functions of $CD4^+$ and $CD8^+$ lymphocytes on PWM-induced B cell differentiation in relation to HNK1 expression

	Helper function assay					Suppressor function assay				
	Numbers of cIg^+ lymphocytes/ 1000 cultured non-T cells				ELISA-spot assay	Numbers of cIg^+ lymphocytes/ 1000 cultured non-T cells				ELISA-spot assay
T cell subset	Donor 1	Donor 2	Donor 3	Donor 4	Donor 4	Donor 1	Donor 2	Donor 3	Donor 4	Donor 4
Labelled and shamsorted*	314	27	25	33	19	587	50	60	53	35
Sorted $CD4^+$. $HNK1^+$	28 (9%)	<1 (2%)	2 (8%)	89† (271%)	54† (284%)	546 (93%)	24‡ (48%)	62 (103%)	64 (120%)	40 (114%)
Sorted $CD4^+$, $HNK1^-$	19 (6%)	82† (302%)	187† (748%)	111† (335%)	ND	376 (64%)	87 (174%)	79 (132%)	231 (436%)	124 (354%)
Labelled & Shamsorted§	342	51	15	46	ND	485	63	68	63	38
Sorted $CD8^+$, $HNK1^+$	3 (1%)	1 (2%)	<1 (1%)	1 (2%)	ND	175‡ (36%)	19‡ (30%)	65 (95%)	18‡ (29%)	26 (68%)
Sorted $CD8^+$, $HNK1^-$	154 (45%)	12 (23%)	2 (14%)	4 (9%)	ND	189‡ (39%)	21‡ (34%)	74 (109%)	23‡ (36%)	20 (53%)
Non-T Cells Only	5	<1	<1	<1	1	6	<1	14	3	10

Experimental results, expressed as fractions of the results obtained with the relevant labelled and sham-sorted T cells, are given in parentheses.
ELISA spot assay expressed as number of immunoglobulin-producing lymphocytes/10 000 cultured T cells.
* Labelled with anti-Leu3/PE and anti-Leu 7/FITC.
† Samples with helper activity (i.e. > 150% of the relevant labelled and sham-sorted controls).
‡ Samples with suppressor activity (i.e. < 50% of the relevant labelled and sham-sorted controls).
§ Labelled with anti-Leu2/PE and anti-Leu7/FITC.

Table 2. Expression of HNK1 and CD11b by CD4+ and CD8+ lymphocytes in relation to CMV carrier status

	CMV status*		
	Seropositive ($n=27$)	Seronegative ($n=42$)	
Lymphocyte subset	Lymphocytes/mm^3	Lymphocytes/mm^3	P†
CD4+, HNK1−, CD11b−	943 (363, 1881)	864 (431, 1408)	NS
CD4+, HNK1−, CD11b+	48 (16, 117)	45 (15, 91)	NS
CD4+, HNK1+, CD11b−	48 (13, 87)	19 (<5, 69)	<0·001
CD4+, HNK1+, CD11b+	10 (<5, 42	5 (<5, 19)	<0·001
CD8+, HNK1−, CD11b−	496 (237, 938)	384 (190, 711)	NS
CD8+, HNK1−, CD11b+	43 (16, 141)	60 (17, 146)	NS
CD8+, HNK1+, CD11b−	61 (18, 135)	30 (8, 70)	<0·001
CD8+, HNK1+, CD11b+	27 (10, 144)	17 (<5, 102)	0·002

* Median, with 5th and 95th percentiles in parentheses.

† Determined using standard regression analyses that controlled for age, gender and CMV carrier status (see Materials & Methods).

NS = not significant.

Table 3. Proportions of CD11b+ cells among HNK1+ and HNK1− fractions of CD4+ and CD8+ lymphocytes

	Percent expressing CD11b*	
Lymphocyte subset	CMV seropositive ($n=27$)	CMV seronegative ($n=42$)
CD4+, HNK1+	20·7 (5·8, 42·9)	20·7 (7·3, 68·4)
CD4+, HNK1−	4·8 (2·5, 9·3)	5·3 (2·5, 9·9)
CD8+, HNK1+	35·1 (12·0, 66·7)	33·4 (10·7, 73·1)
CD8+, HNK1−	10·0 (4·0, 26·0)	12·4 (4·6, 28·3)

* Median (5th and 95th percentiles) percentage of CD11b+ lymphocytes among the CD4+, HNK1+, CD4+, HNK1−, CD8+, HNK1+ and CD8+, HNK1− lymphocyte subsets. None of the differences between the CMV-seropositive and CMV-seronegative groups were significant.

CD8+ enriched lymphocytes had no helper capacity in any of the four cases, irrespective of whether they expressed HNK1.

Among the CD4+, HNK1+ lymphocytes, suppressor function was only weakly present in only one of four cases, and the CD4+, HNK1− enriched lymphocytes did not have suppressor function in any case. In contrast, the CD8+ enriched lymphocytes of three of the four donors showed suppressor function, which was present in both HNK1+ and HNK1− fractions. Subsequent analysis of the CD8+, HNK1+ and CD8+, HNK1− lymphocytes of donor 3 revealed a relatively low expression of CD11b (15% and 8%, respectively). That result may explain the absence of suppressor function of this donor's CD8+ lymphocytes.

