

CD30 Expression by CD8⁺ T Cells Producing Type 2 Helper Cytokines. Evidence for Large Numbers of CD8⁺CD30⁺ T Cell Clones in Human Immunodeficiency Virus Infection

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Summary

A large panel of CD8⁺ T cell clones generated from peripheral blood lymphocytes (PBL) of healthy donors or human immunodeficiency virus (HIV)-infected individuals were assessed for both cytokine secretion profile and CD30 expression and release. The great majority of CD8⁺ T cell clones generated from healthy individuals showed the ability to produce interferon γ (IFN- γ), but not interleukin 4 (IL-4), and none of them either expressed membrane CD30 or released substantial amounts of soluble CD30 (sCD30) in their supernatant. In contrast, high numbers of CD8⁺ T cell clones generated from HIV-infected individuals, which produced IL-4 (and IL-5) in addition to IFN- γ or IL-4 (and IL-5) alone, expressed membrane CD30 and released detectable amounts of sCD30 in their supernatants. Indeed, CD30 expression appeared to be positively correlated with the ability of CD8⁺ T cell clones to produce IL-4 and IL-5 and inversely correlated with their ability to produce IFN- γ , whereas no correlation between CD30 expression and production of IL-10 was observed. These data suggest that CD30 is a marker for CD8⁺ T cells that have switched to the production of type 2 helper cytokines.

CD30 is a 120-kD surface antigen originally identified by the Ki-1 mAb developed against Hodgkin's and Reed-Sternberg (H-RS) cells (1). CD30 antigen expression was then found in various non-Hodgkin's lymphomas (2), as well as on several virally transformed T- (HTLV I and II) and B- (EBV) cell lines (3, 4). That CD30 plays a role in normal lymphoid interaction is suggested by its histological detection on a scanty population of lymphoid cells in reactive lymph nodes and by induced expression on purified T and B cells after lectin activation (5). cDNA cloning studies demonstrated that CD30 belongs to the TNF/nerve growth factor (NGF) receptor family (6, 7), whose ligand is a new membrane-bound cytokine sharing a number of structural and functional similarities with TNF (8). At present, the physiological role of CD30 is unknown. In recent studies, however, we have demonstrated that CD30 is selectively expressed by human CD4⁺ T cells producing type 2 helper (Th2) cytokines (9).

In this report we have asked whether the association between CD30 expression and the ability to produce Th2-type cytokines is restricted to the CD4⁺ T cell subset or also extends to CD8⁺ T lymphocytes. To answer this question we have analyzed large panels of CD8⁺ T cell clones generated from both normal donors and HIV-infected individuals.

CD8⁺ T cell clones producing Th2-type cytokines, which are rare in normal subjects, can be indeed generated in large numbers from some HIV-infected individuals (10). Results showed that CD8⁺ T cell clones producing IFN- γ but not IL-4 or IL-5 (Th1-like) neither expressed membrane CD30 nor released soluble CD30 (sCD30) in their supernatants (SN), whereas both membrane CD30 and sCD30 are consistently found in human CD8⁺ T cells producing IL-4 and IL-5 (Th0- or Th2-like). Thus, CD30 expression appears to be a marker not only for CD4⁺ Th2 cells, but also CD8⁺ T cells that have switched to the production of Th2-type cytokines.

Materials and Methods

Patients. The study was performed in five HIV-1-infected patients and three HIV-1-seronegative healthy volunteers. According to the Centers for Disease Control (CDC) criteria (11), three of the patients were classified in the Group IV Subgroup C-1 because two have suffered from *Pneumocystis carinii* and one from *Toxoplasma gondii* infection; two in the Group IV Subgroup D (one with Kaposi's sarcoma and one with non-Hodgkin's lymphoma). Immunophenotyping of PBMC by flow cytometry revealed very low levels of CD4⁺ T cells (<50/ μ l) in all five HIV-1-infected patients

but normal values in the three HIV-1-seronegative subjects (920, 840, and 1,100 cells/ μ l, respectively). Absolute values of CD8⁺ T cells were within normal values (ranging from 350 to 600 cells/ μ l) in both HIV-1-infected patients and healthy subjects. All patients and controls gave informed consent for the studies.

