Functional Properties of the T Cell Receptor Repertoire in Responding to the Protective Domain of Heat-Shock Protein 60 from *Histoplasma capsulatum*

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Cells expressing the T cell receptor β-chain variable region (Vβ) 6 constitute the majority of T cells responding to the protective domain (F3) of heat-shock protein 60 from *Histoplasma capsulatum*. This subset of cells exhibits a T helper type 1 (Th1) profile and is pivotal in protection. In this study, additional F3-reactive T cell lines were generated, leading to the discovery of a Th2 line. Vβ usage by clones was more diverse than has been previously recognized. Nearly all Th2 clones expressed Vβ8.1/8.2, whereas Th1 clones expressed Vβ11 or Vβ6. In adoptive transfer studies, only the Vβ6+ Th1 clone prolonged survival; all Th2 clones accelerated mortality. The ameliorative effect of the Vβ6+ Th1 clone was abrogated by treatment with monoclonal antibody to interferon-γ. Neutralization of interleukin-4 reversed the shortened survival of mice to which the Vβ6+ Th2 clone was administered. Thus, F3-mediated protection is confined to a defined Vβ population, but exacerbation of disease is mediated by multiple Vβ families.

*Histoplasma capsulatum* is a dimorphic fungal pathogen endemic to the midwestern and southeastern United States. Infection is acquired by accidental inhalation of mycelial fragments or microconidia from disrupted niches that harbor these fungal elements, such as soil containing bird or bat excrement. Although conidia are the infective form of the pathogen, the clinicopathological manifestations of disease are principally caused by yeasts that parasitize mammalian phagocytes [1]. In most cases, the initial illness is clinically inapparent or is characterized by influenza-like symptoms. In a small proportion of cases, inhalation of a large number of fungal particles leads to overwhelming pneumonitis or progressive disseminated disease.

Growth inhibition within the principal effector cell population of monocytes and macrophages requires interaction with T cells and the elaboration of their soluble products [2, 3]. A decrease in T cell number or impairment of their function increases the likelihood of more serious infection with *H. capsulatum* [4–7]. Hence, activation of an effective T cell–mediated immune response is required for reducing the fungal burden in infected organs, including lung, liver, and spleen [8]. The engagement of the interleukin (IL)–12/interferon (IFN)–γ axis also is important in the generation of a protective immune response to this fungus [9–11].

A fragment (F3) of *H. capsulatum* heat-shock protein 60 (hsp60) that spans aa 172–443 can be used to vaccinate mice against a lethal challenge [12]. The efficacy of this immunization has been reported to be crucially dependent on the presence of cells expressing the T cell receptor (TCR) β-chain variable region (Vβ) 6 that generate IFN-γ [13]. Subsequent to that report, additional F3-reactive T cell lines and clones were generated. Unexpectedly, the same lot of F3 used in the original study [13] produced a Th2 line with a completely different profile of Vβ usage. We report our findings here and demonstrate that the same polypeptide can elicit a Th1 or Th2 response in a single mouse strain.

Materials and Methods

Mice. C57BL/6 mice and TCR αβ−/− mice were purchased from Jackson Laboratories. Animals were housed in isolator cages and were maintained by the University of Cincinnati Department of Laboratory Animal Medicine (Cincinnati), which is accredited by the American Association for Accreditation of Laboratory Animal Medicine.

Preparation of *H. capsulatum* and infection of mice. *H. capsulatum* yeasts were prepared as described elsewhere [14]. To produce infection, animals were infected intranasally with yeasts in a 30-μL volume.

Preparation of F3. Cloning and expression of F3 have been described elsewhere [12]. In brief, the gene fragment was cloned into the NdeI and BamHI sites of pET19b. To induce expression of recombinant protein, *Escherichia coli* harboring the plasmid was...
grown at 37°C in Luria-Bertani broth to a final OD at 600 nm (OD$_{600}$) of 0.4–0.5. Subsequently, isopropyl-thiogalactose was added to cultures at a final concentration of 1 mM. Cultures were incubated for 3 h. Cells were harvested by centrifugation at 5000 g. The pellet was suspended in a buffer consisting of 5 mM imidazole, 500 mM NaCl, and 20 mM Tris-Cl (pH 7.9) and lysed by freeze-thaw cycles followed by sonification. Soluble and insoluble fractions were separated by centrifugation at 20,000 g.

