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# Human IL-18 Receptor and ST2L Are Stable and Selective Markers for the Respective Type 1 and Type 2 Circulating Lymphocytes<sup>1</sup>

Woon Ling Chan,<sup>2\*</sup> Nada Pejnovic,<sup>\*</sup> Christine A. Lee,<sup>†</sup> and Nadia A. Al-Ali<sup>\*</sup>

CD4<sup>+</sup> (Th) and CD8<sup>+</sup> (Tc) T and NK lymphocytes can be divided into type 1 and 2 subsets according to their cytokine secretion profile. Studies on the role of lymphocyte subsets in human diseases have been hampered by the lack of stable surface markers to define them. Recently, we reported that ST2L and IL-18R are stably expressed on murine Th2 and Th1 cells, respectively. In this study, we generated Abs to human homologues of ST2L and IL-18R and tested them against Th1/Th2, Tc1/Tc2, and NK1/NK2 lines and PBMCs from healthy individuals. We show for the first time that ST2L and IL-18R are stable selective cell surface markers for human Th2/Tc2/NK2 and Th1/Tc1/NK1 lymphocytes, respectively. We then investigated PBMCs from HIV-infected patients and HIV-negative individuals, to test whether Abs to these two surface markers could be used directly to monitor lymphocyte subset distribution in human diseases. We found a clear Th1 to Th2 shift in the HIV-infected individuals, thus settling a long-standing controversy and include, for the first time, Tc and NK cells as well. Therefore, these cell surface molecules could serve as important determinants of the immune status of human diseases in general, and thereby could be useful for therapeutic monitoring and intervention. *The Journal of Immunology*, 2001, 167: 1238–1244.

Both CD4<sup>+</sup> (Th) and CD8<sup>+</sup> (Tc)<sup>3</sup> T lymphocytes from mice and humans can be divided into type 1 (IFN- $\gamma$ , IL-2) and type 2 (IL-4, IL-5) subsets on the basis of their cytokine secretion profile (1–4). Recently, NK cells were also shown to be divisible into NK1 (IFN- $\gamma$ -producing) and NK2 (IL-5- and IFN- $\gamma$ -producing) subsets (5). Th1 cells promote cell-mediated immune responses and activate macrophages, thus are defensive against intracellular pathogens. In contrast, Th2 are associated with allergic reactions involving IgE, eosinophils, and basophils. However, there are numerous exceptions, and conclusive evidence for this rule is not yet available, especially in clinical diseases. The roles of Tc and NK subsets are even less well defined. Although the balance of Th1 and Th2 is believed to determine the outcome of an infectious, chronic inflammatory, or autoimmune disease (6, 7), studies on the role of lymphocyte subsets in human diseases have been hampered by the lack of stable surface markers to define these cells. We and others have recently reported that ST2L is stably expressed on murine Th2 cells (8–10) and IL-18R on Th1 cells (11), and showed that Abs to these subset-specific surface markers can modulate the outcome of infectious and inflammatory diseases in vivo. Such markers would be useful in monitoring and possibly in therapeutic intervention of diseases in general.

We have now generated Abs against the human homologues of ST2L and IL-18R and report in this work that these are stable cell

surface markers for human Th1/Th2, Tc1/Tc2, and NK1/NK2 cells. Furthermore, we show that these Abs can be valuable tools for identifying type 1 and type 2 lymphocytes in clinical diseases such as AIDS. AIDS is one human disease in which understanding the role of lymphocyte subsets is critical, but it has remained unclear and controversial. It has been suggested that during HIV infection there is a shift from Th1 to Th2 cell balance accompanying the progression of HIV infection (12–22). However, evidence has also been presented that the shift was from Th1 to Th0 phenotype (23). In contrast, it has been reported that there was no evidence of any changes in the Th1:Th2 cytokine pattern (24, 25) during progressive HIV infection. The controversy of Th1:Th2 balance during HIV infection is most certainly due to the different methods used for assessing cytokine expression (ELISA, RT-PCR, T cell cloning). Even intracellular staining (20, 22) may not reflect the in vivo status because the assay involves stimulation with PMA/ionomycin and monensin or brefeldin in vitro.

We now report that the human homologues of ST2L and IL-18R are selectively and stably expressed on human Th2/Tc2/NK2 and Th1/Tc1/NK1 lines, respectively. Furthermore, when fresh ex vivo PBMCs from HIV-1-infected individuals were reacted with Abs to these two cell surface markers, there is a shift from Th1 to Th2 response in HIV-infected individuals compared with HIV-negative controls, and this includes, for the first time, Tc and NK cells as well. Therefore, these cell surface molecules could serve as important determinants of the immune status of human diseases in general and thereby provide a better understanding of disease pathology. Hence, they could be useful for therapeutic monitoring.

## Materials and Methods

### Antibodies

A peptide corresponding to the hydrophilic region of the extracellular domain of human ST2L (hST2L) (26) and conjugated to keyhole limpet hemocyanin was used with CFA to immunize BALB/c mice. The animals were boosted three times with the peptide in the presence of IFA before they were bled out sera collected for staining ST2L and spleen cells used for fusion with the myeloma NS1, as previously described (27). A keyhole

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<sup>3</sup> Abbreviations used in this paper: h, human; NT, natural T; R2, region 2.

limpet hemocyanin-conjugated peptide to hIL-18R extracellular domain (28) was also used similarly to raise anti-IL-18R Abs in rabbits.

### Cell lines and cell culture

Short-term polarized human Th and Tc cell lines were generated as previously described (29–31). PBMCs were separated from cord blood by the standard Lymphoprep (Nycomed Pharma, Oslo, Norway) method. To obtain enriched CD4<sup>+</sup> and CD8<sup>+</sup> T cells, monocytes were depleted by two rounds of plastic adherence, followed by negative selection with anti-hCD56 (AMS Biotechnology Europe, Abingdon, U.K.) and anti-mouse IgG magnetic beads (DynaL Biotech, Oslo, Norway). The remaining unbound cells were further incubated with pan B and CD4 or CD8 magnetic beads (DynaL Biotech) to complete the enrichment process. Then 2 × 10<sup>5</sup>/ml CD4<sup>+</sup> or CD8<sup>+</sup> T cells were stimulated with 1 × 10<sup>6</sup>/ml irradiated (2500 rad) allogenic PBMCs in IMDM (Life Technologies, Paisley, U.K.) supplemented with 10% pooled male human sera, 5% FCS, 2 mM glutamine, 100 μg/ml penicillin/streptomycin, 1 mM pyruvate, and 50 μM 2-ME with the following polarizing conditions: Th1 and Tc1, in the presence of 40 ng/ml hIL-12 (Genetic Institute, Boston, MA) and neutralizing sheep anti-IL-4 (National Institute for Biological Standards & Control (NIBSC) Potters Bar, U.K.); Th2 and Tc2, in the presence of 50 ng/ml IL-4 (Schering-Plough, Madison, NJ) and neutralizing sheep anti-IL-12 and sheep anti-IFN-γ (NIBSC). Cells were expanded by addition of IL-2 (Glaxo, Stevenage, U.K.; 10 U/ml) on day 3 and harvested on day 7 for restimulation. Th2 and Tc2 were restimulated as before, except IL-4 was reduced to 5 ng/ml for Tc2. Both Th1 and Tc1 were restimulated as before with the additional presence of irradiated (4000 rad) B lymphoblastoid cells (EBV transformed) from the same allogenic donor at 6 stimulators:1 responder. IL-2 (10 U/ml for T1; 40 U/ml for T2) was added to all cultures on the day of restimulation. All further restimulations of the four lines were conducted using culture conditions of the second cycle for the respective lines.

