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Rapid Communication

Hairy Cell Leukemia-Associated Antigen LeuM5 (CD11c) Is Preferentially Expressed by Benign Activated and Neoplastic CD8 T Cells

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LeuM5 antigen (CD11c, p150,95) expression, widely used as an immunodiagnostic marker for B-cell hairy cell leukemia, was examined on benign, normal peripheral blood T cells before and after stimulation in vitro ***with phytohemagglutinin and on a large, diverse panel of 73 T-cell neoplasms. Resting T cells lacked LeuM5. Intracytoplasmic LeuM5 was detectable at 3 to 4 days and surface membrane LeuM5 was detectable continuously between 5 and 17 days on ≥20% CD3 cells (maximum, 42% CD3 cells at 10 days) after activation. Two-color flow cytometric analysis of the activated T cells demonstrated that a maximum of 60% CD8 but only 25% CD4 cells expressed LeuM5; the mean percentage of LeuM5$^+$ CD8 cells was 44% compared with 12% LeuM5$^+$ CD4 cells. A variable proportion of the neoplastic T cells in 19 of 73 (26%) T-cell neoplasms were LeuM5$^+$. Twelve of 18 CD4$^-$CD8$^+$ (67%) but only 5 of 40 CD4$^+$CD8$^-$ T-cell neoplasms expressed LeuM5. These studies demonstrate that the LeuM5 antigen is 1) expressed in association with T-cell activation, 2) preferentially expressed by activated CD8 cells, and 3) variably expressed by neoplastic T cells, but particularly by those exhibiting the CD4$^-$CD8$^+$ phenotype. (Am J Pathol 1990, 136:29–37)***

The LeuM5 antigen (CD11c, p150,95) is a heterodimer composed of two noncovalently linked polypeptide chains designated α and β, of 150 and 95 kilodaltons (kd), respectively.[1–4] LeuM5 serves as a receptor molecule for C3bi[4,5] and belongs to the β_2 subfamily of integrin receptor molecules, which includes lymphocyte function-associated antigen (LFA) and OKM1/Mac-1.[4,6–10] These three molecules share an identical β_2 chain. The α chain is recognized by monoclonal antibody SHCL-3, prepared by immunizing with hairy cell leukemia cells,[3] and has been widely used to detect the LeuM5 antigen. This antibody was originally thought to react only with granulocytes, monocytes, macrophages, acute myeloid, and monocytic leukemias and hairy cell leukemias and not with other benign and neoplastic lymphoid cells.[3] These findings have led to the widespread use of LeuM5 as an immunodiagnostic marker for hairy cell leukemia.[3,11]

However, a number of antigens highly associated with non–T-cell lineages and generally absent from benign normal T cells, are expressed by activated and/or neoplastic T cells. For example, the granulocyte/monocyte-associated antigen LeuM1 (CD15), frequently identified on Reed–Sternberg cells,[12–15] and the Reed–Sternberg cell-associated antigen Ki-1 (CD30), recognized by a monoclonal antibody prepared by immunization with a Hodgkin's disease-derived cell line,[16] have been identified on activated and neoplastic T cells.[12,13,17–25] The epithelial membrane antigen (EMA) has been also identified on neoplastic T cells.[20,26–28] In addition, some T-cell activation-associated antigens are expressed by cells of other lineages. For example, the interleukin-2 receptor (IL2-R, the Tac antigen CD25), is expressed by more than 90% of hairy cell leukemias, a B-cell neoplasm.[11]

Recently, the LeuM5 antigen was shown to be expressed by some cytotoxic T-cell clones[6–8] and by occasional peripheral T-cell lymphomas.[17] In addition, we discovered LeuM5 on the neoplastic cells of some cutaneous T-cell lymphomas during the course of our recent

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studies of LFA expression.[10] These findings led us to hypothesize that LeuM5 antigen expression also may be associated with T-cell activation. This hypothesis was investigated by examining LeuM5 antigen expression by benign normal T cells activated with phytohemagglutinin *in vitro* and by neoplastic T cells. The results of those studies are the subject of this report.

