Apoptosis of $\text{Fas}^{\text{high}}$ CD4$^+$ Synovial T Cells by $Borrelia$-reactive Fas-ligand$^{\text{high}}$ $\gamma\delta$ T Cells in Lyme Arthritis

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Summary

The function of the minor subset of T lymphocytes bearing the $\gamma\delta$ T cell antigen receptor is uncertain. Although some $\gamma\delta$ T cells react to microbial products, responsiveness has only rarely been demonstrated toward a bacterial antigen from a naturally occurring human infection. Synovial fluid lymphocytes from patients with Lyme arthritis contain a large proportion of $\gamma\delta$ cells that proliferate in response to the causative spirochete, $Borrelia$ burgdorferi. Furthermore, synovial $\gamma\delta$ T cell clones express elevated and sustained levels of the ligand for Fas (APO-1, CD95) compared to $\alpha\beta$ T cells, and induce apoptosis of $\text{Fas}^{\text{high}}$ CD4$^+$ synovial lymphocytes. The findings suggest that $\gamma\delta$ T cells contribute to defense in human infections, as well as manifest an immunoregulatory function at inflammatory sites by a Fas-dependent process.

*Abbreviation used in this paper: PBL, peripheral blood lymphocytes.

While most T lymphocytes express a TCR composed of $\alpha$ and $\beta$ chains, a subpopulation of T cells bearing alternate $\gamma$ and $\delta$ chains exists as a minor subset of peripheral blood lymphocytes (PBL)$^1$ (1). While the function of $\gamma\delta$ T cells is uncertain, a clue may lie in their increased proportion at epithelial barriers, during certain infections, and at sites of chronic inflammation such as synovial tissue in rheumatoid arthritis (2–7). Some $\gamma\delta$ T cells respond to bacterial products and can be identified after infection of mice with particular bacteria (8–15). However, in humans, leprosy is the only infectious disease to date in which $\gamma\delta$ T cells from affected individuals have been shown to respond to the causative organism (9).

$\gamma\delta$ T cells frequently manifest cytolytic activity toward a broad array of target cells (2, 16). Such a spectrum of cytolysis might occur when a target molecule is widely expressed, such as the Fas antigen (APO-1, CD95) (17). Fas is a 45-kD cell surface molecule that mediates apoptosis and is a member of a family of molecules that includes the type I receptor for TNF. Fas is one of the principle components responsible for T cell–mediated cytotoxicity (18–20). Expression of mRNA for the Fas ligand (FasL) was originally described as being transiently expressed by activated $\alpha\beta$ T cells, although higher mRNA levels were noted in $\gamma\delta$ T cells (21). More recent findings have noted constitutive expression of FasL by nonlymphoid cells, including Sertoli cells of the testis (22) and certain components of the eye (23). FasL expression by these tissues parallels their ability to suppress immune-mediated inflammation. These collective observations suggested that $\gamma\delta$ T cells in Lyme arthritis might respond to $Borrelia$ burgdorferi as well as contribute to regulation of the synovial inflammatory infiltrate.

Materials and Methods

**Patients.** Lyme arthritis patients came from areas endemic for Lyme disease and were followed at the Lyme Disease Clinic at the University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School. All patients had histories, exams, and serologies consistent with Lyme arthritis, including $Borrelia$-specific antibody titers that were higher in synovial fluid relative to serum. Synovial fluid lymphocytes were examined from seven patients with Lyme arthritis of 6-mo to 3.2-yr duration.

**Flow Cytometry.** Lymphocytes were isolated from peripheral blood or synovial fluid by Ficoll–Hypaque centrifugation. Cells were stained with the indicated fluorochrome-conjugated antibody at 4°C for 30 min. Antibodies were specific for TCR-$\alpha\beta$ (JOVI-1; Ancell Corp., Bayport, MN), TCR-$\gamma\delta$ (5A6.E9; T Cell Sciences, Inc., Cambridge, MA), TCR-V61 and TCR-V82 (AB and BB3, respectively, courtesy of Dr. Alessandro Moretta, Uni-