Short-term polarized human NK cell lines were generated as previously described (5, 32). PBMCs were enriched for NK cells by negative selection with CD3 magnetic beads (DynaL Biotech). Then 1 × 10<sup>5</sup>/ml NK cells were stimulated with 7 × 10<sup>5</sup>/ml of a mitomycin C-treated (50 μg/ml, 37°C, 30 min) allogenic B lymphoblastoid cell line (EBV transformed) in RPMI 1640 medium (Life Technologies) supplemented with 15% pooled male human sera, 2 mM glutamine, 100 μg/ml penicillin/streptomycin, 1 mM pyruvate, 100 μM 2-ME, and 200 U/ml IL-2 with the following polarizing conditions: NK1, in the presence of 10 ng/ml hIL-12 (Genetic Institute) and neutralizing sheep anti-IL-4; NK2, in the presence of 50 ng/ml IL-4 (Schering-Plough) and neutralizing sheep anti-IL-12 and sheep anti-IFN-γ. Both NK1 and NK2 were harvested on day 12 and restimulated as before.

In some experiments, ST2L<sup>+</sup>IL-18R<sup>-</sup> and ST2L<sup>-</sup>IL-18R<sup>+</sup> lymphocytes were sorted from PBMCs obtained from healthy individuals. They were then stimulated through two rounds of *in vitro* culture with immobilized anti-CD3 Ab (1 μg/ml; Dako, Carpinteria, CA) plus anti-CD28 Ab (1 μg/ml; Coulter/Immunotech, Luton, U.K.) before they were activated for 4 h and stained for intracellular cytokine, as detailed below.

### Flow cytometric analysis

Three-color flow cytometry with intracellular cytokine staining was used to determine the cytokine profile (IL-4, IL-5, and IFN-γ) of ST2L<sup>+</sup> and IL-18R<sup>+</sup> human Th, Tc, and NK lines, as previously described (8, 33, 34). Alloantigen-specific Th1/Tc1 and Th2/Tc2 lines were activated for 1 and 4 h, respectively, with PMA (50 ng/ml; Sigma, St. Louis, MO) and ionomycin (500 ng/ml; Calbiochem, La Jolla, CA) before staining. Brefeldin (10 μg/ml; Sigma) was present during the final 1–2 h of activation. NK1 and NK2 cells were PMA and ionomycin (1 μg/ml) activated for 2 and 6 h, respectively, in the presence of 2 μM monensin before three-color flow cytometry and intracellular cytokine staining. However, because PMA activation reduces the cell surface expression of both ST2L and IL-18R, their expression was determined simultaneously on activated and unactivated Th, Tc, and NK lines. With the NK2 line, it was necessary to determine their expression on unactivated cells since ST2L expression is also down-regulated by high levels of IFN-γ (our unpublished data). Because of the slower kinetics of IL-4 and IL-5 production, NK2 cells have to be activated for 6 h for detectable production, by which time a high level of IFN-γ has accumulated in the cell. Although both ST2L (using mouse anti-hST2L peptide) and IL-18R (using rabbit anti-hIL-18R or mAb anti-hIL-18R; R&D Systems, Abingdon, U.K.) are detectable at low levels after PMA activation, the histograms in Fig. 1 are derived from data obtained with unactivated cells. Likewise, the distribution of ST2L<sup>+</sup> and IL-18R<sup>+</sup> lymphocytes in HIV-positive and HIV-negative individuals is determined from unactivated PBMCs. Detection was with biotinylated goat anti-mouse Ig

(Harlan Sera Laboratory, Loughborough, U.K.) and PerCP-streptavidin (BD Biosciences, Mountain View, CA). Normal mouse or rabbit sera (Harlan Sera Lab) and normal mouse IgG (BD Biosciences) were used as control. Both anti-hIL-4 and anti-hIL-5 were PE conjugated, whereas anti-hIFN-γ was FITC conjugated (Cambridge Bioscience, Cambridge, U.K.). The lymphocyte markers used were FITC-conjugated mAbs CD3 (Dako) and PE-conjugated mAbs CD4, CD8, and CD56 (BD Pharmingen, San Diego, CA). FITC- and PE-conjugated isotype control Abs were obtained from BD Biosciences. The numerical values in the dot plots denote percentage of cells within gated quadrants. All flow cytometric analysis was conducted with WinMDI version 2.8 or PC LYSYS (BD Biosciences).

### HIV-infected individuals

Blood from HIV-infected individuals was obtained after approval by the local ethical committee and with the patient's consent. Our cohort of 22 HIV-infected individuals included 12 hemophilic patients who were infected in the early 1980s, and the rest were a mixture of seroconvertors, progressors, and asymptomatics.

