

Patterns of Cytokine Production by Mycobacterium-Reactive Human T-Cell Clones

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To gain insight into the functional capacity of human T cells in the immune response against *Mycobacterium tuberculosis*, we evaluated the spectrum of cytokines produced by mycobacterium-reactive human T-cell clones. Nine of 11 T-cell clones bearing $\alpha\beta$ or $\gamma\delta$ T-cell receptors produced both Th1 and Th2 cytokines, a pattern resembling that of murine Th0 clones. The most frequent pattern was secretion of gamma interferon, tumor necrosis factor alpha (TNF), and interleukin-10 (IL-10), in combination with IL-2, IL-5, or both. Two clones produced only Th1 cytokines, and none produced exclusively Th2 cytokines. Although IL-4 was not detected in cell culture supernatants, IL-4 mRNA was detected by polymerase chain reaction amplification in two of six clones. There were no differences between the cytokine profiles of $\alpha\beta$ and $\gamma\delta$ T cells. A striking finding was the markedly elevated concentrations of TNF in clone supernatants, independent of the other cytokines produced. Supernatants from mycobacterium-stimulated T-cell clones, in combination with granulocyte-macrophage colony-stimulating factor, induced aggregation of bone-marrow-derived macrophages, and this effect was abrogated by antibodies to TNF. The addition of recombinant TNF to granulocyte-macrophage colony-stimulating factor markedly enhanced macrophage aggregation, indicating that TNF produced by T cells may be an important costimulus for the granulomatous host response to mycobacteria. The cytokines produced by T cells may exert immunoregulatory and immunopathologic effects and thus mediate some of the clinical manifestations of tuberculosis.

T lymphocytes play a central role in orchestrating cell-mediated and humoral immune responses to microbial pathogens through release of specific cytokines. In animal models, two functional subpopulations of CD4⁺ cells, distinguished by their patterns of cytokine production, contribute to these responses. Th1 cells mediate delayed-type hypersensitivity (DTH) responses through secretion of gamma interferon (IFN- γ) and interleukin-2 (IL-2) (8), and Th2 cells enhance antibody synthesis through production of IL-4, IL-5, and IL-10 (27, 33). The striking contribution of these subpopulations to outcomes of infection is exemplified by *Leishmania*-infected mice, in which Th1 cells producing IFN- γ contribute to control and elimination of infection, whereas Th2 cells producing IL-4 result in progressive and uncontrolled disease (18, 32).

A growing body of evidence suggests that the human immune response to infection is similarly mediated by T-cell subpopulations secreting specific cytokine patterns. At the site of mycobacterial infection, mRNA for Th1 cytokines IFN- γ and IL-2 predominate in tissue lesions of patients with immunologic resistance to *Mycobacterium leprae*, whereas mRNA for Th2 cytokines IL-4, IL-5, and IL-10 are prominent in patients with extensive disease (35). Mycobacterium-reactive CD4⁺ T-cell clones from healthy donors preferentially produce IFN- γ and IL-2 (10, 17), and *M. leprae*-reactive CD4⁺ T-cell clones, thought to mediate DTH, produce IFN- γ (31). CD8⁺ T cells in patients with disseminated *M. leprae* infection may mediate immunosuppression through production of IL-4 (31). The study of other patho-

gens indicates that CD4⁺ clones that recognize *Toxocara canis* secrete IL-4 and IL-5 (10), and CD4⁺ tetanus-toxoid-responsive clones providing B-cell help secrete IL-4 and IL-5 (31). To delineate the spectrum of functional capacities of human T cells that encounter mycobacterial antigen, we investigated the cytokine profiles of *Mycobacterium tuberculosis*-reactive human T-cell clones from healthy tuberculin reactors. Because $\alpha\beta$ and $\gamma\delta$ T cells are thought to contribute to immune defenses against mycobacteria, we compared the cytokine patterns of *M. tuberculosis*-reactive $\alpha\beta$ and $\gamma\delta$ T cells.