vory of Genoa, Genoa, Italy), CD4 (SFC12T4D11; Coulter Corp., Hialeah, FL), CD8 (SFC121Thy2D3; Coulter Corp.), Fas (M38) (24) and FasL (polycyonal C-20; Santa Cruz Biotechnology, Santa Cruz, CA; or monoclonal A11 [25]). Surface staining for Fas was performed by one of three methods. The first approach used a fusion protein composed of the extracellular domain of murine Fas linked to the human Ig Fc portion (Fas-Fc) (26). This was followed by goat anti-human Fc–biotin and then avidin–phycocerythrin. Control staining was accomplished by staining for surface IL4 using an IL4 receptor–Fc fusion protein. Alternatively, surface Fas was measured using either a rabbit anti-serum to the extracellular carboxyl-terminal portion of human FasL and purified on a FasL sepharose column (C-20), or monoclonal antibody A11 that recognizes both mouse and human Fas (25). To measure Fas induction, cells were examined 3 h after stimulation with PMA (10 ng/ml) and ionomycin (250 ng/ml), in the absence or presence of metalloprotease inhibition using 5 mM EDTA (27). Samples were analyzed on a Coulter Elite flow cytometer (Coulter Corp.) and at least 2 × 10^6 events were accumulated for analysis.

**Proliferation Assays and Derivation of Lyme Synovial γδ T Cell Clones.** Synovial fluid lymphocytes were cultured in AIM-V serum-free medium (GIBCO BRL, Gaithersburg, MD) in either bulk cultures (10^5/ml) for phenotyping, or in round-bottomed microtiter wells (10^3/well) for proliferation assays. Cells were stimulated with 3 μg/ml of a sonicate of B. burgdorferi grown in BSK II medium as previously described (28). Triplicate cultures were pulsed with ^3H-TdR during the last 18 h of a 6-d culture, harvested, and counted. From parallel cultures, responding cells were cloned at 0.3 cells/well in AIM-V with 5% FCS in the presence of irradiated PBL (3 × 10^5/well), human recombinant IL2 (10 U/ml), and 3 μg/ml of B. burgdorferi sonicate. Responding wells were phenotyped and the γδ cells expanded by restimulation at 10-d intervals.

**PCR Analysis of Synovial Fluid T Lymphocyte Vδ Repertoire.** Semi-quantitative PCR was performed on samples using cDNA prepared from oligo–dT–primed RNA and reverse transcriptase (GIBCO BRL) as previously described (29). The 5’ Vδ- and Cδ-specific primers are modifications of published sequences (30) as follows: Vδ1: 5’-AGGAAATCCAGCAAAAGG-3’; Vδ2: 5’-AGGAAACCGCCAAGGCTACAA-3’; Vδ3: 5’-CAGTTATCCAAATCAGA-3’; Vδ4: 5’-TGGACACGTGATTCCAAGTT-3’; Vδ5: 5’-CTGTGACTATACTAACAG-3’; Vδ6: 5’-TATCATGATCCGGACCC-3’; Vδ6: 5’-CTCTGCTGGTGCAAG-3’; Vδ3/Cδ: 5’-CTTCACAGACAA-GGCCACAT-3’. A PCR reaction master mix that was common to all samples contained 100 mM Tris HCl, pH 8.3, 500 mM KCl, 2 mM MgCl2, 200 μM dNTPs, with 25 pmoles of 3’ Cδ primer, 2.2 μCi α-32P-dCTP, and 2.5 U Taq polymerase (GIBCO BRL) per tube. The final volume was 100 μl and contained 10 ng cDNA, and 25 pmoles of individual Vδ primer. Samples were run on a thermocycler (model 9600; Perkin-Elmer Corp., Norwalk, CT) for 24 cycles using the parameters: cycle 1: 94°C × 3 min, 50°C × 45 s, 72°C × 1 min; cycles 2–23: 94°C × 30 s, 50°C × 45 s, 72°C × 1 min; cycle 24: 94°C × 30 s, 50°C × 45 s, 72°C × 7 min. Samples were resolved on a 29 cm 10% polyacrylamide gel containing 7 M urea in TBE buffer and electrophoresed at 80 V for 18 h. The gel was dried and developed on an analyzer (Betascope 603; Betagen, Waltham, MA). The percentage expression of each Vδ was assigned by dividing the actual cpm for a specific Vδ by the total cpm for Vδ1–Vδ6 after correction for the total Cδ message in each sample.