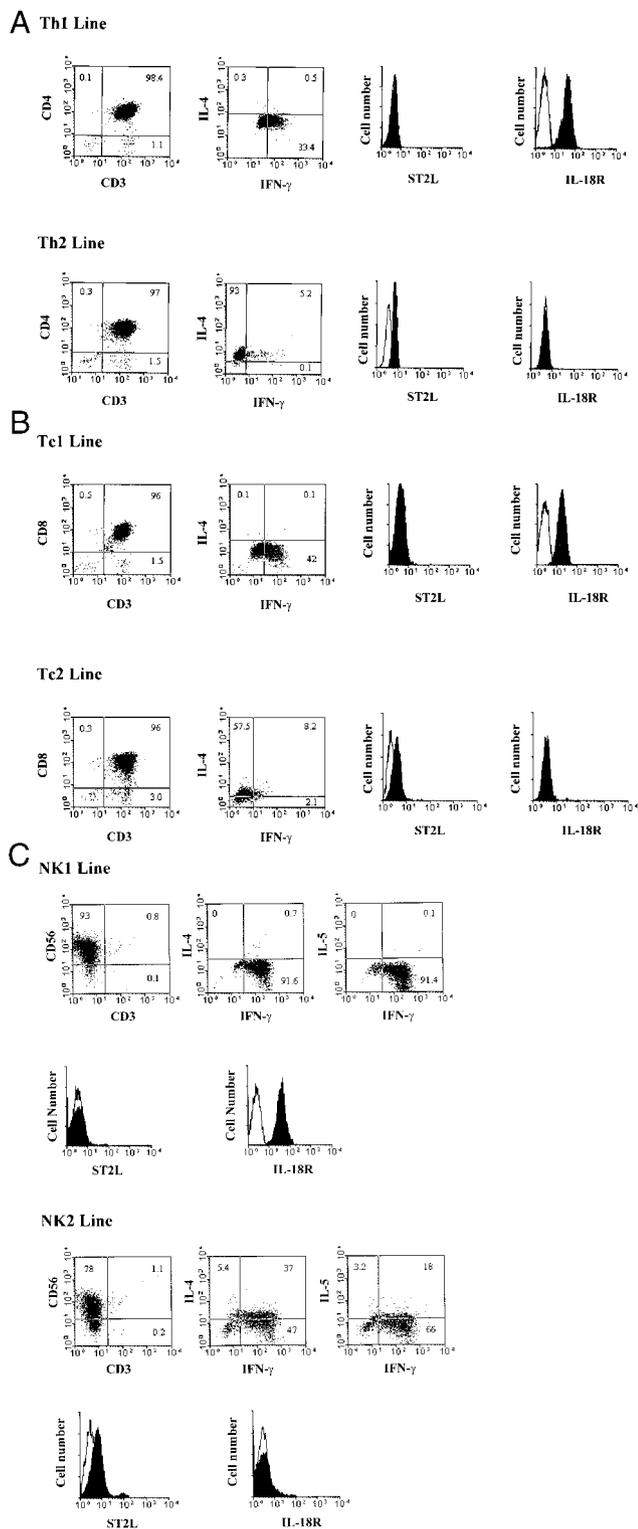
## Results

### ST2L and IL-18R are selectively expressed on Th2/Tc2/NK2 and Th1/Tc1/NK1 lines, respectively

To define hST2L and hIL-18R as stable subset-specific markers of type 2 and 1 human lymphocytes, respectively, we conducted three-color flow cytometry on polarized allogenic Th, Tc, and NK lines by staining them with Abs for cell surface ST2L or IL-18R and intracellular IL-4, IL-5 (NK only), and IFN-γ after PMA and ionomycin activation, as previously described (8, 33, 34). As shown in Fig. 1A, ST2L is expressed on IL-4-producing CD3<sup>+</sup>CD4<sup>+</sup> Th2 cells and not on the IFN-γ-producing Th1 cells. In the predominantly Th2 line, ~5% of the cells are Th0 and coexpress IL-4 with IFN-γ. These Th0 cells were also ST2L<sup>+</sup> when they were electronically gated and examined for ST2L expression (data not shown). In contrast, IL-18R is expressed on Th1, but not Th2 cell lines. Similarly, the CD3<sup>+</sup>CD8<sup>+</sup> IL-4-producing Tc2 cells (Fig. 1B) express ST2L, but not IL-18R, which is expressed only by the IFN-γ-producing Tc1 cells. Th1/Tc1 and Th2/Tc2 lines that were restimulated for up to six rounds remained IL-18R<sup>+</sup> and ST2L<sup>+</sup>, respectively. These markers are also selectively expressed by Th1 and Th2 clones (data not shown). Similar to the Th1 and Tc1 cells, the CD56<sup>+</sup> NK1 cells stained positive for IL-18R and IFN-γ, but negative for ST2L and IL-4 or IL-5 (Fig. 1C). Cell surface expression of IL-18R is influenced by the presence of IL-2 in the culture medium (35) as well as PMA/ionomycin activation, which can down-regulate its expression (our unpublished data). For optimal expression, the cell lines were washed and cultured in the absence of IL-2 overnight before PMA/ionomycin stimulation for 1 h, which was found to be sufficient to activate good detectable IFN-γ production, without much effect on IL-18R expression. NK2 cells were, however, negative for IL-18R, but positive for ST2L, IL-4, IL-5, and IFN-γ (Fig. 1C). Like the Th2 and Tc2 cells, NK2 cells express ST2L, but unlike the T2 lines they also coexpress IFN-γ with IL-4 or IL-5. In contrast to an earlier report (5), we found that NK2 cells produce substantial levels of IL-4, in excess of IL-5 production. This could be attributed to the different assay conditions.

### Ex vivo PBMCs are distinguishable into lymphocyte subsets by their ST2L and IL-18R expression

The specificity of ST2L and IL-18R as markers of T2 vs T1 lines, respectively, suggests that they may have *in vivo* relevance as diagnostic markers for the state of some human diseases such as AIDS. We therefore investigated their ability to distinguish and determine the proportion of specific lymphocyte subsets in *ex vivo* PBMCs. Using three-color flow cytometry, PBMCs from healthy individuals were stained with Abs for cell surface ST2L or IL-18R and intracellular IL-4 or IL-5 and IFN-γ. IL-18R<sup>+</sup> lymphocytes

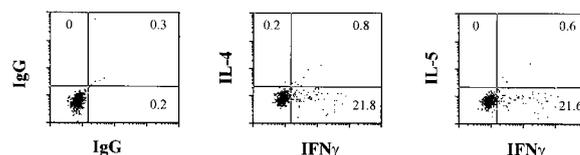
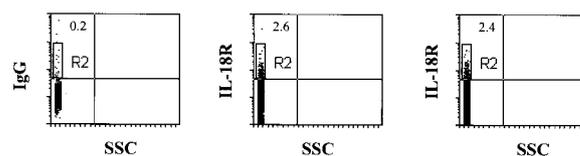


**FIGURE 1.** ST2L is a type 2 marker, while IL-18R is a type 1 marker. Short-term polarized human cell lines were generated as described in *Materials and Methods*. Expression of ST2L and IL-18R on Th1 and Th2 lines (A), Tc1 and Tc2 lines (B), and NK1 and NK2 lines (C) is shown. Intracellular cytokine staining and three-color flow cytometric analysis of PMA- and ionomycin-activated alloantigen-specific cells were conducted as described in *Materials and Methods*. The expression of ST2L and IL-18R on NK cells is shown for unactivated cells. Similar results, but with slightly reduced signal, were also obtained with PMA-activated cells (data not shown). The numerical values in the dot plots denote percentage of cells within gated quadrants that were statistically set with negative control Abs (data not shown). All flow cytometric analyses were conducted with WinMDI version 2.8 or PC LYSYS (BD Biosciences). Results are representative of four experiments.

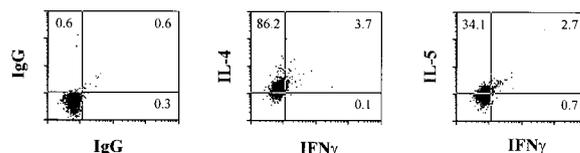
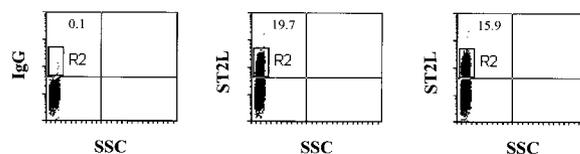
(region 2 (R2)) were IFN- $\gamma$  positive, but IL-4 and IL-5 negative (Fig. 2A). ST2L<sup>+</sup> lymphocytes (R2) shown in Fig. 2B from an individual with hay fever were mainly IL-4 (86%) and IL-5 (34%) positive, but IFN- $\gamma$  negative, although a small proportion of cells coexpresses IFN- $\gamma$  with IL-4 (3%) or IL-5 (2%). This observation indicated that there may be two types of ST2L<sup>+</sup> lymphocytes in peripheral blood: those such as Th2/Tc2 that express only the type 2 cytokines IL-4 or IL-5, and those that coexpress IFN- $\gamma$  with IL-4 or IL-5 as, for example, Th0 (data not shown) and NK2 cells (Fig. 1C).

To identify the two types of ST2L<sup>+</sup> lymphocytes, PBMCs from healthy individuals were stained for cell surface ST2L and CD3 or CD56 and intracellular IL-4, IL-5, or IFN- $\gamma$ . The CD3<sup>+</sup> ST2L<sup>+</sup> lymphocyte-gated population (R2) were IL-4 positive, but IFN- $\gamma$  negative, indicating that they are Th2/Tc2 cells (Fig. 3). Although only 1.7% ST2L<sup>+</sup> T cells from this healthy individual without hay fever or any known allergic condition produce IL-4, 86% ST2L<sup>+</sup> T cells from an individual with hay fever produce IL-4 (Fig. 2B),