The insoluble pellet was suspended in a buffer consisting of 6 M urea, 500 mM NaCl, 5 mM imidazole, and 20 mM Tris-Cl (pH 7.9). The denatured material was recovered in supernatants after centrifugation at 20,000 g and filtered to remove particulate material. The protein was purified by metal chelate chromatography, using Ni$^{2+}$-sepharose affinity column (His-Bind; Novagen). The recombinant protein was eluted with the same buffer described above but with 1 M rather than 5 M imidazole. The eluate was dialyzed against buffer containing decreasing amounts of urea and concentrated by ultrafiltration. The protein concentration was determined. F3 contained <10 pg of lipopolysaccharide/µg of protein.

**Immunization.** Mice were immunized subcutaneously at the base of the tail with F3. The antigen was suspended in adjuvant containing monophosphoryl lipid A, synthetic trehalose dicorynomycolate, and cell-wall skeleton (Corex) at a concentration of 1 mg/mL. Animals were injected subcutaneously with 0.1 mL of emulsion (100 µg of protein) twice. Injections were separated by 2 weeks.

**Splenocyte preparation.** Spleen cells were isolated by teasing apart of spleens between the frosted ends of 2 ground-glass slides. Cells were washed 3 times in Hanks’ balanced salt solution and resuspended in RPMI 1640 medium containing 10% fetal bovine serum, 1% nonessential amino acids, 1% sodium pyruvate, 5 × 10^{-5} M 2-mercaptoethanol, and 10 µg/mL gentamicin (complete medium) if the cells were to be used for establishment of T cell lines.

**Establishment of T cell lines and clones.** T cell lines and clones were initiated and maintained as described elsewhere [13]. T cells (10$^6$) were incubated in 0.2 mL of complete medium, and 50 µL of F3 (final concentration, 20 µg/mL). Cells were incubated for 72 h; 16 h before cell harvest, 1 µCi of [H]$^3$H]thymidine (specific activity, 6.7 Ci/mmol; New England Nuclear) was added to each culture. Cells were collected on glass-fiber filters with a semiautomated harvester, and uptake of radioactivity was measured by a liquid-scintillation counter. The stimulation index was calculated by dividing the count per minute for cells exposed to antigen by the count per minute for cells in medium alone.

**RVA extraction.** T cells (10$^6$) were incubated in 0.2 mL of RNAzol (BIOTECKX). RNA was extracted with chloroform and precipitated following the manufacturer’s protocol. RNA was resuspended in nuclease-free water, and the nucleic acid yield and were determined by the OD$_{260}$ reading and the ratio of OD$_{260}$ to OD$_{280}$ respectively. Samples were kept at −70°C until processing.

**Reverse-transcription–polymerase chain reaction (RT-PCR) of Vβ families.** One microgram of total RNA was annealed with 10 ng of an antisense primer complementary to the TCR Cβ constant region (Cβ) 1. First-strand cDNA synthesis was performed with AMV reverse transcriptase (Promega) and dNTPs. Aliquots of 1 µL of the RT reaction were used as template in 20 parallel PCRs. Each reaction contained a common nested antisense primer specific to Cβ2 and each of 20 Vβ-specific sense primers [15], dNTPs, and Taq polymerase (Invitrogen). Reactions were denatured at 94°C for 45 s, annealed at 60°C for 45 s, and extended at 72°C for 60 s. The number of cycles necessary to produce a visible signal without saturation was determined in preliminary experiments. Most samples required 28–32 cycles. The primers and their sequences have been published previously [16].

The presence of a Vβ-specific PCR product was determined by Southern blot analysis: 5 µL of each PCR was electrophoresed in 1% agarose gel, blotted onto a nylon membrane (Roche Biochemicals), and hybridized with a digoxigenin-labeled DNA probe specific to the TCR Cβ. After samples were washed in 0.1% saline sodium citrate at 65°C, the signal was visualized with alkaline phosphatase-conjugated anti-digoxigenin Fab (Roche) and the chemiluminescence substrate CDP Star (Roche). Light production was measured directly with a ChemiImager 4000 instrument (Alpha Innotech).