MATERIALS AND METHODS

T-cell lines. For the analysis of cytokine production, venous blood was obtained from four healthy tuberculin reactors. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation over Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, N.J.). To isolate antigen-reactive $\alpha\beta$ T cells, PBMC were cultured at 10⁶ cells per ml in RPMI medium (GIBCO Laboratories, Grand Island, N.Y.) with penicillin and streptomycin (GIBCO), 10% human AB serum, and 10 μ g of a protein-peptidoglycan complex derived from the cell wall of *M. tuberculosis* Erdman per ml (5). To expand $\gamma\delta$ T cells, PBMC were cultured under the same conditions except that the mycobacterial antigen preparation used was sonicated, heat-killed *M. tuberculosis* (strain H37Ra; Difco Laboratories, Detroit, Mich.). Cells were maintained at 37°C and 7.5% CO₂. After 5 days, cells were centrifuged over Ficoll-Paque to enrich for lymphoblasts and further expanded with fresh AB serum and IL-2 (Electronu-

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cleonics, Silver Spring, Md.) to establish antigen-reactive T-cell lines.

T-cell clones. $\alpha\beta$ T-cell clones were obtained from T-cell lines established in the presence of the protein-peptidoglycan complex as previously described (5). $\gamma\delta$ T-cell clones were derived from H37Ra-stimulated T-cell lines enriched for $\gamma\delta$ T cells by immunomagnetic depletion of $\alpha\beta$ and CD4⁺ T cells as described previously (4). Clones were established by limiting dilution and plating 0.3 cell per well in 200- μ l round-bottom wells in the presence of 10% IL-2, 10 μ g of H37Ra per ml, 10% AB serum, and 10⁵ irradiated autologous PBMC per well. T-cell clones were expanded by adding fresh AB serum and IL-2 twice a week, irradiated autologous or allogeneic PBMC weekly, and H37Ra every 2 weeks. Proliferative responses of T-cell clones were determined by the incorporation of [³H]thymidine as previously described (5) and were expressed as stimulation indices (counts per minute in the presence of H37Ra divided by counts per minute with media alone).

Immunolabeling. T-cell clones were labeled by standard techniques (28) with anti-CD3 (Leu 4; Becton Dickinson Monoclonal Center, San Jose, Calif.), anti-CD4 (OKT4; Coulter Immunology, Hialeah, Fla.), anti-T-cell receptor $\alpha\beta$ (BMA-031; provided by R. Kurrle, Behringwerke, Marburg, Germany), anti-T-cell receptor $\gamma\delta$ (TCR- δ 1; provided by Michael Brenner, Harvard Medical School, Boston, Mass.), and BB3 (provided by L. Moretta, Istituto Nazionale, Genoa, Italy), which identifies V δ 2⁺ T cells.

Measurement of cytokine concentrations. T-cell clones were tested a minimum of 1 week after the addition of antigen or feeder cells and a minimum of 3 days after the addition of IL-2 to minimize the effects of exogenous IL-2 on cytokine production. For the generation of supernatants, T-cell clones (10⁶ cells) were cultured in 2-ml wells in the presence of 10⁶ irradiated autologous PBMC as antigen-presenting cells and 10 μ g of H37Ra per ml. Control wells contained irradiated PBMC alone or irradiated PBMC with 10 μ g of H37Ra per ml, without cloned T cells. In some experiments, T-cell clones were stimulated by coating wells with 1 μ g of anti-CD3 monoclonal antibody per ml (64.1 [26]) in the absence of mycobacterial antigen or antigen-presenting cells. Supernatants from cell cultures were collected at 24 h and stored at -70°C. An enzyme-linked immunosorbent assay was used to measure the concentrations of IL-2, IL-3, IL-4, IL-5, IL-10, tumor necrosis factor alpha (TNF), and IFN- γ (2, 11).

Cytokine mRNA induction. After the collection of supernatants, total cellular RNA was extracted from pelleted cells, and cDNA was synthesized from RNA by reverse transcription. Aliquots of cDNA were used as a template for amplification by polymerase chain reaction as previously described (35). Polymerase chain reaction product was subjected to electrophoresis on 2% agarose gels and visualized by staining with ethidium bromide.

Macrophage aggregation. Two *M. leprae*-reactive $\gamma\delta$ T-cell lines and one $\alpha\beta$ T-cell line were derived from the lesions of two tuberculoid leprosy patients as described previously (26). These lines were stimulated with 10 μ g of sonicated *M. leprae* per ml (a kind gift of Vijay Mehra, Albert Einstein School of Medicine, New York, N.Y.) and irradiated PBMC as antigen-presenting cells, and supernatants were harvested after 24 h. Four $\gamma\delta$ T-cell lines were derived from PBMC of purified protein derivative-responsive individuals. These lines were stimulated with anti-CD3 or with H37Ra and irradiated PBMC, and supernatants were collected after 24 h.

