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*J Immunol* 2000; 164:488-494; doi: 10.4049/jimmunol.164.1.488

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Up-Regulation of IL-12 in Monocytes: A Fundamental Defect in Common Variable Immunodeficiency

Rosario Cambronero, W. A. Carrock Sewell, Margaret E. North, A. David B. Webster, and John Farrant

We show that LPS-stimulated circulating CD14-positive monocytes from patients with common variable immunodeficiency (CVID) express a higher proportion of intracellular IL-12-positive cells than monocytes from patients with X-linked agammaglobulinemia or normal subjects. We used four-color flow cytometry and measured IL-12 with an Ab to the p40 subunit following stimulation with LPS. The raised IL-12 is associated with an increased frequency of IFN-γ-positive T cells, but not of IFN-γ-positive CD56+ NK cells. These increases in frequency of cytokine-positive cells are due to a decrease in the absolute numbers of circulating monocytes and T cells that are negative for IL-12 and IFN-γ, respectively. The increased frequency of IL-12-positive monocytes appears to be selective because TNF-α was not increased, and is thus unlikely to reflect a general activation. Chronic infection is also unlikely to explain our data since cells from X-linked agammaglobulinemia patients with a similar Ig deficiency do not show these changes. Our data suggest a fundamental abnormality in the IL-12/IFN-γ circuit in CVID, with up-regulation of IL-12 being the “primary” factor. This imbalance is likely to skew the immune response away from Ab production and also explains the failure of CVID T cells to make Ag-specific memory cells and the chronic inflammatory and granulomatous complications that are a feature of CVID. This disease appears to be a rare example of a polarized Th1-type response and may in part be due to a genetic defect in the control of IL-12 production. The Journal of Immunology, 2000, 164: 488–494.

There are persistently low levels of IgG, IgA, and usually IgM in common variable immunodeficiency (CVID) (1). Originally, CVID was considered to be a defect in B cell maturation and function (2), but later it became clear that one reason the B cells did not function was that they were not receiving appropriate signals from T cells. This was shown partly by the restoration of some Ig secretion in vitro by CVID B cells, e.g., after stimulation with anti-CD40 and cytokines such as IL-4 and IL-10 (3). Also, various direct defects of T cells were uncovered that would prevent them from providing the signals needed by B cells to function normally. As an example, we showed that CVID T cells stimulated with Ags such as purified protein derivative, tetanus toxoid, and keyhole limpet hemocyanin (KLH) fail to produce Ag-specific memory cells (4–6). Thus, on challenge with Ag, there is a lack of Ag-specific T cells available to help B cells to make specific Abs.

There has been some debate as to whether the T cell defects originated in the T cells themselves or in APC such as dendritic cells (DC) and monocytes. The weight of evidence has been that the Ag-presenting function is normal (7, 8); however, the simplistic linear view of the immune response proceeding from APC through T cells to B cells is no longer valid. The processes are complex with positive and negative feedback between cells, including the regulation of surface-signaling molecules (9) and the production of soluble signals such as cytokines. One of the most defined cytokine circuits is that of IL-12 produced by APC such as DC and monocytes inducing the secretion of IFN-γ in T cells (the classical Th1 cytokine), which in turn induces expression of the proinflammatory cytokine TNF-α (10, 11). In addition, IFN-γ up-regulates IL-12 production (positive feedback) (12).

Although IL-2 deficiency has been proposed for CVID (13), recent data from our laboratory show up-regulation of the Th1 cytokine IFN-γ in T cells, especially in subsets of CD8+ lymphocytes defined by their expression of the costimulatory molecule CD28 (14, 15). Shifts of cytokine profiles toward “Th1” (e.g., IFN-γ) implies shifts away from “Th2” (e.g., IL-4) which would normally support B cell secretion of Ig (16). Strictly speaking, Th1 and Th2 should not include CD8 lymphocytes but it is clear that these cells contribute to the skewing of the cytokine patterns.

We have recently developed four-color flow cytometric methods to measure cytokines within defined lymphocyte subsets in mononuclear cell (14) and whole-blood samples (17). The aim of the present study was to develop a whole-blood method to measure intracellular cytokines in DC and monocytes and then to see whether the raised IFN-γ in T cell subpopulations (15) could be due to a raised expression of IL-12.

