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C1.7 Antigen Expression on CD8+ T Cells Is Activation Dependent: Increased Proportion of C1.7+CD8+ T Cells in HIV-1-Infected Patients with Progressing Disease[1]

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The C1.7 Ag is a surface marker previously shown to be expressed on all NK cells and on a subset of CD8+ T cells. We report in this study that C1.7 Ag expression on peripheral blood-derived CD8+ T cells overlaps with activation markers S6F1^{high} and CD29^{high} and is reciprocally expressed with CD62L. C1.7 Ag expression can be induced in vitro on CD8+ T cells by anti-CD3 cross-linking, suggesting that C1.7 Ag is activation dependent. In contrast to NK cells, C1.7 Ag does not signal on CD8+ T cells, nor does it induce redirected lysis upon ligation. The proportion of C1.7 Ag+CD8+ T cells is increased in HIV-infected patients compared with healthy donors. In 69 HIV-infected patients, we observed a significant inverse correlation between the percentage of C1.7 Ag-expressing CD8+ T cells and the absolute CD4+ T cell count. Two-year clinical follow-up of patients with initial CD4+ T cell count of >400 cells/mm^3 and a normal proportion of C1.7 Ag+CD8+ T cells revealed that these patients were clinically stable with minimal HIV-associated symptoms. In contrast, 10 of 12 patients with CD4+ T cell counts of >400 cells/mm^3 and an elevated proportion of C1.7 Ag+CD8+ T cells were symptomatic. ANOVA analysis of patients indicates that C1.7 Ag is a better predictor of disease progression than CD4 count. Overall, our findings indicate that C1.7 Ag is the first described marker for activated/memory CD8+ T cells and a useful parameter for evaluating the level of CD8+ T cell activation in vivo. *The Journal of Immunology,* 1999, 162: 7563–7568.

T he C1.7 Ag is a cell surface marker identified by a mAb generated by immunization of BALB/c mice with human NK cells. The C1.7 Ab was selected by screening the hybridomas generated for the ability to be lysed by NK cells. Ab cross-linking of C1.7 Ag on NK cell surface initiates polyphosphoinositol turnover and intracellular Ca^{2+} flux, induces Ab-redirected cytotoxicity and lymphokine secretion, while C1.7 F(ab′)$_2$ Ab blocks NK cell-mediated cytolysis (1). C1.7 Ag is expressed on virtually all human peripheral blood NK cells, approximately one-half of CD8+ T cells (TCR α/β), and the majority of γ/δ T cells (1). Although we have not observed C1.7 Ag expression on PBL-derived CD4+ T cells, it has been reported to be present on high avidity cytotoxic CD4+ T cells involved in heart graft rejection (2) and on activated CD4+CD8+ T cells from an HIV-infected individual producing high levels of IFN-γ and TNF-α (3). No other Ab submitted to the V International Workshops on Human Leukocyte Differentiation Antigens was observed to have the same phenotypic distribution and function (4).

The C1.7 Ag and its counter-receptor have recently been cloned[5]. The C1.7 Ag is a variably glycosylated molecule of 39–69 kDa and is a member of the Ig superfamily with homology with CD84 and CD48[5]. It is the human homologue of the murine 2B4 Ag, found on NK and non-MHC-restricted cytotoxic T cells (5). Similar to C1.7 Ag, Abs to 2B4 activate NK cytotoxic activity and secretion of IFN-γ. The ligand for C1.7 Ag is CD48, an Ag found primarily on B and dendritic cells, whose function is still unclear.

Within IL-2-activated CD8+ T cells, non-MHC-restricted cytotoxic activity is mediated exclusively by the C1.7 Ag-expressing CD8+ T cells in that depletion of C1.7 Ag+CD8+ T cells totally abolished the ability of CD8+ T cells to lyse a variety of sensitive targets (1). A potential role for C1.7 Ag in cytotoxicity was further suggested by the fact that C1.7 Ab inhibited non-MHC-restricted cytotoxicity mediated by IL-2-activated CD8+ T cells (1).

In HIV-1-infected patients, a progressive increase in CD8+ T cell activation has been documented extensively by analyzing the expression of activation-dependent surface Ags (6–16). CD8+ T cells may control HIV infection via cytotoxic activity against infected cells and inhibition of viral infection via soluble mediators (16). Elevated levels of CD8+ T cell activation markers such as CD38 have been shown to have prognostic value in determining progression to AIDS (17). The ability to characterize CD8+ T cell function and activation is therefore an important aspect of HIV disease analysis.