The capacity of T-cell supernatants and cytokines to induce macrophage aggregation was assessed as previously described (26). Briefly, freshly isolated bone marrow cells from normal donors were isolated by centrifugation over Ficoll-Paque. Bone marrow cells (10⁵) were cultured in 0.3-ml LabTek chamber wells (Nunc, Inc., Naperville, Ill.) in RPMI medium containing 10% human AB serum. To detect macrophage aggregation, granulocyte-macrophage colony-stimulating factor (GM-CSF, 150 U/ml; Genzyme, Boston, Mass.), recombinant TNF (Genzyme), polyclonal goat anti-human TNF antibodies (10 μ g/ml; R & D Systems, Minneapolis, Minn.), rabbit anti-human TNF antibodies (1:50; Genzyme), and T-cell supernatants (33%) were added at the onset of culture. After 5 days, the number of aggregates of 20 or more cells per well was recorded. We have previously shown that the aggregating cells are monocytes by using immunolabeling with monoclonal antibodies (26).

RESULTS

Cytokine patterns of $\alpha\beta$ and $\gamma\delta$ T-cell clones. $\alpha\beta$ T-cell clones ($n = 9$) were established from three donors, and $\gamma\delta$ T-cell clones ($n = 5$) were established from one donor. The $\alpha\beta$ T-cell clones were CD3⁺ BMA031⁺ TCR δ 1⁻ CD4⁺, and the $\gamma\delta$ clones were CD3⁺ BMA031⁻ TCR δ 1⁺ V δ 2⁺. The mean counts per minute with media alone for the $\alpha\beta$ T-cell clones was 1,329 \pm 754 versus 647 \pm 333 for the $\gamma\delta$ T-cell clones. The mean stimulation index of the $\alpha\beta$ T-cell clones to *M. tuberculosis* was 81.7 \pm 28.6 versus 20.3 \pm 7.2 for the $\gamma\delta$ T-cell clones.

The specific contribution of the T-cell clones to cytokine production was estimated by subtracting cytokine concentrations in supernatants generated by antigen-presenting cells and H37Ra from those generated by the clone, antigen-presenting cells, and H37Ra. In most cases, irradiated PBMC (which served as antigen-presenting cells) and *M. tuberculosis* (without cloned T cells) produced low but detectable levels of IL-2, IL-10, and TNF. IL-3 was detected in 4 of 10 cases, and IFN- γ was detected in 5 of 11 cases. Cytokine concentrations were increased in cultures containing $\alpha\beta$ or $\gamma\delta$ T-cell clones, antigen-presenting cells, and *M. tuberculosis*. Results of two representative experiments are shown in Table 1.

Specific patterns of cytokine production by 11 clones in response to *M. tuberculosis* are shown in Table 2. The dominant cytokine pattern in nine clones (six of eight $\alpha\beta$ and three of three $\gamma\delta$) was a combination of Th1 and Th2 cytokines. We considered these nine clones to be Th0-like, although the classic Th0 cytokine pattern described for murine clones includes IL-3 and IL-4. Of the nine Th0-like clones, seven (five $\alpha\beta$ and two $\gamma\delta$) produced IL-10, TNF, and IFN- γ . Some of these seven clones secreted IL-2 and/or IL-5 as well. One $\alpha\beta$ and one $\gamma\delta$ clone produced IFN- γ , TNF, and IL-5. Only two $\alpha\beta$ clones produced the Th1 cytokine IFN- γ but no Th2 cytokines and were considered to be Th1-like. The concentrations of IL-2 in supernatants of these clones were lower than those described for murine Th1 clones. Of all the cytokines tested, the concentrations of TNF in supernatants were substantially higher than those of other cytokines, including IFN- γ .