**Sequencing the TCR.** PCR products from T cells were cloned into pCR2.1 TOPO TA and transformed in TOP10F (Invitrogen). Plasmid was sequenced in both directions.

**Flow cytometry.** To confirm the identity of the Vβ family, cells were adjusted to a concentration of 5 × 10$^5$ cells/200 µL of PBS containing 2% bovine serum albumin and 0.02% sodium azide; stained with 0.5 µg of a biotin-labeled monoclonal antibody (Mab) (PharMingen) to Vβ6 (clone RR4-7), or Vβ11 (clone RR3-15), or Vβ14 (clone 14-2); and incubated with streptavidin-phycoerythrin. Cells were washed and fixed in 1% paraformaldehyde until flow cytometric analysis was done.

**Adoptive transfer.** Resting T cells were suspended in Hanks’ balanced salt solution and injected intravenously into mice 8 h before intranasal infection. Injections contained 2 × 10$^6$ cells in 0.5 mL of solution per recipient.

**Generation of cytokine-containing supernatants.** To generate supernatants from T cell clones, 10$^6$ T cells were incubated with 2.0 × 10$^5$ irradiated splenocytes in the presence or absence of 20 µg of F3/mL of cells. One milliliter of this solution was dispensed into each well of a 24-well plate. All cultures were incubated for 48 h at 37°C in 5% CO$_2$. Supernatants were harvested, filtered, and stored at −70°C until assays were done.

**Cytokine analysis.** Commercially available ELISA kits were used to measure IFN-γ, IL-4, tumor necrosis factor (TNF)–α, granulocyte-macrophage colony-stimulating factor (GM-CSF) (Endogen), IL-10 (PharMingen), and IL-13 (R&D Systems). The data for cytokine measurements were expressed as the change in the level of a particular cytokine (calculated by subtracting the amount of cytokine detected in medium alone from the amount found in supernatants of antigen-stimulated cells).
Among clones derived from line 10, all but 1 expressed V\(b\) by ELISA. Data are expressed as the change (\(\Delta\)) in cytokine level, calculated as described in Materials and Methods.

### Results

#### T cell lines reactive to F3.
We previously reported that the vast majority of T cell clones that were reactive to F3 expressed V\(\beta6\) and released IFN-\(\gamma\) but did not release IL-4. This V\(\beta\) family was essential to the success of F3 as a vaccine for H. capsulatum infection [13]. Subsequently, we continued to generate F3-reactive T cell lines and to assess their cytokine profile. Cell lines 9 and 11 were biased to Th1. One of the lines, line 10, generated a brisk IL-4 response, and the amount of this cytokine exceeded that of other F3-reactive lines that had been created during that time period (figure 1). This finding was unexpected, because the same lot of F3 was used in the present study as in the previous study [13]. Accordingly, we sought to determine whether the TCR usage of clones derived from a line that produced a larger quantity of IL-4 differed from what we had reported.

#### T cell clones from cell lines 10 and 11.
We generated a panel of T cell clones from lines 10 and 11 to determine whether there were differences in V\(\beta\) expression. All clones were generated by limiting dilution of lines 10 and 11. Phenotyping of expression of CD4 and CD8 revealed the clones from each line to be CD4\(^+\). Among clones derived from line 10, all but 1 expressed V\(\beta8.1\)/

8.2. The exception expressed V\(\beta14\) (table 1). The dominant \(\beta\)-chain joining region (J\(\beta\)) usage was 1.1, and its expression was linked to the sequence VGTP in the complementarity-determining region 3 (CDR3). J\(\beta2.1\) was associated with INRGG in the CDR3 region.

Clones from line 11 expressed either V\(\beta11\) or V\(\beta6\). The V\(\beta11\) clones predominantly expressed J\(\beta2.5\), and the CDR3 contained an SPQR motif (table 2). The V\(\beta6\) clones expressed 1 of 3 different J\(\beta\)s, 2.3, 2.4, or 1.2. SPQR was the dominant sequence in the CDR3.