In this study, we demonstrated that C1.7 Ag on CD8+ T cells is activation dependent and its expression overlaps with other activation markers. On freshly isolated PBL, C1.7 Ag is the first described marker characterizing the activated/memory CD8+ T cells

characterized by low expression of CD62L. A pilot study examining the peripheral blood from HIV-1-infected patients determined that the proportion of C1.7 expressing CD8$^+$ T cells increases in HIV patients and correlates with clinical outcome.

Materials and Methods

Reagents

PHA and PMA were purchased from Sigma (L-9132, P-8139; St. Louis, MO). Anti-CD3 (OKT3) was obtained from American Type Culture Collection (Manassas, VA). Anti-CD16 (3G8) was a kind gift of Dr. J. Unkeless (Mount Sinai School of Medicine, New York, NY). Fluorochrome-conjugated anti-CD8, anti-CD28, and anti-CD62L were purchased from Immunotech (Marseille, France). S6F1 (anti-LFA-1 epitope (18)) and anti-CD29 (15) were kind gifts from Dr. R. Raynor (Coulter, Homestead, FL). B33.1 (anti-HLA-DR) was generated in our laboratory and directly conjugated with FITC. C1.7 Ab, generated in our laboratory, was used either biotinylated and developed by streptavidin PE-Cy5 (Life Technologies, Gaithersburg, MD) or directly conjugated with PE-Cy5 (IM1609; Immunotech). Human rIL-12 was kindly provided by Dr. S. Stan Wolf (Genetics Institute, Cambridge, MA). ^{51}Cr was obtained from DuPont-NEN (NEZ 030S; Wilmington, DE).

Patients and healthy controls

Blood was collected from 69 randomly selected HIV-1-infected patients and 14 healthy controls at the Hospital of the University of Pennsylvania (Philadelphia, PA). The blood samples from the patients were leftover material from determination of CD4$^+$ T cell count. 70% of the patients were on ACTG or industry-sponsored antiviral therapy, most of which involved the use of two or more agents. The remainder of patients represents a mix of clinic patients, 50% of whom were on no therapy and had a high CD4 count. Fixty-six patients (44 male, 9 female) were clinically followed for 2 yr. Thirteen patients either moved from the area or were not being cared for at the University of Pennsylvania for the complete 2-yr clinical analysis. Patients' clinical stage was classified at the end of 2 yr using the Centers for Disease Control (CDC) clinical classification, as described (19, 20). Briefly, CDC classification A are patients who are asymptomatic, acute primary HIV, or persistent generalized lymphadenopathy. Classification B are those patients who are symptomatic with conditions that are attributable directly to the HIV infection itself or due to dysfunction of the cell-mediated immune response such as Thrush, shingles, neuropathy, listeriosis, or bacillary angiomatosis. Classification C included clinical conditions such as Kaposi's sarcoma and *Pneumocystis carinii* pneumonia.

For in vitro experiments, healthy donor PBL, cord blood, or excess thymic tissue from neonatal heart surgery was obtained, and mononuclear cells were separated by Ficoll density-gradient centrifugation, followed by 1-h adherence on plastic at 37°C, stained, and analyzed on a Coulter XL flow cytofluorometer.

Flow cytometry and cell sorting

PBMC from HIV patients and controls were analyzed using three-parameter analysis from a whole blood preparation on a Becton Dickinson (Franklin Lakes, NJ) FACSort (FITC-CD3/PE-CD8α/biotinylated C1.7 Ag developed with streptavidin PE-Cy5). Isotype controls were used to establish cross-fluorochrome compensation.

Sterile sorting was conducted on a Coulter Elite using multiparameter analysis. Cell phenotypic analysis was conducted on >10,000 cells using a Coulter XL utilizing a single 488-nm excitation laser and channels scatter (488 nm), FITC (525 nm), PE (575 nm), PE-Texas Red (615 nm), PE-Cy5 (675 nm), and Abs described.

Cytotoxicity

NK and CD8$^+$ T cells were tested for cytotoxic potential by addition of 0.1 μg/ml of anti-CD16 or anti-CD3 to 10,000 ^{51}Cr-labeled P815 $\times$ 2 FcR$^+$ target cells/well with varying concentrations of effector cells. At the end of a 4-h incubation, 100 μl of supernatant was tested for ^{51}Cr release and percent cytotoxicity was determined according to the formula (cpm sample − cpm spontaneous)/(cpm maximal − cpm spontaneous) $\times$ 100.

