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Lyme Arthritis Synovial γδ T Cells Instruct Dendritic Cells via Fas Ligand

Cheryl Collins,* Julie Wolfe,* Karen Roessner,* Cuixia Shi,* Leonard H. Sigal,† and Ralph C. Budd2*†

γδ T cells participate in the innate immune response to a variety of infectious microorganisms. They also link to the adaptive immune response through their induction of maturation of dendritic cells (DC) during the early phase of an immune response when the frequency of Ag-specific T cells is very low. We observe that in the presence of *Borrelia burgdorferi*, synovial Vδ1 T cells from Lyme arthritis synovial fluid potently induce maturation of DC, including production of IL-12, and increased surface expression of CD40 and CD86. The activated DC are then able to stimulate the Vδ1 T cells to up-regulate CD25. Both of these processes are initiated primarily by Fas stimulation rather than CD40 activation of DC via high expression of Fas ligand by the Vδ1 T cells. DC are resistant to Fas-induced death due to expression of high levels of the Fas inhibitor c-FLIP. This effect serves to divert Fas-mediated signals from the caspase cascade to the ERK MAPK and NF-κB pathways. The findings affirm the importance of the interaction of certain T cell populations with DC during the early phases of the innate immune response. They also underscore the view that as levels of c-FLIP increase, Fas signaling can be diverted from induction of apoptosis to pathways leading to cell effector function. The Journal of Immunology, 2005, 175: 5656–5665.

The innate immune response is designed to respond rapidly to infection as a first line of defense. To accomplish this, it uses an array of nonpolymorphic receptors, such as TLR and CD1 molecules, which are designed to recognize conserved motifs of microorganisms (1–5). Among the cell types that express these receptors are dendritic cells (DC), which are critical for linking innate immunity to the second phase adaptive immune response (6, 7). Immature DC are programmed for uptake of foreign Ags for processing, whereas mature DC are highly efficient at presentation of Ags and costimulatory molecules that are necessary for the activation of naive T cells (1, 8–10). Maturation of DC results in up-regulation of surface MHC, costimulatory molecules CD80 and CD86, and secretion of IL-12 in response to IFN-γ (11). In vitro, this mature stage may be as brief as 12 h, after which mature DC are no longer able to secrete IL-12 (11, 12). Members of the TNF-α superfamily can promote DC maturation, including CD40L and TNF-α, which are derived from certain activated T cells in addition to other cell types (11). However, these maturation cues are not likely to be provided by naïve Th cells, as both the frequency of Ag-specific naïve T cells and their effector function are too limited at the initiation of an immune response to promote rapid DC maturation.

A link between the innate and adaptive immune responses can be provided by T lymphocytes that react to the CD1 family or directly to microbial products. This includes NKT cells that respond to CD1d and human γδ T cells of the Vδ1 subset that recognize CD1c (3, 4, 13–15). In addition, the Vδ2 subset can recognize small nonpeptidic phosphorylated Ags derived from *Mycobacterium* (16, 17), certain bishophophonates (18), alkylamines (17), and the association of membrane F1-ATPase with apolipoprotein A-I (19) in the absence of classical MHC molecules. The contribution of γδ T cells to defense from infection has been examined in mice in a number of model systems including *Listeria* (20), *Leishmania* (21), *Mycobacterium* (22), *Plasmodium* (23), and *Salmonella* (24). All of these studies have shown a protective role for γδ T cells. However, in other infectious models subsets of γδ T cells can have opposing effects. Thus, in murine *Coxackievirus*-induced myocarditis, the Vγ1 subset was protective, whereas the Vγ4 subset promoted disease (25).

γδ T cells also accumulate at inflammatory sites in autoimmune disorders such as rheumatoid arthritis (26), celiac disease (27), and sarcoidosis (28). The reason for this effect remains largely unknown. Some evidence suggests that γδ T cells may have a regulatory role in certain autoimmune models to suppress inflammation. Collagen-induced arthritis in mice (29), adjuvant arthritis in rats (30), and a murine model of orchitis (31) are all worse after depletion of γδ T cells. Similarly, MRL/lpr mice lacking γδ T cells develop a more aggressive lupus-like illness (32).