Materials and Methods

T-Cell Purification and Culture

Leukocytes were obtained from buffy coats of units of whole blood freshly collected from healthy normal donors at the Greater New York Blood Center, New York, NY. Viable mononuclear cells were immediately separated by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation.[29] The mononuclear cells were washed twice in phosphate-buffered saline (PBS) and the T cells were separated from other mononuclear cells on a Ficoll-Hypaque density gradient according to their ability to form sheep erythrocyte (E) rosettes with Vibrio cholerae neuraminidase (VCN type V, Sigma Chemical Co, St. Louis MO)-treated sheep erythrocytes.[30] After washing in PBS, an aliquot of purified resting T cells was removed for baseline staining. The remaining purified T cells were cultured at a concentration of 1×10^6 cells/ml in RPMI-1640 (Cellgro, Washington, D.C.) containing 10% bovine serum albumin (BSA, Flow Laboratories, McLean, VA), 1% penicillin and gentamycin and 2ug/ml phytohemagglutinin (Wellcome Diagnostics, Research Triangle, NC) in a humidified 5% CO_2 atmosphere at 37 C for up to 28 days.

Pathologic Specimens

A panel of 73 T-cell neoplasms consisting of 27 peripheral T-cell lymphomas (PTCL), 26 cutaneous T-cell lymphomas (CTCL), 15 T-cell chronic lymphocytic leukemias (CLL), and 5 precursor T-cell acute lymphoblastic leukemias (ALL) and lymphoblastic lymphomas (LBL) were included in this study. These neoplasms were randomly selected from cases that had been previously well-characterized histologically and immunophenotypically on the basis of having adequate numbers of cryopreserved viable cells or tissue blocks available to perform the studies described here.

Monoclonal Antibodies

The monoclonal antibodies used in these studies included: OKT3 (CD3), OKT4 (CD4), OKT6 (CD1), OKT8 (CD8), OKT9, OKT10 (CD38), OKT11 (CD2) (Ortho Diagnostics, Raritan, NJ), IL2-R (CD25), HLA-DR (United Biomedical, Lake Success, NY), Leul (CD5), Leu2a (CD8), Leu3a (CD4), LeuM3 (CD14), LeuM5 (CD11c) (Becton–Dickinson, Mountain View, CA), Ki-67 (Dako Corporation, Santa Barbara, CA), and B1 (CD20) (Coulter Immunology, Hialeah, FL). The following isotype-matched monoclonal antibodies with irrelevant specificity were used as negative controls: MOPC21 (IgG_1), UPC-10 (IgG_{2a}), MOPC141 (IgG_{2b}), and MOPC104 (IgM) (Sigma).

Immunophenotypic Analysis of Cultured T Cells

One-and two-color indirect immunofluorescent cytofluorometric analyses were performed using the FACScan fluorescent activated cell sorter (Becton–Dickinson, Mountain View, CA) as previously described in detail.[31] A minimum of 1.5×10^4 events in each sample were collected, stored, and analyzed using the CD30 Hewlett Packard system (Mountain View, CA). Dead cells and debris were excluded by conventional scatter gating and propidium iodide staining. Fluoroisothiocyanate and phycoerythrin emission signals were collected using appropriate filters at 530 and at 575 nm, respectively, after logarithmic amplification. Isotype-matched monoclonal antibodies with irrelevant specificity were used as negative controls.

Intracytoplasmic LeuM5 was detected by immunoperoxidase staining of cytopsin slide preparations. Aliquots of the cultured T cells were removed at time 0 and at each 24-hour culture interval for 7 days. The cells were placed onto glass microscopic slides by cytocentrifugation (Shandon-Elliot Cytocentrifuge, Pittsburgh, PA), immediately fixed in cold 70% ethanol for 30 minutes at 4 C, and then washed in PBS overnight. The slides were overlaid with 100 ul of anti-LeuM5 diluted 1:20 in horse serum, incubated in a moist chamber for 15 minutes at 37 C, washed in PBS at room temperature, and returned to the moist chamber where they were overlaid with biotinylated goat anti-mouse IgG immunoglobulin (1:200, Vector Laboratories, Burlingame, CA) for 10 minutes at 37 C. They were then washed in PBS, incubated with peroxidase conjugated avidin-biotin complex (1:100, Vector), developed with DAB (1:100, Sigma) as previously described,[31] and counterstained with hematoxylin.