### Cytokine analysis of T cell clones from lines 10 and 11.
Clones from T cell lines 10 and 11 were stimulated with F3, and the supernatants were assayed for release of IFN-\(\gamma\), IL-4, IL-10, IL-13, GM-CSF, and TNF-\(\alpha\). The results are depicted in figure 2. Only 4 clones derived from line 10 released IFN-\(\gamma\) in response to F3, and, of those 4, 10.17 was the only clone that released IFN-\(\gamma\) but did not release IL-4. The remaining 10 clones produced IL-4 in response to F3. IL-10 was produced by 5 of 14 clones, and all clones released IL-13 and GM-CSF. Analysis of the response by clones from line 11 revealed that 6 of 11 generated IFN-\(\gamma\) and all but 1 secreted IL-4. Only 1 V\(\beta6\) clone, 11.19, did not release IFN-\(\gamma\). Four of 11 clones from line 11 generated IL-10, and 9 of 10 released IL-13. All secreted GM-CSF. None of the clones stimulated with F3 expressed amounts of TNF-\(\alpha\) that were higher than the levels expressed by unstimulated cells.

### In vivo activity of T cell clones.
To determine whether the cytokine profile and V\(\beta\) expression were associated with in vivo biological activity, we selected clones from V\(\beta6\), V\(\beta8.1\)/8.2, and V\(\beta11\) families and adoptively transferred them into TCR a\(\beta\)\(^{-/-}\) mice. We chose clones that produced predominantly IFN-\(\gamma\) or IL-4 to represent Th1 or Th2 cells, respectively. Eight hours after intravenous transfer, mice were infected with 5 \(\times\) 10\(^4\) yeasts intranasally. Mice were injected with V\(\beta8.1\)/8.2\(^+\) (clone

### Statistical analysis.
Survival data were analyzed using the log-rank test. \(P < 0.05\) was considered to be statistically significant.

### Table 1. T cell receptor analysis of clones from the F3-reactive T cell line 10.

<table>
<thead>
<tr>
<th>Clone</th>
<th>V(\beta)</th>
<th>SI</th>
<th>J(\beta)</th>
<th>CDR3</th>
</tr>
</thead>
<tbody>
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<td>7</td>
<td>1.1</td>
<td>VGTP</td>
</tr>
<tr>
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<td>8.1/8.2</td>
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<td>1.1</td>
<td>VGTP</td>
</tr>
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<td>2.6</td>
<td>INRGG</td>
</tr>
<tr>
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<td>8.1/8.2</td>
<td>6</td>
<td>2.1</td>
<td>INRGG</td>
</tr>
<tr>
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<td>8.1/8.2</td>
<td>3</td>
<td>1.1</td>
<td>VGTP</td>
</tr>
<tr>
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<td>8.1/8.2</td>
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<td>1.1</td>
<td>VGTP</td>
</tr>
<tr>
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<td>8.1/8.2</td>
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<td>INRGG</td>
</tr>
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<td>VGTP</td>
</tr>
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<td>2.1</td>
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</tr>
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<td>3</td>
<td>1.2</td>
<td>SFQP</td>
</tr>
</tbody>
</table>

**NOTE.** Stimulation index (SI) was calculated by dividing the counts per minute for cells incubated with antigen by the response to medium alone.

CDR3, complementarity-determining region 3; J\(\beta\), \(\beta\)-chain joining region; V\(\beta\), \(\beta\)-chain variable region.
were given V clones of TCR selected, one that was Th1 and one that was Th2. Separate that were Th1 (clone 11.11) was prolonged (log-rank test) from that of mice that received the V clone or the V, or an MAb to the respective cytokine. Another group received no cells. Treatment with MAb to IFN-γ demonstrated enhanced survival (log-rank test), compared with controls (figure 3A). Survival of mice that did not receive any cells or to cells (clone 11.21, Th1) cells, and the course of infection was monitored. Mice injected with clones 10.22 and 11.10 (Th2) died earlier in the course of infection (P < 0.01; log-rank test), compared with mice that did not receive any cells (figure 3A). Survival of mice that were injected with Vb8.1/8.2 or Vβ11 Th1 clones was not significantly different (P > 0.05; log-rank test) from that of mice that received no cells (figure 3A).