Increased cytokine concentrations in wells containing T-cell clones may have resulted from enhanced cytokine production by irradiated PBMC after interaction with the clones rather than from the clones themselves. To more specifically measure cytokine concentrations by T-cell clones, we stimulated clones with anti-CD3 in the absence of

TABLE 1. Cytokine production by T-cell clones in response to *M. tuberculosis*

APC ^b	Culture ^a		Cytokine concn (pg/ml)						
	H37Ra	Clone ^c	IL-2	IL-3	IL-4	IL-5	IL-10	TNF	IFN- γ
+	-	-	131	186	<40	<20	<40	161	<100
+	+	-	191	210	<40	<20	66	598	137
+	+	+ (S1 [$\alpha\beta$])	224	216	<40	<20	304	15,039	3,003
+	-	-	46	<40	<40	<20	<40	263	<100
+	+	-	76	<40	<40	22	1,053	4,219	<100
+	+	+ (G4 [$\gamma\delta$])	80	<40	<40	1,390	873	23,480	3,426

^a +, present; -, absent.

^b APC, antigen-presenting cells.

^c T-cell receptor is shown in brackets.

irradiated PBMC directly through the T-cell receptor (Table 3). Patterns of cytokine production by $\alpha\beta$ and $\gamma\delta$ clones were similar to those for clones stimulated with *M. tuberculosis* (Table 3). Six of seven clones (two $\alpha\beta$ and four $\gamma\delta$) produced a Th0-like pattern. Five clones secreted IL-10, TNF, and IFN- γ . Some of these five clones secreted a combination of IL-2, IL-4, and/or IL-5. One $\alpha\beta$ clone produced the Th1 cytokines IFN- γ and IL-2 but no Th2 cytokines. Of the four clones stimulated both with *M. tuberculosis* and with anti-CD3 (clones D3, G3, G4, and G5), all produced a Th0-like pattern under both conditions.

Our Th0-like clones differed from classic murine Th0 clones in that IL-4 was not detected in most culture supernatants. The failure to detect IL-4 may have resulted from the concomitant production and uptake of this cytokine by the T-cell clones. To investigate this possibility, we used polymerase chain reaction amplification to evaluate the transcription of IL-4 mRNA by T-cell clones in which IL-4 was not detected in supernatants. IL-4 mRNA was not detectable in antigen-presenting cells stimulated with H37Ra in the absence of T-cell clones (Table 4). In contrast, IL-4 mRNA was detectable in two of five clones stimulated with mycobacterial antigen and one of two clones stimulated with anti-CD3.

Capacity of T-cell supernatants to promote macrophage aggregation. Supernatants of $\gamma\delta$ T cells from lesions of leishmaniasis have been shown to enhance macrophage aggregation in combination with GM-CSF (26). In the present study, we established *M. leprae*-reactive $\gamma\delta$ and $\alpha\beta$

T-cell lines from leprosy lesions and *M. tuberculosis*-reactive $\gamma\delta$ T-cell lines from PBMC. The addition of recombinant GM-CSF alone to bone marrow macrophages caused minimal aggregation. Supernatants were generated from the following: (i) two $\gamma\delta$ lines from leprosy lesions, stimulated with *M. leprae* and irradiated PBMC as antigen-presenting cells; (ii) three $\gamma\delta$ *M. tuberculosis*-reactive lines from PBMC, stimulated with *M. tuberculosis* and irradiated PBMC; and (iii) four $\gamma\delta$ *M. tuberculosis*-reactive lines from PBMC, stimulated with anti-CD3. These supernatants, when added to GM-CSF, markedly enhanced macrophage aggregation. Supernatants from several lines were tested on two to three occasions, and aggregation was consistently observed. A representative result is shown in Fig. 1. The addition of supernatants from irradiated PBMC, cultured in either the presence or absence of mycobacterial antigen, minimally enhanced macrophage aggregation. No aggregation was seen in the absence of GM-CSF (26). Supernatants from a *M. leprae*-stimulated $\alpha\beta$ T-cell line derived from a leprosy lesion exhibited similar effects (data not shown).

Because supernatants of stimulated $\gamma\delta$ T cells in the current study contained markedly elevated concentrations of TNF, we hypothesized that this cytokine might induce clustering of macrophages. To assess the contribution of TNF to macrophage aggregation, supernatants from stimulated $\gamma\delta$ T cells were assayed for their capacity to induce macrophage aggregation in the presence of anti-TNF antibodies. Anti-TNF antibodies markedly reduced the ability of $\gamma\delta$ T-cell supernatants to synergize with GM-CSF in the

