The Journal of Immunology

RESEARCH ARTICLE | SEPTEMBER 15 2001

Selective Expansion and Partial Activation of Human NK Cells and NK Receptor-Positive T Cells by IL-2 and IL-15[1] FREE

Jean Dunne; ... et. al

J Immunol (2001) 167 (6): 3129–3138.
https://doi.org/10.4049/jimmunol.167.6.3129

Selective Expansion and Partial Activation of Human NK Cells and NK Receptor-Positive T Cells by IL-2 and IL-15[1]

Jean Dunne,* Sara Lynch,* Cliona O'Farrelly,† Stephen Todryk,§ John E. Hegarty,‡ Conleth Feighery,* and Derek G. Doherty[2†§]

IL-2 and IL-15 are lymphocyte growth factors produced by different cell types with overlapping functions in immune responses. Both cytokines costimulate lymphocyte proliferation and activation, while IL-15 additionally promotes the development and survival of NK cells, NKT cells, and intraepithelial lymphocytes. We have investigated the effects of IL-2 and IL-15 on proliferation, cytotoxicity, and cytokine secretion by human PBMC subpopulations in vitro. Both cytokines selectively induced the proliferation of NK cells and $CD56^+$ T cells, but not $CD56^-$ lymphocytes. All NK and $CD56^+$ T cell subpopulations tested ($CD4^+$, $CD8^+$, $CD4^-CD8^-$, $\alpha\beta TCR^+$, $\gamma\delta TCR^+$, $CD16^+$, $CD161^+$, $CD158a^+$, $CD158b^+$, $KIR3DL1^+$, and $CD94^+$) expanded in response to both cytokines, whereas all $CD56^-$ cell subpopulations did not. Therefore, previously reported IL-15-induced $\gamma\delta$ and $CD8^+$ T cell expansions reflect proliferations of NK and $CD56^+$ T cells that most frequently express these phenotypes. IL-15 also expanded $CD8\alpha^+\beta^-$ and $V\alpha24V\beta11$ TCR^+ T cells. Both cytokines stimulated cytotoxicity by NK and $CD56^+$ T cells against K562 targets, but not the production of IFN-γ, TNF-α, IL-2, or IL-4. However, they augmented cytokine production in response to phorbol ester stimulation or CD3 cross-linking by inducing the proliferation of NK cells and $CD56^+$ T cells that produce these cytokines at greater frequencies than other T cells. These results indicate that IL-2 and IL-15 act at different stages of the immune response by expanding and partially activating NK receptor-positive lymphocytes, but, on their own, do not influence the Th1/Th2 balance of adaptive immune responses. *The Journal of Immunology,* **2001, 167: 3129–3138.**

Interleukin-2 and IL-15 are structurally related cytokines with growth factor activity for T cells, B cells, NK cells, and NKT cells (1–3). Both cytokines belong to the four-helix bundle family of proteins and bind to receptors with shared signaling components, but they differ in their cellular sites of synthesis and in their regulation of expression. IL-2 is produced by activated T cells and binds to a heterotrimeric receptor (IL-2R) consisting of the IL-2R α-, β-, and γ-chains. In contrast, IL-15 is produced by many cell types in most tissues and binds to two different receptor complexes: on lymphocytes a trimeric receptor (IL-15R) consisting of the IL-15R α-chain and the β- and γ-chains shared by IL-2R (1, 3, 4), and on mast cells a distinct receptor, IL-15RX (5). Studies in vivo and in vitro have demonstrated overlapping functions as well as distinct roles for IL-2 and IL-15 in lymphocyte development, homing, proliferation, and survival. Comparative studies of knockout mice deficient in IL-2Rα, IL-15Rα, IL-2/15Rβ, IL-2, and IL-15 have demonstrated that IL-15/IL-15R signaling, but not IL-2/IL-2R signaling, is required for the development and homing of NK cells, NKT cells, and subsets of $CD8^+$ T cells, $\gamma\delta$ T cells, and intestinal intraepithelial lymphocytes (IELs)[3] (6–11). IL-2 and IL-15 added in vitro promote the survival and proliferation of memory lymphocytes (1, 10, 12) and the induction of cytolytic effector cells (13), but while IL-2 predisposes activated T cells to die by apoptosis, IL-15 promotes their survival (14). Exogenous IL-15 most notably induces the proliferation, survival, and effector functions of resting NK cells (13, 15, 16), NKT cells (9), $\gamma\delta$ T cells (17, 18), and IELs (18–20), suggesting that it has a more general role in the activation of innate and tissue-associated immune responses.

In humans local immune responses are thought to be mediated in part by NK cells, $\gamma\delta$ T cells, and T cells that express activating receptors that are typically found on NK cells (21–25). IL-2 and IL-15 can stimulate or augment human NK cell (13, 15, 16) and $\gamma\delta$ T cell (17) activation, but little is known about their effects on human NK receptor-positive (NKR^+) T cells. Human NKR^+ T cells are predominantly $CD8^+$, they express $\alpha\beta$ or $\gamma\delta$ TCRs and activated/memory $CD28^-CD45RA^-CD45RO^+CD69^+$ T cell phenotypes and possess a variety of NKRs, including CD16, CD56, CD161 (NKR-P1A), NKG2D, and receptors for MHC class I molecules (killer Ig-like receptors, KIRs), such as CD158a, CD158b, and KIR3DL1, and CD94) (22, 24, 26–32). Of these, $CD56^+$ T cells have been most extensively characterized with regard to phenotype and function. $CD56^+$ T cells can be induced to lyse NK-sensitive target cell lines in vitro (26, 27, 33), and their activities are regulated by inhibitory KIR and CD94 molecules (22, 31). $CD56^+$ T cells can also be activated by TCR ligation or in response to cytokines in the microenvironment and stress-inducible proteins present on target cells (33–35). A small proportion ($<1\%$) of peripheral $CD56^+$ T cells express an invariant $V\alpha24J\alpha Q$ TCR α-chain that preferentially pairs with a $V\beta11$

*Department of Immunology, St. James's Hospital, Dublin, Ireland; †Education and Research Center and ‡Liver Unit, St. Vincent's University Hospital and Conway Institute, University College Dublin, Dublin, Ireland; and §Institute of Immunology, Department of Biology, National University of Ireland, Maynooth, Ireland

Received for publication December 18, 2000. Accepted for publication July 6, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by grants awarded by the Irish Health Research Board the Higher Education Authority of Ireland, and the Irish National Liver Transplant Centre.

[2] Address correspondence and reprint requests to Dr. Derek G. Doherty, Institute of Immunology, Department of Biology, National University of Ireland, Maynooth, County Kildare, Ireland. E-mail address: derek.g.doherty@may.ie

[3] Abbreviations used in this paper: IEL, intraepithelial lymphocyte; NKR^+, NK receptor-positive; KIR, killer Ig-like receptor; MNC, mononuclear cell; LAK, lymphokine-activated killing; DP, double-positive; DN, double-negative.

0022-1767/01/$02.00

β-chain and recognizes glycolipid Ags presented by CD1d (32, 36). Upon activation, CD56$^+$ T cells can rapidly produce proinflammatory (Th1-type) and Th2-type cytokines, suggesting roles for these cells both in innate immunity and in the regulation of adaptive immune responses (33, 37, 38).

CD56$^+$ T cells account for a small percentage (~5%) of PBL, but they can expand rapidly in response to various stimuli (22, 24, 34). They are also present in remarkably high numbers in the liver and bone marrow of healthy adults, accounting for 15–55% of all T cells in these organs (24, 32, 33). The factors responsible for the selective accumulation of CD56$^+$ T cells at particular locations are unknown, but are likely to involve chemokines that preferentially recruit these cells from the circulation (39) and/or cytokines and that induce their local expansion (34). Although IL-15 stimulates the growth of murine NKT cells (9), little is known about its influence on the development and function of resting human NKR$^+$ T cell subsets. Therefore, we have examined the relative effects of this cytokine on the proliferation, cytotoxic function, and cytokine secretion profiles of human lymphocyte subpopulations in the absence of stimulation and compared these effects with those of IL-2. We found that either IL-2 or IL-15 induced the selective expansion of resting human NK cells, CD56$^+$ T cells, other NKR$^+$ T cells, $\gamma\delta$ T cells, and CD8$^+$ cells, whereas only IL-15 expanded CD8$\alpha^+\beta^-$ cells and Vα24$^+$Vβ11$^+$ T cells. Importantly, all cell expansions were due to expansions of CD56$^+$ cells, whereas CD56-negative $\gamma\delta^+$ and CD8$^+$ cells showed no proliferation. IL-2 and IL-15 potently induced cytotoxicity by NK and CD56$^+$ T cells, but not the secretion of IFN-γ, TNF-α, IL-2, or IL-4, which required cell activation. However, IL-2 and IL-15 indirectly enhanced the production of these cytokines by expanding the cells that most readily produced cytokines in response to activation. Thus, IL-2 and IL-15 initiate immune responses by selectively expanding, but only partially activating, NK cells and CD56$^+$ T cells.

Materials and Methods

Reagents

Human rIL-15 was purchased from R&D Systems (Abingdon, U.K.). Human rIL-2, BSA, sodium azide, PMA, ionomycin, saponin, and brefeldin A were purchased from Sigma (Poole, U.K.). CFSE was purchased from Molecular Probes (Leiden, The Netherlands).

Cells

PBMC were prepared from 15 healthy adults by Lymphoprep density gradient centrifugation (Nycomed, Oslo, Norway). Cells were cultured for 1–7 days in complete RPMI medium (RPMI 1640 containing 25 mM HEPES, 2 mM L-glutamine, 50 μg/ml streptomycin, 50 U/ml penicillin, and 10% heat-inactivated FCS; Life Technologies, Paisley, U.K.) in the presence or the absence of the indicated amounts of human rIL-15 or IL-2. Cells were then analyzed phenotypically and in functional assays.