DISCUSSION

We have analysed the effects of CMV carrier status on peripheral CD4+ and CD8+ lymphocytes in relation to their expression of HNK1 and CD11b. CMV carriers have significantly higher numbers of CD4+, HNK1+ and CD8+, HNK1+ lymphocytes than do non-carriers, but the numbers of CD4+, CD11b+ and CD8+, CD11b+ lymphocytes in both groups are similar. The effects of CMV carrier status are not only evident on the majority of CD4+, HNK1+ and CD8+, HNK1+ lymphocytes which are CD11b−, but also on the minority of CD4+, HNK1+ and CD8, HNK1+ lymphocytes which are CD11b+.

CD11b, the third receptor for complement, belongs to a group of cell surface molecules, termed integrins, that facilitate intercellular contacts either by increasing the avidity of binding between effector and target cell, or by triggering cells with lytic potential (Hynes, 1987). The binding of these receptors on effector cells to C3 fragments attached to target cells increases the efficiency of lysis (Ramos *et al.*, 1985). HNK1 is an epitope on the carbohydrate moiety of several neural cell adhesion molecules and is involved in the interactions between those cells (Künemund *et al.*, 1988). The expression of HNK1 on the surface of lymphocytes, which is closely related with the presence of cytoplasmatic azurophilic granules (Abo *et al.*, 1983; Gratama *et al.*, 1988), may serve a similar function, i.e. lymphocyte–target cell interaction.

CD8+, CD11b+ lymphocytes show numerous electron-dense azurophilic granules (Clement, Grossi & Gartland, 1984) and a scattered staining pattern for non-specific esterase activity (Landay, Clement & Grossi, 1984). In contrast, CD8+, HNK1+ lymphocytes are in a less active phase of granule formation and lack non-specific esterase activity (Gratama *et al.*, 1987b). The latter observation suggests that the CD8+, HNK1+ lymphocytes have returned to a resting state after previous activation. Phillips & Lanier (1985) reported that a proportion of CD16+, HNK1− lymphocytes acquired HNK1 after a 6-day stimulation with K562 erythroleukaemia cells, and suggested that HNK1 expression was a late event in the differentiation of CD16+ lymphocytes. These authors also noted a decrease of CD11b expression by the CD16+ lymphocytes during such cultures.

We propose the following model, based on the combined results of several investigators (Clement, Grossi & Gartland,

1984; Landay, Clement & Grossi, 1984; Phillips & Lanier, 1985; Gratama *et al.*, 1987b) and our present studies. The $CD4^+$ and, particularly, $CD8^+$ lymphocytes that are activated by CMV infection develop cytoplasmatic granulation, and acquire HNK1 and CD11b. The molecules carrying those epitopes may serve as interaction structures with (virally infected) target cells. When those lymphocytes have returned to a resting state, such as in healthy CMV carriers, they have lost most of their granules, retain HNK1, but have down-regulated their CD11b expression.

Among peripheral blood $CD4^+$ lymphocytes, HNK1 identifies a small subset that provides generally neither help nor suppression to PWM-driven B cell differentiation. Similarly, Velardi *et al.* (1986b) showed that $CD4^+$, $HNK1^+$ lymphocytes obtained from reactive tonsils have no helper function in that assay, and their study of clones derived from those cells revealed that $CD4^+$, $HNK1^+$ lymphocytes have only a very limited capability to produce helper factors (Velardi *et al.*, 1986b). Both peripheral blood $CD8^+$, $HNK1^+$ and $CD8^+$, $HNK1^-$ lymphocytes in our study can suppress PWM-driven B cell differentiation. Thus, neither the HNK1 (this study) nor the CD11b markers (Takeuchi *et al.*, 1988) allow the exclusive identification of suppressor cells among the $CD8^+$ lymphocytes. The failure of HNK1 to do so can be explained by our observation that both $CD8^+$, $HNK1^+$ and $CD8^+$, $HNK1^-$ fractions contain $CD11b^+$ and $CD11b^-$ lymphocytes, and by the situation that our PWM-driven B cell differentiation assay detects the activities of the two types of suppressor cells as distinguished by Takeuchi *et al.* (1988): the $CD11b^-$ ones, that require the presence of $CD4^+$, $CD45R^+$ suppressor inducer cells, and the $CD11b^+$ ones, that may exert their immunosuppressive activities using lectin-dependent cytotoxicity (Phillips & Lanier, 1986).

The occurrence of the immunosuppressive effect of active CMV infection in otherwise healthy individuals (Carney, Iacoviello & Hirsch, 1985) and in bone marrow transplant recipients (Verdonck & De Gast, 1984) coincides with the appearance of large numbers of $CD8^+$ lymphocytes in the perpheral blood, of which the majority express HNK1 (Würsch *et al.*, 1985). The results of the present study suggest that $CD8^+$, $HNK1^+$ lymphocytes contribute to CMV-associated immunosuppression, but are not exclusively responsible for this effect.

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