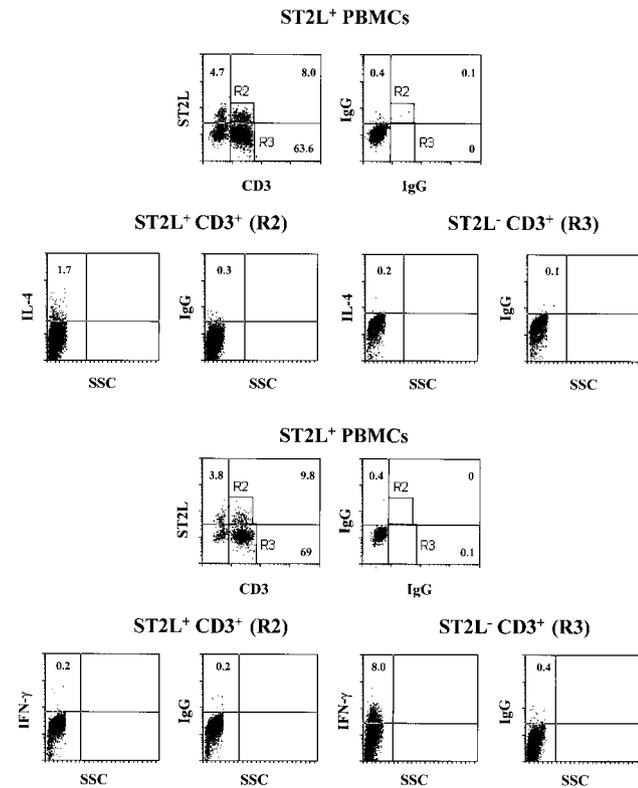
### (A) IL-18R<sup>+</sup> PBMCs



### (B) ST2L<sup>+</sup> PBMCs



**FIGURE 2.** Cytokine secretion profile of ST2L<sup>+</sup> and IL-18R<sup>+</sup> PBMCs from healthy individuals. A, IL-18R<sup>+</sup> lymphocytes produce only IFN- $\gamma$ . Cytokine production was determined after 6-h activation with PMA/ionomycin in the presence of monensin. Three-color flow cytometric analysis of IFN- $\gamma$ -positive cells was conducted on the IL-18R<sup>+</sup>-gated population (R2). B, The majority of ST2L<sup>+</sup> lymphocytes produce only IL-4 or IL-5 and no IFN- $\gamma$ , whereas a very small proportion coexpresses IFN- $\gamma$  with IL-4 or IL-5. Three-color flow cytometric analysis of IFN- $\gamma$  and IL-4 or IL-5 production by the ST2L<sup>+</sup>-gated population (R2) of PBMCs from an individual with hay fever after 6-h in vitro activation with PMA/ionomycin. The numerical values in the dot plots denote the percentage of cells within the respective gated quadrants that were statistically set with negative control Abs. The reagents used are the same as in Fig. 1. FACS data were analyzed from the 35,000 events acquired in the lymphocyte-gated population. Results are representative of three experiments.



**FIGURE 3.** Within the ST2L<sup>+</sup> lymphocyte population in PBMCs, T (CD3<sup>+</sup>) lymphocytes do not produce IFN- $\gamma$ . Three-color flow cytometric analysis using the same reagents as in Fig. 1, with the exception of PE-conjugated anti-hIFN- $\gamma$ . R2 and R3 denote the respective ST2L<sup>+</sup> CD3<sup>+</sup> and ST2L<sup>-</sup> CD3<sup>+</sup>-gated populations that were analyzed for IL-4 and IFN- $\gamma$  production. The PBMCs were PMA/ionomycin activated for 6 h (*upper panel*) and 1 h (*lower panel*) before staining for IL-4 and IFN- $\gamma$ , respectively. A total of 35,000 events in the gated lymphocyte population is shown. Results are representative of three experiments.

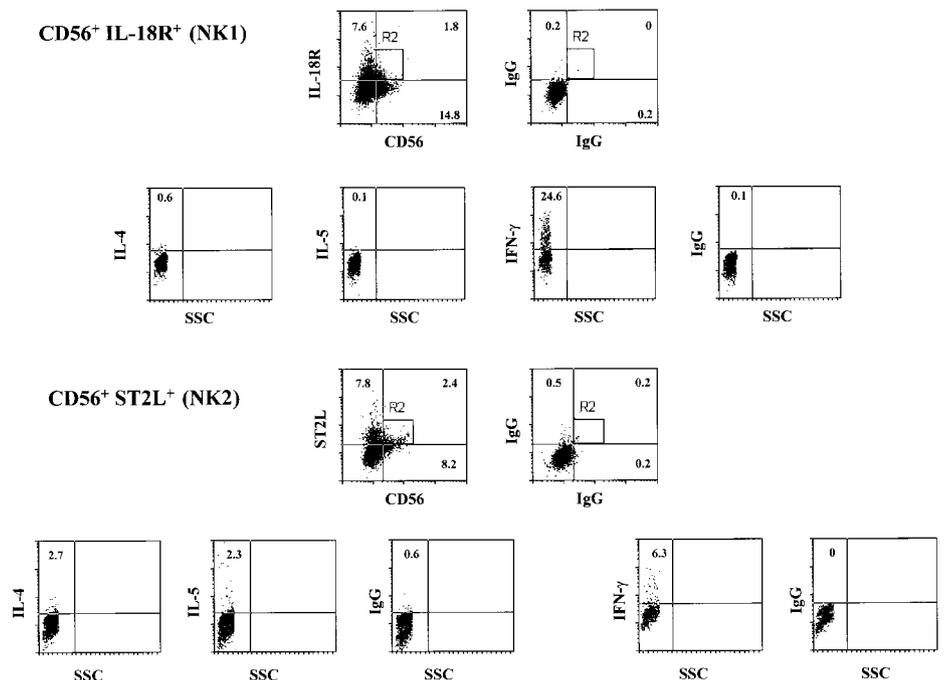
suggesting that *in vivo* activated T cells produce IL-4 more readily than resting memory T cells on *in vitro* activation. It is conceivable that a small proportion of ST2L<sup>+</sup> cells that coexpress IL-4 and IFN- $\gamma$  (Th0), as in Fig. 2B, will not be detected in this study since these cells were activated for only 1 h before staining for IFN- $\gamma$ . The CD3<sup>+</sup> ST2L<sup>-</sup> lymphocyte-gated population (R3) was IL-4 negative, but IFN- $\gamma$  positive, indicating that they are Th1/Tc1 cells (Fig. 3). In contrast to the CD3<sup>+</sup> ST2L<sup>+</sup> cells, the CD56<sup>+</sup> ST2L<sup>+</sup>-gated cells (R2) produced IFN- $\gamma$  and IL-4 or IL-5, indicating that they are NK2 cells (Fig. 4, *lower panel*). CD56<sup>+</sup> IL-18R<sup>+</sup> cells (R2), on the other hand, produce only IFN- $\gamma$  and no IL-4 or IL-5, demonstrating that they are NK1 cells (Fig. 4, *upper panel*). These results show that ST2L is expressed not only by lymphocytes that produce IL-4 or IL-5 alone (Th2/Tc2), but also by lymphocytes that coexpress IFN- $\gamma$  with IL-4 or IL-5, such as Th0/Tc0 and NK2. To date, our results show clearly that ST2L and IL-18R are stable cell surface markers selectively expressed on Th2/Tc2/NK2 and Th1/Tc1/NK1 lymphocytes, respectively, and are detectable in fresh *ex vivo* PBMCs from healthy individuals.