Abs and flow cytometry

Monoclonal Abs specific for CD3, CD4, CD8α, CD16, CD56, CD161, CD158a, KIR3DL1, $\alpha\beta$TCR, $\gamma\delta$TCR, IFN-γ, TNF-α, and IL-2 conjugated with FITC; anti-CD56, -CD4, -CD8α, -CD25, and -CD122 conjugated with PE; anti-CD3, -CD8α, -CD45, and -CD19 conjugated with PerCP; and isotype-matched anti-IgG controls (FITC, PE, and PerCP) were purchased from BD Biosciences (Oxford, U.K.). Unconjugated anti-CD3 (clone HIT3a) and anti-IL-4 FITC were obtained from BD PharMingen (Oxford, U.K.). Anti-Vα24 TCR biotin, anti-Vβ11 PE, and unconjugated anti-CD94 and anti-CD8β were obtained from Coulter-Immunotech (Marseilles, France). The CD158b mAb was provided by Dr. L. Moretta (Instituto Nazionale per la Ricerca Sul Cancro Genova, Italy). Phenotypic analysis of lymphocyte subsets was performed by mAb staining and flow cytometry using a FACScan (BD Biosciences, Mountain View, CA) and analysis using CellQuest software (BD Biosciences) (32, 33). Four-color flow cytometry was performed on a FACStar (BD Biosciences) with Multimate software (BD Biosciences).

Assessment of cell expansions

Cell numbers were determined before and after culture in the presence or the absence of IL-2 or IL-15 by staining with ethidium bromide and acridine orange and quantification of viable mononuclear cells (MNC) by fluorescent microscopy. The proportions of MNC that were positive for combinations of CD3, CD4, CD8α, CD8β, CD16, CD19, CD56, CD158a, CD158b, CD161, $\alpha\beta$TCR, $\gamma\delta$TCR, CD25, CD122, CD94, KIR3DL1, and the Vα24 and Vβ11 TCR chains were determined by mAb staining and flow cytometry. Absolute numbers of lymphocyte subpopulations were calculated from the viable MNC counts. Changes in cell numbers over time in culture are expressed as ratios of the numbers of viable cells of a particular phenotype that were present after culture to the numbers of those cells that were present before culture (factorial changes in cell numbers).

Analysis of cell division

PBMC were either untreated or labeled before culture with CFSE as described by Lyons and Parish (40). Cells were washed and suspended at a density of 5×10^6 cells/ml in PBS containing 5 μM CFSE for 10 min at 37°C, followed by washing with cold culture medium. The cells were then cultured in the absence or the presence of IL-2 or IL-15 as described above. Cells stimulated with 5 μg/ml PHA and 25 ng/ml IL-2 were used as positive controls. Cell division of CFSE-labeled PBMC subpopulations was assessed by flow cytometry after labeling with CD3 and CD56.

Cytotoxicity assays

In vitro-expanded PBMC were separated into CD3$^+$ and CD3$^-$ cell fractions using anti-CD3 mAb-coated magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) (33). The purity of isolated fractions was assessed by flow cytometry, and preparations whose purities were >95% were used as effectors in cytotoxicity assays. NK cytotoxicity and lymphokine-activated killing (LAK) of K562 target cells by NK cells and CD56$^+$ T cells was assayed in 4-h ^{51}Cr release assays using the CD3$^-$ cells and CD3$^+$ cells as effectors, respectively, at E:T ratios of 1:1 to 1:500. We and others have previously found that IL-2-induced LAK activity of human PBMC against K562 cells resides in CD56$^+$, but not CD56$^-$, T cells (26, 33); therefore, the cytotoxic activity of NK cells and CD56$^+$ T cells was measured using the CD3$^+$ and CD3$^-$ cell fractions, respectively.

Analysis of cytokine production

The effects of IL-2 and IL-15 on the production of IFN-γ, TNF-α, IL-2, and IL-4 by stimulated peripheral blood NK cells, CD56$^-$ T cells, and CD56$^+$ T cells was measured by a combination of cell surface and intracytoplasmic mAb staining and analysis by flow cytometry as described previously (33). Briefly, human PBMC were cultured for 7 days in the absence or the presence of 25 ng/ml (50 U/ml) IL-2 or 10 ng/ml IL-15. The cells were then stimulated for 4 h with 10 ng/ml PMA plus 1 μg/ml ionomycin or with plate-bound anti-CD3 mAb (10 μg/ml HIT3a bound to plates by incubation for 6 h at 37°C in 0.1 M Na_2HPO_4) in the presence of 1 ng/ml PMA and 10 μg/ml brefeldin A. As controls, unstimulated cells were treated similarly. The cells were then stained with mAbs against cell surface CD3 and CD56, followed by fixing with 4% paraformaldehyde, permeabilizing with 0.2% saponin, and intracytoplasmic mAb staining for IFN-γ, TNF-α, IL-2, or IL-4 (33). Cytokine production by CD3$^-$CD56$^+$ NK and by CD3$^+$CD56$^-$ and CD3$^+$CD56$^+$ T cells was detected by three-color flow cytometry.

Results

Lymphocyte subpopulations in human peripheral blood

Flow cytometry was used to determine the proportions of freshly isolated PBMC that expressed B cell (CD19$^+$), T cell (CD3$^+$CD56$^-$, and CD3$^+$CD56$^+$), NK cell (CD3$^-$CD56$^+$ and CD3$^-$CD16$^+$), $\alpha\beta$TCR$^+$, CD4$^+$, CD8$^+$, CD8$\alpha^+\beta^-$, double-positive (DP) CD4$^+$ CD8$^+$, and double-negative (DN) CD4$^-$CD8$^-$ phenotypes. The cell frequencies are shown in Table I. The frequencies of CD3$^-$ and CD3$^+$ cells that expressed the NK cell markers CD16, CD161, CD56, CD158a, CD158b, KIR3DL1, and CD94 are shown in Table II. The frequencies of NK cells, CD56$^-$ T cells, and CD56$^+$ T cells that expressed $\alpha\beta$, $\gamma\delta$, Vα24 and Vα24Vβ11 TCRs, CD4, CD8, DN, CD25, and CD122 are shown in Table III. Two subpopulations of CD56$^+$ NK cells were seen: 12.4% expressed CD56^{bright} phenotypes, whereas the remainder were CD56^{dim}. All CD56$^+$ T cells were CD56^{dim}. CD16 was expressed by 40–45% of CD56$^+$ cells, but was

Table I. *Distribution of lymphocyte subpopulations (% ± SD) in fresh PBMC*[a]

Cell Population (phenotype)	% of PBMC
B cells ($CD19^+$)	4.5 ± 0.5
T cells ($CD3^+CD56^-$)	73.9 ± 2.9
T cells ($CD3^+CD56^+$)	2.8 ± 0.5
NK cells ($CD3^-CD56^+$)	8.6 ± 1.7
NK cells ($CD3^-CD16^+$)	4.5 ± 0.9
$\alpha\beta TCR^+$ cells	79.5 ± 1.4
$\gamma\delta TCR^+$ cells	1.7 ± 0.8
$CD4^+$ cells	47.5 ± 3.0
$CD8^+$ cells ($CD8\alpha^+$)	19.3 ± 2.7
$CD8\alpha^+\beta^-$ cells	2.9 ± 0.2
DP cells ($CD4^+CD8^+$)	0.95 ± 0.1
DN cells ($CD4^-CD8^-$)	34.3 ± 2.4

[a] All values are means of at least 4 and up to 15 samples.

rarely found on $CD56^-$ cells. Ninety-eight to 100% of $CD16^+$ cells were negative for CD3 (data not shown).

Human NK and NKR$^+$ T cells are selectively expanded by IL-2 and IL-15

Dose- and time-dependent expansion of NKR^+ PBMC in response to culture with IL-15 and IL-2 added once at time zero, indicated that maximal changes in cell numbers occurred ~7 days after the addition of these cytokines. One to 10 ng/ml IL-15 (Fig. 1*A*) and 25 ng/ml IL-2 (data not shown) induced maximal expansion of cells expressing the NKRs, CD161, CD56, KIR3DL1, and CD94. Culturing of PBMC for 7 days in medium only, 25 ng/ml IL-2, or 10 ng/ml IL-15 resulted in mean 0.64-, 1.14-, and 1.26-fold changes in total lymphocyte numbers, respectively. Fig. 1*B* shows that IL-2 and IL-15 induced significant expansion of NK cells and $CD56^+$ T cells, but not B cells or conventional $CD56^-$ T cells. IL-15 was more potent than IL-2 at expanding these cells even at concentrations >500 ng/ml. $CD56^+$ T cells were consistently more responsive than NK cells, exhibiting mean expansions of 8.8-fold for IL-15 and 7.6-fold for IL-2 compared with 2.3- and 3.7-fold for NK cells. After incubation with IL-2, 71.8% of NK cells and 38.2% of $CD56^+$ T cells expressed $CD56^{bright}$ phenotypes, while IL-15 induced this phenotype on 76.7% of NK cells and 27.3% of $CD56^+$ T cells. Culture in medium only resulted in reductions in cell numbers (Fig. 1*B*). The simultaneous addition of IL-2 and IL-15 did not promote cell expansion beyond that of IL-15 alone.

The effects of culturing PBMC in the presence or the absence of 25 ng/ml IL-2 or 10 ng/ml IL-15 on the expansion of NK cells and T cells expressing different NKRs are compared in Fig. 1, *C* and *D*. IL-15 consistently induced the expansion of both $CD3^+$ and $CD3^-$ cells expressing CD16, CD56, CD158a, CD158b, and CD94 and, to a lesser degree, CD161 and KIR3DL1. IL-2 expanded NK cells and T cells expressing CD16, CD56, CD158a, CD158b, and, to a lesser degree, $CD161^+$, $KIR3DL1^+$, and $CD94^+$ cells. Both $CD16^-$ and $CD16^+$ NK cells proliferated in response to IL-2 or IL-15 at the concentrations used. IL-15 was consistently more potent than IL-2, and $CD3^+CD56^+$ cells expanded to a greater degree than $CD3^+$ cells bearing the other NKRs (Fig. 1, *C* and *D*).

Table II. *Distribution of NK cell surface receptors (% positivity ± SD) among freshly isolated CD3$^-$ and CD3$^+$ PBMC*[a]

NK Receptor	% of $CD3^-$ PBMC	% of $CD3^+$ PBMC
CD16	13.1 ± 2.7	1.0 ± 0.4
CD161	19.2 ± 2.8	15.4 ± 2.0
CD56	28.9 ± 5.6	4.0 ± 0.7
CD158a	10.5 ± 3.2	1.1 ± 0.4
CD158b	45.5 ± 8.1	2.6 ± 0.4
KIR3DL1	7.1 ± 0.8	1.1 ± 0.3
CD94	22.0 ± 0.9	4.8 ± 0.4

[a] All values are means of at least 4 and up to 15 samples.