**Assay of Cytolytic Activity.** Fas<sup>low</sup> variants of the wild-type Jurkat T cell line, H7 (3% normal surface Fas levels) and B4 (1% normal Fas levels), were derived through irradiation mutagenesis using five doses of 200 Rads each, delivered at 5-d intervals. After each irradiation, cells were cultured in wells coated with hytic anti-Fas antibody (M2, 3 μg/ml)(24). The Fas<sup>low</sup> variants and wild-type Jurkat cells were incubated with ^51Cr Chromium (65Cr) for 1 h, washed, and then mixed at various effector/target ratios with cloned Vδ1 cells in a total volume of 200 μl. After a 4-h incubation at 37°C, 100 μl of supernatant were removed and counted for γ emission. Spontaneous release was determined from labeled targets in the absence of effector cells. Maximum release was determined by lysing target cells with 1.0 N HCl. The percentage of specific ^51Cr release was calculated as:

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\text{% Specific ^51Cr release} = \frac{\text{experimental cpm} - \text{spontaneous cpm}}{\text{maximal cpm} - \text{spontaneous cpm}} 
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Blocking studies of cytolysis were performed using either specific antibodies at the concentrations indicated, or Fas-Fc fusion protein (10 μg/ml) preincubated with appropriate cells for 30 min before beginning the cytolysis assay. Antibodies used were specific for TCR-γδ (5A6.E9), HLA class I (W6/32; Accurate Chemical and Science Corp., Westbury, NY), HLA class II (L243; Becton Dickinson & Co., Immunocytometry, Sys., Mountain-view, CA), LFA-1 (R7.1; Biosource International, Camarillo, CA), or Fas (M38).

**TUNEL Assay for Apoptosis.** Cells were initially stained for expression of surface γδ, CD4, or CD8 and then fixed for 15 min in 1% paraformaldehyde. Cell membranes were then permeabilized for 15 min using 70% ethanol at 4°C. Samples were incubated at 37°C for 1 h in 100 μl containing 0.5 μM dUTP-biotin (Boehringer Mannheim Biochemicals Corp., Indianapolis, IN) (31, 32). Specimens were washed twice with PBS/1% BSA and incubated with a 1:50 dilution of avidin-tricolor (Caltag Labs., South San Francisco, CA) at 4°C for 30 min. Cells were washed twice and analyzed by flow cytometry.

**Results**

**Reciprocal Changes in Synovial Fluid CD4<sup>+</sup> and γδ T Cells with Borrelia Stimulation.** Synovial fluid lymphocytes were examined from seven patients with Lyme arthritis of 6 mo to 3.2-y duration. These contained a predominance of CD4<sup>+</sup> over CD8<sup>+</sup> αβ T cells in only four of seven cases (Fig. 1 A, Table 1), compared to a consistent CD4 predominance in PBL. Also present in the synovial mononuclear cells was a remarkable percentage of γδ T cells (18.9 ± 6.8%) (Fig. 1 A, Table 1), compared to ~1–5% in PBL (Reference 1 and see Fig. 3). The synovial γδ population was largely devoid of surface CD4, and only a minor proportion (~20% on average) expressed low to intermediate levels of CD8 (Fig. 1 B).

In addition, whereas γδ T cells from PBL express predominantly the Vδ2 gene product (33), Lyme arthritis synovial fluid γδ cells were primarily of the Vδ1 subset, with lesser proportions of Vδ2 and Vδ3 cells. This was determined by both flow cytometry using Vδ-specific antibodies (Fig. 1 A), and semi-quantitative PCR using specific Vδ primers (Fig. 2).