Materials and Methods

Donor groups

These were patients with CVID, as defined by the World Health Organization criteria (1), attending the clinic for Ig replacement therapy (n = 12); a control group of patients with X-linked agammaglobulinemia (XLA; confirmed by defects in the Btk gene) having the same therapy (n = 6); and normal donors (laboratory personnel, n = 12). The CVID patients were selected randomly from those attending the clinic. The division of the sexes was equal. Only 1 patient of the 12 had granulomatous disease, and 1 other patient had raised alkaline phosphatase suggestive of liver granulomas. Four of the patients were familial in that they had first-degree relatives with CVID.
either CVID or IgA deficiency. The numbers were insufficient to show any clustering of the data due to these different characteristics. All donors gave their informed consent. Lithium heparin blood (1–2 ml) was taken, and, for the patients’ groups, this was before their routine Ig infusions were started.

**Cells and cell culture**

The method of whole-blood culture to induce cytokines in monocytes was modified from our procedure for measuring intracellular cytokines in lymphocytes (17). Briefly, aliquots (250 μl) of whole blood were diluted 1/2 with RPMI 1640 (Life Technologies, Paisley, U.K.). Cells were stimulated with either LPS (0.1 μg/ml; Sigma, Poole, U.K.) for 5 h (for monocytes and DC), or with PMA (10 ng/ml; Calbiochem, Nottingham, U.K.) and ionomycin (free acid, 2 μmol/L; Calbiochem) for 2 h (for the lymphocytes and NK cells). Both the stimulated cultures and control unstimulated cultures contained monensin (sodium salt, 3 μmol/L; Calbiochem) from the start of culture. Control experiments showed negligible direct effects of LPS on T cells and negligible effects of PMA and ionomycin on DC and monocytes.

**Cell staining**

After culture, harvesting, and washing, the cells were resuspended in 250 μl of medium. Cell aliquots (50 μl, equivalent to 50 μl of the original whole-blood sample) were added for 15 min to the conjugated Abs directed against cell surface markers. This was done before fixation because the CD14 and CD56 Abs did not stain well after the fixation and permeabilization steps. RBC were lysed with OptiLyse C (500 μl; Beckman Coulter, High Wycombe, U.K.) and the preparation was washed again. To each tube, Lysormer A (50 μl; Serotec, Kidlington, U.K.) was added for fixation. After a wash, Lysormer B (50 μl; Serotec) was added for permeabilization along with the anti-cytokine Ab. After 30 min and another wash, the cells were kept at 4°C in paraformaldehyde (500 μl, 0.5% in PBS) until analysis within 18 h.

**Flow cytometry**

The samples were read on a four-color Epics MCL flow cytometer (Beckman Coulter). The tubes for each Ab combination were read in pairs with an unstimulated sample (cultured with monensin alone) preceding a stimulated sample. The combinations of directly conjugated Abs were as follows in the sequence of FL1(FTTC/FL2/PE)/FL3(EDC)/FL4(PE5Cy5): for monocyte/DC, CD14/IL-12/CD3/HLA-DR; and TNF-α/CD4/CD3/HLA-DR; for T cells IFN-γ/CD28/CD8/CD3; and for NK cells IFN-γ/CD56/CD69/CD53. The definition of CD4⁺ T cells was done without a CD4⁺ Ab: they were defined as CD3⁺-positive cells and CD8α bright and dim negative.

Anti-IFN-γ, anti-TNF-α, and anti-CD14 FITC Abs were obtained from Serotec. The anti-IL-12 PE (anti-p40) Ab was purchased from Cambridge Bioscience (Cambridge, U.K.) and the anti-CD14 PE and anti-CD28 PE Abs were purchased from Becton Dickinson (Oxford, U.K.). All other Abs against surface markers were obtained from Beckman Coulter. Just before data acquisition, constant aliquots of a known concentration of fluorescent beads (Flow-count beads; Beckman Coulter) were added to allow absolute cell concentrations of cell populations to be calculated (18). It is important to measure absolute cell numbers as well as percentages since this allows changes in percentages of positive cells to be explained in terms of independent alterations to the numbers of cytokine-positive and cytokine-negative cells.