Human γδ T cells and invariant αβ T cells are selectively expanded by IL-2 and/or IL-15

The expansions of $\alpha\beta$ T cells, $\gamma\delta$ T cells, and T cells expressing the $V\alpha24$ and $V\alpha24V\beta11$ TCRs that are associated with invariant $V\alpha24J\alpha Q$ TCR^+ cells (38, 41) were compared after culture in medium only or with 25 ng/ml IL-2 or 10 ng/ml IL-15. Fig. 2*A* shows that both IL-2 and IL-15 induced expansions of $\gamma\delta$ T cells, but not $\alpha\beta$ T cells. $\gamma\delta$ T cells expanded from being 2–4% of $CD3^+$ PBMC to 10–15%, and to 40% in one individual. IL-15, but not IL-2, induced the expansion of $V\alpha24$ and $V\alpha24V\beta11$ TCR-bearing cells. $V\alpha24V\beta11^+$ T cells generally represent 0.1–0.2% of peripheral T cells (Table III), but IL-15 expanded them to >1%, and in one case 4%. Because $\gamma\delta$ TCRs are present on greater proportions of $CD56^+$ T cells than $CD56^-$ T cells (Table III), the effects of culturing PBMC in medium alone, IL-2, or IL-15 on $\alpha\beta$ and $\gamma\delta$ $CD56^-$ and $CD56^+$ T cell expansions were compared. Fig. 2*B* shows that these cytokines induced only moderate proliferation of both $\alpha\beta$ and $\gamma\delta$ $CD56^-$ T cells, but $\alpha\beta$ and $\gamma\delta$ $CD56^+$ T cells were significantly expanded by both IL-2 and IL-15. Thus, the expansions of $\gamma\delta$ T cells by these cytokines, shown in Fig. 2*A*, are mainly due to the expansion of $CD56^+$ T cells.

Expansions of CD8$^+$ and DN cells by IL-2 and IL-15 are due to NK cell and CD56$^+$ T cell expansions

PBMC were cultured for 7 days in medium only or in 25 ng/ml IL-2 or 10 ng/ml IL-15 and changes in the numbers of cells expressing CD4, CD8, $CD8\alpha^+\beta^-$, and DN and DP phenotypes were

Table III. *Distribution of lymphocyte receptors (% positivity ± SD) among freshly isolated NK cells (CD3$^-$CD56$^+$ cells), CD56$^-$ T cells (CD3$^+$CD56$^-$), and CD56$^+$ T cells (CD3$^+$CD56$^+$)*[a]

Receptor	% of NK Cells	% of $CD56^-$ T Cells	% of $CD56^+$ T Cells
$\alpha\beta$ TCR	0	95.5 ± 1.1	55.5 ± 14.4
$\gamma\delta$ TCR	0	2.4 ± 0.7	37.6 ± 12.2
$V\alpha24$ TCR	0	0.7 ± 0.3	5.1 ± 2.8
$V\alpha24V\beta11$ TCR	0	0.19 ± 0.05[b]	
CD4	0	77.0 ± 2.7	7.9 ± 2.3
CD8	40.8 ± 5.5	16.5 ± 2.6	67.1 ± 14.8
DN	60.2 ± 4.1	0.1 ± 0.05	26.8 ± 13.4
CD25	2.1 ± 0.2	26.9 ± 5.6	6.9 ± 3.5
CD122	10.2 ± 1.9	98.8 ± 0.4	73.8 ± 6.8

[a] All values are means of at least 4 and up to 15 samples.
[b] $V\alpha24V\beta11$ TCR^+ cells were analyzed as a proportion of the total $CD3^+$ cell compartment only.

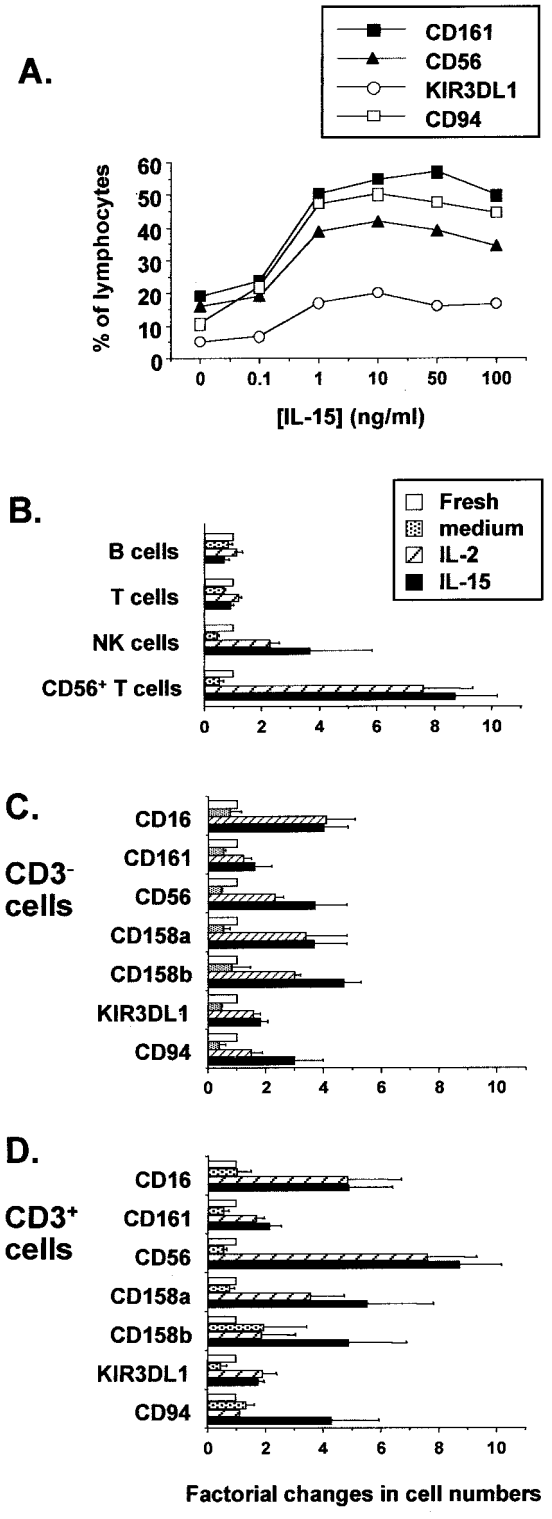

FIGURE 1. Expansion of human peripheral blood lymphocyte populations cells by IL-2 or IL-15. *A*, Dose-dependent expansion of PBMC expressing the NK receptors CD161, CD56, KIR3DL1, and CD94 following culture for 7 days in the absence or the presence of 0.1–100 ng/ml IL-15. *B*, Absolute expansions of B cells (CD19$^+$), CD56$^-$ T cells (CD3$^+$CD56$^-$), NK cells (CD3$^-$CD56$^+$), and CD56$^+$ T cells (CD3$^+$CD56$^+$) following culture for 7 days in 25 ng/ml IL-2 or 10 ng/ml IL-15. *C* and *D*, Absolute expansions of CD16-, CD161-, CD56-, CD158a-, CD158b-, KIR3DL1-, and CD94-positive CD3$^-$ (*C*) and CD3$^+$ (*D*) cells following culture for 7 days in the absence or the presence of IL-2 or IL-15. Cell expansions in *B*, *C*, and *D* are expressed as the ratios of the numbers of viable cells present after culture to the numbers present before culture shown in Tables I and II (□). Results are means of four to eight (*A*, *C*, and *D*) and nine (*B*) experiments.

determined. As shown in Fig. 3*A*, IL-2 and IL-15 induced a moderate (2- to 4-fold) expansion of CD8$^+$ cells, but CD4$^+$ cell numbers were not changed. As previously reported (20), CD8$\alpha^+\beta^-$ cells were significantly expanded by IL-15. The numbers of DN cells were increased 2-fold with IL-15, but DP cell numbers remained unchanged. To identify the CD8$^+$ and DN MNC subpopulations that expanded in response to IL-15, the changes in the numbers of CD4$^+$, CD8$^+$, and DN NK cells and CD56$^-$ and CD56$^+$ T cells upon culture with medium, IL-2, or IL-15 were determined. Fig. 3, *B–D*, shows that both CD8$^+$ NK cells and CD56$^+$ T cells were significantly expanded by IL-2 and/or IL-15, but CD8$^+$CD56$^-$ T cells were not. Thus, as for $\gamma\delta$ T cells, the expansion of CD8$^+$ cells shown in Fig. 3*A* was due to expansions of NK cells and CD56$^+$ T cells only. Surprisingly, CD4$^+$ T cells were also expanded to a similar degree to CD8 T cells within the CD56$^+$ T cell population (Fig. 3*D*). Total CD4$^+$ cell expansions were not noted (Fig. 3*A*), because all NK cells are negative for CD4, and only 8% of CD56$^+$ T cells are CD4$^+$ (Table III). Similarly, NK and CD56$^+$ T cells bearing DN phenotypes were significantly expanded by IL-2 and IL-15, but because only 0.1% of CD56$^-$ T cells are DN (Table III), the overall increase in DN cells was only marginal. Thus, Fig. 3 indicates that the increases in the numbers of CD8$^+$ and DN cells in response to IL-2 and IL-15 reflect increases in NK cells and CD56$^+$ T cells, both populations that are predominantly CD8$^+$ or DN.

IL-2 and IL-15 induce proliferation of CD56$^+$ MNC

The expansions of CD56$^+$ cells in response to IL-2 or IL-15 appear to be due to the induction of both proliferation and survival, because cell numbers increased after culture with IL-2 or IL-15, but decreased after culture in medium alone (Figs. 1–3). To confirm that proliferation of CD56$^+$ cells takes place, PBMC were labeled with CFSE and cultured for 7 days in medium alone or in 25 ng/ml IL-2 or 10 ng/ml IL-15, and changes in the intensities of CFSE staining of CD56$^+$ and CD56$^-$ cells were examined by flow cytometry. CFSE-labeled CD56$^+$ cells (Fig. 4*D*), but not CD56$^-$ cells (Fig. 4*C*), underwent sequential halving of CFSE fluorescence intensity upon culture with IL-15. Similar results were obtained with PBMC cultured with IL-2 (data not shown). This halving of fluorescence intensity was comparable to, but less marked than, the profile seen with CD3$^+$ PBMC stimulated with PHA and cultured with IL-2 as a positive control (Fig. 4*B*).