Stimulation of Lyme arthritis synovial fluid mononuclear cells with a sonicate of B. burgdorferi (strain N40) induced vigorous proliferation (Table 1), yielding a two- to three-
fold increase in cell number over 6 d. During this period, the composition of T cell subsets shifted considerably. Although the percentage of CD8<sup>+</sup> cells changed only slightly, there was frequently a striking loss in the proportion of CD4<sup>+</sup> cells by as much as threefold. Thus, despite the increase in total lymphocyte number during the 6-d culture, there was frequently little change or even a decrease in the absolute number of CD4<sup>+</sup> cells, as illustrated by patient no. 2 in Table 2. This was paralleled by a reciprocal increase in γδ T cells, in some cases to as much as 50% of the cultured synovial lymphocytes (Fig. 1, Table 1). These continued to be mostly Vδ1 cells as determined by both antibody (Fig. 1A) and PCR (Fig. 2) analysis.

The loss of CD4<sup>+</sup> synovial cells might have resulted from unresponsiveness of this subset to <i>B. burgdorferi</i>, and hence overgrowth by the CD8<sup>+</sup> and γδ<sup>+</sup> subsets. However, this seems unlikely since we have previously observed that PBL also proliferate strongly to <i>B. burgdorferi</i> with an expansion of predominantly CD4<sup>+</sup> cells (28). Alternatively, because PBL contain only a small proportion of γδ cells (1), the γδ subset might be responsible for the loss of CD4<sup>+</sup> cells in <i>Borrelia</i>-activated synovial cultures. Consistent with this notion was the one case (patient no. 6) where the per-

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**Table 1. Phenotypic Changes in Lyme Arthritis Synovial Fluid T Cells following Borrelia Stimulation**

| Patient No. | Before Borrelia stimulation | After Borrelia stimulation | Proliferation |
|-------------|-----------------------------|---------------------------|---------------|
|             | %CD4/%CD8 | %TCR-γδ | %CD4/%CD8 | %TCR-γδ | Medium | + Borrelia |
| 1           | 29.7/45.3 | 23.8   | 9.0/39.8 | 56.8  | 5,316   | 178,964 |
| 2           | 52.6/28.2 | 20.0   | 18.0/29.6 | 45.8  | 526     | 19,122  |
| 3           | 27.8/45.7 | 15.7   | 29.0/33.2 | 29.9  | 17,369  | 82,440  |
| 4           | 64.4/16.6 | 11.1   | 55.1/8.8 | 11.8  | 2,791   | 32,418  |
| 5           | 25.4/39.5 | 13.5   | 7.4/38.3 | 41.5  | 4,516   | 51,278  |
| 6           | 36.5/18.8 | 30.8   | 51.4/28.9 | 2.9   | 2,937   | 44,474  |
| 7           | 45.4/35.3 | 11.6   | 14.0/23.6 | 25.7  | 30,230  | 89,533  |

Synovial fluid lymphocytes were isolated by Ficoll-Hypaque. Specimens were analyzed freshly isolated, or placed in serum-free AIM-V medium (GIBCO BRL) with a sonicate of <i>B. burgdorferi</i> (3 μg/ml) and re-phenotyped after 7 d. Proliferation of 5 × 10<sup>5</sup> cells/well was measured by ³H-TdR uptake during the last 18 h of culture. cpm, mean of triplicate cultures. Standard deviations were <15%.

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Figure 1. Reciprocal shifts in the percentages of γδ versus CD4<sup>+</sup> T cells after stimulation by <i>B. burgdorferi</i> of Lyme arthritis synovial fluid T cells. Synovial fluid mononuclear cells, isolated by Ficoll-Hypaque centrifugation, were analyzed either freshly isolated or 6 d after stimulation with a 3 μg/ml sonicate of <i>B. burgdorferi</i>. (A) Flow cytometric analysis of synovial fluid mononuclear cells reveals a prominent population of γδ cells that expresses mostly Vδ1 and expands dramatically following stimulation with <i>B. burgdorferi</i>. The numbers in the histograms indicate the percent of positively stained cells. (B) Synovial fluid γδ cells are predominantly CD4<sup>+</sup>-CD8<sup>-</sup>. FACS<sup>®</sup> staining demonstrates that γδ cells are largely devoid of CD4 and only a minor subset expresses low to intermediate levels of surface CD8.
percentage of γδ T cells did not increase following stimulation with *B. burgdorferi*. In this instance, the proportion of CD4+ cells actually increased from 36.5 to 51.4% (Table 1).