All samples were acquired uncompensated with the experimental samples preceded by single- and two-color standards. The single-color standards were usually anti-CD8α with each of the four fluorochromes, and the two color standards were the Cytoomp reagents from Beckman Coulter. Acquisition was ungated (10,000 events). The listmode files obtained were transferred to offline PCs using Zip disks (Omega) and analyzed with Winlist ver 4.0 (Verity, Topsham, ME). Using the standard samples, four-color compensation was applied by Winlist. Regions were drawn to enable gates to define different cell populations. Fig. 1 shows an example for IL-12 (using the combination CD14/FTTC/IL-12PE/CD8α/EDC/CD3/PeCy5). First, regions were defined in an ungated light scatter plot for lymphocytes and monocytes (Fig. 1a). The number of Flow-count beads was measured in another ungated dot plot (Fig. 1b). Fluorescence plots gated on the combined regions of lymphocytes and monocytes were used to define regions for CD14 and CD3 (Fig. 1c) and HLA-DR (Fig. 1d). IL-12 was then measured in the monocytes in a quadrant dot-plot gated as being in the “monocyte scatter region” and also being CD14⁺, HLA-DR⁺, and CD3⁺. The quadrant plot allowed the CD14⁺ (monocyte) and CD14⁻ (“dendritic”) populations to be distinguished on the ordinate and the IL-12-positive and -negative cells on the abscissa (see Fig. 1, e and f, for unstimulated and stimulated cells, respectively). Similar strategies of regions and gates were used for the other combinations of Abs. These included measuring TNF-α in monocytes and DC, IFN-γ in CD28-positive and -negative subsets of CD4⁺, CD8⁺, and total CD3⁺ T cells, as well as IFN-γ in the CD3⁺ and CD3⁻ subsets of CD56⁺ NK cells. The discrimination between cytokine-negative and cytokine-positive cells was done using the control conditions of culture without stimulus but in the presence of monensin.

**Data analysis**

The data were transferred by dynamic data exchange from the Winlist program to Microsoft Excel spreadsheets for analysis. The number of events for all populations was converted to the absolute concentration per ml calibrated using the Flow-count bead data. Percentage values were also calculated. The significance of differences between mean data from donor groups was assessed by Student’s unpaired t test.

**Results**

**Stimulation of monocytes and DC within whole-blood samples with LPS to induce IL-12 and TNF-α**

**Conditions for measuring intracellular IL-12 in monocytes and DC.** Preliminary experiments showed that optimal conditions for inducing IL-12 expression in monocytes and DC in our system was LPS (0.1 μg/ml) for 5 h. Although some monocytes may be lost to analysis by adherence during culture, the mean concentration of nonadherent monocytes measured by the absolute bead method after a 5-h stimulation in the presence of LPS (0.6 × 10⁹/L) was in the middle of the normal range (0.1–1.1 × 10⁹/L of whole blood). Therefore, the monocyte data represent the large majority of cells that are not initially adherent after 5 h with LPS.

**The proportion of IL-12-positive monocytes is higher in CVID than in normal or XLA donors.** Fig. 2 shows for all groups of donors a powerful induction of IL-12 in monocytes following a 5-h stimulation with LPS. The increase in mean percentage of IL-12 on stimulation with LPS was significant at p < 0.0001 for both the normal and CVID groups and at p < 0.01 for the XLA group. For all donor groups, there was a low level of intrinsic IL-12 in unstimulated monocytes cultured for 5 h with monensin but without LPS (mean of 5.0% IL-12-positive cells in normal donors, 6.2% in CVID patients, and 3.0% in XLA patients). Intrinsic IL-12 in monocytes in the XLA group was significantly less than in normal cells (p < 0.05, see Fig. 2). However, the most important finding in Fig. 2 is that after LPS stimulation the CVID patients had a significantly larger increase in the mean percentage level of IL-12 compared with normal donors (p < 0.01). The mean IL-12 percentage value in the stimulated XLA monocytes was slightly reduced but not significantly different from the normal expression.