Expression of CD25 and CD122 by human peripheral blood CD56$^-$ T cells, NK cells, and CD56$^+$ T cells

Flow cytometry revealed that the IL-2R α-chain, CD25, was expressed by a mean of 27% of freshly isolated human CD56$^-$ T cells, but only by 2 and 7% of NK cells and CD56$^+$ T cells, respectively (Fig. 5*A*). In contrast, the IL-2R/IL-15R β-chain, CD122, was constitutively expressed by NK cells (99%) and most CD56$^+$ T cells (74%), but only 10% of CD56$^-$ T cells expressed this receptor (Table III and Fig. 5*B*). All CD56^{bright} and most CD56^{dim} cells expressed CD122. Interestingly, the intensity of staining for CD122 was 10-fold higher on NK cells than on CD56$^+$ T cells (Fig. 5*B*). Expression of the IL-15R α-chain or the common γ-chain was not tested.

IL-2 and IL-15 induce spontaneous cytotoxicity by human peripheral NK cells and CD56$^+$ T cells

PBMC were cultured for 7 days in medium only or in 25 ng/ml IL-2 or 10 ng/ml IL-15 and separated into CD3$^-$ and CD3$^+$ fractions using mAb-coated magnetic beads. The purities of the isolated fractions were shown by flow cytometry to be >95% (data

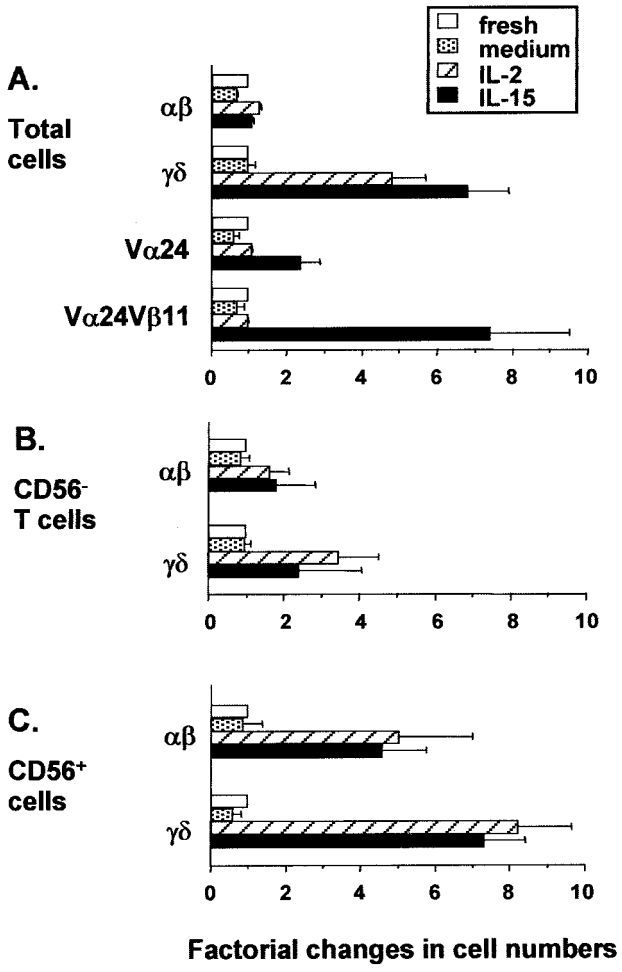

FIGURE 2. Expansion of human peripheral blood $\alpha\beta$, $\gamma\delta$, and Vα24Vβ11 cells by IL-2 or IL-15. *A*, Absolute expansions of $\alpha\beta$, $\gamma\delta$, Vα24, and Vα24Vβ11 cells following culture for 7 days in the absence or the presence of 25 ng/ml IL-2 or 10 ng/ml IL-15. *B* and *C*, Absolute expansions of $\alpha\beta$ and $\gamma\delta$ CD56$^-$ (*B*) and CD56$^+$ T (*C*) cells. Cell expansions are expressed as the ratios of the numbers of viable cells present after culture to the numbers present before culture shown in Tables I and III (□). Results are means of seven to nine (*A*) and four (*B* and *C*) experiments.

not shown). Isolated CD3$^-$ and CD3$^+$ PBMC were used as effectors in cytotoxicity assays against K562 target cells. Fig. 6 shows that cytotoxic activity was present in CD3$^-$ cells that were freshly isolated or cultured in medium alone, giving about 20% specific lysis at E:T ratios of 100:1. No cytotoxicity was detected in the CD3$^+$ fraction even at E:T ratios of 500:1. However, after incubation with either IL-2 or IL-15, both fractions demonstrated 55–75% specific lysis of K562 targets (Fig. 6). CD56$^-$ T cells are incapable of killing K562 cells (26, 33), so the cytotoxicity induced in the CD3$^+$ cells is attributable to the CD56$^+$ T cells. Therefore, CD56$^+$ T cells can be induced by either IL-2 or IL-15 to mature into LAK cells.

IL-2 and IL-15 do not induce cytokine production by resting human PBMC, but can augment cytokine production by activated PBMC

The effect of culturing PBMC for 7 days in medium only or in 25 ng/ml IL-2 or 10 ng/ml IL-15 on the production of IFN-γ, TNF-α, IL-2, and IL-4 in the absence of stimulation or upon stimulation with PMA and ionomycin or anti-CD3 mAb was examined by intracellular cytokine staining and flow cytometry (Fig. 7*A*). As shown in Fig. 7 (*B* and *C*) for IFN-γ and IL-4, no cytokines were produced in the absence of stimulation even after culture with IL-2 or IL-15. However, compared with PBMC cultured in medium only and stimulated with either PMA and ionomycin or anti-CD3 mAb, the numbers of cells capable of producing IFN-γ and IL-4 were dramatically increased by preculturing with IL-2 or IL-15 (Fig. 7, *B* and *C*). Similar increases in TNF-α- and IL-2-expressing PBMC were found in two experiments (data not shown).

IL-2 and IL-15 augment cytokine production by human PBMC by selectively expanding NK and CD56$^+$ T cells

The augmentation of cytokine production by IL-2 and IL-15 (Fig. 7) could be due either to costimulation of IFN-γ and IL-4 production by PBMC or to a selective expansion of IFN-γ- and IL-4-secreting cell subpopulations. To address this question we used flow cytometry to determine the percentages of NK cells, CD56$^-$ T cells, and CD56$^+$ T cells incubated with medium only, IL-2, or IL-15 that produce IFN-γ and IL-4 upon stimulation. Fig. 8 shows that after incubation in medium alone, 47% of NK cells and 86% of CD56$^+$ T cells, but only 12% of CD56$^-$ T cells, produced IFN-γ, and 1% of T cells and 13% of CD56$^+$ T cells produced IL-4 upon stimulation with PMA and ionomycin. Slightly lower frequencies of these lymphocyte subpopulations produced cytokines upon stimulation with anti-CD3 mAb. Interestingly, 20% of NK cells (CD3-negative) produced IFN-γ after anti-CD3 mAb stimulation, presumably due to factors produced by activated T cells or to stimulation of NK cells through binding of the Fc portions of the anti-CD3 mAb to CD16. IFN-γ was produced by both CD56^{bright} and CD56^{dim} NK cells. Incubation with IL-2 or IL-15 did not significantly augment the proportions of NK cells, T cells, and CD56$^+$ T cells expressing these cytokines (Fig. 8). These results show that greater proportions of CD56$^+$ T cells produce IFN-γ and IL-4 upon stimulation than NK and T cells, and that augmentation of cytokine production by IL-2 and IL-15 seen in Fig. 7 is due to selective expansion of CD56$^+$ T cells and, to a lesser degree, NK cells. Thus, IL-2 and IL-15 do not induce or costimulate cytokine production by PBMC, but they selectively expand IFN-γ- and IL-4-producing NK and CD56$^+$ T cells.

Discussion

Intracellular communications involved in immune responses are often mediated by cytokines that show a high degree of redundancy and pleiotropy. In this respect, IL-2 and IL-15 are structurally related cytokines that bind to receptors with shared signaling components and stimulate lymphocyte development, homing, proliferation, and survival (1–3). Differences in the cellular sites of synthesis and regulation of IL-2 and IL-15 production point to distinct roles for these cytokines in immune activation. IL-2 and IL-2Rα appear only to be expressed by activated T cells, suggesting a role for this cytokine/receptor system in the perpetuation of adaptive immune responses. In contrast, IL-15 is produced by many cell types, including kidney, lung, heart, liver, placenta, skeletal muscle, epithelial cells, and monocytes, suggesting a broader role for this cytokine in innate immune responses and in the homeostatic maintenance of effector cells in various tissues (1, 4).

The multiplicity and selectivity of the stimulatory properties of IL-2 and IL-15 for different lymphocyte subpopulations prompted us to quantify the lymphocyte subpopulations present in human blood and to examine the effects of these cytokines on their proliferation, cytotoxic function, and cytokine secretion in vitro. We found that the effects of IL-2 and IL-15 on resting human lymphocyte subpopulations are essentially the same. Addition of either