Immunophenotypic Analysis of T-Cell Neoplasms

The immunophenotype of all 73 T-cell neoplasms had been determined at the time of diagnosis using previously described cell-suspension immunofluorescent cytofluo-

rometric and/or immunohistochemical techniques.[31] LeuM5 antigen expression by the 17 T-cell leukemias was determined by single-color, and in some instances, by two-color flow cytometry.[31] LeuM5 antigen expression by the 56 T-cell lymphomas was determined by single-color immunohistochemical analysis on cryostat tissue sections using a three-step avidin-biotin immunoperoxidase technique.[31]

Many LeuM5-positive macrophages were present in the tissue sections of some T-cell lymphomas, rendering accurate assessment of LeuM5 antigen expression by neoplastic T cells in these cases difficult. The proliferation-associated antigen Ki-67 is detectable in the nuclei of proliferating neoplastic cells but not in macrophages.[32-34] Therefore, in these cases double immuno-enzymatic staining with monoclonal antibodies Ki-67 and LeuM5 was performed by immunoperoxidase and alkaline phosphatase, respectively, to detect LeuM5 on the surface membrane of Ki-67-positive proliferating, neoplastic T cells. After fixation, the cryostat sections were overlaid with Ki-67 (diluted 1:20) for 15 minutes at 37 C, washed in PBS, and reacted with horse anti-mouse IgG immunoglobulin (diluted 1:200, Vector). Peroxidase conjugated avidin-biotin complex was applied and developed with DAB.[31] The sections were then overlaid with LeuM5 (diluted 1:240), incubated for 35 minutes at 37 C, washed in PBS, covered with goat anti-mouse IgG_{2b} (diluted 1:100, Fisher) and incubated for 20 minutes at 37 C. The sections were covered with alkaline phosphatase anti-alkaline phosphatase (APAAP) complex (diluted 1:25, Dako Corporation, Santa Barbara CA), incubated for 20 minutes at 37 C, washed three times in PBS, and developed for 20 minutes at room temperature on a shaker using Fast Blue BB (Sigma) as substrate.

Results

Activated Normal T Cells in Culture

The purified T cells were examined before culture, at time 0, and at each 24-hour interval during culture for 7 consecutive days using one-color (12 experiments) and two-color (5 experiments) flow cytometry. The cells were also analyzed on culture days 10, 14, 17, 21, and 28 in those experiments in which sufficient numbers of viable cells were available. The purified T cells were examined before culture, time 0, and at random time points during culture for their expression of pan T-cell antigen CD3, pan B-cell antigen CD20, and monocyte-associated antigen CD14 so as to monitor their purity. The cells were always ≥95% $CD3^+$, ≤2% $CD20^+$, and ≤1% $CD14^+$. They were also examined for CD3, the helper and suppressor/cytotoxic T-cell subset-associated antigens CD4 and CD8, respectively, and for the LeuM5 antigen at each time point. The cultured T cells were double stained for LeuM5 and CD4 or CD8 in five experiments to determine LeuM5 antigen expression by the CD4 and CD8 subpopulations.

There was no evidence of intracytoplasmic or surface membrane LeuM5 antigen expression on resting, unstimulated T cells. Intracytoplasmic LeuM5 was initially detected at 72 to 96 hours after stimulation where it was identified primarily in the Golgi region of the cultured T cells (Figure 1). Surface membrane LeuM5 was initially detected on ≥20% $CD3^+$ cells at 120 hours; this expression was maintained continuously in culture until after 17 days (Figures 1, 2). The peak expression of LeuM5 occurred at 240 hours (10 days) when LeuM5 was detected on the surface membrane of 42% CD3 cells. Cytoplasmic and surface membrane expression were retained simultaneously at least until 168 hours (7 days) in culture.

Two-color cytometric analysis of the cultured T cells with LeuM5 and CD4 and LeuM5 and CD8 showed that LeuM5 was preferentially expressed by the $CD4^-CD8^+$ (suppressor/cytotoxic) T-cell subset (Figure 3). Twenty percent or more of the CD8 cells expressed LeuM5 at 96 hours (4 days) and continued to express this antigen until after 14 days in culture. In contrast, as many as 20% of the CD4 T cells expressed LeuM5 only at one time point, 240 hours (10 days), after stimulation (Figure 2). Furthermore, a larger proportion of CD8 cells than CD4 cells expressed LeuM5. At its maximum peak of expression, nearly 60% of the CD8 but only 25% of the CD4 cells expressed LeuM5 (Figure 2). During a 14-day culture period, the mean percentage of LeuM5-positive CD8 cells was 44% compared with only 12% CD4 cells.