In parallel experiments, we tested whether the freshly created Vβ6 clones could modulate infection. Two Vβ6 clones were selected, one that was Th1 and one that was Th2. Separate groups of Vb6+/8.2– mice that received either the Vβ6 Th1 clone or the Vβ6 Th2 clone were given MAb to IFN-γ or to IL-4, respectively. As a control, an equal amount of rat IgG was given to mice that did not receive the MAb. Mice were infected with H. capsulatum yeasts and subsequently injected with the Vβ6 clone 11.11 (Th1) or the Vβ6 clone 11.19 (Th2) and with rat IgG or an MAb to the respective cytokine. Another group of mice received no cells. The survival of mice given Vβ6+ cells that were Th1 (clone 11.11) was prolonged (P < 0.01; log-rank test), compared with infected control mice, and mice that received the Vβ6 Th2 clone (clone 11.19) died earlier in the course of infection (P < 0.01; log-rank test) than mice that received no cells. Treatment with MAb to IFN-γ of mice that were given Vβ6 Th1 cells reversed the salutary effect of these cells (figure 3B). In fact, such mice manifested a survival curve similar to those given Th2 cells. Conversely, mice treated with MAb to IL-4 demonstrated enhanced survival (P < 0.01; log-rank test), compared with controls (figure 3B). The survival profile for that group of mice did not differ (P > 0.05; log-rank test) from that of mice that received the Vβ6 Th1 clone, 11.11.

### Table 2. T cell receptor analysis of clones from the F3-reactive T cell line 11.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Vδ</th>
<th>SI</th>
<th>Iβ</th>
<th>CDR3</th>
</tr>
</thead>
<tbody>
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<td>2.5</td>
<td>SGQR</td>
</tr>
<tr>
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</tr>
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<td>SGQR</td>
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<td>9</td>
<td>1.2</td>
<td>SFQP</td>
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</tbody>
</table>

**NOTE.** Stimulation index (SI) was calculated by dividing the counts per minute for cells incubated with antigen by the response to medium alone. CDR3, complementarity-determining region 3; Iδ, β-chain joining region; Vδ, β-chain variable region.

10.22, Th2), Vβ8.1/8.2– (clone 10.11, Th1), Vβ11+ (clone 11.10, Th2), or Vβ11– (clone 11.21, Th1) cells, and the course of infection was monitored. Mice injected with clones 10.22 and 11.10 (Th2) died earlier in the course of infection (P < 0.01; log-rank test), compared with mice that did not receive any cells (figure 3A). Survival of mice that were injected with Vb8.1/8.2 or Vβ11 Th1 clones was not significantly different (P > 0.05; log-rank test) from that of mice that received no cells (figure 3A).

To explore the organization of the T cell response to F3, we generated 28 F3-reactive T cell clones, and all but 3 bore Vb6. These cells exhibited a strong Th1 phenotype and were critical to the efficacy of vaccination [13]. In the present study, we build on our previous observations concerning the clonal T cell response to vaccination with F3. The creation of additional F3-reactive T cell lines produced 2 unanticipated results. One was that a much higher proportion of T cell clones expressed a Vδ family other than Vβ6, and the other was the discovery of T cell clones with a Th2 phenotype that had a modestly diverse repertoire of Vb8.1/8.2, Vβ11, Vβ14, or Vb6. This finding demonstrated that a polypeptide could induce production of both Th1 and Th2 cells in C57BL/6 mice. In this regard, injection of a peptide consisting only of 15 aa from the G protein of respiratory syncytial virus in BALB/c mice can elicit CD4+ T cells that are either Th1 or Th2 [28]. In other models, the same antigen stimulated either Th1 or Th2 cells, depending on the genetic background of the host. For example, the LACK protein from Leishmania major induces production of Th1 cells in a resistant strain of mouse and Th2 cells in susceptible mice [29–31].

The CDR3 of the TCR α and β chains serves as a point of contact with peptide/major histocompatibility complex molecules. This region of the TCR is critically important for the generation of diversity in antigenic recognition by T cells [32]. Among the Vb8.1/8.2– clones, the CDR3 motif of INRGG was directly correlated with a Th2 phenotype. Alternatively, expression of a CDR3 motif of VGTP was present in Vb8.1/8.2+ cells that were either Th1 or Th2. There was a highly conserved motif, SGQR, in both Vβ11+ and Vb6+ cells. Of the 5 Vβ11+ T cell clones, 4 were Th2, and of the 6 Vb6+ T cell clones, only l was Th2. Thus, in the clones derived from line 11, there was no correlation between Vδ expression and CDR3 sequence. These findings support the contention that Th1 and Th2 cells may express identical TCR and CDR3.