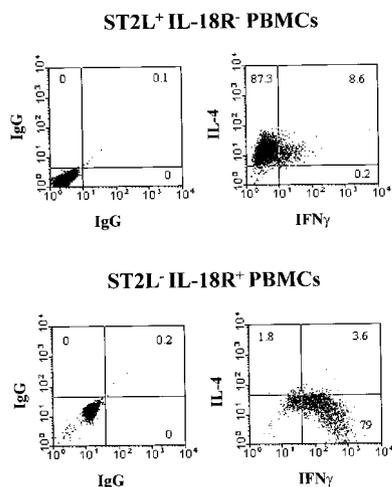
To further assess whether ST2L or IL-18R are appropriate markers for stably committed peripheral blood T2 and T1 lymphocytes, respectively, ST2L<sup>+</sup>IL-18R<sup>-</sup> and ST2L<sup>-</sup>IL-18R<sup>+</sup> cells were sorted from PBMCs obtained from healthy individuals and stimulated through two rounds of *in vitro* culture with immobilized anti-CD3 Ab plus anti-CD28 Ab before intracellular cytokine analysis. Our data show clearly that despite repeated restimulation, 87.3% of ST2L<sup>+</sup>IL-18R<sup>-</sup>-sorted cells express only IL-4, whereas 79% of ST2L<sup>-</sup>IL-18R<sup>+</sup> cells express only IFN- $\gamma$  (Fig. 5). Similar levels of IL-4 and IFN- $\gamma$  expression were obtained in the two respective sorted populations before stimulation. Hence, ST2L and IL-18R are useful markers for peripheral blood memory lymphocytes that stably express T2 and T1 phenotypes, respectively.

*HIV patients have more lymphocytes that express ST2L than IL-18R*

To assess whether these markers can be used to monitor lymphocyte subset distribution in human disease, PBMCs from 22 HIV-1-infected and 21 HIV-negative individuals were stained for cell surface ST2L or IL-18R along with CD3 and CD4, CD8, or CD56.

**FIGURE 4.** CD56<sup>+</sup> lymphocytes that express ST2L produce IL-4, IL-5, and IFN- $\gamma$ , whereas the IL-18R<sup>+</sup> cells produce only IFN- $\gamma$ . Three-color flow cytometric analysis was performed on PBMCs that have been activated with PMA/ionomycin for 6 h (IL-4 and IL-5) and 1 h (IFN- $\gamma$ ), using the same reagents as in Fig. 1, with the exception of FITC-conjugated mAb CD56 (Serotec, Oxford, U.K.) and PE-conjugated anti-hIFN- $\gamma$ . The cytokine-producing CD56<sup>+</sup> lymphocytes were either ST2L<sup>+</sup> or IL-18R<sup>+</sup>-gated cells in R2. A total of 35,000 events in the gated lymphocyte population is shown. Results are representative of three experiments.





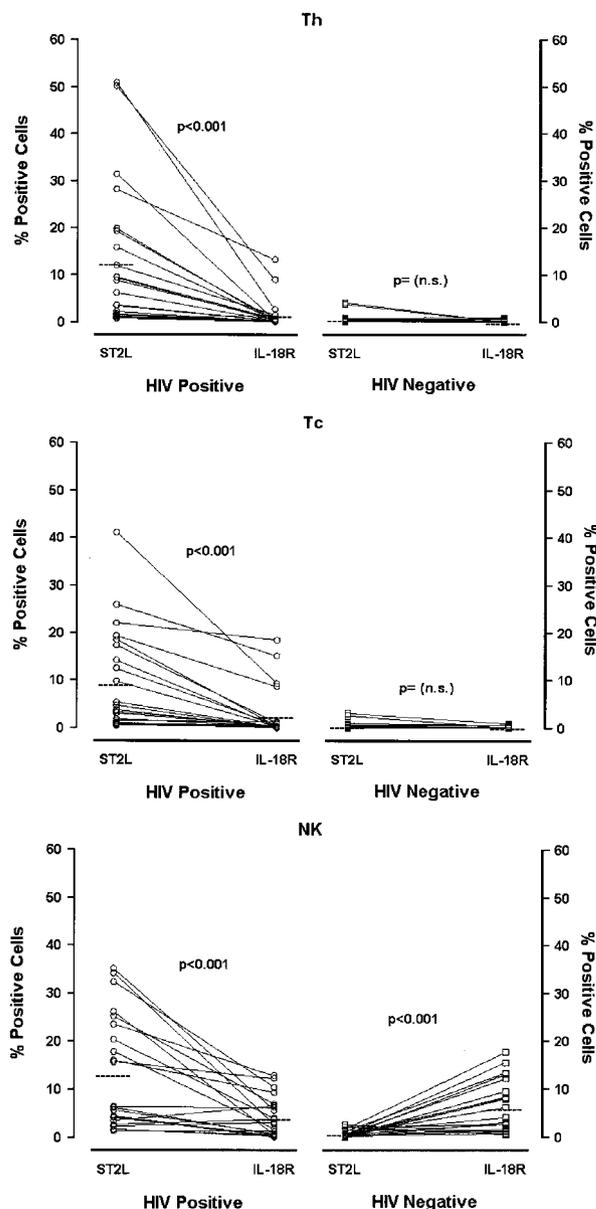
**FIGURE 5.** ST2L and IL-18R are stable markers for memory T2 and T1 PBMCs. ST2L<sup>+</sup>IL-18R<sup>-</sup> and ST2L<sup>-</sup>IL-18R<sup>+</sup> cells sorted from PBMCs and stimulated through two rounds of in vitro culture were PMA/ionomycin activated before two-color flow cytometric analysis. Results are representative of three experiments.

PBMCs from HIV-negative individuals were obtained either from blood donation by healthy volunteers in the laboratory or from the blood bank, avoiding people with hay fever or blood bank donation during the hay fever season. The results in Fig. 6 show clearly that there is a highly significant shift of type 1 to type 2 lymphocytes among the HIV-1-infected individuals. This is especially evident from the slope of the graph joining percentage of ST2L<sup>+</sup> to percentage of IL-18R<sup>+</sup> cells for each of the individuals tested within the Th, Tc, and NK cell populations of the patient cohort, and the difference of the mean values obtained is highly significant with *p* values of 0.001. There was no significant difference in the proportion of type 2 to type 1 Th and Tc cells in the control group. However, in contrast to HIV-positive individuals, HIV-negative individuals have a significantly higher (*p* = 0.001) level of NK1 compared with NK2 cells, perhaps reflecting the importance of NK1 cells as a first line of defense in normal individuals. Our results therefore provide direct evidence for a Th1 to Th2 shift in HIV infection, and include, for the first time, a Tc1 to Tc2 and NK1 to NK2 shift as well.

## Discussion

Several surface molecules that preferentially associate with human Th1 or Th2 cells have been described recently. Th1-associated molecules include membrane IFN- $\gamma$ , LAG-3, IL-12R $\beta_2$ , CCR5, and CXCR3 (36–39), while CD62L, CD30, CCR3, CCR4, CCR8, CXCR4, and CRTH2 are associated with human Th2 cells (40–44) during certain phases of their differentiation/activation process. However, the stability of the expression of these molecules in vivo is unclear, although CCR5<sup>+</sup> T cells were reported to be increased in the blood of individuals with progressive multiple sclerosis, and these cells secreted high levels of IFN- $\gamma$  (45).