TABLE 2. Cytokine production by T-cell clones in response to *M. tuberculosis*

Clone (T-cell receptor)	Cytokine concn (pg/ml) ^a							Designation
	IL-2	IL-3	IL-4	IL-5	IL-10	TNF	IFN- γ	
S1 ($\alpha\beta$)	33	<40	<40	<20	<u>238</u>	<u>14,478</u>	<u>2,864</u>	Th0-like
S2 ($\alpha\beta$)	<u>45</u>	<u>140</u>	<40	<20	<40	<u>26,117</u>	<u>4,216</u>	Th1-like
T3 ($\alpha\beta$)	<u>452</u>	<40	<40	<u>1,200</u>	<u>1,935</u>	<u>10,567</u>	<u>6,120</u>	Th0-like
T6 ($\alpha\beta$)	<20	<40	<40	<20	<40	<100	<u>323</u>	Th1-like
M2 ($\alpha\beta$)	<20	<40	<40	<u>100</u>	<40	<u>1,339</u>	<u>362</u>	Th0-like
M3 ($\alpha\beta$)	<u>1,363</u>	<40	<u>237</u>	<u>2,194</u>	<u>3,343</u>	<u>28,768</u>	<u>6,299</u>	Th0-like
D2 ($\alpha\beta$)	<u>155</u>	<40	<40	<20	<u>5,866</u>	<u>35,603</u>	<u>6,270</u>	Th0-like
D3 ($\alpha\beta$)	<u>828</u>	<40	<40	<20	<u>10,913</u>	<u>43,643</u>	<u>6,270</u>	Th0-like
G3 ($\gamma\delta$)	<u>121</u>	ND ^b	67	<u>198</u>	<u>299</u>	<u>1,879</u>	<u>7,860</u>	Th0-like
G4 ($\gamma\delta$)	<20	<20	<40	<u>1,368</u>	<40	<u>19,261</u>	<u>3,426</u>	Th0-like
G5 ($\gamma\delta$)	<20	<40	<40	<u>192</u>	<u>412</u>	<u>15,005</u>	<u>2,234</u>	Th0-like
Mean \pm SE ^c	272 \pm 134	14 \pm 14	28 \pm 22	477 \pm 228	2,091 \pm 1,047	17,878 \pm 4,358	4,204 \pm 775	

^a Values underlined are at least twice the lower limit of detectability.

^b ND, not done.

^c Mean values are based on the assumption that values lower than the limit of detectability are equal to zero.

TABLE 3. Cytokine production by T-cell clones in response to anti-CD3 monoclonal antibody

Clone (T-cell receptor)	Cytokine concn (pg/ml) ^a						Designation
	IL-2	IL-4	IL-5	IL-10	TNF	IFN- γ	
D3 ($\alpha\beta$)	<20	<40	<20	<u>233</u>	<u>748</u>	<u>384</u>	Th0-like
D6 ($\alpha\beta$)	<u>315</u>	<40	<20	<u>853</u>	<u>3,003</u>	<u>39,781</u>	Th0-like
G2 ($\gamma\delta$)	<u>98</u>	<40	<20	<40	<u>198</u>	<u>4319</u>	Th1-like
G3 ($\gamma\delta$)	<u>163</u>	<u>1,190</u>	<u>493</u>	<u>84</u>	<u>484</u>	<u>31,802</u>	Th0-like
G4 ($\gamma\delta$)	<u>1,810</u>	<40	<u>37</u>	<u>92</u>	<u>737</u>	<u>2,082</u>	Th0-like
G5 ($\gamma\delta$)	<u>183</u>	<40	<u>1,011</u>	<40	<u>460</u>	<u>1,945</u>	Th0-like
G8 ($\gamma\delta$)	110	<u>650</u>	<u>55</u>	<u>804</u>	<u>533</u>	<u>28,771</u>	Th0-like

^a Values underlined are at least twice the lower limit of detectability.

induction of macrophage aggregation (Fig. 2). Control antibodies did not neutralize aggregation. To confirm the contribution of TNF to macrophage aggregation, the effect of recombinant TNF alone was assessed (Fig. 3). In the absence of GM-CSF, TNF caused minimal macrophage clustering at a concentration of 10 ng/ml. However, when GM-CSF was present, TNF induced significant macrophage aggregation at 100-fold-lower concentrations of 0.1 ng/ml.