Neoplastic T Cells

Nineteen of the 73 T-cell neoplasms (26%) contained LeuM5-positive neoplastic T cells. This included 5 of 15 CLLs (33%), 8 of 26 CTCLs (32%), and 6 of 27 (22%) PTCLs, but none of the 5 lymphoblastic malignancies. Among those PTCLs in which numerous macrophages were present, two-color immunohistochemical staining with monoclonal anti-Ki-67 and anti-LeuM5 showed that a variable proportion of the Ki-67-positive, actively proliferating, presumably malignant T cells, and not the Ki-67-negative, inactive macrophages, truly expressed LeuM5 (Figure 1). The percentage of LeuM5-positive neoplastic T cells in each case ranged from 20% to 90%. Twenty percent to 30% of the neoplastic T cells in 15 cases and more than 50% of the neoplastic T cells in 4 cases expressed LeuM5.

LeuM5 was preferentially expressed by the $CD4^-CD8^+$ neoplasms (Table 1). Specifically, 12 of 18 $CD4^-CD8^+$ (67%), but only 5 of 40 $CD4^+CD8^-$ (13%), 2 of 8

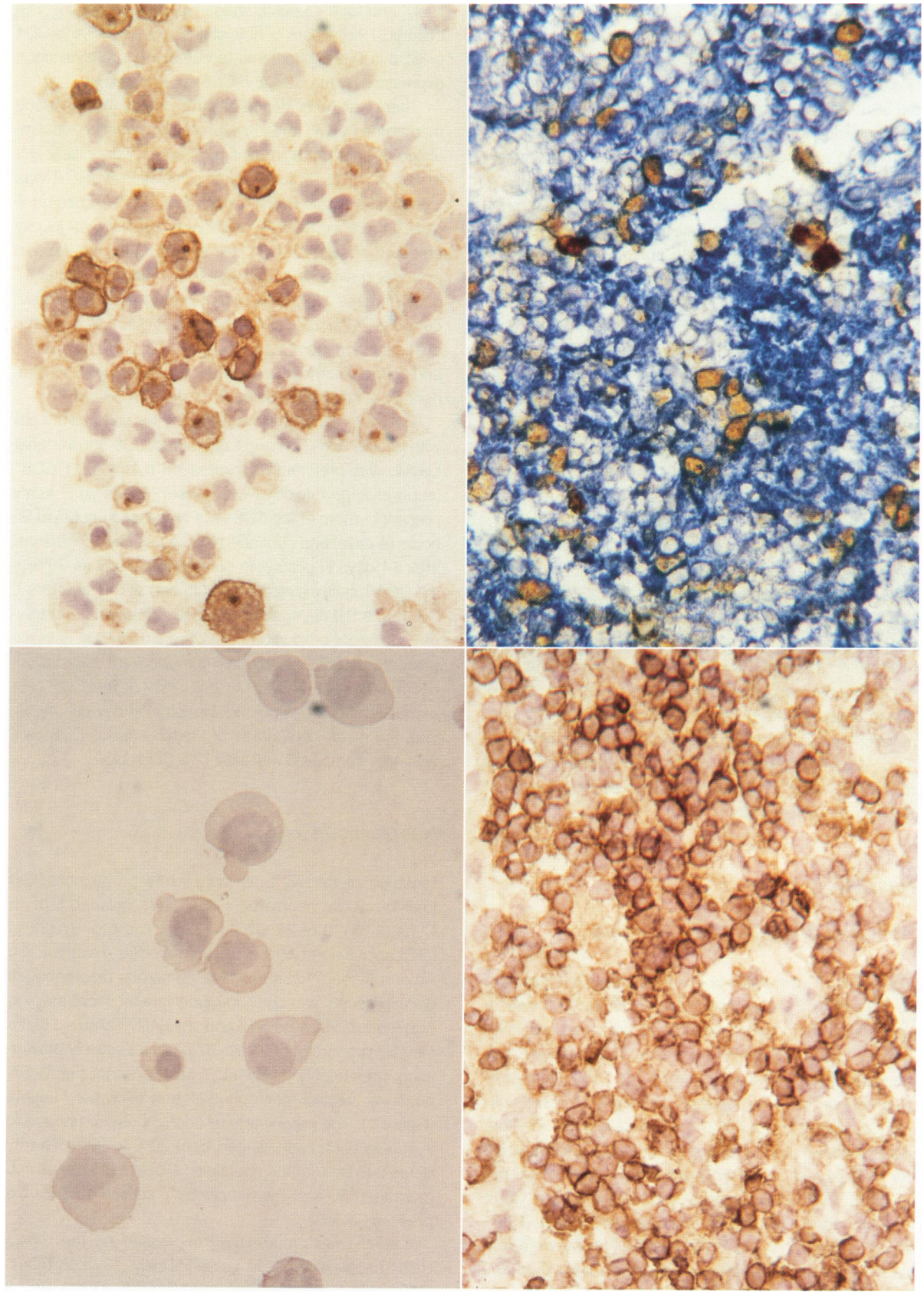

Figure 1. *(With journal held vertically) Cytocentrifuge slide preparations of purified CD3 positive (T) cells cultured in the presence of PHA and stained by immunoperoxidase for the presence of Leu M5.* **A** *(top left): At 48 hours after stimulation with PHA, the T cells lack cytoplasmic and surface membrane Leu M5.* **B** *(top right): At 120 hours following stimulation with PHA, many T cells express cytoplasmic Leu M5 (solitary focus of positive staining in the Golgi region) or a combination of cytoplasmic and surface membrane Leu M5.