Adoptive transfer of Th2 clones significantly shortened the survival of Vαβ8.2– mice, regardless of Vβ family or CDR3 sequence. By contrast, the only T cell clone that mediated protection was a Th1 Vβ6+ T cell clone. This result extends a previous finding that only a Vβ6+ T cell clone conferred protection in TCR αβ8.2– mice after adoptive transfer [13]. A sharp

**Discussion**

Clearance of the pathogenic fungus *H. capsulatum* from lymphoid and visceral organs requires an intricate series of interaction among T cells, macrophages, and dendritic cells [2, 3, 17]. T cells are instrumental in the optimal expression of protective immunity both in response to infection with viable yeasts and in vaccination with either hsp60 or the polypeptide F3 [3, 6, 7, 13, 18, 19]. The principal, if not only, effector mechanism of *H. capsulatum*–reactive T cells is cytokine elaboration. Attempts to identify additional effector properties, such as cytolytic activity, have not been successful [20]. Several endogenous cytokines released by T cells, including IFN-γ, GM-CSF, and TNF-α, promote protective immunity in response to primary or secondary infection with *H. capsulatum* yeasts [9–11, 21–27]. At present, the only T cell–derived cytokine essential for immunity that is associated with F3 vaccination is IFN-γ [13].

To explore the organization of the T cell response to F3, we generated 28 F3-reactive T cell clones, and all but 3 bore Vb6. These cells exhibited a strong Th1 phenotype and were critical to the efficacy of vaccination [13]. In the present study, we build on our previous observations concerning the clonal T cell response to vaccination with F3. The creation of additional F3-reactive T cell lines produced 2 unanticipated results. One was that a much higher proportion of T cell clones expressed a Vδ family other than Vb6, and the other was the discovery of T cell clones with a Th2 phenotype that had a modestly diverse repertoire of Vb8.1/8.2, Vβ11, Vβ14, or Vb6. This finding demonstrated that a polypeptide could induce production of both Th1 and Th2 cells in C57BL/6 mice. In this regard, injection of a peptide consisting only of 15 aa from the G protein of respiratory syncytial virus in BALB/c mice can elicit CD4+ T cells that are either Th1 or Th2 [28]. In other models, the same antigen stimulated either Th1 or Th2 cells, depending on the genetic background of the host. For example, the LACK protein from *Leishmania major* induces production of Th1 cells in a resistant strain of mouse and Th2 cells in susceptible mice [29–31].

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Figure 2. Cytokine profile for T cell clones. Each clone was stimulated with 20 μg/mL F3 for 48 h or incubated in medium only, and supernatants from stimulated and unstimulated cells were harvested and assayed for interferon (IFN)–γ, interleukin (IL)–4, IL-10, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-13. Data are expressed as the change (Δ) in cytokine level, calculated as described in Materials and Methods. Results from 1 of 2 experiments are shown.
Groups of mice injected with a Th1 or Th2 Vβ, the CDR3 sequence SHAGG protected TCRα. This finding has been observed most notably in mice infected with \( H. \) capsulatum \([9, 10, 14, 25, 33–35]\). In murine histoplasmosis, the inimical effects of endogenous IL-4 have been associated with a lack of cytokines that are involved in the protective immune response, including IFN-γ, TNF-α, and GM-CSF \([10, 20, 24]\). Our study has provided additional evidence that IL-4 is deleterious to the protective immune response. Adoptive transfer of T cell clones that produced IL-4 and not IFN-γ diminished the length of survival of TCR αβ−/− mice. Conversely, neutralization of IL-4 prevented the detrimental effects of this cytokine, although it did not lead to sterilizing immunity.