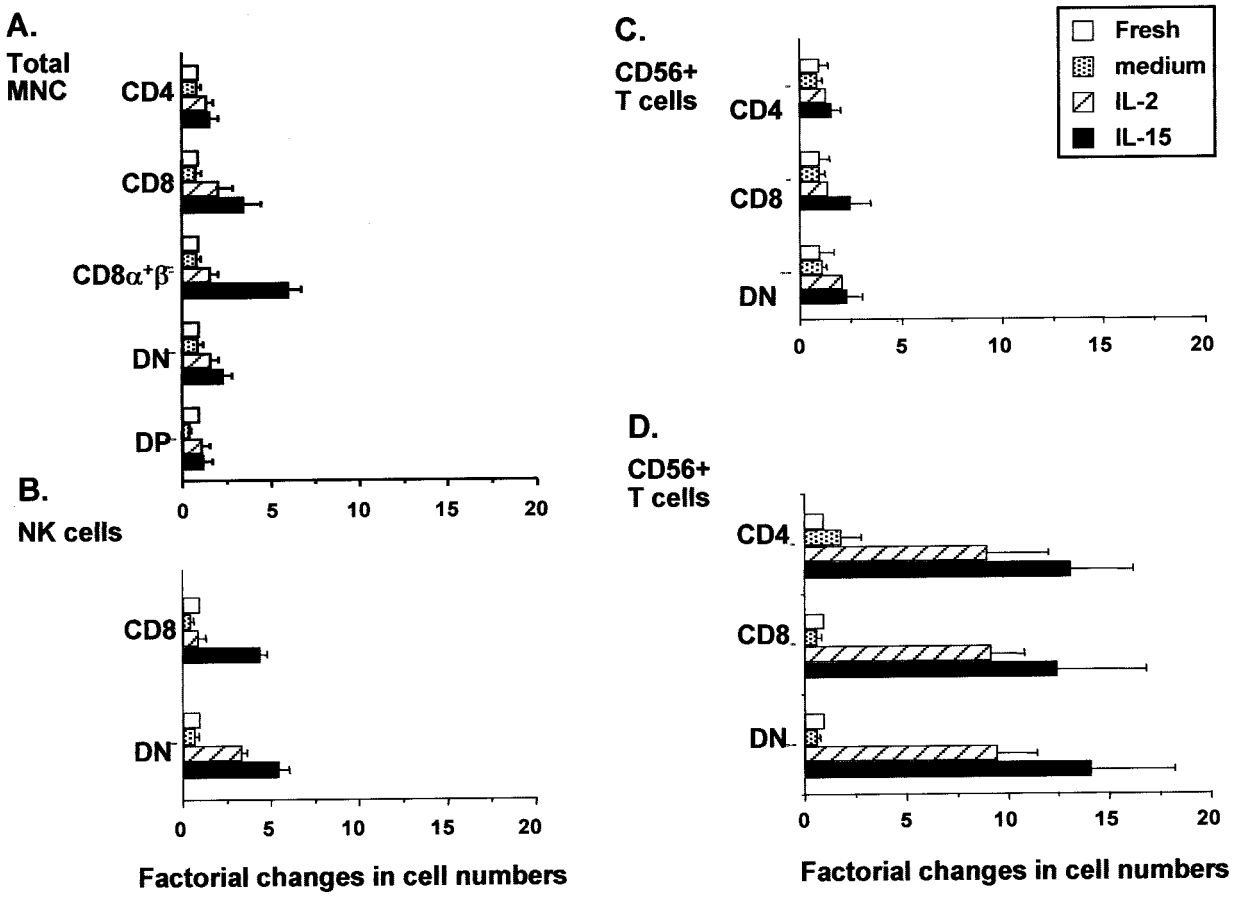

FIGURE 3. Expansion of human peripheral blood CD4$^+$, CD8$^+$, CD8$\alpha^+\beta^-$, CD4$^-$CD8$^-$ (DN), and CD4$^+$CD8$^+$ (DP) cells by IL-2 or IL-15. *A*, Absolute expansions of CD4$^+$, CD8$^+$, CD8$\alpha^+\beta^-$, DN, and DP cells following culture for 7 days in the absence or the presence of 25 ng/ml IL-2 or 10 ng/ml IL-15. *B–D*, Absolute expansions of CD4$^+$, CD8$^+$, and DN CD3$^-$CD56$^+$ NK cells (*B*); CD56$^-$ T cells (*C*); and CD56$^+$ T cells (*D*). Cell expansions are expressed as the ratios of the numbers of viable cells present after culture to the numbers present before culture shown in Tables I and II (□). Results are the means of four experiments.

cytokine to human PBMC in the absence of prior activation resulted in selective expansions of NK cells and T cells expressing various NKRs (CD16, CD161, CD158a, CD158b, KIR3DL1, and CD94), but not of conventional T cells or B cells. CD56$^+$ T cells, which almost invariably coexpress one or another of these NKRs, were the most responsive, exhibiting 7- to 10-fold expansions in 1 wk. These expansions are due both to proliferation, because cell numbers increased, and cell division was observed as a sequential reduction of CFSE fluorescence, and to survival, because CD56$^+$ cells cultured in the absence of these cytokines were depleted. As reported previously (13, 42, 43), only CD56^{bright} NK cells proliferated significantly in response to IL-2 or IL-15. CD56^{bright} cells are absent among fresh T cells, but both IL-2 and IL-15 up-regulated CD56 expression to levels comparable to those of CD56^{bright} NK cells. Although CD16$^-$ NK cells are more responsive to low concentrations of IL-2 than CD16$^+$ NK cells (44), we found that both NK subsets responded similarly to 25 ng/ml IL-2 or 10 ng/ml IL-15.

Several studies have demonstrated that IL-15 preferentially promotes the survival and proliferation of $\gamma\delta$ over $\alpha\beta$ T cells (17–19) and CD8$^+$ over CD4$^+$ T cells (20, 45), which has led Ma et al. (4) to suggest that $\gamma\delta$ and CD8$^+$ T cells resemble innate immune cells. These findings were confirmed in the present study, but we have further demonstrated that $\gamma\delta$ and CD8$^+$ T cell expansions in response to both IL-2 and IL-15 are due to expansions of CD56$^+$ T cells, of which 30–50% express $\gamma\delta$ TCRs and 50–80% express CD8. Both $\alpha\beta$ and $\gamma\delta$ T cells expressing CD56 were similarly expanded by IL-2 or IL-15, but conventional CD56$^-$ T cells with either $\alpha\beta$ or $\gamma\delta$ TCRs were negligibly expanded. Similarly, proliferative responses of CD56$^+$ cells occurred regardless of CD4/CD8/DN phenotypes, but minimal expansions of CD56$^-$ T cells bearing CD4$^+$, CD8$^+$, or DN phenotypes were observed. CD8$^+$ and DN, but not CD4$^+$, phenotypes are expressed by significant proportions of CD56$^+$ T cells and NK cells; therefore, expansions of CD4$^+$ cells are not detected when total MNC were examined. Thus, the previous data that indicate that $\gamma\delta$, CD8$^+$, and DN lymphocytes proliferate in response to IL-15 (17–20, 45) most likely reflect expansions of CD56$^+$ T cells and NK cells.

Two lymphocyte subpopulations were found in the present study to proliferate differentially in response to IL-15 and IL-2. Lymphocytes expressing the CD8 α-chain in the absence of the CD8 β-chain, which are commonly found among intestinal IELs (18, 20), and T cells expressing the Vα24Vβ11 TCR, which would include invariant Vα24JαQ$^+$ NKT cells (36, 38, 41), exhibited strong proliferative responses to IL-15, but not IL-2. CD8$\alpha^+\beta^-$ cells have previously been shown to define lymphocyte subpopulations that are maintained by IL-15 (20). The lack of Vα24Vβ11$^+$ cell expansions in response to IL-2 in the absence of stimulation is surprising, because either IL-2 or IL-15 can support the expansion of these cells following stimulation (37, 46). It is possible that only a small proportion of the Vα24Vβ11$^+$ T cells express the invariant Vα24JαQ TCR chain.

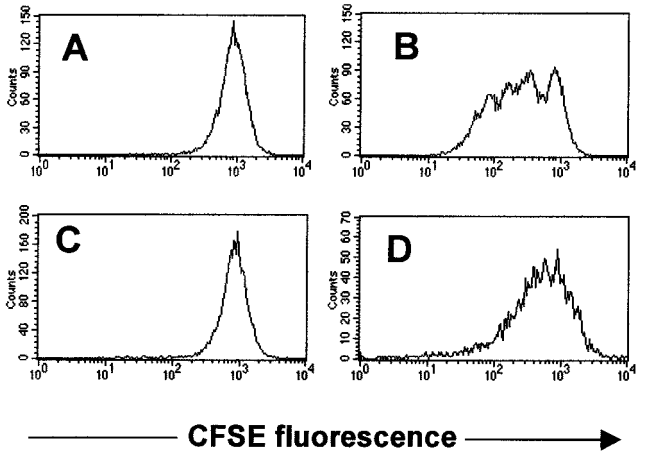

FIGURE 4. Proliferation of CD56$^+$ PBMC in response to IL-15. Flow cytometric analysis of CFSE-stained PBMC after culturing for 7 days in the absence or the presence of 5 μg/ml PHA and 25 ng/ml IL-2 or 10 ng/ml IL-15. *A*, PBMC cultured in medium alone. *B*, Gated CD3$^+$ PBMC after culture with PHA and IL-2. *C*, Gated CD56$^-$ PBMC after culture with IL-15. *D*, Gated CD56$^+$ PBMC after with IL-15. Results are representative of eight samples.

Our results indicate that in the absence of antigenic stimulation, IL-2 and IL-15 selectively promote the survival and proliferation of NK cells and T cells that express CD56. Although CD56$^+$ cells clearly proliferate in response to IL-2 or IL-15, as evidenced by our observations of CFSE-stained cell division, it remains to be determined whether these cytokine-induced expansions involve de novo induction of CD56 expression by CD56-negative cells. IL-2

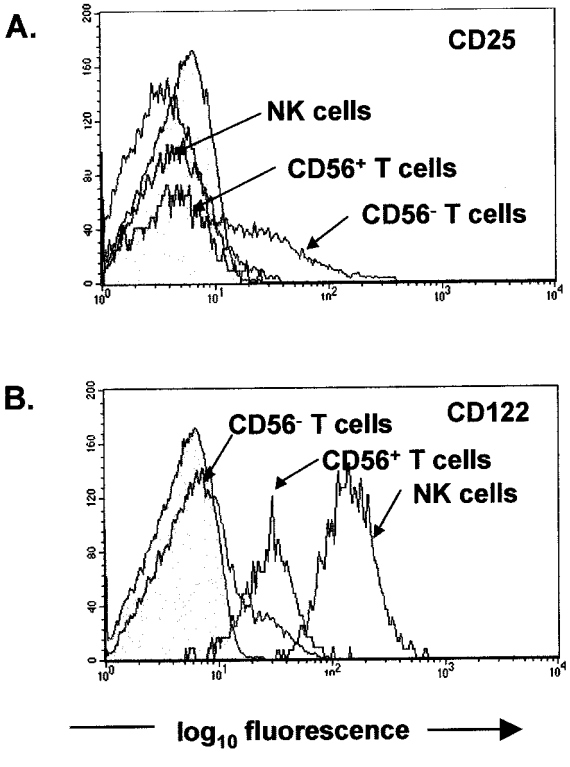

FIGURE 5. Expression of IL-2R α-chain, CD25 (*A*), and IL-2/IL-15R β-chain, CD122 (*B*), by human peripheral blood NK cells (CD3$^-$CD56$^+$), CD56$^-$ T cells (CD3$^+$CD56$^-$), and CD56$^+$ T cells (CD3$^+$CD56$^+$). Staining by isotype-matched anti-IgG mAbs is shown by the shaded histograms. Flow cytometry histograms are representative of eight experiments.