The present study set out to examine whether human ST2L and IL-18R are the respective stable specific markers for in vitro driven T2/NK2 and T1/NK1 lines as well as lymphocytes from ex vivo PBMCs, and subsequently to test the applicability of these surface markers to monitor lymphocyte subset changes in human diseases. Thus, AIDS is chosen because of its substantial, yet controversial Th2 shift in HIV patients. Our results presented in this study demonstrate clearly that ST2L and IL-18R are stable cell surface markers for human Th2/Tc2/NK2 and Th1/Tc1/NK1 cells, respectively,



**FIGURE 6.** Predominance of type 2 lymphocytes in HIV-1-infected individuals. PBMCs were obtained from normal healthy controls (HIV<sup>-</sup>, *n* = 21) and HIV-1-infected individuals (HIV<sup>+</sup>, *n* = 22). The distribution of type 1 and 2 lymphocytes in the Th, Tc, and NK cell populations was determined by three-color flow cytometry using FITC-conjugated anti-CD3, with PE-conjugated anti-CD4, anti-CD8, or anti-CD56 and anti-hIL-18R, anti-hST2L, or control sera. hIL-18R and hST2L expression were detected with biotinylated goat anti-rabbit Ig or goat anti-mouse Ig and PerCP-streptavidin. PE- or FITC-conjugated isotype control Ig were used. The proportion of ST2L<sup>+</sup> or IL-18R<sup>+</sup> cells in the gated CD3<sup>+</sup>CD4<sup>+</sup> (Th), CD3<sup>+</sup>CD8<sup>+</sup> (Tc), or CD3<sup>-</sup>CD56<sup>+</sup> (NK) populations was quantitated within gated quadrants that were statistically set with negative control Abs such that the PerCP-positive control cells were  $\leq$ 0.3%. To ensure an unbiased estimation of the proportions of PerCP-stained IL-18R<sup>+</sup> and ST2L<sup>+</sup> cells within any one of the gated Th, Tc, or NK cell populations, similar levels of percentage of PerCP-positive control cells were set for both. Each  $\circ$  represents one HIV-positive individual, and each  $\square$  represents one HIV-negative individual. To show the T1:T2 relationship, the percentages of ST2L<sup>+</sup> and IL-18R<sup>+</sup> cells within each of the Th, Tc, and NK cell populations of an individual are connected by lines. Dashed lines represent the mean. The significance of the difference in distribution of type 1 vs type 2 lymphocytes was determined using the paired sample *t* test. A total of 25,000 events in the gated lymphocyte population is shown.

in vitro as well as in vivo, and that there is a clear shift from Th1 to Th2 during HIV infection. Information on the balance between Th1 and Th2 cell function during HIV infection is of considerable importance in our understanding of the pathogenesis of this disease. CD4<sup>+</sup> T cells play a central role in immune regulation, including helping the expansion of CD8<sup>+</sup> cells (46, 47). Furthermore, dysregulation of Th1 and Th2 cytokine expression may be a mechanism for T cell loss via induction of apoptosis in HIV infection (48).

That ST2L is a stable and suitable marker for ex vivo T2 lymphocytes is shown in Fig. 2B. In this study, the circulating ST2L<sup>+</sup> lymphocytes obtained from an individual with hay fever comprise a high percentage of lymphocytes that produce only IL-4 or IL-5, but no IFN- $\gamma$ , indicating that the latter are T2 cells. Consistent with reports that Th2 are important mediators of allergic reactions (6, 7), they are likely to be mainly Th2 cells. In addition, there is a small proportion (~3%) of ST2L<sup>+</sup> lymphocytes that coexpress IL-4 and IFN- $\gamma$ . This could be attributed to the presence of Th0, NK2, or natural T (NT) cells that are known to produce IFN- $\gamma$  and IL-4 (49), and we have shown that NK2 cells (Figs. 1C and 4) and Th0 cell lines (our unpublished data) express ST2L. In addition, our current ongoing studies with a defined patient population show that some CD56<sup>+</sup>CD3<sup>+</sup> NT cells express ST2L (data not shown). An even smaller proportion (2%) of ST2L<sup>+</sup> cells shown in Fig. 2B also coexpresses IFN- $\gamma$  and IL-5. Since only rarely do peripheral blood CD4<sup>+</sup> T cells (0.03–1.4%) coexpress IL-5 and IFN- $\gamma$  (50) and NT cells do not (49), they could be due to the presence of NK2 cells (Figs. 1C and 4). Therefore, ex vivo Th, Tc, NK, and NT subsets in human peripheral blood can be defined and monitored by multiple color flow cytometry involving the use of ST2L or IL-18R with combinations of lymphocyte markers. Furthermore, the usefulness of ST2L and IL-18R as stable markers for memory T2 and T1 cells, respectively, is further illustrated by the maintained predominance of the respective phenotypes even after two rounds of in vitro stimulation (Fig. 5).

The in vivo relevance of these findings in humans is clearly demonstrated in our ex vivo observation on the shifting of T1/NK1:T2/NK2 balance during HIV infection. As shown in Fig. 6, in all the patients studied, there is in most cases more T2/NK2 compared with T1/NK1 cells, as shown by the slope of the graph joining percentage of ST2L<sup>+</sup> and percentage of IL-18R<sup>+</sup> cells for each and every individual. This is confirmed by the highly significant difference between the two mean values ( $p = 0.001$ ) for the Th1, Tc1, and NK cell populations. Although the shift from type 1 to type 2 lymphocyte is highly significant, the percentage of type 2 lymphocytes is, as expected, variable among HIV-1-infected individuals. In addition, as our study group comprises infected individuals at different stages of disease (long-term nonprogressors, asymptomatics, and progressors), it is conceivable that there will be variable levels of ST2L and IL-18R among the patients. Such variability will no doubt be evident even within a specific cohort such as progressors. Thus, although the percentage of IL-18R<sup>+</sup> cells in one individual could be higher than the percentage of ST2L<sup>+</sup> cells in another individual, this has no bearing on the T2/NK2 vs T1/NK1 shift within a particular individual, as shown by the slope of the graph joining percentage of ST2L<sup>+</sup> to percentage of IL-18R<sup>+</sup> cells for each individual (Fig. 6). Furthermore, although HIV-positive individuals express a relatively higher percentage of IL-18R on Th and Tc cells compared with that in HIV-negative individuals, this is to be expected in people with virus infections in which an initial Tc1/Th1 response is mounted by the host to combat the disease (6, 7), and will generally persist until the infection is cleared. However, unlike other viral infections, others (18–22) and we show in this study that with HIV infection,

in addition to the initial Tc1/Th1 response, there is an increasing abundance of Tc2/Th2, which is reflected in the highly variable slope of the IL-18R/ST2L graphs and levels of ST2L expressed by the individuals from our heterogenous cohort of patients at different stages of disease. It is presently not entirely clear whether the T1 to T2 shift is due to the loss of T1, the progressive increase of T2, or a combination of both.

Apart from Th2/Tc2, ST2L is also expressed by Th0/Tc0. However, the proportion of Th0/Tc0 in vivo in healthy people is generally very low compared with Th2/Tc2, with the latter being ~20- to 25-fold more (Fig. 2B). Since the cells in Fig. 2B were activated for 6 h in vitro to allow for the slower kinetics of IL-4 expression, the proportion of Th0/Tc0 (3%) shown in Fig. 2B could be an overestimation because of the extended activation time for IFN- $\gamma$ , which displays peak expression at 1–2 h (47). Therefore, it is unlikely that the presence of Th0/Tc0 among ST2L<sup>+</sup> cells could significantly affect the overall predominance of T2 in HIV patients. It is currently not clear from our cohort of individuals at different stages of disease how the magnitude of the shift or the absolute percentage of type 2 lymphocytes relates to disease progression. This is currently being addressed with larger cohorts in different stages of HIV disease.

Our data also indicate that the controversial results reported previously could have arisen from the different experimental methods used. The earlier proposed shift of Th1 to Th2 by Clerici and Shearer (18) was based on decreased IL-2 in the presence of increased IL-4 production by bulk cultures of PBMCs from HIV-1-infected individuals. Other studies (23–25) on the Th1/Th2 balance were based on in vitro IFN- $\gamma$  vs IL-4 production by PBMCs from HIV-infected individuals, and these studies have mostly contradicted the proposed shift. The problems associated with assays using IFN- $\gamma$  and IL-4 production following in vitro stimulation as an indicator of Th1 and Th2 cells are several: 1) The kinetics of IL-4 and IFN- $\gamma$  secretion differ, and results would be biased toward IFN- $\gamma$  if both cytokines are quantitated over the same stimulation period. 2) IL-4 is not as easily detectable as IFN- $\gamma$ . 3) NK2 and NT cells also produce IFN- $\gamma$ .