Fig. 3A shows for both stimulated and unstimulated samples the mean absolute concentrations of CD14⁺ monocytes positive and negative for IL-12 and also the mean percentage values for IL-12 derived from them. The reduction in the percentage of intrinsic IL-12 in the unstimulated XLA monocytes was due to the higher absolute numbers of monocytes in XLA rather than to a decrease in the IL-12-positive monocytes. It can be seen that after stimulation with LPS the CVID patients had significantly lower numbers of monocytes compared with normal donors. Surprisingly, the significant mean increase in percentage of IL-12-positive cells induced by LPS in CVID compared with the normal group was not due to an increase in the concentration of IL-12-positive cells. It was instead due to the reduced absolute number of IL-12-negative monocytes in CVID compared with the normal group. There was a significant increase in the total number of CD14⁺ monocytes in the XLA donor group, but again the number of IL-12-positive cells was not significantly different from the normal values.
The proportion of IL-12-positive DC in CVID is the same as in normal and XLA donors. Fig. 3B shows that, for all donor groups, LPS induced IL-12 expression in CD14-negative HLA-DR-positive DC just as it did in CD14+ monocytes. The significance of the increase in mean percentage of IL-12 between unstimulated and stimulated samples was \( p < 0.001 \) for the normal group, \( p < 0.0001 \) for the CVID group, and \( p < 0.01 \) for the XLA group. As with monocytes, there was a low level of intrinsic IL-12. However, unlike the situation with monocytes, the DC of the CVID patients had a mean percentage of IL-12 that was not significantly different from that of the normal group. The XLA data were also not different from the normal value.

TNF-\( \alpha \) in CVID monocytes and DC is at normal levels. On activation, a higher percentage of monocytes was positive for TNF-\( \alpha \) than for IL-12. However, unlike IL-12, there were no significant differences between normal and CVID donors in the mean expression of TNF-\( \alpha \) in the CD14+ monocytes measured either in percentage or absolute terms. The values for TNF-\( \alpha \)-positive cells before and after stimulation with LPS were 1.2 ± 0.2% and 44.7 ± 2.5% for normal donors and 1.6 ± 0.2% and 48.5 ± 4.9% for the CVID patients, respectively. TNF-\( \alpha \) was not measured in cells from XLA patients. As with the CD14+ monocytes, there were no significant differences between normal donors and CVID patients in the mean expression of TNF-\( \alpha \) in the HLA-DR+CD14- DC
measured either as a percentage or in absolute terms. The values for TNF-α-positive cells before and after stimulation with LPS were 2.3 ± 0.5% and 22.1 ± 2.5% for normal donors and 3.0 ± 1.0% and 27.5 ± 3.7% for the CVID patients, respectively.

Stimulation of lymphocytes and NK cells within whole-blood samples with PMA and ionomycin to induce IFN-γ

The proportion of IFN-γ-positive cells in CD3+, CD4+, and CD8+ T cells and their CD28 subsets is generally higher in CVID than in normal or XLA donors. Table I records for all three donor groups the percentage of IFN-γ-positive cells in the T cell populations of CD3+, CD4+, and CD8+ (and their subsets positive and negative for CD28) after stimulation with PMA and ionomycin for 2 h. The population of CD4+CD28− cells was too small to be studied. For all of the cell populations in Table I, there was a significantly greater percentage of IFN-γ-positive cells in CVID patients than in T cells from normal donors. The control group of patients with XLA had normal levels of IFN-γ expression. Fig. 4A shows, using the total CD3+ cell population as an example, how the mean IFN-γ percentage values are derived from the absolute concentrations of cells positive and negative for IFN-γ under unstimulated or stimulated conditions. It can be seen that there is no intrinsic expression of IFN-γ in unstimulated CD3− cells. As with the IL-12 data in monocytes, the increase in percentage of IFN-γ-positive CD3+ cells induced by PMA and ionomycin in CVID patients compared with the normal group was due to the reduced absolute number of IFN-γ-negative lymphocytes in CVID patients rather than to an increase in IFN-γ-positive cells. The XLA values were similar to normal levels.

The CVID data for CD4+ and CD4+CD28− were similar to that for the total CD3+ cells, in that the significant increase in the percentage of IFN-γ in these populations (Table I) was also due to a reduction in the number of the IFN-γ-negative cells (data not shown). However, the situation is different for CD8+ cells and its subsets defined by CD28. For total CD8+ and for CD8+CD28− cells, the mean absolute numbers of the cytokine-positive and -negative cells in these populations are not significantly different between CVID patients and the normal group (data not shown). This is despite the significant increase in the percentage of IFN-γ-positive cells of these populations in CVID patients on stimulation (see Table I).