Statistics

Univariant and multivariant ANOVA analyses were employed to compare the ability of C1.7 Ag and CD4 counts to predict clinical HIV disease severity (CDC classification A vs B or C). Two-way ANOVA was conducted on C1.7 Ag expression and CD4 count as a predictor of disease
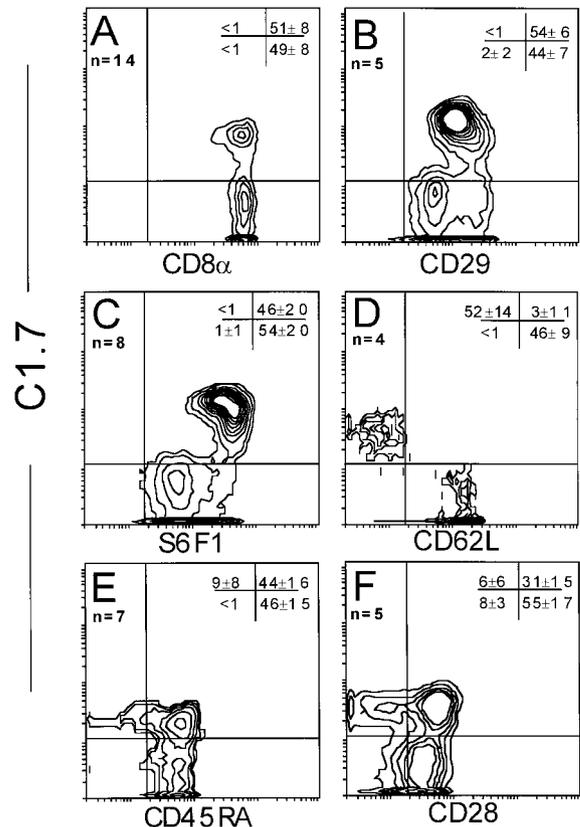

FIGURE 1. Comparison of C1.7 Ag expression and various surface markers on peripheral blood CD8$^+$ T cells. PBL were stained with biotin-conjugated C1.7 Ab developed with either PE-Cy5-streptavidin or PE-CD8α and compared with several FITC-conjugated surface proteins used to delineate activated states (*x*-axis). Results from PBL of a representative donor, gated on CD8α^{high}, are depicted with an average $\pm$ SD for all donors tested shown in cross-hair quadrant insets.

progression for all patients and then independently for patients with CD4 counts >400 cells/mm^3 and CD4 counts <400 cells/mm^3.

To compare cytotoxic activities of C1.7$^+$ Ag and C1.7$^-$ Ag populations in vitro, an additive two-way ANOVA model was fit to the data to estimate the common difference between C1.7 Ag$^+$ and C1.7 Ag$^-$ groups across all six E:T ratios.

Results

Expression of C1.7 Ag on CD8$^+$ T cells

C1.7 Ag, in agreement with previously reported data (1), was observed to be expressed on 51 $\pm$ 8% of PBL-derived CD3$^+$CD8$^+$ cells from 14 donors (Fig. 1A). C1.7 Ag expression was compared with that of other surface Ags in CD8$^+$ T cells by multicolor flow cytometry. PBL from healthy donors were stained with triple combination of PE or FITC anti-CD8α, biotinylated C1.7 Ab (developed with streptavidin PE-Cy5), and FITC-conjugated anti-CD29, anti-CD28, anti-CD45RA, and anti-CD62L, and PE-conjugated S6F1. CD8^{high} cells were electronically gated, and the staining with FITC-conjugated Ab (Fig. 1, *x*-axis) was correlated to C1.7 Ag staining (Fig. 1, *y*-axis).

C1.7 Ag was expressed on most CD8$^+$ T cells expressing CD29^{high} (Fig. 1B) and S6F1^{high} (Fig. 1C), although a small number of C1.7 Ag-negative, CD29^{high}, or S6F1^{high} cells were detected. Both CD29 and the S6F1 are expressed at intermediate levels on naive CD8$^+$ T cells and at high levels on activated T cells (21). Only a small percentage of CD8$^+$ T cells expressed the acute activation markers CD69 and HLA-DR: these cells all expressed C1.7 Ag (not shown).

C1.7 Ag expression negatively correlates with CD62L expression defining two mutually exclusive CD8$^+$ T cell subsets (Fig. 1D). CD62L is expressed on naive T cells and is lost upon activation (22). C1.7 Ag expression was found on all CD45RA$^-$CD8$^+$ T cells and on approximately one-half of the CD45RA$^+$CD8$^+$ T cell population (Fig. 1E) consistent with its expression on all CD62L$^-$-activated CD8$^+$ T cells. Whereas on CD4$^+$ T cells CD45RA expression is considered a marker for naive cells (23), CD45RA$^+$CD8$^+$ T cells, in addition to naive T cells, also comprise effector cells with high cytotoxic activity and elevated levels of CD11a, CD11b, CD18, and CD49d, and absence of CD62L (22, 24, 25). All CD45RA$^-$CD8$^+$ T cells have an activated phenotype and uniformly express C1.7 Ag.