**Table 2.** Changes in Absolute Numbers of Synovial T Cell Subsets with *Borrelia* Stimulation

| Subset | Percent | Absolute No. (×10⁶) | Percent | Absolute No. (×10⁶) |
|--------|---------|---------------------|---------|---------------------|
| CD4+   | 52.6    | 5.8                 | 18.0    | 4.1                 |
| CD8+   | 28.2    | 3.1                 | 29.6    | 6.8                 |
| γδ+    | 20.0    | 2.2                 | 45.8    | 10.5                |

Synovial fluid lymphocytes from patient No. 2 were analyzed for surface phenotype and absolute counts were determined when freshly isolated and after seven days of stimulation with *B. burgdorferi*.

that synovial γδ cells might be cytolytic toward the CD4+ subset, γδ T cell clones were derived from synovial fluids of two Lyme arthritis patients using a sonicate of *B. burgdorferi* and irradiated autologous PBL. A panel of 18 Borrelia-responsive γδ clones was established, the majority of which express Vδ1 and lack surface CD4 and CD8. DNA sequencing of the \( \delta \) chain from seven clones confirmed that they all express Vδ1, but were otherwise each unique and contained varying degrees of N region diversity (Roessner, K., manuscript in preparation).

γδ T cells frequently manifest cytolytic activity toward a broad array of target cells (2, 16). Such a spectrum of cytolyis might occur when a target molecule is widely expressed, as is the case with the apoptosis-inducing molecule, Fas (17). As shown in Fig. 3, Fas expression by fresh CD4+ PBL was low to negligible, but was present on a large proportion of CD4+ synovial lymphocytes. By contrast, the CD8+ and γδ+ subsets of PBL or synovial lymphocytes displayed considerably lower levels of Fas.

Surface expression of Fas ligand protein by *B. burgdorferi*-reactive γδ and CD4+ αβ T cell clones was examined by flow cytometry using two methods, a Fas-Fc fusion protein as well as a purified anti-human Fas rabbit antiserum. Con-
trol staining for Fas–Fc was determined using a human IL4 receptor–Fc (IL4R–Fc) fusion protein (as surface-bound IL4 would not be anticipated for a secreted cytokine). Fig. 4 A (column 3) illustrates results of staining using the Fas–Fc fusion protein, on representative αβ (114B) and γδ (2.11) synovial T cell clones. By this method, surface FasL protein was expressed on a considerably higher proportion of the γδ cells than on the B. burgdorferi–reactive αβ T cell clones seven days after the last stimulation. Similar findings were seen with an additional two αβ and two γδ synovial T cell clones. In contrast, the levels of surface Fas antigen on the γδ clones were somewhat less than on the αβ clones, (Fig. 4 A, column 4).

The anti–FasL antibody confirmed the disparity in surface FasL expression between synovial γδ versus αβ T cell clones. Fig. 4 B (column 1) shows that 7 d after antigenic stimulation of the Borrelia–reactive αβ (114B) and γδ (2.11) clones, surface FasL was present on the γδ clone, but was only marginally detectable on the αβ clone. This finding was consistent for three αβ and three γδ clones studied. However, the αβ clones were capable of induction of FasL upon stimulation, as shown after 3 h of activation with PMA and ionomycin. In agreement with a recent report (27), FasL expression on the T cell line, Molt 4, was enhanced by blocking metalloprotease activity with EDTA (Fig. 4 B, column 4). This was less consistently observed for the αβ T cell clones, and was not observed for the γδ clones. It was particularly striking that the levels of FasL on the γδ clones remained detectable for at least 10 d following stimulation with B. burgdorferi (unstimulated), or 3 h after activation with PMA and ionomycin (iono). Cells were also analyzed in the absence or presence of the metalloprotease inhibitor EDTA in an effort to block degradation of surface FasL (27). Open numbers in histograms represent the percent of positive cells above background staining. Numbers in parentheses indicate the mean fluorescence intensity of the positively stained cells.