![FIGURE 2. IL-12-positive cells (percent) in CD14+ monocytes with and without stimulation with LPS for 5 h. Each symbol represents an individual donor in the three groups of normal (n = 12), CVID (n = 12), and XLA (n = 6) patients. The horizontal lines represent the mean percent values. Significant differences between the patient data and the normal values (unstimulated or stimulated) are shown by asterisks as for Fig. 2. The mean patient data and the mean normal values (unstimulated or stimulated) in this and subsequent figures are shown as follows: *, p < 0.05; **, p < 0.01; and ***, p < 0.001.](Image 54x579 to 274x734)

![FIGURE 3. IL-12 expression in monocytes (A, CD14+HLA-DR+ cells) and DC (B, CD14- HLA-DR+ cells). The mean data are given for the three donor groups: normal, CVID, and XLA. In both A and B, the lower graphs represent the percentage of IL-12 expression (mean ± SE) in each cell type without stimulation (monensin) or with stimulation (LPS + monensin). The upper graphs show the absolute cell concentrations of each cell population (A, monocytes; B, DC) for the three donor groups with and without LPS stimulation. The solid components of the absolute count bars represent the concentrations of the IL-12-positive monocytes (A) or DC (B). Significant differences between the mean patient data and the mean normal values (unstimulated or stimulated) are shown by asterisks as for Fig. 2.](Image 307x306 to 537x734)
The proportion of IFN-γ-positive CD56+CD3−NK cells is at normal levels in CVID patients despite the depletion of these NK cells. Fig. 5 shows that the absolute concentrations of the principal population of NK cells (CD56+CD3−) in the two disease groups (CVID and XLA) were significantly lower at only one-third of the level of that of normal donors. This was true both for samples stimulated for 2 h with PMA and ionomycin in the presence of monensin and for samples cultured for 2 h without stimulation but also with monensin. Fig. 5 shows that the depletion of these NK cells in CVID patients occurred in both the IFN-γ-positive and -negative populations, thus leaving the cytokine percentage expression unchanged from normal. Fig. 5 shows that for all three donor groups there is no intrinsic IFN-γ expression in the stimulated samples unchanged from normal. Fig. 5 shows that the depletion of these NK cells in CVID patients occurred in both the IFN-γ-positive and -negative populations, thus leaving the cytokine percentage expression in the stimulated samples unchanged from normal. Fig. 5 shows that for all three donor groups there is no intrinsic IFN-γ but that the NK cells became positive for intracellular expression of IFN-γ at a level of 50% or more on stimulation. In the smaller CD56+CD3+ population of cells (about 20% of the total CD56+ cells), again there was no significant difference in IFN-γ expression (percent) in the three donor groups. Neither were there any differences in the absolute numbers of IFN-γ-positive or -negative cells between the donor groups (data not shown).

### Table I. Significant increases in the percentage of IFN-γ-positive T lymphocyte subpopulations in CVID compared with normal donors*

<table>
<thead>
<tr>
<th>Cell Population</th>
<th>Normal Donors (n = 12)</th>
<th>CVID (n = 12)</th>
<th>XLA (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>SE (%)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>CD3+</td>
<td>19.5 2.0</td>
<td>33.7 3.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4+</td>
<td>11.9 1.5</td>
<td>21.6 2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4+CD28+</td>
<td>10.4 1.2</td>
<td>20.4 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD8+</td>
<td>39.1 2.8</td>
<td>60.7 4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD8+CD28+</td>
<td>32.2 2.5</td>
<td>49.2 4.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CD8−CD28−</td>
<td>66.8 4.0</td>
<td>78.5 4.0</td>
<td>=0.05</td>
</tr>
</tbody>
</table>

* Cultures were stimulated with PMA and ionomycin for 2 h.

**Discussion**

CVID is a disease characterized by low levels of serum IgGs due to a failure of B cells to produce Abs. Although all three main Ig classes are usually low, the finding that many patients can make IgM IgGs in vivo and in vitro (19) suggests that there is a defect at the stage of B cell class switching and affinity maturation in the lymphoid germinal center. The recent demonstration of reduced hypermutations in the V regions of circulating CVID B cells is compatible with this scenario (20). There is good evidence that the B cells in this condition are intrinsically normal, and that the defect lies in the inability of T cells to provide the appropriate signals. Circulating CVID B cells from many patients can be induced in vitro to produce all classes of IgGs in the presence of anti-CD40 and cytokines known to stimulate a Th2-type response (3, 21). However, in CVID the appropriate T cells are not available. We have shown by limiting dilution (6) that functional Ag-specific memory T cells are not produced in a secondary response in CVID. This explains why T cell proliferation induced by Ags (e.g., tetanus toxoid, purified protein derivative, or the neoantigen KLH) is low or absent (4–6). A reasonable hypothesis is that the microenvironment for Ag-specific CD4+ T cell maturation in the lymphoid apparatus is suppressive, possibly due to a marked skewing toward a Th1-type response by overproduction of cytokines such as IL-12 and IFN-γ. Although we have previously shown an up-regulation of IFN-γ in some T cell subsets in CVID, especially in subsets of CD8+ cells (14, 15), the present work provides evidence for the first time that the skewing toward Th1 within T lymphocytes in CVID is linked to an overexpression of IL-12 in monocytes.