Expression of CD28, a costimulatory receptor for T cell activation, is found on the majority of CD8$^+$ T cells of which approximately one-third of CD28$^+$CD8$^+$ T cells expressed C1.7 Ag. Upon in vivo activation, a subset of CD8$^+$ T cells lose CD28 expression (26). Although a proportion of both the CD28^{dim} and CD28^{high} population is C1.7$^-$, it is noteworthy that all of the cells of the small CD8$^+$ T cell subset that is completely CD28$^-$ are C1.7 Ag$^+$ (Fig. 1F).

In vitro activation with anti-CD3 mAb induces C1.7 Ag expression on CD8$^+$ T cells

To assess the effect of in vitro activation on C1.7 Ag expression, PBL-derived CD8$^+$ T cells were sorted by flow cytometry into C1.7 Ag$^+$ and C1.7$^-$ populations and cultured in the presence of either PHA or plastic-bound anti-CD3 (OKT3) Ab for 2 wk in medium containing 50 U/ml rIL-2. Fresh medium and IL-2 were added every 4 days. Both PHA- and anti-CD3-activated CD8$^+$ T cells developed a typical phenotype of activated CD8$^+$ T cells (HLA-DR$^+$, CD62L$^-$, S6F1^{high}, and CD29^{high}, not shown). However, whereas PHA-activated C1.7 Ag$^-$CD8$^+$ T cells maintained the C1.7$^-$ phenotype even if the culture was continued for over 1 mo (data not shown), anti-CD3 stimulation reproducibly resulted in expression of C1.7 Ag on the majority of CD8$^+$ T cells in three experiments conducted (Fig. 2). C1.7 Ag expression on C1.7 Ag$^+$CD8$^+$ T cells was unaffected by these culture conditions (data not shown).

C1.7 Ag is expressed on neither thymocytes nor cord blood CD8$^+$ T cells (data not shown and Fig. 2). Similar to PBL-derived CD8$^+$ T cells, activation of cord blood-derived CD8$^+$ T cells with PHA did not induce C1.7 Ag expression (not shown); however, C1.7 Ag expression was induced on these cells by stimulation with plate-bound anti-CD3 (Fig. 2).

Cytotoxic activity of C1.7 Ag$^+$CD8$^+$ T cells

It was shown previously that following culture in IL-2, CD8$^+$ T cell-mediated non-MHC-restricted killing was found exclusively in the C1.7 Ag$^+$ subset population (1). To investigate whether this was true for TCR-mediated killing, we used the experimental model of CD3 cross-linking (27) to induce redirected lysis in 5-day PHA-activated CD8$^+$ T cells. Although C1.7$^-$CD8$^+$ T cells had cytolytic activity, it was consistently lower at all E:T ratios than that of the C1.7-expressing population in eight experiments conducted (Fig. 3A). Additive ANOVA fit to the data to estimate a common difference between C1.7$^+$ and C1.7$^-$ cytotoxicity across all E:T ratios revealed a 15.64% difference in y-intercepts with a p value of 0.0001.

Differential function of C1.7 Ag on NK and CD8$^+$ T cells

C1.7 Ag cross-linking on NK cells induces intracellular Ca^{2+} flux, polyphosphoinositol hydrolysis, cytokine secretion, and redirected lysis (Fig. 3B) (1). To explore the function of C1.7 Ag on CD8$^+$
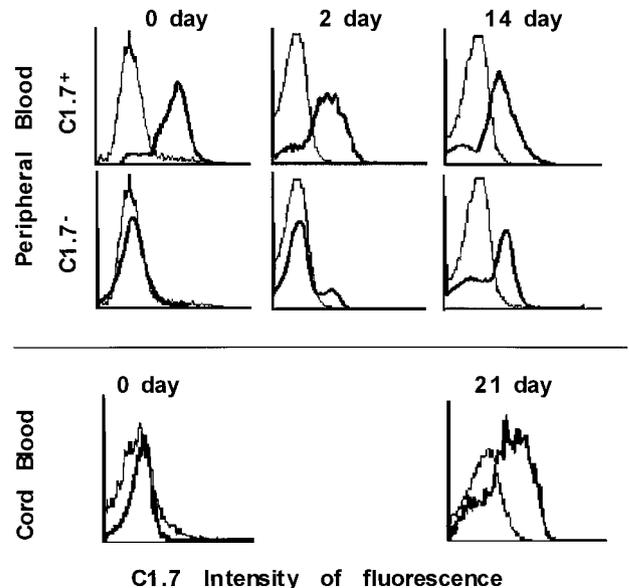