Reagents. PHA was purchased from GIBCO BRL (Gaithersburg, MD) and PMA from Sigma Chemical Co. (St. Louis, MO). Recombinant IL-2 was a kind gift of Eurocetus (Milan, Italy). Recombinant IL-5 and IL-10 were purchased from Amersham International (Amersham, Bucks, UK) and Genzyme Corp. (Cambridge, MA), respectively. Anti-CD3, anti-CD4, anti-CD8, anti- α/β -TCR mAbs were purchased from Becton Dickinson & Co. (Mountain View, CA). Anti-CD30 mAb (Ber-H2) was purchased by Dako (Glostrup, Denmark).

T Cell Cloning System. PBMC were obtained from the four HIV-1-infected patients and the two HIV-seronegative healthy volunteers after Ficoll-Hypaque gradient centrifugation, and T cells cloned according to a previously described technique that allows the clonal expansion of virtually every T cell regardless of its antigen specificity (10). Briefly, PBMC were seeded under limiting dilution conditions (0.3 cells/well) in round-bottomed microwells containing 10⁵ irradiated allogeneic spleen cells (as feeder cells) and PHA (1% vol/vol) in a final volume of 0.2 ml RPMI 1640 medium supplemented with 2 mM L-glutamine, 2 \times 10⁻⁵ M 2-ME (complete medium) containing human recombinant IL-2 (20 U/ml) and 10% FCS (Hyclone Laboratories, Logan, UT). Growing microcultures were then supplemented at weekly intervals with IL-2 (20 U/ml) and 10⁵ irradiated feeder cells.

Immunophenotyping of T Cell Clones. Cell surface marker anal-

ysis of T cell clones was performed on a Cytoron Absolute cytofluorimeter (Ortho Pharmaceuticals, Raritan, NJ) by using fluoresceinated (FITC) or phycoerythrinated (PE) anti-CD3, anti-CD4, anti-CD8, anti- α/β -TCR, or anti-CD30, as described (9, 10).

Assessment of Cytokine Secretion Profile of T Cell Clones. The cytokine secretion profile of T cell clones was evaluated by stimulating 10⁶ T cell blasts from each clone in 1 ml complete medium with PMA plus anti-CD3 Ab, as reported elsewhere (10). After 24 h, culture supernatants were collected and stored at -70°C until used. The determination of IFN- γ and IL-4 was performed by a commercial RIA (Centocor Inc., Malvern, PA) and a commercial ELISA (Quantikine; R & D Systems, Inc., Minneapolis, MN), respectively. IL-5 and IL-10 were quantified by in-house-made capture ELISAs using anti-IL-5 or anti-IL-10 mAb bound to microwell plates, and biotinylated anti-IL-5 or anti-IL-10 mAbs (PharMingen, San Diego, CA) as revealing antibodies, respectively. T cell clone SN showing IFN- γ , IL-4, IL-5, or IL-10 levels 5 SD over the mean levels in control SN derived from irradiated feeder cells alone were regarded as positive.

Assessment of sCD30. sCD30 was measured in the SN of CD8⁺ T cell clones stimulated for 24 h with PMA plus anti-CD3 mAb by a CD30 ELISA test kit (Dakopatts, Glostrup, Denmark), based on the use of two mAbs reacting with two different epitopes of the 88-kD soluble form of CD30 molecule. The standard curve was prepared from six sCD30 calibrators and the concentration of sCD30 in SN was determined by interpolation of values detected in duplicate samples. The detection limit of the assay was estimated to be 1 U/ml by determination of the mean absorbance (\pm 2 SD) of 20 measurements of the 0 U/ml standard (12).

Table 1. Cytokine Production Profile of CD8⁺ T Cell Clones Generated from Peripheral Blood of HIV-seronegative and HIV-seropositive Individuals

Subjects	No. of CD8 ⁺ clones tested	No. of clones showing the indicated profile		
		Th1-like	Th0-like	Th2-like
HIV-seropositive				
SC	21	2	9	10
GC	19	15	1	3
AM	54	24	18	12
SR	112	36	55	21
TM	37	28	4	5
Total clones	243	105 (43%)*	87 (36%) [†]	51 (21%) [§]
HIV-seronegative				
PP	84	70	14	0
MA	39	38	1	0
AM	21	14	7	0
All clones	144	122 (85%)*	22 (15%) [†]	0

CD8⁺ T cell clones were stimulated for 24 h with PMA plus anti-CD3 Ab and assessed for the ability to produce IL-4 and IFN- γ in their supernatants as described in Materials and Methods. Clones producing IFN- γ , but not IL-4, were classified as Th1-like, clones producing both IFN- γ and IL-4 were classified as Th0-like, and clones producing IL-4, but not IFN- γ , were classified as Th2-like.