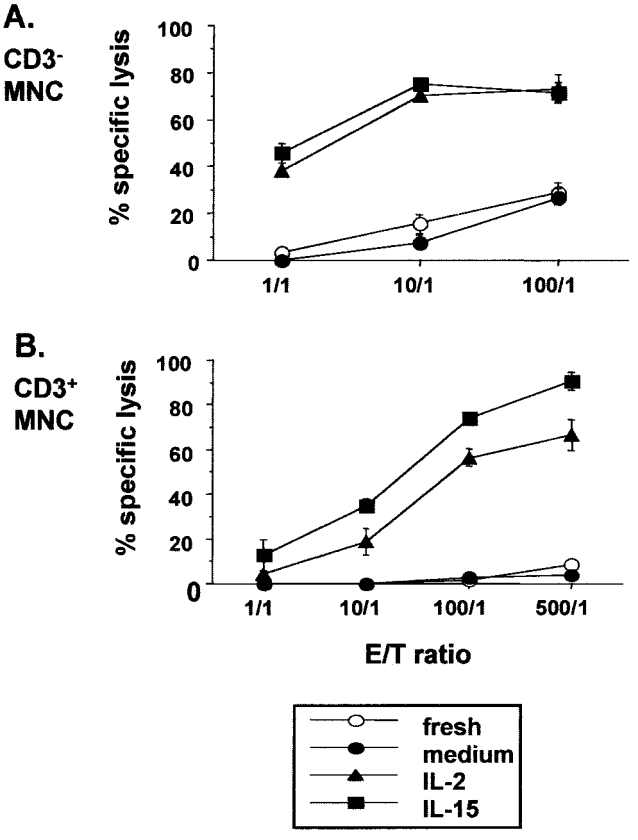

FIGURE 6. Effects of IL-2 and IL-15 on the cytotoxic activities of human peripheral blood CD3$^-$ (*A*) and CD3$^+$ (*B*) cells against K562 target cells. Freshly isolated PBMC or PBMC that were cultured for 7 days in the absence or the presence of 25 ng/ml IL-2 or 10 ng/ml IL-15 were separated into CD3$^-$ and CD3$^+$ fractions using mAb-coated magnetic beads and were used as effectors in chromium release assays at the indicated E:T ratios. Results are representative of four triplicate samples.

and IL-15 promote the development of CD56$^+$ NK cells, but not T cells, from CD56$^-$CD34$^+$ hemopoietic stem cell precursors in vitro (47). The expression of other NKRs by CD8$^+$ T cells can be induced de novo or up-regulated by T cell activation. In mice, activation of IL-2/15Rβ^+CD8$^+$ T cells results in the induction of NK1.1 and Ly49 expression (48). KIR expression by human CD8$^+$ T cells can be up-regulated by T cell activation (49), and de novo expression of CD94 can be induced by IL-15 in the presence of TCR stimulation, but not in the absence of TCR stimulation (50). We found that IL-2 or IL-15 on their own induced both proliferation (cell division) and up-regulation (from CD56^{dim} to CD56^{bright}) of CD56 expression by T cells in the absence of antigenic stimulation, but studies of clonal populations of resting CD56$^-$ T cells are needed to resolve whether de novo induction of CD56 expression by CD56$^-$ T cells occurs.

Because IL-2 and IL-15 are potent stimulators of NK and CD56$^+$ T cell survival and proliferation, we further investigated whether these cytokines could activate these cells in cytotoxicity and cytokine production assays. Both CD56^{dim} and CD56^{bright} NK cells can spontaneously lyse a range of tumor cell lines in vitro (26–28, 33), and this cytotoxicity is enhanced by IL-2 and IL-15 (13, 43). CD56$^+$ T cells can be induced to kill K562 targets by preincubation with IL-2 (LAK activity) (26–28, 32, 33). In the present study IL-15 had effects comparable to those of IL-2 in potently inducing cytotoxicity against K562 cells by both NK cells and CD56$^+$ T cells, but not by CD56$^-$ T cells. Thus, IL-2 and

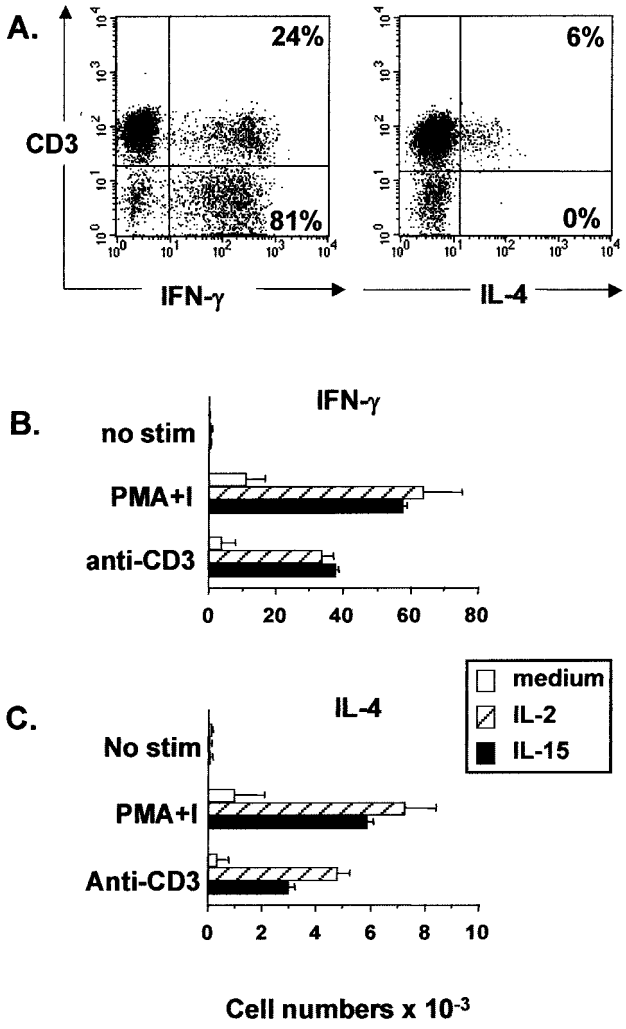

FIGURE 7. Effects of IL-2 and IL-15 on IFN-γ and IL-4 production by human PBMC. *A*, Flow cytometric detection of CD3 expression and IFN-γ (*left*) and IL-4 (*right*) production by human PBMC after incubation for 7 days in 10 ng/ml IL-15 and stimulation for 4 h with 10 ng/ml PMA plus 1 μg/ml ionomycin. The numbers denote the percentage of CD3$^+$ cells (*top* quadrants) and CD3$^-$ cells (*bottom* quadrants) that are positive for IFN-γ (*left*) and IL-4 (*right*). *B* and *C*, Absolute PBMC numbers producing IFN-γ (*B*) and IL-4 (*C*) after incubating 100,000 PBMC for 7 days in medium only, 25 ng/ml IL-2, or 10 ng/ml IL-15 and stimulating for 4 h with 10 ng/ml PMA plus 1 μg/ml ionomycin or with plate-bound anti-CD3 mAb. Cell numbers are expressed as products of the percentages of total PBMC expressing the cytokines and the total PBMC numbers. All results are the means of four experiments.

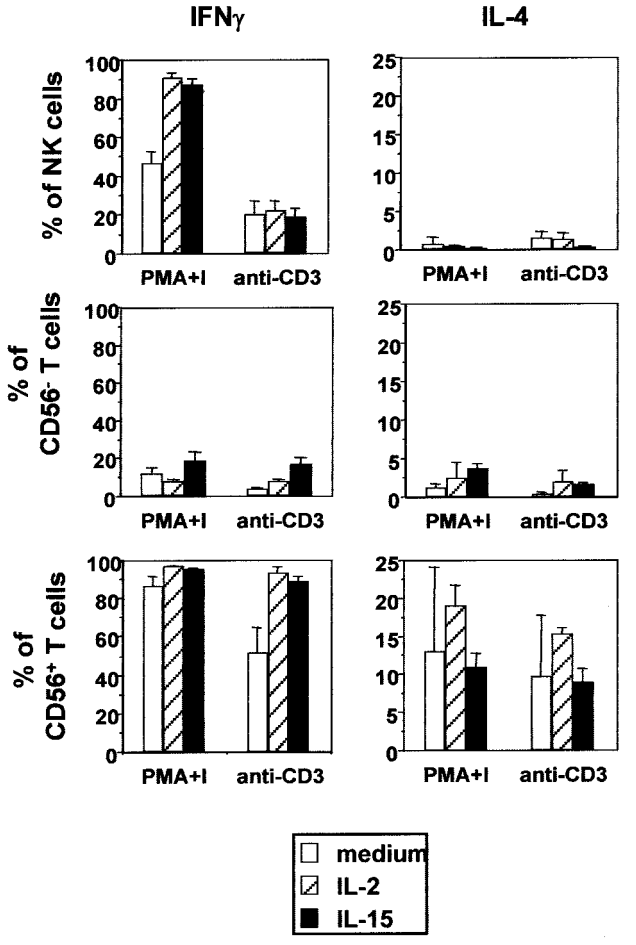

FIGURE 8. Effects of IL-2 and IL-15 on IFN-γ and IL-4 production by human NK cells, T cells, and CD56$^+$ T cells. PBMC were incubated for 7 days in medium only, 25 ng/ml IL-2, or 10 ng/ml IL-15 and stimulated for 4 h with 10 ng/ml PMA plus 1 μg/ml ionomycin or with plate-bound anti-CD3 mAb. The percentages of NK cells (CD3$^-$CD56$^+$), CD56$^-$ T cells (CD3$^+$CD56$^-$), and CD56$^+$ T cells (CD3$^+$CD56$^+$) producing IFN-γ (*left*) and IL-4 (*right*) were determined by three-color flow cytometry. All results are the means of four experiments.

IL-15 can similarly activate both proliferation and cytotoxicity by NK cells and CD56$^+$ T cells.