FIGURE 2. Expression of C1.7 Ag on peripheral blood- and cord blood-derived CD8$^+$ T cells. Peripheral blood CD8$^+$ T cells from three donors (representative donor shown) were sorted into C1.7$^+$ and C1.7$^-$ populations and stimulated with immobilized anti-CD3 or PHA for 14 days in the presence of 50 U/ml of rIL-2. Cells were stained with C1.7 Ab (bold line) or isotype control Ab (fine line). A total of 67.3% of the C1.7$^-$CD8$^+$ T cells expressed C1.7 Ag after 2 wk of anti-CD3 activation cells (*upper panel*). Cord blood-derived CD8$^+$ T cells, which are 100% C1.7$^-$, were stimulated with immobilized anti-CD3 in the presence of 50 U/ml rIL-2 for 3 wk before conducting two-color flow-cytometric analysis gating on CD8$^+$ T cells and analyzing C1.7 Ag expression.

T cells, we cross-linked C1.7 Ag on freshly isolated and 5-day PHA-treated CD8$^+$ T cells. Unlike on NK cells, C1.7 Ag cross-linking on CD8$^+$ T cells did not induce Ca^{2+} flux, secretion of cytokines (not shown), or Ab-redirected lysis of P815 target cells (Fig. 3C). However, CD8$^+$ T cells secreted IFN-γ and lysed P815 targets when treated with anti-CD3 Ab (Fig. 3C).

The proportion of CD8$^+$ T cells expressing C1.7 Ag$^+$ correlates with disease progression in HIV-infected patients

We quantified the proportion of C1.7 Ag-expressing CD8$^+$ T cells by gating on CD3$^+$CD8$^+$ T cells from 69 HIV-1-infected patients and 14 healthy controls using three-color flow cytometry. Fig. 4a depicts the percentage of CD8$^+$ T cells expressing C1.7 Ag (x-axis) plotted against absolute PBL CD4$^+$ T cell count/mm^3. The HIV-negative donors had 42.8 $\pm$ 9.6% (X $\pm$ SD) (range = 21–63%) of CD8$^+$ T cells expressing C1.7 Ag and 928 $\pm$ 353 CD4$^+$ T cells/mm^3. HIV-infected patients had a significantly higher (62.6 $\pm$ 20.8%, p = 0.0001; range = 7–93%) percentage of C1.7$^+$ Ag CD8$^+$ T cells and lower CD4 counts (337 $\pm$ 308 cells/mm^3, p = 0.0002) with a significant (p < 0.001) negative linear correlation (y = -6.2X + 720) between absolute CD4$^+$ T cell count and C1.7 Ag expression on CD8$^+$ T cells (Fig. 4B). However, a small number of HIV-infected patients with very low absolute CD4$^+$ T cell counts did not show an increased expression of C1.7 Ag on their CD8$^+$ T cells. These advanced patients also have a severe reduction in the number of CD8 T cells, perhaps resulting in a decreased level of activation in the surviving CD8$^+$ T cells.