DISCUSSION

Because specific cytokines produced by T lymphocytes exert striking and contrasting immunologic effects in the response to pathogens, it is important to characterize the profile of cytokines secreted by mycobacterium-reactive human T cells. Our results indicate that *M. tuberculosis*-reactive human T-cell clones bearing the $\alpha\beta$ or $\gamma\delta$ T-cell receptor secrete a wide variety of cytokines in response to *M. tuberculosis*. Most clones produced IFN- γ , TNF, and IL-10, together with IL-2 and/or IL-5, a pattern that resembles the cytokine profile of murine Th0 cells. The cytokine produced in the highest concentration by $\alpha\beta$ and $\gamma\delta$ T cells was TNF, a potent stimulus for macrophage aggregation, which may be pivotal in facilitating macrophage aggregation and granuloma formation to contain mycobacterial infection.

Human T-cell subpopulations defined by cytokine patterns analogous to murine Th1 and Th2 cells are thought to play a central role in infectious disease and allergic disorders (27, 29, 30, 33). Several groups have investigated the cytokine patterns of mycobacterium-reactive human T cells (7, 10, 16, 17, 24, 31). Mycobacterium-reactive T-cell clones from

healthy tuberculin reactors exhibit a Th1-like pattern of cytokine production, characterized by high concentrations of IFN- γ but low concentrations of IL-4 and IL-5 (10, 17). In contrast, our results and those of Boom et al. (7) indicate that *M. tuberculosis*-reactive T cells can secrete a less-restricted Th0-like pattern of cytokines. Salgame et al. (31) recently divided CD4⁺ and CD8⁺ cells into type 1 and type 2 subpopulations, on the basis of distinct patterns of cytokine production. Type 1 CD4⁺ *M. leprae*-reactive T-cell clones that mediate DTH and type 1 CD8⁺ cytotoxic cells secrete IFN- γ but not IL-4. In contrast, type 2 CD4⁺ cells that provide B-cell help and type 2 CD8⁺ suppressor cells produce IL-4 but not IFN- γ .

Differences in cytokine patterns of clones derived from different laboratories may result from variability in antigen fine specificity, as distinct microbial antigens can favor preferential development of Th1 or Th2 cells (21, 23). We used heat-killed H37Ra as the antigen, and our clones did not proliferate in response to the mycobacterial 65-kDa heat shock protein (data not shown), whereas many clones generated by Haanen et al. recognize this protein (17). Purified protein derivative of *M. tuberculosis* was used to obtain T-cell clones in two other reports (7, 10). Variations in T-cell

TABLE 4. Detection of IL-4 mRNA by polymerase chain reaction amplification^a

APC ^b + H37Ra	Anti-CD3	Clone	IL-4 mRNA
+	-	-	-
+	-	+(S1 [$\alpha\beta$]) ^c	-
+	-	-	-
+	-	+(T6 [$\alpha\beta$])	-
+	-	-	-
+	-	+(M2 [$\alpha\beta$])	+
+	-	-	-
+	-	+(G4 [$\gamma\delta$])	+
+	-	-	-
+	-	+(G5 [$\gamma\delta$])	-
-	+	+(G4 [$\gamma\delta$])	+
-	+	+(D6 [$\alpha\beta$])	-

^a +, present; -, absent.

^b Antigen-presenting cells.

^c Type of clone is shown in parentheses; T-cell receptor is shown in brackets.

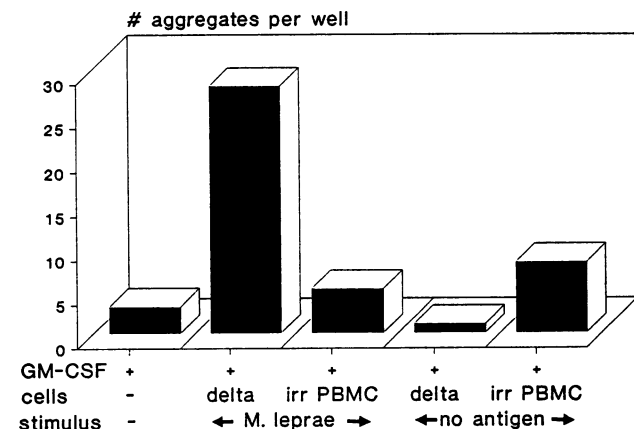


FIG. 1. Macrophage aggregation induced by supernatants from mycobacterium-stimulated $\gamma\delta$ T cells. The aggregation of bone marrow macrophages was assessed after the addition of GM-CSF, with or without cell culture supernatants. Supernatants were generated from a $\gamma\delta$ T-cell line from a tuberculoid leprosy lesion, unstimulated or stimulated with *M. leprae* in the presence of irradiated PBMC as antigen-presenting cells. Additional controls included supernatants from unstimulated or *M. leprae*-stimulated irradiated PBMC.