NK cells and CD56$^+$ T cells can contribute to the activation and regulation of T cells, B cells, and other cells of the adaptive immune system via the production of cytokines. NK cells can secrete IFN-γ, TNF-α, and IL-5 (15, 33, 42), while CD56$^+$ T cells can secrete IFN-γ, TNF-α, IL-2, and IL-4 (33, 37, 38). Thus, both cell types can induce Th1 (IFN-γ or TNF-α) or Th2 (IL-4 or IL-5) cell activation. The selective secretion of Th1- or Th2-type cytokines by NK cells and murine NKT cells is controlled in part by cytokines in the microenvironment, such as IL-2, IL-7, IL-10, and IL-12 (51, 52). Our data indicate that neither IL-2 nor IL-15 induced the production of IFN-γ, TNF-α, IL-4, or IL-2 by resting PBMC, but, as reported previously (53, 54), both cytokines augmented the production of all these cytokines in response to phorbol ester or anti-CD3 mAb stimulation. This augmentation was not the result of an induction of cytokine production by NK cells or T cells, but was due to proliferation of CD56$^+$ T cells subpopulations that most frequently responded to these stimuli. Thus, while $<$20% of T cells cultured in medium alone could produce IFN-γ or IL-4 in response to phorbol ester stimulation or CD3 cross-linking, 50–100% of NK cells and CD56$^+$ T cells in the same cultures produced IFN-γ and/or IL-4. IL-2 or IL-15 did not enhance these proportions, but induced the survival and proliferation of the most "trigger-happy" effectors, NK cells and CD56$^+$ T cells. Neither IL-2 nor IL-15 polarized lymphocyte activation to Th1- or Th2-type responses via the selective stimulation of IFN-γ, TNF-α, or IL-4 production. The combination of IL-2 and IL-15 has been reported to selectively induce IL-5 production by resting human NK cells (42), and both cytokines can synergize with IL-12 and IL-18 in inducing IFN-γ production (15, 44, 45, 55). Therefore, IL-2 and IL-15 promote cytokine production by CD56$^+$ cells, but other factors are required to selectively activate Th1- or Th2-type responses.

The selective responsiveness of cells to various cytokines is governed by their expression of the appropriate receptors. As previously reported (43), we found that the majority of NK cells express the IL-2/15R β-chain, CD122, but not the IL-2R α-chain,

CD25, suggesting that NK responses to IL-2 are mediated through a receptor complex consisting of CD122 and γc, which can participate in low affinity binding of IL-2 (3, 43). All $CD56^{bright}$ and most $CD56^{dim}$ cells expressed CD122. We also found that CD25 expression by NK cells was induced by IL-2 (data not shown), confirming that both the low affinity IL-2Rβγ and high affinity IL-2Rαβγ receptors can be used by NK cells (13, 43). Similarly, $CD56^+$ T cells were found to express CD122, but not CD25, but, interestingly, NK cells expressed ~10-fold higher levels of CD122 than $CD56^+$ T cells, yet $CD56^+$ T cells proliferated more vigorously than NK cells in response to IL-2 or IL-15. The CD122/γc receptor also mediates low affinity IL-15 signaling (13), thus explaining the similarities of NK and $CD56^+$ T cell responses to both IL-2 and IL-15. Resting $CD56^-$ T cells are negative for CD122 and do not respond to these cytokines. mAbs specific for IL-15Rα were not available for analysis of the expression of this receptor chain, but previous studies have shown that IL-15Rα is expressed by several nonlymphoid and lymphoid cell types, and IL-15Rα mRNA expression by lymphocytes can be up-regulated by activation (56).

Although IL-2 and IL-15 differ in their cellular sites of synthesis (1–3) and have distinct roles in lymphocyte development (6–11), we have found that these cytokines have almost identical effects on resting lymphocyte proliferation, cytotoxicity, and cytokine secretion. IL-15 is produced by several nonlymphoid cell types and is likely to contribute to the initiation of innate immune responses via the partial activation of NK cells, which, in turn, regulate T cell differentiation into Th1 or Th2 cells. IL-2 is produced by activated T cells in the adaptive immune response and induces the proliferation of IL-2R-positive activated T cells, thus amplifying the response until a point at which IL-2 induces apoptosis. The partial activation of NK cells by IL-2 indicates that it also influences innate immune responses, and this feedback mechanism may serve to enhance or substitute for the first-line defense mechanisms involving IL-15-secreting cells. $CD56^+$ T cells also display properties of innate lymphocytes, having invariant receptors for stimulatory ligands present on target cells (24, 35, 37, 41) and the capacity to rapidly kill tumor cells (26–31, 33, 34), to produce cytokines (22, 24, 33, 37, 38), and to respond to IL-12 (34). Alternatively, $CD56^+$ T cells may constitute a subset of memory cells. They express activated/memory $CD45RA^-CD45RO^+CD28^-CD122^+CD69^+$ phenotypes (22, 32) and homing chemokine receptors (39). They have a predominant localization in peripheral tissues (24, 32, 33) and can rapidly acquire effector functions (22, 26–31, 33, 34). A role for IL-15 in inducing proliferation of memory $CD8^+$ T cells has previously been reported (1, 10, 12) and CD56 has been reported to be expressed by most IL-2-dependent Ag-specific CTL lines in long term culture (28). T cell expression of other NKRs, including KIRs and CD94, can be induced by activation (48, 49), and KIR and CD94 induction correlates with the transition from effector to memory CTLs (30, 57). Therefore, IL-2 and IL-15 appear to have roles in immune activation at the innate, adaptive, and memory stages of an immune response.

References

1. Grabstein, K. H., J. Eisenman, K. Shanebeck, C. Rauch, S. Srinivasan, V. Fung, C. Beers, J. Richardson, M. A. Schoenborn, M. Aldieh, et al. 1994. Cloning of a T cell growth factor that interacts with the β chain of the interleukin-2 receptor. *Science 264:965.*
2. Waldmann, T. A., and Y. Tagaya. 1999. The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. *Annu. Rev. Immunol. 17:19.*
3. Waldman, T. A., Y. Tagaya, and R. Bamford. 1998. Interleukin-2, interleukin-15, and their receptors. *Int. Rev. Immunol. 16:205.*
4. Ma, A., D. L. Boone, and J. P. Lodolce. 2000. The pleotropic functions of interleukin 15: not so interleukin 2-like after all. *J. Exp. Med. 191:753.*
5. Tagaya, Y., J. D. Burton, Y. Miyamoto, and T. A. Waldmann. 1996. Identification of a novel receptor/signal transduction pathway for IL-15/T in mast cells. *EMBO J. 15:4928.*
6. Sadlock, B. H. Merz, H. Schorle, A. Schimpl, A. C. Feller, and I. Horak. 1993. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell 75:253.*
7. Willerford, D. M., J. Chen, J. A. Ferry, L. Davidsin, A. Ma, and F. W. Alt. 1995. Interleukin-2 receptor α chain regulates the size and content of the peripheral lymphoid compartment. *Immunity 3:521.*
8. Suzuki, H., G. S. Duncan, H. Takimoto, and T. W. Mak. 1997. Abnormal development of intestinal intraepithelial lymphocytes and peripheral natural killer cells in mice lacking the IL-2 receptor β chain. *J. Exp. Med. 185:499.*
9. Ohteki, T., S. Ho, H. Suzuki, T. W. Mak, and P. S. Ohashi. 1997. Role for IL-15/IL-15 receptor β-chain in natural killer 1.1^+ T cell receptor-$\alpha\beta^+$ cell development. *J. Immunol. 159:5931.*
10. Lodolce, J. P., D. L. Boone, S. Chai, R. E. Swain, T. Dassopoulos, S. Trettin, and A. Ma. 1998. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity 9:669.*
11. Kennedy, M. K., M. Glaccum, S. N. Brown, E. A. Butz, J. L. Viney, M. Embers, N. Matsuki, K. Charrier, L. Sedger, C. R. Willis, et al. 2000. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J. Exp. Med. 191:771.*
12. Zhang, X., S. Sun, I. Hwang, D. F. Tough, and J. Sprent. 1998. Potent and selective stimulation of memory-phenotype $CD8^+$ T cells in vivo by IL-15. *Immunity 8:591.*
13. Carson, W. E., J. G. Giri, M. J. Lindemann, M. L. Linett, M. Ahdieh, R. Paxton, D. Anderson, J. Eisenmann, K. Grabstein, and M. A. Caligiuri. 1994. Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. *J. Exp. Med. 180:1395.*
14. Bulfone-Paus, S., D. Ungureanu, T. Pohl, G. Lindner, R. Paus, R. Rückert, H. Krause, and U. Kunzendorf. 1997. Interleukin-15 protects from lethal apoptosis in vivo. *Nat. Med. 3:1124.*
15. Carson, W. E., M. E. Ross, R. A. Baiocchi, M. J. Marien, N. Boiani, K. Grabstein, and M. A. Caligiuri. 1995. Endogenous production of interleukin 15 by activated human monocytes is critical for optimal production of interferon-γ by natural killer cells in vitro. *J. Clin. Invest. 96:2578.*
16. Carson, W. E., T. A. Fehniger, S. Haldar, K. Eckhert, M. J. Lindemann, C.-F. Lai, C. M. Croce, H. Baumann, and M. A. Caligiuri. 1997. A potential role for interleukin-15 in the regulation of human natural killer cell survival. *J. Clin. Invest. 99:937.*
17. García, V. E., D. Jullien, M. Song, K. Uyemura, K. Shuai, C. T. Morita, and R. L. Modlin. 1998. IL-15 enhances the response of human γδ T cells to nonpeptide microbial antigens. *J. Immunol. 160:4322.*
18. Chu, C. L., S.-S. Chen, T.-S. Wu, S.-C. Kuo, and N.-S. Liao. 1999. Differential effects of IL-2 and IL-15 on the death and survival of activated $TCR\gamma\delta^+$ intestinal intraepithelial lymphocytes. *J. Immunol. 162:1896.*
19. Inagaki-Ohara, K., H. Nishimura, A. Mitani, and Y. Yoshikai. 1997. Interleukin-15 preferentially promotes the growth of intestinal intraepithelial lymphocytes bearing γδ T cell receptor in mice. *Eur. J. Immunol. 27:2885.*
20. Lai, Y.-G., V. Gelfanov, V. Gelfanova, L. Kulik, C. L. Chu, S.-W. Jeng, and N.-S. Liao. 1999. IL-15 promotes survival but not effector function of $CD8^+$ $TCR\alpha\beta^+$ intestinal intraepithelial lymphocytes. *J. Immunol. 163:5843.*
21. Bancroft, G. J. 1993. The role of natural killer cells in innate resistence to infection. *Curr. Opin. Immunol. 5:503.*
22. Mingari, M. C., C. Vitale, A. Cambiaggi, F. Schiavetti, G. Melioli, S. Ferrini, and A. Poggi. 1995. Cytolytic T lymphocytes displaying natural killer (NK)-like activity: expression of NK-related functional receptors for HLA class I molecules (p58 and CD94) and inhibitory effect on the TCR-mediated target cell lysis or lymphokine production. *Int. Immunol. 7:697.*
23. Lanier, L. L., B. Corliss, and J. H. Phillips. 1997. Arousal and inhibition of human NK cells. *Immunol. Rev. 155:145.*
24. Doherty, D. G., and C. O'Farrelly. 2000. Innate and adaptive lymphoid cells in the human liver. *Immunol. Rev. 174:5.*
25. Hayday, A. C. 2000. γδ T cells: a right time and a right place for a conserved third way of protection. *Annu. Rev. Immunol. 18:975.*
26. Schmidt, R. E., C. Murray, J. F. Daley, S. F. Schlossman, and J. Ritz. 1986. A subset of natural killer cells in peripheral blood displays a mature T cell phenotype. *J. Exp. Med. 164:351.*
27. Lanier, L. L., A. M. Le, C. I. Civin, M. R. Loken, and J. H. Phillips. 1986. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral NK cells and cytotoxic T lymphocytes. *J. Immunol. 136:4480.*
28. Lanier, L. L., A. M. Le, A. Ding, E. L. Evans, A. M. Krensky, C. Clayberger, and J. H. Phillips. 1987. Expression of Leu-19 (NKH-1) antigen on IL-2-dependent cytotoxic and non-cytotoxic T cell lines. *J. Immunol. 138:2019.*
29. Ortaldo, J. R., R.T. Winkler-Pickett, H. Yagita, and H. A. Young. 1991. Comparative studies of $CD3^-$ and $CD3^+CD56^+$ cells: examination of morphology, functions, T cell receptor rearrangement, and pore-forming protein expression. *Cell. Immunol. 136:486.*
30. Mingari, M. C., F. Schiavetti, M. Ponte, C. Vitale, E. Maggi, S. Romagnani, J. Demarest, G. Pantaleo, A. S. Fauci, and L. Moretta. 1996. Human $CD8^+$ T lymphocyte subsets that express HLA class I-specific inhibitory receptors represent oligoclonally or monoclonally expanded cell populations. *Proc. Natl. Acad. Sci. USA 93:12433.*
31. Moretta, A., R. Biassoni, C. Bottino, D. Pende, M. Vitale, A. Poggi, M. C. Mingari, and L. Moretta. 1997. Major histocompatibility complex class I-specific receptors on human natural killer and T lymphocytes. *Immunol. Rev. 155:105.*