After the initial flow-cytometric analysis, 56 patients were followed clinically for approximately 2 yr. ANOVA statistical analysis applied to the percentage of C1.7$^+$Ag CD8$^+$ T cells and absolute CD4 counts indicates a strong two-factor iteration
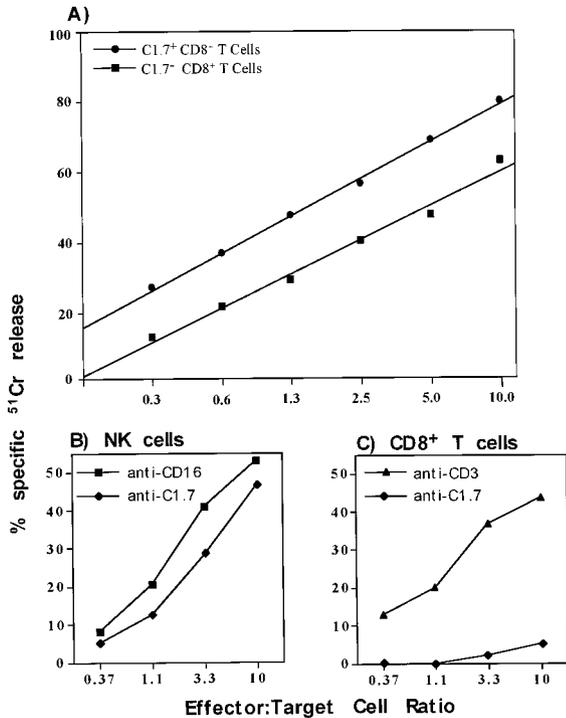
FIGURE 3. Cytotoxic activity of ex vivo and in vitro activated CD8^{+} T cells. *A*, C1.7^{+} and C1.7^{−} sorted CD8^{+} T cells from 5-day PHA-activated PBL were titrated with FcR^{+} P815 cells + anti-CD3 Ab (OKT3). Represented is linear regression of average of eight experiments. *B*, Redirected lysis of anti-CD16 and anti-C1.7 Abs on cultured NK cells, and *C*, anti-CD3 and anti-C1.7 Abs on cultured C1.7[+] PHA blasts. Cytotoxic activity was measured using a 4-h ^{51}Cr release assay.
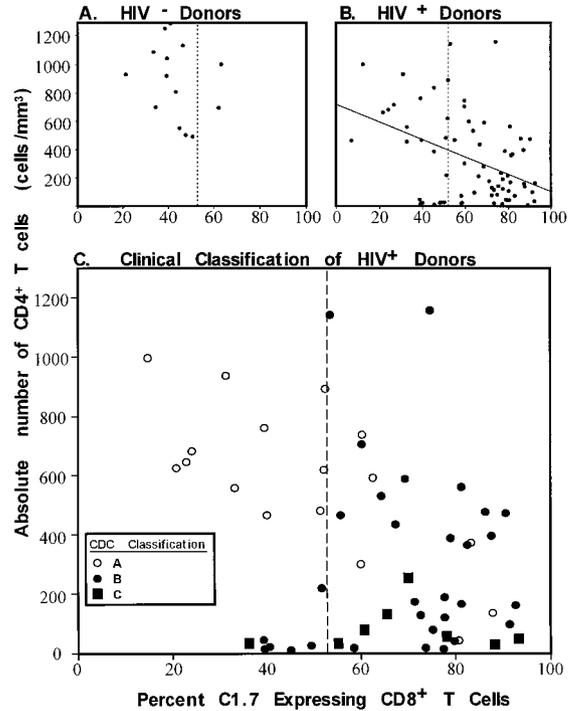

FIGURE 4. Correlation of peripheral blood absolute CD4^{+} T cell count and C1.7 Ag expression on CD8^{+} T cells from HIV-1-infected patients and healthy controls. PBL from 14 controls (*A*) and 69 HIV-1-infected patients (*B*) were stained with FITC anti-CD3, PE anti-CD8, and biotin anti-C1.7 Ab developed with PE-Cy5-streptavidin. The *x*-axis denotes the percentage of CD3^{+}CD8^{+} cells, which are C1.7 Ag positive. The dotted line represents average percentage of control donor C1.7 Ag expression plus 1 SD. Linear regression analysis of HIV-infected patients (*B*) reveals a significant ($p < 0.001$) correlation between C1.7 Ag expression and absolute CD4 cells/mm^3 ($r^2 = 0.4147$). *C*, Represents a plot of absolute CD4^{+} T cell count (*y*-axis) and percentage of C1.7^{+}CD8^{+} T cells (*x*-axis) at the initiation of the study. Fifty-six patients were scored using the CDC clinical classification (A, B, and C) for symptoms that were developed during the 2-yr follow-up. Thirteen of 69 patients had no follow-up since they were no longer cared for at the Hospital of the University of Pennsylvania.