**Figure 4.** Lyme arthritis synovial γδ clones express high levels of FasL. (A) Surface FasL expression using a Fas–Fc fusion protein (column 3) is shown for a B. burgdorferi–reactive αβ T cell clone (114B) and a synovial γδ clone (2.11). Negative staining controls included either second step fluorescein–anti-Fc antibody alone (column 1), or initial staining with IL4R–Fc followed by second step anti-Fc antibody (column 2). Column 4 indicates the levels of Fas expression by the same clones. (B) Surface FasL expression detected by an anti–human FasL rabbit antiserum (C-20) purified using a FasL–sepharose column. Cell lines included the T cell leukemic line, molt 4, and the αβ and γδ T cell clones used in A. Shown is the control staining using rabbit immunoglobulin (faint line) superimposed on the staining with the anti–FasL antibody (dark line). T cell clones were examined either seven days after the last stimulation with B. burgdorferi (unstimulated), or 3 h after activation with PMA and ionomycin (iono). Cells were also analyzed in the absence or presence of the metalloprotease inhibitor EDTA in an effort to block degradation of surface FasL (27). Open numbers in histograms represent the percent of positive cells above background staining. Numbers in parentheses indicate the mean fluorescence intensity of the positively stained cells.
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was examined using three approaches. Initially, two Faslow variants of Jurkat cells, H7 and B4, were independently derived by radiation mutagenesis followed by selection with lytic anti-Fas antibody, M2. H7 expresses 3% of the levels of Fas found on wild-type Jurkat cells, whereas B4 displays 1% (Fig. 5B). Fig. 5A demonstrates that the efficiency of cytolysis of both Faslow variants was diminished approximately two- to threefold compared to that observed with wild-type Jurkat cells. However, lysis of the Jurkat Faslow variants was not completely eliminated, suggesting that part of the cytolytic activity of the γδ clones was independent of Fas. This was supported by anti-Fas antibody blocking studies.

Inhibition of Jurkat cell cytolysis by the γδ clones was also achieved using a nonlytic anti-Fas antibody, M38 (24). Fig. 5D shows that the blocking of cytolysis with M38 was partial, achieving 30–50% inhibition at the highest concentration of antibody (10 μg/ml), whereas control mouse Ig did not block cytolysis. In vitro cytolysis consists of a calcium-independent component mediated by Fas and a calcium-dependent component delivered by perforin (18–20). Blocking perforin action by chelation of calcium with EGTA also resulted in partial inhibition of Jurkat cytolysis, which could then be blocked almost completely by the further addition of anti-Fas antibody (Fig. 5D). A third method of disrupting Fas–FasL interaction used the Fas-Fc fusion protein. Fig. 5E shows that Fas–Fc, but not IL4R–Fc, partially blocked cytolysis of Jurkat cells by the γδ clones, though to a slightly lesser extent than did nonlytic anti-Fas antibody.