* **C** *and* **D** *illustrate representative immunohistochemical staining of cryostat tissue sections of a peripheral T-cell lymphoma (PTCL) containing Leu M5 positive neoplastic CD3 (T) cells.* **C** *(bottom left): The majority of the neoplastic T cells express CD3 by immunoperoxidase staining.* **D** *(bottom right): Immunohistochemical staining of the same PTCL for the intranuclear, proliferation-associated antigen Ki-67 by immunoperoxidase (brown) and for Leu M5 by alkaline phosphatase anti-alkaline phosphatase (blue) show that the majority of the cells, including the proliferating, presumably neoplastic Ki-67 positive cells (brown) are Leu M5 positive (blue) (×400).*

$CD4^-CD8^-$ (25%), and none of 6 $CD4^+CD8^+$ neoplasms expressed LeuM5. In summary, 12 of the 19 LeuM5-positive T-cell neoplasms (63%) displayed the $CD4^-CD8^+$ phenotype. Furthermore, two-color flow cytometric analysis for LeuM5 and CD3, CD4, CD8, and CD14 was performed in 4 $CD4^+CD8^-$, 7 $CD4^-CD8^+$, and 1 $CD4^+CD8^+$ CLLs to specifically determine the cell population expressing LeuM5 in each neoplasm. Neoplastic T cells in 4 of the 7 $CD4^-CD8^+$ CLLs but not in any of the other CLLs expressed LeuM5. Furthermore, only the $CD3^+$ and $CD8^+$, and not the $CD4^+$ cells in these 4 cases coexpressed LeuM5 (Table 2).

The 19 T-cell neoplasms expressing LeuM5 were also examined for their expression of those antigens most commonly associated with T-cell activation, namely T9, T10, IL2-R, and HLA-DR. All 19 LeuM5-positive T-cell neoplasms also expressed at least one of these activation antigéns; the majority, 15 cases, expressed two, three, or