32. Norris, S., D. G. Doherty, C. Collins, G. McEntee, O. Traynor, J. E. Hegarty, and C. O'Farrelly. 1999. Natural T cells in the human liver: cytotoxic lymphocytes with dual T cell and natural killer cell phenotype and function are phenotypically heterogeneous and include Vα24-JαQ and $\gamma\delta$ T cell receptor bearing cells. *Hum. Immunol. 60:20.*
33. Doherty, D., S. Norris, L. Madrigal-Estebas, G. McEntee, O. Traynor, J. Hegarty, C. O'Farrelly. 1999. The human liver contains multiple populations of NK cells, T cells and CD356$^+$ natural T cells with distinct cytotoxic activities and Th1, Th2 and Th0 cytokine secretion patterns. *J. Immunol. 163:2314.*
34. Satoh, M., S. Seki, W. Hashimoto, K. Ogasawara, T. Kobayashi, K. Kumagai, S. Matsuno, and K. Takeda. 1996. Cytotoxic $\gamma\delta$ or $\alpha\beta$ T cells with a natural killer cell marker, CD56, induced from human peripheral lymphocytes by a combination of IL-2 and IL-12. *J. Immunol. 157:3886.*
35. Bauer, S., V. Groh, J. Wu, A. Steinle, J. H. Phillips, L. L. Lanier, and T. Spies. 1999. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science 285:727.*
36. Dellabona, P., G. Casorati, B. Friedli, L. Angman, F. Sallusto, A. Tunnacliffe, E. Roosneek, and A. Lanzavecchia. 1993. In vivo persistence of expanded clones specific for bacterial antigens within the human T cell receptor $\alpha\beta$ CD4$^-$8$^-$ subset. *J. Exp. Med. 177:1763.*
37. Exley, M., J. Garcia, S. P. Balk, and S. Porcelli. 1997. Requirements for CD1d recognition by human invariant Vα24$^+$CD4$^-$CD8$^-$ T cells. *J. Exp. Med. 186: 109.*
38. Prussin, C., and B. Foster. 1997. TCR Vα24 and Vβ11 coexpression defines a unique NK1 T cell analog containing a unique Th0 population. *J. Immunol. 159:5862.*
39. Campbell, J. J., S. Qin, D. Unutmaz, D. Soler, K. E. Murphy, M. R. Hodge, L. Wu, and E. C. Butcher. 2001. Unique subpopulations of CD56$^+$ NK and NK-T peripheral blood lymphocytes identified by chemokine receptor expression repertoire. *J. Immunol. 166:6477.*
40. Lyons, A. B., and C. R. Parish. 1994. Determination of lymphocyte division by flow cytometry. *J. Immunol. Methods 171:131.*
41. Porcelli, S. A., B. W. Segelke, M. Sugita, I. A. Wilson, M. B. Brenner. 1998. The CD1 family of lipid antigen-presenting molecules. *Immunol. Today 19:362.*
42. Warren, H. S., B. F. Kinnear, R. L. Kastelein, and L. L. Lanier. 1996. Analysis of the costimulatory role of IL-2 and IL-15 in initiating proliferation of resting (CD56^{dim}) human NK cells. *J. Immunol. 156:3254.*
43. Caligiuri, M. J., A. Zmuidinas, T. J. Manley, H. Levine, K. A. Smith, and J. Ritz. 1990. Functional consequences of interleukin 2 receptor expression on resting human lymphocytes: identification of a novel natural killer with high affinity receptors. *J. Exp. Med. 171:1509.*
44. Nagler, A., L. L. Lanier, S. Cwirla, and J. H. Phillips. 1989. Comparative studies of human FcRIII-positive and negative natural killer cells. *J. Immunol. 143:3183.*
45. Khan, I. A., and L. H. Kasper. 1996. 1996. IL-15 augments CD8$^+$ T cell-mediated immunity against *Toxoplasma gondii* infection in mice. *J. Immunol. 157: 2103.*
46. Van der Vliet, H. J. J., N. Nishi, Y. Koezuka, B. M. E. von Blomberg, A. J. M. van den Eertwegh, S. A. Porcelli, H. M. Pinedo, R. J. Scheper, and G. Ciaccone. 2001. Potent expansion of human natural killer T cells using α-galactosylceramide (KRN7000)-loaded monocyte-derived dendritic cells, cultured in the presence of IL-7 and IL-15. *J. Immunol. Methods 247:61.*
47. Mrózek, E., P. Anderson, and M. A. Caligiuri. 1996. Role of interleukin-15 in the development of human CD56$^+$ natural killer cells from CD34$^+$ hematopoietic progenitor cells. *Blood 87:2632.*
48. Assarsson, E., T. Kambayashi, J. K. Sandberg, S. Hong, M. Taniguchi, L. Van Kaer, H.-G. Ljunggren, and B. J. Chambers. 2000. CD8$^+$ T cells rapidly acquire NK1.1 and NK cell-associated molecules upon stimulation in vitro and in vivo. *J. Immunol. 165:3673.*
49. Huard, B., and L. Karlsson. 2000. KIR expression on self-reactive CD8$^+$ T cells is controlled by T-cell receptor engagement. *Nature 403:325.*
50. Mingari, M. C., M. Ponte, S. Bertone, F. Schiavetti, C. Vitale, R. Bellomo, A. Moretta, and L. Moretta. 1998. HLA class I-specific inhibitory receptors in human T lymphocytes: interleukin 15-induced expression of CD94/NKG2A in superantigen- or alloantigen-activated CD8$^+$ T cells. *Proc. Natl. Acad. Sci. USA 95:1172.*
51. Leite-De-Moraes, M. C., A. Herbelin, J. M. Gombert, A. Vicari, M. Papiernik, and M. Dy. 1997. Requirement of IL-7 for IL-4-producing potential of MHC class I-selected CD4$^-$CD8$^-$TCR$\alpha\beta^+$ thymocytes. *Int. Immunol. 9:73.*
52. Leite-De-Moraes, M. C., G. Moreau, A. Arnould, F. Machavoine, C. Garcia, M. Papiernik, and M. Dy. 1998. IL-4-producing NK T cells are biased towards IFN-γ production by IL-12: influence of the microenvironment on the functional capacities of NK T cells. *Eur. J. Immunol. 28:1507.*
53. Körholz, D., U. Banning, H. Bönnig, M. Grewe, M. Schnieder, C. Mauz-Körholz, A. Klein-Vehne, J. Krutmann, and S. Burdach. 1997. The role of interleukin-10 (IL-10) in IL-15-mediated T-cell responses. *Blood 90:4513.*
54. Borger, P., H. F. Kauffman, D. S. Postma, M. T. Esselink, and E. Vellenga. 1999. Interleukin-15 differentially enhances the expression of interferon-γ and interleukin-4 in activated human (CD4$^+$) T lymphocytes. *Immunology 96:207.*
55. Lauwerys, B. R., N. Garot, J.-C. Renauld, and F. A. Houssiau. 2000. Cytokine production and killer activity of NK/T-NK cells derived with IL-2, IL-15, or the combination of IL-12 and IL-18. *J. Immunol. 165:1847.*
56. Giri, J. G., S. Kumaki, M. Ahdieh, D. J. Friend, A. Loomis, K. Shanebeck, R. DuBose, D. Cosman, L. S. Park, and D. M. Anderson. 1995. Identification and cloning of a novel IL-15 binding protein that is structurally related to the α chain of the IL-2 receptor. *EMBO J. 14:3654.*
57. Young, N. T., M. Uhrberg, J. H. Phillips, L. L. Lanier, and P. Parham. 2001. Differential expression of leukocyte receptor complex-encoded Ig-like receptors correlates with the transition from effector to memory CTL. *J. Immunol. 166:3933.*