The above findings show that γδ clones derived from synovial fluid express prolonged and high levels of FasL and suggest that γδ cells preferentially lyse Fashigh cells. To directly assess whether uncloned synovial γδ cells function in a similar manner, FasLhigh γδ T cells were combined at the ratios indicated with [3H]-labeled Jurkat target T cells in a four h cytolytic assay. (A) Comparison of cytolytic activity toward wild-type Fashigh Jurkat T cells (closed squares) compared with two Faslow Jurkat variants, H7 (open squares) and B4 (open circles), which express, respectively, 3 and 1% of surface Fas levels observed on wild-type Jurkat cells. (B) Level of Fas expression on wild-type Jurkat T cells and two variants, H7 and B4, selected by repeated irradiation and culture in the presence of lytic anti-Fas antibody, M2. Number insets indicate the mean fluorescence intensity of the gated area. (C) Attempts to block γδ cytolytic activity using antibodies to HLA class I (closed squares), HLA class II (open squares), TCR-γδ (open circles), and anti-LFA-1 (closed triangles). The anti–LFA-1 study was part of a separate experiment in which the baseline cytolysis was 42%. (D) Ability of various concentrations of nonlytic anti-Fas antibody M38 to inhibit cytolysis of wild-type Jurkat cells by the Vβ1 clones. Cytolysis assay was also performed in the absence (closed square) or presence (open square) of 2.5 mM EGTA, an inhibitor of calcium-dependent perforin activity (18). Lysis in the presence of control IgG antibody (10 μg/ml) is shown by the closed triangle. (E) Inhibition of Jurkat cytolysis by the Vβ1 clones 16 and 2.11 in the presence of 10 μg/ml of either anti-Fas antibody M38, Fas-Fc fusion protein, both, or IL4R–Fc fusion protein.
sample of the same specimen after five days of stimulation with *B. burgdorferi*. During this period, the γδ cells in the nondepleted synovial sample expanded from 4.3% to 11% (Fig. 6 B). This was accompanied by a decreased proportion of CD4\(^+\) cells, from 35.6 to 25.3%. In striking contrast, the γδ-depleted population contained only 4% γδ cells after 5 d and manifested a predominance of CD4\(^+\) cells (40.8%) (Fig. 6 B). In addition, the CD4\(^+\) cells in the 4-day cultures contained a subpopulation of CD4\(^{low}\) cells which comprised a greater proportion of the total CD4\(^+\) subset in the γδ-replete than the γδ-depleted specimen (Fig. 6 B, arrow inset). These CD4\(^{low}\) cells represented apoptotic cells, as determined by the TUNEL assay combined with surface staining and analyzed by flow cytometry (Fig. 6 C).

To assess whether the appearance of the apoptotic CD4\(^{low}\) subset in the γδ-replete cultures was in part Fas-mediated, FasL was blocked using the Fas-Fc fusion protein. Synovial fluid mononuclear cells were stimulated with *B. burgdorferi* \((B.b.)\) in the presence of no additives \((\text{closed circles})\), or 10 μg/ml of either Fas-Fc \((\text{closed squares})\), or mouse IgG \((\text{open circles})\). After the indicated day of stimulation, cultures were analyzed for expression of CD4, CD8, and γδ. Shown is the expression of apoptotic CD4\(^{low}\) cells \((\text{as identified by TUNEL in Fig. 6 C})\) as a percentage of the total CD4\(^+\) cells. The results are representative of two experiments.
Discussion

The collective observations suggest an immunoregulatory circuit whereby synovial V81 T cells bearing high levels of FasL selectively restrict the expansion of infiltrating inflammatory FasLhigh CD4+ lymphocytes through cytolytic in a Fas-dependent manner. The findings are in agreement with recent studies showing that FasL mRNA expression by T cells is highest in the γδ subset (21). Not only were levels of surface FasL high on the V61 clones, they remained elevated for considerably longer periods than similarly activated αβ T cells. This may serve to explain the broad spectrum of cytolytic activity that has frequently been observed for many γδ cells (2, 16). The results parallel other recent descriptions of immunosuppression resulting from constitutive expression of FasL by Sertoli cells in the testis (22), and by components of the eye (23).

The current findings may also bear on observations that collagen-induced arthritis in mice (34) and adjuvant arthritis in rats (35) are both more severe following administration of anti-γδ antibody. Collagen-induced arthritis is also more aggressive in mice bearing a genetic deletion of the δ locus (Lefrancois, L., personal communication). Similar results have been observed in a model of orchitis in which reactive CD4+ T cells have also been observed for many γδ cells (2, 16). The results parallel other recent descriptions of immunosuppression resulting from constitutive expression of FasL by Sertoli cells in the testis (22), and by components of the eye (23). The current findings may also bear on observations that collagen-induced arthritis in mice (34) and adjuvant arthritis in rats (35) are both more severe following administration of anti-γδ antibody. Collagen-induced arthritis is also more aggressive in mice bearing a genetic deletion of the δ locus (Lefrancois, L., personal communication). Similar results have been observed in a model of orchitis in which reactive CD4+ T cells have also been observed for many γδ cells (2, 16). The results parallel other recent descriptions of immunosuppression resulting from constitutive expression of FasL by Sertoli cells in the testis (22), and by components of the eye (23).

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