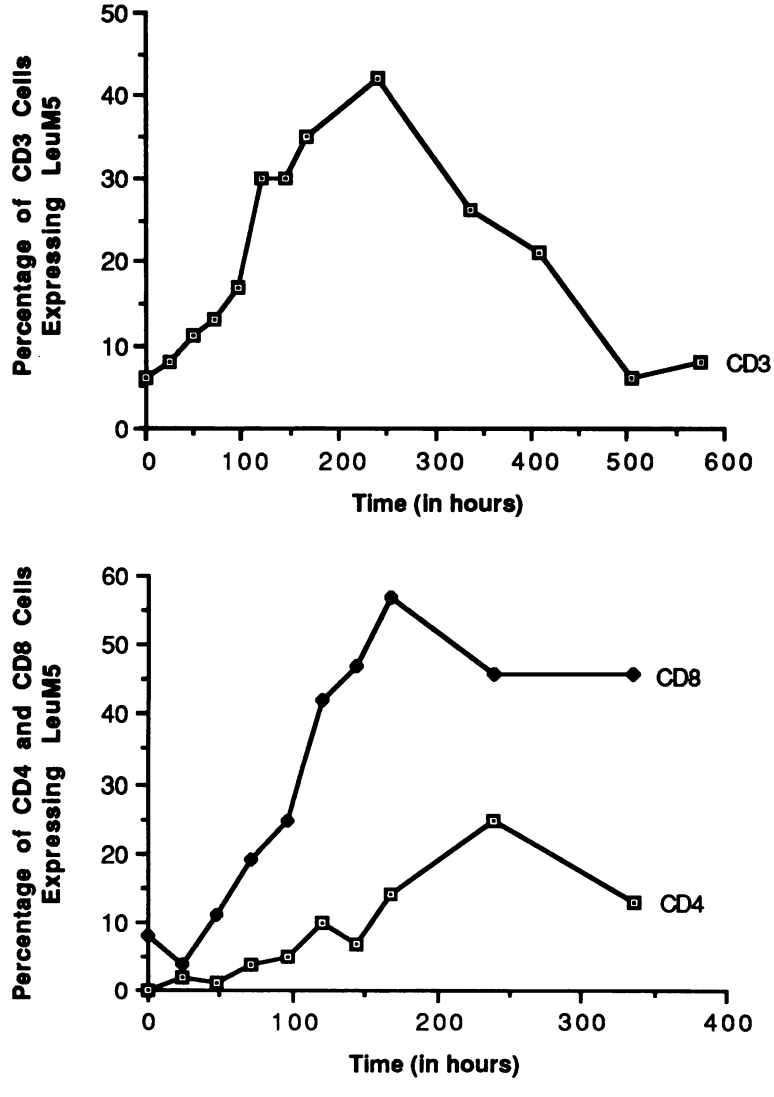

Figure 2. *The percentage of cultured CD3 (top), CD4 and CD8 (bottom) cells expressing Leu M5 at specific points in time following stimulation with PHA.*

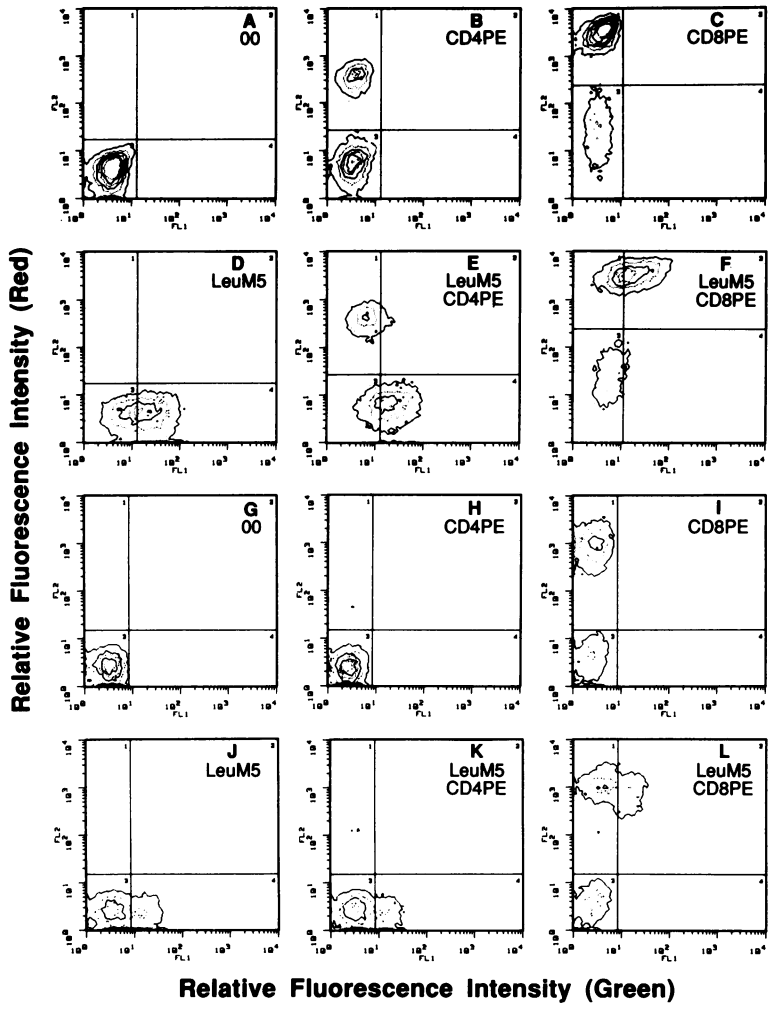

Figure 3. *Leu M5 antigen expression by benign and malignant CD4 and CD8 T lymphocytes based upon indirect immunofluorescent flow cytometric analysis. Contour maps A-F represent analysis of PHA-stimulated benign, peripheral blood T lymphocytes after 2 weeks in culture. Contour maps G–L represent analysis of a $CD4^-CD8^+$ T-cell chronic lymphocytic leukemia. The thresholds of positivity for green (Leu M5, vertical line) and for red (CD4 and CD8, horizontal line) fluorescence intensities were established with unstained controls (A and G), negative controls stained with unrelated antibodies (not shown) and single staining with CD4PE (B and H) and CD8PE (C and I).*