IL-12 made by DC and monocytes is an important cytokine that up-regulates IFN-γ in lymphocytes once the lymphocytes are activated sufficiently to express the IL-12 receptor. The principal objective of the present work was to see whether the raised IFN-γ in CVID was associated with an up-regulation of IL-12 in monocytes and DC. We approached this problem not by the isolation of different cell populations but by using whole-blood cultures and identifying the cytokines and cell populations within them by flow cytometry.

We detected potent up-regulation of IL-12 expression on stimulation with LPS in both DC and monocytes from all donor groups (normal, CVID, and XLA). The most important finding was that the mean expression of IL-12 in CD14-positive monocytes in CVID was up-regulated significantly more than in the normal group or the XLA control group. It is probable that this is the cause of the raised IFN-γ in lymphocyte subsets in CVID. The cytokine network between IL-12 produced in APC, inducing IFN-γ in T cells is well established, with positive feedback of IFN-γ inducing IL-12 (12).

Interestingly, the up-regulation of IL-12 did not occur in CVID in the CD14-negative DC population. The normal IL-12 in CVID DC does correlate with normal DC function in CVID. These are the only cells capable of initiating a primary immune response, and we have previously reported that a primary response in T cells (as represented by an allogeneic MLR) is able to function normally in CVID (4).

The up-regulation of IFN-γ in CD4+ T cells skews the system away from the Th2 cytokine pattern needed for B cell Ab production (16). Raised IFN-γ production by CD8+ T cells, previously reported by us (14, 15), probably contributes to this skewed response. Thus, our finding of both raised IL-12 in monocytes and IFN-γ in T cells provides a mechanistic explanation for the failure of Ab production in CVID. It is known that the IL-12/IFN-γ circuit is crucial for protection against mycobacteria (22–24). The up-regulation of this circuit in CVID explains why those patients with CD4+ T cell lymphopenia do not suffer from infection with these organisms. This contrasts with AIDS where the combination of CD4+ T cell lymphopenia and apparent down-regulation of IL-12 is probably the major predisposing factor for the marked susceptibility to mycobacterial disease (25). HIV infection is associated with a skewing toward a Th2-type response (26), in extreme cases causing very high levels of IgE. We can speculate that this is mediated by a down-regulation of IL-12 and that this also occurs in those rare CVID patients who normalize their IgG Ab production after HIV infection (27, 28).

The normal levels of TNF-α in both DC and monocytes in CVID show the specificity of the up-regulation of IL-12 that could be expected to up-regulate IFN-γ not only in T cells but also in NK cells (11), although we found normal percentage levels of IFN-γ in...
CD56-positive NK cells in CVID. This shows the specificity of the abnormal cytokine patterns in CVID. However, we did find a marked depletion in the CD56 NK cell population in both the CVID and XLA patient groups. Because this occurred in both patient groups, it could be due to recurrent infections from which both groups suffer or to therapy with i.v. Ig. We are studying this possibility.

There are three pieces of evidence that suggest that the failure of T cell responses in CVID are due to the Th1-skewed T cells rather than to a failure in the Ag-presenting ability of the monocytes. First, there are reports with allogenic MLC experiments with siblings with and without CVID that suggest that the dysfunctional cell is the T cell (8). Second, our limiting dilution experiments with and without CVID that suggest that the dysfunctional cell is the T cell (8). Second, our limiting dilution experiments using the neoantigen KLH show that there is a failure in the production of functional Ag-specific T memory cells (6). Third, in the mouse, adding IFN-γ inhibits the proliferative response to KLH-pulsed monocytes by KLH-primed T cells (29).

There is still a question as to whether the primary abnormality in CVID is in the monocyte up-regulation of IL-12 or in the raised IFN-γ in T cells that might itself increase IL-12 production by LPS-stimulated monocytes in our in vitro system. The latter is unlikely because we saw no increase in monocyte IL-12 in unstimulated blood from CVID patients. Moreover, although chronic infection in Ab-deficient patients might lead to raised T cell IFN-γ production, our finding of normal levels in XLA patients suggests that the abnormalities we describe are specific to CVID and are involved in its mechanism.