One candidate cytokine that may have prolonged the survival of mice given an MAb to IL-4 in conjunction with a Th2 clone is GM-CSF. GM-CSF is a key regulator of the protective immune response to this fungus. Neutralization of endogenous GM-CSF vitiates host resistance to \( H. \) capsulatum, and administration of recombinant GM-CSF improves host resistance in experimental pulmonary histoplasmosis \([21, 36]\). The release of GM-CSF by T cells may be one cause of the observed effect. TNF-α, which is also necessary for the full expression of the protective immune response to \( H. \) capsulatum \([26]\), was not released, and, hence, it is unlikely that it contributed to the prolonged survival of mice that received the Th2 clone and MAb to IL-4.

Pulmonary infection with \( H. \) capsulatum induces the production of IFN-γ in the lungs of mice, and host control of the infectious process is dependent on IFN-γ expression \([10, 14, 20]\). This mediator is principally generated by CD4+ T cells after infection \([6]\), and this cell population is requisite for control of primary infection and for the efficacy of vaccine in inducing immunity to this fungus \([6, 19]\). In this study and in previous work, transfer of F3-reactive Vβ6+ cells that secreted IFN-γ enhanced the protective immune response in TCR αβ−/− mice \([13]\). We also validated that IFN-γ was key for the biological action of the transferred clone. Recipients of the Vβ6+ T cell clone survived longer than those given no cells or those given a Th2 clone. Neutralization of IFN-γ in mice receiving the Vβ6+ clone shortened survival, and the survival curve of these mice was not dissimilar from the survival of animals that received a Th2 clone. These results establish the central influence of IFN-γ in T cell–mediated immunity to this fungus. More important, they suggest that another cytokine may be operative in dampening protective immunity. Because the particular clone that was used in the present study did not release IL-4 on antigenic stimulation (clone 11.11), this cytokine is not responsible for the accelerated death of mice.

A cytokine generated by clone 11.11 that may have blunted immunity is IL-10. IL-10 is known to down-regulate protective immunity in murine histoplasmosis, but only in mice that are deficient in either GM-CSF or TNF-α \([21, 25]\). Clone 11.11 also generated a substantial amount of IL-13 in response to F3. This cytokine expresses many biological activities and is known to...
promote fibrosis [37]. In addition, it can modulate immunity to intracellular parasites, in particular L. major. Resistant mice that have been genetically modified to overexpress this cytokine become more susceptible to infection, and, conversely, susceptible mice that lack IL-13 exhibit resistance [38]. It is conceivable that IL-13, like IL-4, may be detrimental to host defenses to H. capsulatum, especially if production of IFN-γ is blunted or nonexistent.

Another explanation for reversal of function by the Th1 and Th2 clones that were transferred into mice that received MAb to IFN-γ or to IL-4, respectively, is that these cells may have switched phenotypes. IL-12 and IL-4 are key cytokines that drive the differentiation of naive T cells into Th1 and Th2 phenotypes [39]. However, less is known about the reversal potential of mature cells, although conversion is possible [39]. Hence, neutralization of IFN-γ or IL-4 may have created an environment in which the stability of the phenotype could not be maintained and the polar phenotype emerged.

Analysis of the cytokine responses of T cell clones revealed that IL-10 generation was closely linked with IFN-γ release. Of the 11 clones that released IFN-γ, 7 (64%) generated IL-10. On the other hand, of the 14 clones that produced IL-4 and IFN-γ, only 2 (14%) expressed IL-10. Simultaneous production of both IL-10 and IFN-γ has been demonstrated in experimental leishmaniasis, in which the majority of CD4⁺ cells that generated the former also released the latter [40]. This population appears to be important in sterilizing immunity, and the authors of that study [40] hypothesized that the release of IL-10, which is a deactivating cytokine [41], mutes the response of cells to high levels of IFN-γ, thus counterregulating the Th1 response. IL-10- and IFN-γ-producing T cells also have been detected in human infections, including tuberculosis and borreliosis [42, 43].

In summary, we have identified regulatory T cell populations from a polypeptide that vaccinates mice against H. capsulatum. Both Th1 and Th2 subsets were obtained by T cell cloning. No correlation could be drawn between TCR expression and capacity to cause accelerated mortality among mice. The capacity to induce a more severe disease by a Th2 clone was blocked when IL-4 activity was neutralized in vivo. Transfer of protection resided in a single population of Vβ6⁺ cells that generated IFN-γ. These results provide additional insights into the control of the host infection and the organization of the T cell response after vaccination with the protective fragment F3.

References
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