four of them. Notably, HLA-DR was expressed by 17 of the 19 cases. T10 was present on all 5 CLLs, but was present on only 6 PTCLs and CTCLs. However, when LeuM5-positive and LeuM5-negative T-cell neoplasms were compared, no distinct pattern or clustering of T-cell activation-associated antigen expression appeared to correlate with LeuM5 antigen expression.

Discussion

Early observations, using monoclonal antibody SHCL-3, suggested that peripheral blood granuloctyes, monocytes, tissue macrophages, and the malignancies derived from these cell populations, acute myeloid and monocytic leukemias, express the LeuM5 antigen.[3,6,9,35–39] In contrast, it was observed that ≥95% of peripheral blood lymphocytes and all B- and T-cell-derived leukemias and lymphomas lack the LeuM5 antigen.[3] The sole exception was hairy cell leukemia,[3] which has been consistently found to be LeuM5 positive.[3,11] However, more recently, LeuM5 antigen expression by occasional B-cell CLLs, both freshly isolated[40] and after stimulation with TPA,[41] occasional CTCLs[10] and some large-cell lymphomas of uncertain lineage[17] has been described. Finally, some cytotoxic T-lymphocyte (CTL) clones expressing either the $CD4^-CD8^+$ or the $CD4^+CD8^-$ phenotype also have been shown to express LeuM5.[7]

In the studies described here, the LeuM5 antigen was not detectable in the cytoplasm or on the surface membrane of resting, unstimulated T cells. However, intracytoplasmic LeuM5 was detectable at 3 to 4 days and surface membrane LeuM5 was detectable 24 to 48 hours later, at 5 days, in ≥20% of T cells after activation with PHA and this expression was continuously maintained for more than 17 days in culture. Twenty-five percent to 57%, mean 44%, of the $CD4^-CD8^+$ T cells expressed LeuM5 continuously between culture days 4 and 14 while 5% to 25%, mean 12%, of the $CD4^+CD8^-$ T cells expressed LeuM5 during this same period. A variable proportion of the neoplastic cells comprising 19 of 73 (26%) T-cell neoplasms, including CLLs, CTCLs, and PTCLs expressed

the LeuM5 antigen. However, 12 of 18 $CD4^-CD8^+$ neoplasms (67%) but only 5 of 40 $CD4^+CD8^-$ neoplasms (13%) contained ≥20% LeuM5 antigen-positive neoplastic T cells. Therefore, the results of our studies demonstrate that the LeuM5 antigen is 1) expressed in association with T-cell activation, 2) preferentially expressed by activated $CD4^-CD8^+$ T cells, and 3) variably expressed by neoplastic T cells, but particularly by those exhibiting the $CD4^-CD8^+$ phenotype.

Interestingly, the mechanism of surface membrane expression of the LeuM5 antigen appears to be similar in granulocytes, monocytes/macrophages, and T cells. Granulocytes and monocytes/macrophages express surface membrane LeuM5 antigen in relatively low density until stimulated.[2,6–8,36–38] Our results demonstrate that T cells also require stimulation to express detectable amounts of surface membrane LeuM5. However, the time course of LeuM5 antigen expression by granulocytes, monocytes/macrophages, and T cells after activation is quite different. LeuM5 appears on the surface membrane of granulocytes and monocytes within minutes after stimulation.[35,37,42] In contrast, significant levels of LeuM5 do not appear on the surface membrane of T cells until 5 days after activation. The temporal difference in surface membrane expression of LeuM5 is probably due to the readily available stores of intracytoplasmic LeuM5 in unstimulated granulocytes and monocytes/macrophages.[37] In contrast, it appears that only activated and not resting T cells synthesize LeuM5 because intracytoplasmic LeuM5 is not detectable in T cells until 3 to 4 days after stimulation.