The inclusion of Flow-count beads that allowed us to measure absolute numbers of all cell populations produced an unexpected result. The raised percentage of IL-12-positive monocytes and the raised percentage of IFN-γ-positive CD4+ T cell populations in CVID was found not to be due to an increase in the absolute concentration of cytokine-positive cells. It was instead due to a significantly decreased number of cytokine-negative cells in the

**FIGURE 4.** IFN-γ expression in total CD3+ lymphocytes (A) and the subset CD3+CD8+ T cells (B). The mean data are given for the three donor groups: normal, CVID, and XLA. In both A and B, the lower graphs represent the percentage IFN-γ expression (mean ± SE) in each cell type without stimulation (monensin) or with stimulation (PMA + ionomycin + monensin). The upper graphs show the absolute cell concentrations of each cell population (A, CD3+ cells), (B, CD8+CD28− cells) for the three donor groups with and without stimulation with PMA + ionomycin. The solid components of the absolute count bars represent the IFN-γ-positive cells. Significant differences between the patient data and the normal values (unstimulated or stimulated) are shown by asterisks as for Fig. 2. The significant increase in percentage of IFN-γ in CVID CD3+CD8+ T cells above normal levels following stimulation (lower graph) is observed to be due to a significant reduction in the concentration of IFN-γ-negative monocytes in CVID because there is no significant increase in the number of IFN-γ-positive CD3+ cells (A, lower graph). However, by contrast, in Fig. 4B (CD8+CD28− subset) there is a significant increase in both the percentage and the number of IFN-γ-positive cells.

**FIGURE 5.** IFN-γ expression in CD56+CD3+ NK cells. The mean data are given for the three donor groups: normal, CVID, and XLA. The lower graph represents the percentage IFN-γ expression (mean ± SE) without stimulation (monensin) or with stimulation (PMA + ionomycin + monensin). The upper graph shows the absolute cell concentrations of CD56+CD3+ NK cells for the three donor groups with and without stimulation with PMA + ionomycin. The solid components of the absolute count bars represent the IFN-γ-positive cells. Significant differences between the patient data and the normal values (unstimulated or stimulated) are shown by asterisks as for Fig. 2. There is no significant increase in percentage of IFN-γ in CVID CD56+CD3+ NK cells above normal levels after stimulation (lower graph). From the upper graph, it is clear that there is a profound and significant depletion of CD56+CD3− NK cells in both the CVID and XLA patient groups compared with the normal donors.
CVID donor group. There is monocyte depletion in CVID just as there is CD4+ T cell depletion. It seems that monocytes and CD4+ lymphocytes capable of making IL-12 and IFN-γ, respectively, are less liable to be depleted in CVID than cells that do not make these cytokines. The depletions seem to occur only in the cell subsets that are incapable of making these cytokines.

One speculation from this could be that in CVID, there is an increased susceptibility to cell death of cells unable to make certain cytokines compared with those that can. An increased apoptosis may occur in CVID and part of the underlying mechanism for CVID may relate to a defect in preventing apoptosis of both T cells and monocytes.

The anti-IL12 Ab that we have used is against the inducible p40 chain. Although only a proportion of the induced p40 is incorporated into the active p70 molecule (30–32), we are encouraged that the use of the anti-p40 Ab is valid. This is because of the report that free p40 does not inhibit the function of IL-12 (33).

In conclusion, we provide evidence of dysregulation in the monocyte/T cell interactions involving IL-12 and IFN-γ in CVID. This is likely to compromise the ability of the monocyte, when it presents Ag, to provide the relevant signals to CD4+ cell Ab production. It is now appropriate to focus research on the regulation of IL-12 in CVID monocytes and investigate the transcriptional and translational control of this cytokine. Because it is possible that CVID is a polygenic disorder linked closely to selective IgA deficiency, a susceptibility gene involving IL-12 production is a candidate for inhibiting production of all classes of Ab. Finally, we suggest that CVID is a valuable model for pathological mechanisms involving altered cytokine profiles since we are not aware of other diseases reported to be associated with an up-regulation of IL-12.

Acknowledgments

We thank Dr. Andrew Lane of Serotech who helped in many ways including the provision of anti-cytokine reagents.

References