($p = 0.0009$). A total of 85% of the patients with original CD4 counts less than 400 cells/mm^3 were symptomatic (CDC clinical classification B and C) 2 yr following initial screening, whereas only 41% of patients with CD4 counts >400 were symptomatic and had fewer and less severe AIDS-associated disease (CDC clinical classification A and B). Most striking was the fact that 2 yr after the T cell subset analysis, all of the patients with greater than 400 CD4^{+} T cells/mm^3 and a proportion of C1.7 Ag^{+} CD8^{+} T cells less than 1 SD above healthy controls were CDC clinical stage A, whereas 82% of patients with initial elevated C1.7^{+}CD8^{+} T cells had progressed to CDC clinical stage B. ANOVA analysis applied to percentage of C1.7 Ag-expressing CD8^{+} T cells and disease severity indicated a positive correlation ($p = 0.017$) between the two. Two one-way ANOVAs were also fitted separately to the patient groups with CD4 <400 and >400 cells/mm^3. For the latter group, the percentage of C1.7 Ag^{+} cells is highly predictive of disease severity, yielding a p value of 0.0002. This same variable, however, is not predictive of disease when patients have less than 400 CD4 T cells/mm^3 ($p = 0.486$). ANOVA analysis using CD4 counts indicated a significant correlation ($p = 0.0001$) between CD4 count and disease severity. However, the one-way ANOVA comparing CD4 counts and disease severity conducted on patients with CD4 counts <400 ($p = 0.686$) or >400 ($p = 0.19$) was not predictive of disease. These data suggest that a higher percentage of C1.7 Ag-expressing CD8^{+} T cells correlates with an increased severity of HIV-associated disease, and it is a better predictor than CD4 counts early in disease.

Discussion

C1.7 Ag expression on CD8^{+} T cells is dependent on activation. C1.7 Ag expression on PBL CD8^{+} T cells largely overlaps with that of the activation markers S6F1^{high} and CD29^{high}, while reciprocal with that of the CD62L marker of naive CD8^{+} T cells. The expression of C1.7 Ag on naive CD8^{+} T cells can, however, be induced by anti-CD3 stimulation.

Elevated CD29 and S6F1 expression has been used as marker for activated CD8^{+} T cells, while the low expressing cells are primarily naive. There is a good correlation between C1.7 Ag expression and the CD29^{high}/S6F1^{high} phenotype on CD8^{+} T cells. The acquisition of CD29^{high} and S6F1^{high} expression is slightly different from that of C1.7 expression since a small population (<5%) of CD8^{+} T cells that are CD29^{high} and S6F1^{high} do not express C1.7 Ag. S6F1 and CD29 are expressed at intermediate levels on all T cells, and upon activation their expression is increased. Therefore, the need for subjective gating and ability to interpret quantitative differences in flow-cytometric analysis limit the usefulness of these markers. We found that C1.7 Ag expression in CD8^{+} T cells defined two clearly defined subsets with homogeneous expression of C1.7 Ag or lack thereof, as opposed to the continuous heterogeneous quantities expressed by other activation-dependent markers.

The majority of naive and activated CD8^{+} T cells express CD28. On naive CD8^{+} T cells, CD28 stimulation is essential for acquisition of cytotoxic activity and proliferation (26, 28). C1.7 Ag is expressed only on the activated population and, therefore, it does

not correlate with CD28 expression. However, there is a small population of highly activated CD8$^+$ T cells (CD11a^{high}, CD58$^+$, CD54$^+$, morphologically large, with short telomeres (29), and high perforin levels (30, 31)) that completely loses CD28 expression (32). These cells have high cytotoxic activity and cytokine secretion and are found at high concentrations in diseases such as HIV infection (33, 34). We found that the CD8$^+$ T cells that completely lacked CD28 expression were uniformly C1.7 Ag$^+$.

CD45RA has long been used as a surface marker for naive CD4$^+$ T cells (24). The alternative splice that leads to CD45RO from CD45RA appears mostly stable in CD4$^+$ T cells, but reverts easily in CD8$^+$ T cells (25). Thus, the CD45RA$^+$CD8$^+$ T cell population comprises both activated and naive CD8$^+$ T cells. These two CD8$^+$ T cell subsets are easily distinguished using C1.7 and CD62L expression as a marker for activated and naive cells, respectively. As expected, all of the CD45RA$^-$CD8$^+$ T cells, which have been shown to be previously activated cells (22), are C1.7 Ag$^+$, while the CD45RA$^+$CD8$^+$ T cells have approximate equal proportions of C1.7 Ag$^+$ and Ag$^-$ cells.

Our findings suggest that the use of C1.7 Ag expression in conjunction with these other surface Ags may allow for a better dissection of CD8$^+$ T cell subsets from peripheral blood. The observation that CD8$^+$ T cells lacking C1.7 Ag are naive cells expressing CD62L^{high} suggests that C1.7 Ag expression is a marker for activated cells, both memory and effector. Using C1.7 in conjunction with CD28, CD45RA, HLA-DR, and CD69 may be used to discern effector populations from activated CD8$^+$ T cells defining the three major CD8$^+$ T cell subsets in human peripheral blood: naive, activated, and effector cells. Thus, our results show that C1.7 Ag is the first described activation marker expressed on all activated/memory CD8$^+$ T cells lacking the naive T cell marker CD62L^{high}, and that it can be used in flow cytometry to identify this subset, similar to the use of the CD45RO marker for CD4$^+$ T cells.