* $p < 0.0005$.

[†] $p < 0.0005$.

[§] $p < 0.0005$.

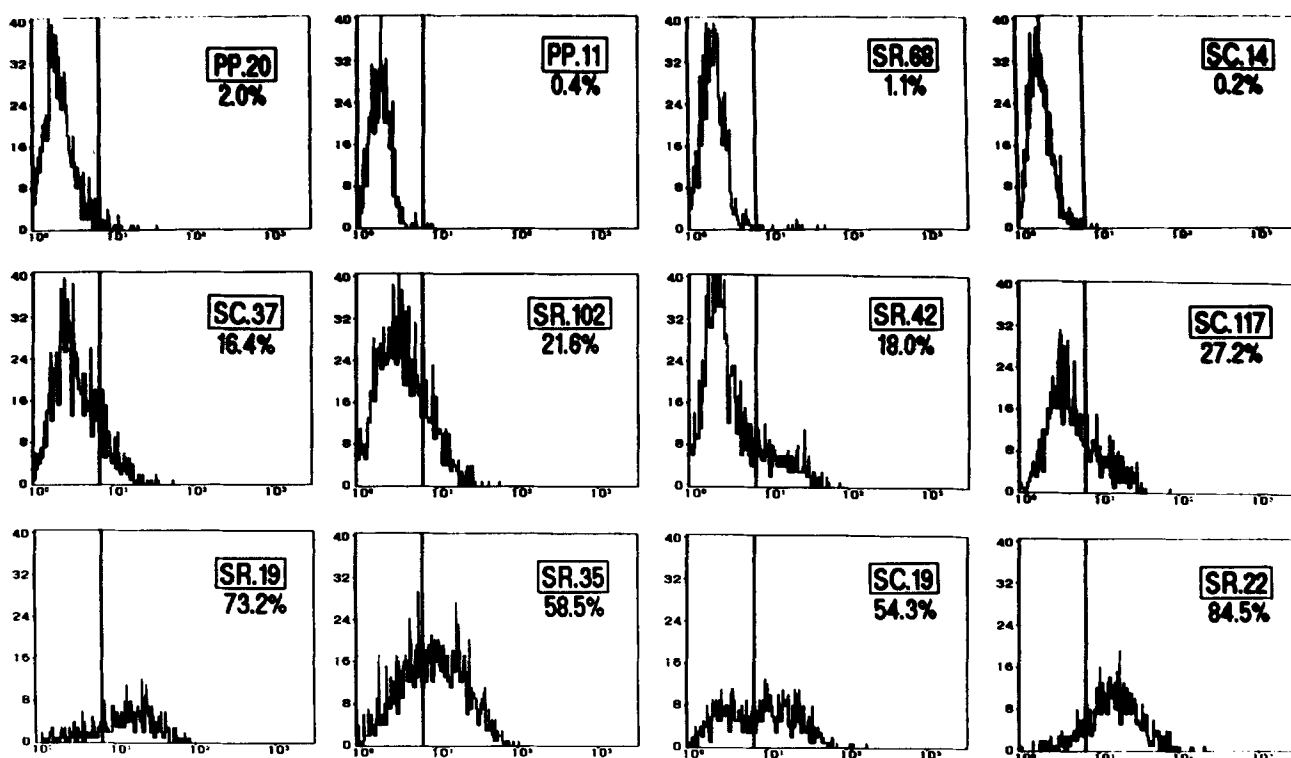


Figure 1. Expression of membrane CD30 by four representative Th1-like (PP.20, PP.11, SR.68, and SC.14) four representative Th0-like (SC.37, SR.102, SR.42, and SC.117), and four representative Th2-like (SR.19, SR.35, SC.19, and SR.22) CD8⁺ T cell clones. T cell blasts from each clone were stained with the FITC-conjugated anti-CD30 mAb, and membrane CD30 expression was determined by flow cytometry. Values indicate the percentage of positive T blasts from each clone.

Results

A total number of 144 CD8⁺ α/β -TCR⁺ T cell clones generated from three normal donors and of 243 CD8⁺ α/β -TCR⁺ T cell clones generated from five HIV-infected individuals were assessed for ability to produce IFN- γ and/or IL-4 in response to stimulation with PMA plus anti-CD3 mAb. As shown in Table 1, 85% of CD8⁺ T cell clones generated from normal donors produced IFN- γ but not IL-4 (Th1-like), whereas the other 15% produced both IFN- γ and IL-4 (Th0-like). In contrast, only 43% of CD8⁺ T cell clones generated from HIV-infected individuals showed a Th1-like profile, whereas 36% produced IL-4 in addition to IFN- γ (Th0-like) and 21% produced IL-4 but not IFN- γ (Th2-like).