The reason(s) why LeuM5 is expressed on activated T cells, especially those belonging to the $CD4^-CD8^+$ subset, and the functional role(s) of LeuM5 on these T cells is unclear. However, the expression and function of the LeuM5 molecule on granulocytes and monocytes/macrophages is understood reasonably well. LeuM5 is probably identical to CR4,[5] which binds C3bi.[5,43,44] Therefore, one of the primary functions of the LeuM5 molecule on these phagocytic cells is probably to clear opsonized particles and immune complexes.[5] The LeuM5 molecule on granulocytes and monocytes/macrophages is also involved in chemotaxis, phagocytosis, and cell adhesion, especially with endothelial cells and other granulocytes and monocytes/macrophages.[35–37,45,46] The lack of LeuM5 and its related antigens, LFA-1 and Mo-1, results in a rare genetic disorder, leukocyte adhesion deficiency (LAD).[42,47,48] Patients with LAD produce abnormal or very small amounts of the 95-kd β chain. These individuals develop recurrent and life-threatening bacterial infections and often die at an early age.[42,47,48] By extrapolation, we can speculate that the functional role(s) of the LeuM5 antigen on T cells may be analogous to its role(s) on granulocytes and monocytes/macrophages, ie, that by binding C3bi, LeuM5 is involved in the removal and eventual destruction of immune complexes, bacteria, viruses, virally infected cells, and tumor cells. This hypothesis is supported by the fact that the LeuM5 molecule on cytotoxic T-cell clones is functionally involved in cell-mediated cytotoxicity by forming conjugates with the target cells.[7] Therefore, these different cell populations may employ similar mechanisms to help eliminate those substances that the body recognizes as foreign.

The hypothesis that LeuM5 antigen expression and function on T cells is analogous to that of granulocytes and monocytes/macrophages may also explain the preferential expression of LeuM5 by $CD4^-CD8^+$ T cells. This T-cell subset is commonly associated with suppressor and cytotoxic functions, some of which are similar to those displayed by monocytes/macrophages, such as conjugate formation with target cells.[7,46] Some $CD4^+CD8^-$ T cells are also associated with cytotoxic functions.[7] Perhaps this explains LeuM5 antigen expression by these T cells as well.

Analogous to benign normal T cells, LeuM5 was expressed by variable proportions of the neoplastic T cells comprising 67% of $CD4^-CD8^+$ but by only 13% of $CD4^+CD8^-$ T-cell neoplasms. All of the LeuM5-positive T-

Table 1. *LeuM5 Antigen Expression on Neoplastic T Cells According to Phenotype*

Phenotype	Number of cases	Number positive	Percentage positive
$CD4^-$ $CD8^+$	18	12	67
ALL/LBL	0	0	0
CTCL	5	4	80
PTCL	5	3	60
CLL	8	5	63
$CD4^+$ $CD8^-$	40	5	13
ALL/LBL	0	0	0
CTCL	16	3	19
PTCL	19	2	16
CLL	5	0	0
$CD4^+$ $CD8^+$	7	0	0
ALL/LBL	3	0	0
CTCL	1	0	0
PTCL	1	0	0
CLL	2	0	0
$CD4^-$ $CD8^-$	8	2	25
ALL/LBL	2	0	0
CTCL	4	1	25
PTCL	2	1	50
CLL	0	0	0

Table 2. *Dual Expression of LeuM5 and CD4, CD8, and CD14 by T-CLLs*

Antigens	Case 1	Case 2	Case 3	Case 4
$LeuM5^+$	21*	76	20	20
$LeuM5^+$ $CD4^+$	<1	<1	12	16
$LeuM5^+$ $CD8^+$	20	67	23	21
$LeuM5^+$ $CD14^+$	<1	<1	1	1

* Percentage positive cells.

cell neoplasms also expressed other antigens commonly associated with T-cell activation, such as T9, T10, IL2-R, and HLA-DR. Similarly, Weiss et al[17] has shown that four of five LeuM5-positive large-cell lymphomas of probable T-cell origin, based on the results of antigen receptor gene rearrangement analysis, also expressed T9, IL2-R, and/or HLA-DR; the fifth case expressed Ki-1 (CD30), another T-cell activation-associated antigen. Therefore, it is highly probable that LeuM5 antigen expression on neoplastic T cells is related to its association with a state of T-cell activation. LeuM5 antigen positive neoplastic T cells may represent the malignant counterpart of activated benign T cells frozen at one or more points after stimulation. *In vitro* functional analyses of LeuM5 antigen positive neoplastic T cells may provide a useful model for improving our understanding of the functional role of the LeuM5 molecule on T cells.

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