Together, our results demonstrate that ST2L and IL-18R are stable cell surface markers for human type 2 and type 1 lymphocytes, respectively. Using Abs against these markers, we may have settled the longstanding controversy concerning the shift from Th1 to Th2 cells in HIV infection. In the mouse, anti-ST2L and anti-IL-18R Ab administered in vivo could markedly modulate the T1:T2 balance, leading to resolution or exacerbation of disease (8, 10, 11). This strongly suggests that this pair of stable markers are not only a powerful tool for determining immune status and progress of human diseases in general, they could also be important targets for therapeutic intervention of a range of clinical infectious, chronic inflammatory, and autoimmune diseases.

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## References

- Mosmann, T. R., H. Cherwinski, M. W. Bond, M. A. Giedlin, and R. L. Coffman. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 136:2348.
- Croft, M., L. Carter, S. L. Swain, and R. W. Dutton. 1994. Generation of polarized antigen-specific CD8 effector populations: reciprocal action of interleukin (IL)-4 and IL-12 in promoting type 2 versus type 1 cytokine profiles. *J. Exp. Med.* 180:1715.
- Salgame, P., J. S. Abrams, C. Clayberger, H. Goldstein, J. Convit, R. L. Modlin, and B. R. Bloom. 1991. Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. *Science* 254:279.