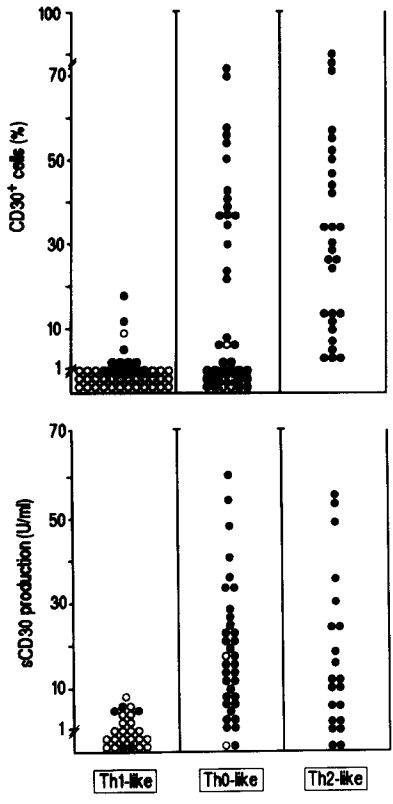
A number of randomly selected CD8⁺ T cell clones from both normal donors and HIV-infected individuals were then assessed for membrane CD30 expression (112 T cell clones) and sCD30 release (84 T cell clones). Th1-like CD8⁺ T cell clones showed low or undetectable expression of membrane CD30 and did not release sCD30, whereas CD30 expression was found in virtually all Th2-like CD8⁺ T cell clones. Th0-like CD8⁺ T cell clones showed an intermediate pattern of CD30 expression (Figs. 1 and 2). Likewise, high proportions of both Th2- and Th0-like clones also produced substantial amounts of sCD30 in their supernatants (Fig. 2). As shown in Fig. 3, CD30 expression was positively correlated with the ability of CD8⁺ T cell clones to produce IL-4 and IL-5 and inversely correlated with their ability to

produce IFN- γ , whereas no correlation between the expression of CD30 and the ability of CD8⁺ clones to produce IL-10 was observed.

Discussion

In a recent study, we have shown that CD30, a member of the TNF receptor superfamily (6, 7) is selectively expressed by human CD4⁺ T cells producing Th2-type cytokines (9). Here, we demonstrate that even on activated CD8⁺ T cells CD30 is selectively expressed by those producing Th2-type cytokines. In fact, we found an inverse correlation of CD30 expression with the production of IFN- γ and positive correlation with the production of IL-4 and IL-5 even on activated CD8⁺ T cells. No correlation was found between CD30 expression and production of IL-10 by CD8⁺ T cell clones. This finding is not surprising since IL-10 production in humans is not restricted to Th2 cells (13, 14), as it is in the mouse (15). Thus, these data strongly support the view that the expression of CD30 reflects and/or defines a selective differentiation and/or activation pathway of T cells (both CD4⁺ and CD8⁺) producing Th2-type cytokines. So far, however, the molecular mechanisms responsible for this association remain unclear.

We have previously demonstrated the presence of Th2-like CD8⁺ T cells showing B cell helper activity and reduced cytolytic potential in two HIV-infected patients with a Job's-



like syndrome (10). The results here reported support this finding and suggest that the switch of CD8⁺ T cells is not limited to a few patients suffering from Job's-like syndrome but may occur, even if to lower extent, in higher proportions of HIV-infected individuals. At present, however, the reason why so high numbers of CD8⁺ T cells in HIV-infected individuals differentiate in vitro into clones showing a Th2-type profile of cytokine production is unclear.

Recently, it has been shown that serum levels of sCD30 are elevated in the large majority of HIV-infected individuals and represent an indicator of progression to AIDS indepen-

Figure 2. Expression of membrane CD30 (*top*) and production of soluble CD30 (*bottom*) by CD8⁺ T cell clones with established Th1-, Th0-, or Th2-like profile of cytokine secretion. CD8⁺ T cell clones were generated from PBMC of HIV-seronegative healthy donors (○) or HIV-infected individuals (●), as described in Materials and Methods, and assessed for their cytokine secretion profile by 24-h stimulation with PMA plus anti-CD3 mAb. Clones producing IFN- γ but not IL-4 were classified as Th1-like, clones producing both IFN- γ and IL-4 were classified as Th0-like, and clones producing IL-4 but not IFN- γ were classified as Th2-like. Membrane CD30 expression was evaluated by flow cytometry on CD8⁺ T cell clones on day 7 after the last stimulation when they were growing in the presence of IL-2 alone. sCD30 production was assessed by a CD30 ELISA kit assay in culture SN of the same clones after 24-h stimulation with PMA plus anti-CD3 mAb.