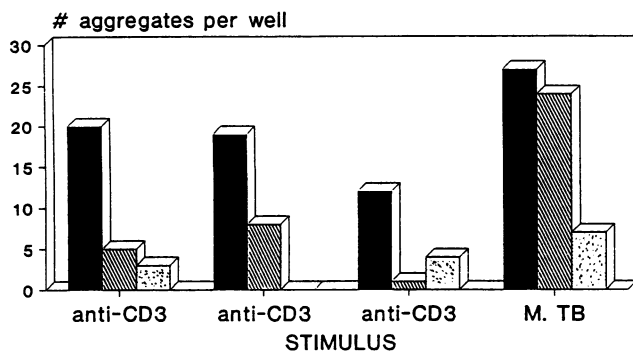


FIG. 2. Macrophage aggregation induced by supernatants from $\gamma\delta$ T cells in the presence of anti-TNF antibodies. $\gamma\delta$ T-cell lines were generated from PBMC of purified protein derivative-responsive individuals, and supernatants were collected after stimulation with anti-CD3 or *M. tuberculosis*. The aggregation by bone marrow macrophages was determined after the addition of supernatants alone (solid bars), supernatants with goat anti-human TNF antibodies (hatched bars), or supernatants with rabbit anti-human TNF antibodies (stippled bars).

cytokine profiles may also result from differences in culture conditions. For example, the addition of IL-4 favors the development of purified protein derivative-reactive clones with Th0- and Th2-like cytokine profiles (24). In addition, different methods of stimulating clones and differential IL-2 receptor expression by the clone at the time of stimulation may yield disparate results. For example, clone D3 in the current study produced IL-2 in response to antigen and irradiated PBMC but not with anti-CD3, whereas the reverse was true for clone G4. Another issue may be the ability to measure IL-4 secretion by T-cell clones. The current and prior reports (17) indicate that Th1-like mycobacterium-reactive T-cell clones, for which IL-4 is undetectable in supernatants, can express IL-4 mRNA, suggesting that IL-4 may be produced and simultaneously utilized by these T cells. In support of our findings, a Th0-like pattern of mRNA for IFN- γ , IL-2, IL-4, and IL-5 was found in some persons at the site of tuberculin skin tests (34), suggesting that both Th1 and Th2 cells, or perhaps Th0 cells, respond to the administration of mycobacterial antigen.

An interesting finding of the current study was the pro-

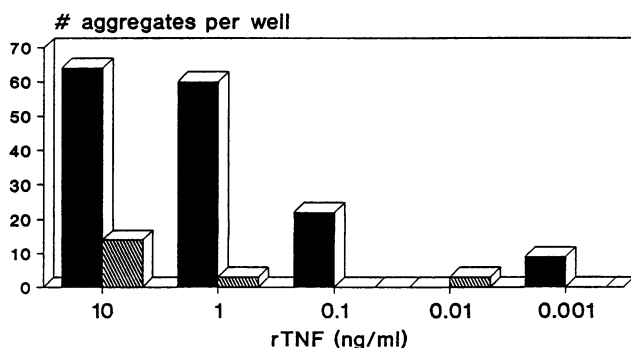


FIG. 3. Macrophage aggregation induced by recombinant TNF. Macrophage aggregation was evaluated after the addition of increasing concentrations of recombinant TNF, in the presence or absence of GM-CSF.

duction of IL-10 by T-cell clones in response to mycobacteria. IL-10 was initially described as a product of murine Th2 cells which inhibits cytokine production by Th1 cells (13) and depresses antigen-induced proliferation by interference with macrophage activation through downregulation of major histocompatibility complex class II expression and inhibition of monokine synthesis (6, 11, 12, 14). In humans, IL-10 is produced by macrophages (11), CD8⁺ cytotoxic T cells (31), and Th0-, Th1-, and Th2-like CD4⁺ T-cell clones (36). This and prior reports indicate that IL-10 is released by both macrophages and CD4⁺ T cells in response to *M. tuberculosis* (3). It is intriguing to speculate that production of IL-10 by macrophages and T cells in response to mycobacterial antigen may downregulate the DTH response, induce immunosuppression, and/or reduce tissue damage from an unrestrained inflammatory response.