4. Del Prete, G. F., M. De Carli, C. Mastromauro, R. Biagiotti, D. Macchia, P. Falagiani, M. Ricci, and S. Romagnani. 1991. Purified protein derivative of *Mycobacterium tuberculosis* and excretory-secretory antigen(s) of *Toxocara canis* expand in vitro human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. *J. Clin. Invest.* 88:346.
5. Peritt, D., S. Robertson, G. Gri, L. Showe, M. Aste-Amezaga, and G. Trinchieri. 1998. Differentiation of human NK cells into NK1 and NK2 subsets. *J. Immunol.* 161:5821.
6. Mosmann, T. R., and R. L. Coffman. 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 7:145.
7. Romagnani, S. 1994. Lymphokine production by human T cells in disease states. *Annu. Rev. Immunol.* 12:227.
8. Xu, D., W. L. Chan, B. P. Leung, F.-p. Huang, R. Wheeler, D. Piedrafita, J. H. Robinson, and F. Y. Liew. 1998. Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. *J. Exp. Med.* 187:787.
9. Löhnig, M., A. Stroehmann, A. J. Coyle, J. L. Grogan, S. Lin, J.-C. Gutierrez-Ramos, D. Levinson, A. Radbruch, and T. Kamradt. 1998. T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10 and important for Th2 effector function. *Proc. Natl. Acad. Sci. USA* 95:6930.
10. Coyle, A. J., C. Lloyd, J. Tian, T. Nguyen, C. Eriksson, L. Wang, P. Ottoson, O. Persson, T. Delaney, S. Lehar, et al. 1999. Crucial role of the interleukin 1 receptor family member T1/ST2 in T helper cell type 2-mediated lung mucosal immune responses. *J. Exp. Med.* 190:895.
11. Xu, D., W. L. Chan, B. P. Leung, D. Hunter, K. Schulz, R. W. Carter, I. B. McInnes, J. H. Robinson, and F. Y. Liew. 1998. Selective expression and functions of interleukin 18 receptor on T helper (Th) type 1 but not Th2 cells. *J. Exp. Med.* 188:1485.
12. Gruters, R. A., F. G. Terpstra, R. De Jong, C. J. Van Noesel, R. A. Van Lier, and F. Miedema. 1990. Selective loss of T cell functions in different stages of HIV infection: early loss of anti-CD3-induced T cell proliferation followed by decreased anti-CD3-induced cytotoxic T lymphocyte generation in AIDS-related complex and AIDS. *Eur. J. Immunol.* 20:1039.
13. Clerici, M., C. S. Via, D. R. Lucey, E. Roilides, P. A. Pizzo, and G. M. Shearer. 1991. Functional dichotomy of CD4<sup>+</sup> T helper lymphocytes in asymptomatic human immunodeficiency virus infection. *Eur. J. Immunol.* 21:665.
14. Giorgi, J. V., J. L. Fahey, D. C. Smith, L. E. Hultin, H. L. Cheng, R. T. Mitsuyasu, and R. Detels. 1987. Early effects of HIV on CD4 lymphocytes in vivo. *J. Immunol.* 138:3725.
15. Ballet, J. J., L. J. Couderc, C. Rabian-Herzog, C. Duval-Roy, M. Janier, F. Danon, J. P. Clauvel, and M. Seligmann. 1988. Impaired T-lymphocyte-dependent immune responses to microbial antigens in patients with HIV-1-associated persistent generalized lymphadenopathy. *AIDS* 2:291.
16. Blatt, S. P., C. W. Hendrix, C. A. Butzin, T. M. Freeman, W. W. Ward, R. E. Hensley, G. P. Melcher, D. J. Donovan, and R. N. Boswell. 1993. Delayed-type hypersensitivity skin testing predicts progression to AIDS in HIV-infected patients. *Ann. Intern. Med.* 119:177.
17. Wright, D. N., R. P. Nelson, Jr., D. K. Ledford, E. Fernandez-Caldas, W. L. Trudeau, and R. F. Lockey. 1990. Serum IgE and human immunodeficiency virus (HIV) infection. *J. Allergy Clin. Immunol.* 85:445.
18. Clerici, M., and G. M. Shearer. 1993. A TH1→TH2 switch is a critical step in the etiology of HIV infection. *Immunol. Today* 14:107.
19. Barcellini, W., G. P. Rizzardi, M. O. Borghi, C. Fain, A. Lazzarin, and P. L. Meroni. 1994. TH1 and TH2 cytokine production by peripheral blood mononuclear cells from HIV-infected patients. *AIDS* 8:757.
20. Klein, S. A., J. M. Dohmeyer, T. S. Dohmeyer, M. Pape, O. G. Ottmann, E. B. Helm, D. Hoelzer, and R. Rossol. 1997. Demonstration of the Th1 to Th2 cytokine shift during the course of HIV-1 infection using cytoplasmic cytokine detection on single cell level by flow cytometry. *AIDS* 11:1111.
21. Meynard, L., S. A. Otto, I. P. Keet, R. A. van Lier, and F. Miedema. 1994. Changes in cytokine secretion patterns of CD4<sup>+</sup> T-cell clones in human immunodeficiency virus infection. *Blood* 84:4262.
22. Meynard, L., E. Hovenkamp, I. P. Keet, B. Hooibrink, I. H. de Jong, S. A. Otto, and F. Miedema. 1996. Single cell analysis of IL-4 and IFN- $\gamma$  production by T cells from HIV-infected individuals: decreased IFN- $\gamma$  in the presence of preserved IL-4 production. *J. Immunol.* 157:2712.
23. Maggi, E., M. Mazzetti, A. Ravina, F. Annunziato, M. de Carli, M. P. Piccinni, R. Manetti, M. Carbonari, A. M. Pesce, G. del Prete, et al. 1994. Ability of HIV to promote a TH1 to TH0 shift and to replicate preferentially in TH2 and TH0 cells. *Science* 265:244.
24. Graziosi, C., G. Pantaleo, K. R. Gant, J. P. Fortin, J. F. Demarest, O. J. Cohen, R. P. Sekaly, and A. S. Fauci. 1994. Lack of evidence for the dichotomy of TH1 and TH2 predominance in HIV-infected individuals. *Science* 265:248.
25. Fakoya, A., P. M. Matear, E. Filley, G. A. Rook, J. Stanford, R. J. Gilson, N. Beecham, I. V. Weller, and A. Vyakarnam. 1997. HIV infection alters the production of both type 1 and 2 cytokines but does not induce a polarized type 1 or 2 state. *AIDS* 11:1445.
26. Tominaga, S., T. Yokota, K. Yanagisawa, T. Tsukamoto, T. Takagi, and T. Tetsuka. 1992. Nucleotide sequence of a complementary DNA for human ST2. *Biochim. Biophys. Acta* 1171:215.
27. Chan, W. L., M. L. Lukig, and F. Y. Liew. 1985. Helper T cells induced by an immunopurified herpes simplex virus type 1 (HSV-1) 115 kilodalton glycoprotein (gB) protect mice against HSV-1 infection. *J. Exp. Med.* 162:1304.
28. Torigoe, K., S. Ushio, T. Okura, S. Kobayashi, M. Taniai, T. Kunikata, T. Murakami, O. Sanou, H. Kojima, M. Fujii, et al. 1997. Purification and characterization of the human interleukin-18 receptor. *J. Biol. Chem.* 272:25737.
29. Hsieh, C. S., S. E. Macatonia, C. S. Tripp, S. F. Wolf, A. O'Garra, and K. M. Murphy. 1993. Development of TH1 CD4<sup>+</sup> T cells through IL-12 produced by *Listeria*-induced macrophages. *Science* 260:547.
30. Sornasse, T., P. V. Larenas, K. A. Davis, J. E. de Vries, and H. Yssel. 1996. Differentiation and stability of T helper 1 and 2 cells derived from naive human neonatal CD4<sup>+</sup> T cells, analyzed at the single-cell level. *J. Exp. Med.* 184:473.
31. Sallusto, F., D. Lenig, C. R. Mackay, and A. Lanzavecchia. 1998. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. *J. Exp. Med.* 187:875.
32. Warren, H. S., B. F. Kinnear, J. H. Phillips, and L. L. Lanier. 1995. Production of IL-5 by human NK cells and regulation of IL-5 secretion by IL-4, IL-10, and IL-12. *J. Immunol.* 154:5144.
33. Jung, T., U. Schauer, C. Heusser, C. Neumann, and C. Rieger. 1993. Detection of intracellular cytokines by flow cytometry. *J. Immunol. Methods* 159:197.
34. Openshaw, P., E. E. Murphy, N. A. Hosken, V. Maino, K. Davis, K. M. Murphy, and A. O'Garra. 1995. Heterogeneity of intracellular cytokine synthesis at the single-cell level in polarized T helper 1 and T helper 2 populations. *J. Exp. Med.* 182:1357.
35. Tomura, M., S. Maruo, J. Mu, X. Y. Zhou, H. J. Ahn, T. Hamaoka, H. Okamura, K. Nakanishi, S. Clark, M. Kurimoto, and H. Fujiwara. 1998. Differential capacities of CD4<sup>+</sup>, CD8<sup>+</sup>, and CD4<sup>+</sup>CD8<sup>-</sup> T cell subsets to express IL-18 receptor and produce IFN- $\gamma$  in response to IL-18. *J. Immunol.* 160:3759.
36. Assenmacher, M., A. Scheffold, J. Schmitz, J. A. Segura Checa, S. Miltenyi, and A. Radbruch. 1996. Specific expression of surface interferon- $\gamma$  on interferon- $\gamma$  producing T cells from mouse and man. *Eur. J. Immunol.* 26:263.
37. Annunziato, F., R. Manetti, I. Tomasevic, M. G. Guidizi, R. Biagiotti, V. Gianno, P. Germano, C. Mavilia, E. Maggi, and S. Romagnani. 1996. Expression and release of LAG-3-encoded protein by human CD4<sup>+</sup> T cells are associated with IFN- $\gamma$  production. *FASEB J.* 10:769.
38. Qin, S., J. B. Rottman, P. Myers, N. Kassam, M. Weinblatt, M. Loetscher, A. E. Koch, B. Moser, and C. R. Mackay. 1998. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *J. Clin. Invest.* 101:746.
39. Rogge, L., A. Papi, D. H. Presky, M. Biffi, L. J. Minetti, D. Miotto, C. Agostini, G. Semenzato, L. M. Fabbri, and F. Sinigaglia. 1999. Antibodies to the IL-12 receptor  $\beta$ 2 chain mark human Th1 but not Th2 cells in vitro and in vivo. *J. Immunol.* 162:3926.
40. Del Prete, G., M. De Carli, F. Almerigogna, C. K. Daniel, M. M. D'Elisio, G. Zancuoghi, F. Vinante, G. Pizzolo, and S. Romagnani. 1995. Preferential expression of CD30 by human CD4<sup>+</sup> T cells producing Th2-type cytokines. *FASEB J.* 9:81.
41. Kanegane, H., Y. Kasahara, Y. Niida, A. Yachie, S. Sughii, K. Takatsu, N. Taniguchi, and T. Miyawaki. 1996. Expression of L-selectin (CD62L) discriminates Th1- and Th2-like cytokine-producing memory CD4<sup>+</sup> T cells. *Immunology* 87:186.
42. Sallusto, F., C. R. Mackay, and A. Lanzavecchia. 1997. Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. *Science* 277:2005.
43. Bonecchi, R., G. Bianchi, P. P. Bordignon, D. D'Ambrósio, R. Lang, A. Borsatti, S. Sozzani, P. Allavena, P. A. Gray, A. Mantovani, and F. Sinigaglia. 1998. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J. Exp. Med.* 187:129.
44. Nagata, K., K. Tanaka, K. Ogawa, K. Kemmotsu, T. Imai, O. Yoshie, H. Abe, K. Tada, M. Nakamura, K. Sugamura, and S. Takano. 1999. Selective expression of a novel surface molecule by human Th2 cells in vivo. *J. Immunol.* 162:1278.
45. Balashov, K. E., J. B. Rottman, H. L. Weiner, and W. W. Hancock. 1999. CCR5<sup>+</sup> and CXCR3<sup>+</sup> T cells are increased in multiple sclerosis and their ligands MIP-1 $\alpha$  and IP-10 are expressed in demyelinating brain lesions. *Proc. Natl. Acad. Sci. USA* 96:6873.
46. Bennett, S. R., F. R. Carbone, F. Karamalis, R. A. Flavell, J. F. Miller, and W. R. Heath. 1998. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* 393:478.
47. Schoenberger, S. P., R. E. Toes, E. I. van der Voort, R. Ofringa, and C. J. Melief. 1998. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature* 393:480.
48. Clerici, M., A. Sarin, R. L. Coffman, T. A. Wynn, S. P. Blatt, C. W. Hendrix, S. F. Wolf, G. M. Shearer, and P. A. Henkart. 1994. Type 1/type 2 cytokine modulation of T-cell programmed cell death as a model for human immunodeficiency virus pathogenesis. *Proc. Natl. Acad. Sci. USA* 91:11811.
49. Prussin, C., and B. Foster. 1997. TCR V $\alpha$ 24 and V $\beta$ 11 coexpression defines a human NK1 T cell analog containing a unique Th0 subpopulation. *J. Immunol.* 159:5862.
50. Elson, L. H., T. B. Nutman, D. D. Metcalfe, and C. Prussin. 1995. Flow cytometric analysis for cytokine production identifies T helper 1, T helper 2, and T helper 0 cells within the human CD4<sup>+</sup>CD27<sup>-</sup> lymphocyte subpopulation. *J. Immunol.* 154:4294.