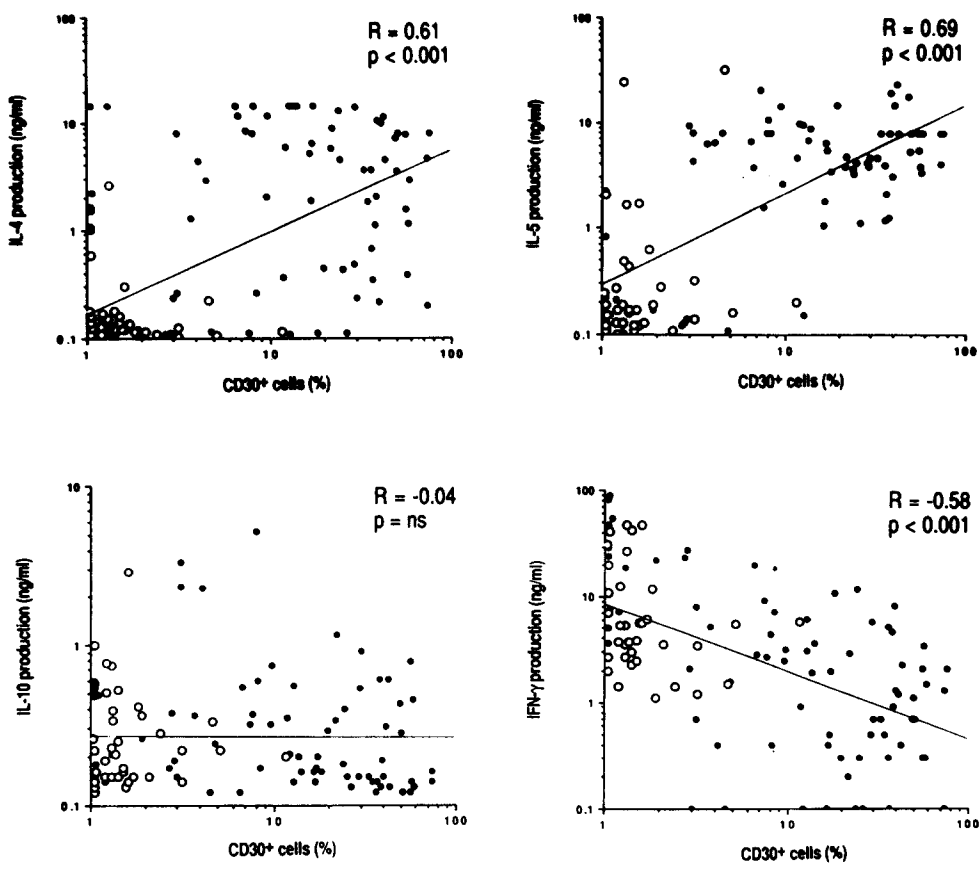


Figure 3. Correlation between membrane CD30 expression and production of different cytokines by CD8⁺ T cell clones generated from HIV-seronegative healthy donors (○) or HIV-infected individuals (●). Membrane CD30 expression was assessed as described in the legend of Fig. 1. Production of IFN- γ , IL-4, IL-5, and IL-10 was evaluated in the SN of T cell clones after 24-h stimulation with PMA plus anti-CD3 mAb by appropriate ELISA, as described in Materials and Methods.

dent of other prognostic parameters (12). The present demonstration of high numbers of CD8⁺ Th2-like clones that express membrane CD30 and can release detectable amounts of sCD30 may provide a reasonable explanation for this finding. Whether elevated serum levels of sCD30 in HIV infection reflect cleavage of membrane CD30 on activated

Th2-like (CD4⁺ and CD8⁺) T and/or B cells or result from HIV-mediated apoptosis of both CD4⁺ and CD8⁺ T cells remains to be established. Whatever mechanism is the possible role of CD30 in the immunopathogenesis of HIV infection deserves further investigation.

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