In contrast to NK cells, C1.7 Ag cross-linking on CD8$^+$ T cells does not induce PIP$_2$ hydrolysis, Ca^{2+} flux, or Ab-redirected lysis of target cells (1). However, C1.7 Ab does block non-MHC-restricted killing by IL-2-activated CD8$^+$ T cells (1). These data suggest that C1.7 Ag plays a role in target cell conjugation or recognition without an associated signal. Several reasons may account for the difference in C1.7 Ag activity on NK and T cells. The C1.7 molecule on CD8$^+$ T cells could be a different isoform expressing the C1.7 Ab epitope or the C1.7 Ag could be alternatively spliced generating a non-signaling receptor or signaling molecules with different signaling abilities. Also, CD8$^+$ T cells may not possess C1.7 Ag-associated signaling structures, which may be utilized in NK cells.

Our finding that the proportion of C1.7 Ag-expressing CD8$^+$ T cells is increased in symptomatic HIV infection suggests that the C1.7 Ag expression may be regulated in vivo in response to Ag stimulation. Previous reports have demonstrated that the immunologic response to HIV infection leads to activation and expansion of the CD8$^+$ T cell compartment, which is responsible for MHC-restricted killing of virally infected cells and the suppression of HIV replication via soluble mediators (35, 36). Numerous reports have shown an association between various activation markers and HIV disease progression (6–16). Activated CD8$^+$ T cells have been found to be predominantly responsible for the virus-specific cytotoxic activity (33, 37, 38). In this study, we show that the expression of C1.7 Ag correlates well with the presence of activation markers on peripheral blood CD8$^+$ T cells and is increased in HIV-infected patients.

Although CD4$^+$ T cell numbers decrease with the onset of AIDS, the CD8$^+$ T cell population remains relatively stable until late stages of AIDS development. The increase in CD8$^+$ T cell activation, considered critical for maintaining clinical latency and low viral load, occurs soon after seroconversion and continues throughout disease (6, 39, 40). Comparison of absolute CD4$^+$ T cells/mm^3 and C1.7 Ag expression on CD8$^+$ T cells showed a significant reverse correlation. To determine relevance of C1.7 Ag expression in disease progression, we clinically followed for approximately 2 yr the patients who were initially tested for C1.7 Ag expression. Patients with elevated proportions of C1.7 Ag$^+$CD8$^+$ T cells (>1 SD above the average expression in healthy donors), including those with normal CD4$^+$ T cell counts, were primarily CDC classification B or C, while the majority of patients with normal levels of C1.7 Ag$^+$CD8$^+$ T cells had higher CD4 counts and were CDC classification A. Most striking were our findings with patients with CD4 counts >400 cells/mm^3. All of these patients with normal proportions of C1.7$^+$CD8$^+$ T were CDC classification A, while the majority of those with elevated proportion of C1.7 Ag$^+$CD8$^+$ T cells were CDC classification B. ANOVA analysis determined that CD4 counts were not a significant predictor of disease progression, while the proportion of C1.7 Ag-expressing CD8$^+$ T cells was highly correlative. Indeed, 75% of patients with CD4 counts >400 and elevated C1.7 Ag expression were on AZT, while only 32% of those patients with normal levels of C1.7 Ag were on anti-retroviral therapy. It is unclear why patients with low C1.7 Ag$^+$CD8$^+$ T cell proportion are fairing better clinically. C1.7 Ag expression is indicative of CD8$^+$ T cell activation. Therefore, a lower proportion of C1.7 Ag$^+$CD8$^+$ T cells may indicate a combination of a less aggressive viral infection and fewer opportunistic infections, which both increase CD8$^+$ T cell activation.

Taken together, these data suggest that the C1.7 molecule may be an important marker for CD8$^+$ T cell activation in response to antigenic stimulation in vivo. Furthermore, our data indicate that the proportion of C1.7 Ag$^+$CD8$^+$ T cells determined in HIV-infected patients is a prognostic indicator of HIV progression. Statistical analysis suggests that elevated proportion of C1.7 Ag-expressing CD8$^+$ T cells early in disease is a better indicator of disease progression than CD4$^+$ T cell count and should be considered as a marker for monitoring disease in patients infected with HIV-1. The recent cloning of the gene encoding this molecule should help us further investigate the biological function of C1.7 Ag on CD8$^+$ T and NK cells.

Acknowledgments

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