Both $\alpha\beta$ and $\gamma\delta$ T cells are likely to play complementary roles in the immune response to mycobacterial infection. Depletion of CD4⁺ $\alpha\beta$ T cells by human immunodeficiency virus infection is the most potent known risk factor for the development of tuberculosis, enhancing susceptibility 25- to 40-fold (9). On the other hand, $\gamma\delta$ T cells are concentrated at the site of inflammation during primary tuberculous infection in animals (19, 20) and in tissue lesions characterized by active granuloma formation in leprosy patients (26). In tuberculosis and leprosy, expansion of $\gamma\delta$ T cells by mycobacterial antigen is greater in patients with limited disease and immunologic resistance than in those with extensive disease and ineffective immunity (4). However, the functional contribution of these cells to the antimycobacterial immune response remains enigmatic. We have previously observed that $\alpha\beta$ and $\gamma\delta$ mycobacterium-reactive T-cell lines produce GM-CSF and IFN- γ in response to *M. tuberculosis* (4). Other investigators have suggested that $\gamma\delta$ T-cell lines produce a Th1-like pattern of cytokines, including IFN- γ and IL-2 but not IL-4 (4). Our current investigation of a broader spectrum of cytokines at the T-cell clonal level indicates that $\gamma\delta$ T cells, like $\alpha\beta$ T cells, produce a cytokine profile that closely resembles that of Th0 cells. Although $\gamma\delta$ and $\alpha\beta$ T cells produce similar cytokines, they may play complementary roles in the antimycobacterial immune response. Perhaps $\gamma\delta$ T cells are dominant in the early response to infection, whereas $\alpha\beta$ T cells predominate at a later phase of the immune response.

An unexpected finding of the current study was the high concentrations of TNF detected in supernatants upon addition of both $\alpha\beta$ and $\gamma\delta$ mycobacterium-reactive T cells. These findings could result from secretion of TNF by the T cells or by T-cell-derived factors that enhanced TNF production by antigen-presenting cells. We have previously observed that $\gamma\delta$ T cells from leishmaniasis lesions produce a soluble factor(s) that, in combination with GM-CSF, greatly enhances macrophage aggregation (26). The current report demonstrates that this effect, exhibited by both $\gamma\delta$ and $\alpha\beta$ T cells, may be mediated by TNF. Induction of macrophage aggregation by TNF may explain the central contribution of this cytokine to granuloma formation in the immune response to parasitic and mycobacterial infection (1, 22). The mechanism(s) underlying the effects of TNF remain uncertain but may involve the capacity to induce macrophage chemotaxis and enhance major histocompatibility complex class II expression (15, 25). T cells can produce TNF in response to tetanus toxoid (31), indicating that secretion of this cytokine does not occur specifically in response to mycobacterial antigens.

The clinical manifestations of tuberculosis suggest that the

pattern of cytokines produced in response to infection is complex and not attributable exclusively to Th1- or Th2-like cells. Tuberculosis patients may have positive or negative tuberculin skin tests, with or without hyperglobulinemia from polyclonal B-cell activation, indicating that individuals express a wide range of immune responses that may include both DTH mediated by Th1-like cells and B-cell activation mediated by Th2-like cells. Our data provide evidence that the clinical manifestations of *M. tuberculosis* infection may reflect the effects of T cells producing a Th0-like cytokine pattern. IFN- γ may contribute to DTH and perhaps to protective immunity. TNF may contribute to antimycobacterial defenses by mediating granuloma formation but may induce immunopathologic effects such as fever, weight loss, and tissue necrosis, which are characteristic of tuberculosis. IL-10 may limit damage from an excessive inflammatory response but depress DTH and result in anergy in some patients. Thus, cytokines that contribute to the containment of mycobacterial infection may cause immunopathology, and those that downregulate the immune response may result in immunosuppression. A more detailed understanding of the immunoregulatory circuits that control cytokine production in human mycobacterial infection in vivo is essential to understand the pathogenesis of tuberculosis and to develop effective immunotherapeutic strategies against this disease.

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