Lyme arthritis represents an inflammatory synovitis caused by infection with *Borrelia burgdorferi*, but can also result in a persistent antibiotic-resistant arthritis after *B. burgdorferi* has been eradicated (33). This can manifest features of an autoimmune arthritis resembling rheumatoid arthritis (34). In addition, although γδ T cells are found at low levels in peripheral blood, the Vδ1 subset is increased to considerable levels in Lyme arthritis synovial fluid (35). We have previously observed that these synovial...
V61 cells are intensely cytolytic due to expression of high and sustained levels of Fas ligand (Fasl) (35). However, we now observe that monocyte-derived DC are resistant to lysis by V61 clones. In exploring the mechanism for this Fas resistance we find that DC express high levels of the Fas inhibitor, c-FLIP, whereas the monocytes manifest very low c-FLIP expression.

c-FLIP is a homologue of caspase-8 but bears a mutation in the caspase domain that renders it enzymatically inactive (36, 37). As such, c-FLIP acts as a competitive inhibitor for recruitment of caspase-8 to Fas-associated death domain (FADD) protein following Fas ligation (38). c-FLIP has additional functions given its ability to associate with TNFR-associated factor (TRAF1), TRAF2 and RIP1, which activate the NF-κB pathway, as well as with Raf-1, which activates the ERK MAPK pathway (39). Increased expression of c-FLIP can thus not only inhibit Fas-induced caspase-8 activation, it can also divert Fas signals toward the Raf-1, which activates the ERK MAPK pathway (39). Increased expression of c-FLIP can thus not only inhibit Fas-induced caspase-8 activation, it can also divert Fas signals toward the Raf-1, which activates the ERK MAPK pathway (39).

**Materials and Methods**

**Patients**

Lyme arthritis patients were followed at the Lyme Disease Clinic at the University of Medicine and Dentistry of New Jersey (Robert Wood Johnson Medical School, New Brunswick, NJ). All patients had histories, examinations, and serologies consistent with Lyme arthritis. Each had Abs to *B. burgdorferi* in both synovial fluid and serum detected by ELISA and confirmed by immunoblot.

**Derivation of synovial fluid lymphocytes and T cell clones and monoocyte-derived DC**

Lymphocytes were purified from synovial fluid by Ficoll-Hypaque centrifugation (Sigma-Aldrich), and cultured in AIM-V medium (Invitrogen Life Technologies) containing 2% FBS (HyClone) and 50 U/ml human rIL-2 (Costar). Complete medium was supplemented with 10 μg/ml sonicate of *B. burgdorferi* strain N40, grown in BSK II medium (Barbour-Stoenner-Kelly II medium; Sigma-Aldrich) as previously described (35, 45). From these bulk cultures, responding cells were cloned at 0.3 cells/well in complete medium, responding cells were cloned at 0.3 cells/well in complete medium, responding cells were cloned at 0.3 cells/well in complete medium, responding cells were cloned at 0.3 cells/well in complete medium, responding cells were cloned at 0.3 cells/well in complete medium.

**Abs and flow cytometry**

Abs were to the determinants CD4 (S3.5; Caltag Laboratories), CD8 (53-1; Alexis), CD40L (24-31; a gift of Dr. R. Noelle, Dartmouth Medical School, Hanover, NH), IFN-γ (MAB25718; R&D Systems), IL-12 (clone 8.6; Endogen), and TNF-α (MAB1; BD Pharmingen).

Chemical fixation of DC was performed by incubating the cells in the presence of *B. burgdorferi* at 10 μg/ml at 37°C in 7% CO2 overnight, then washed in 5% FBS/RPMI 1640 (Mediatech), and fixed by the addition of ice-cold EDCI (1-ethyl-3-(dimethylamino)propyl)-carbodiimide (Pierce) at 75 mM in PBS for 60 min on ice. Following fixation the cells were extensively washed with 5% FBS/RPMI 1640.

**Cytokine quantitation**

Quantitation of specific cytokines (IL-12p70, IFN-γ, and IL-1β) in cell culture supernatants was performed using sandwich ELISA kits (Biosource International) according to the manufacturer’s protocol. Briefly, specific supernatants, along with appropriate standards and controls, were added to microtiter wells previously coated with a mAb specific for each cytokine. A second cytokine-specific biotinylated mAb was added at the same time. The mixture was then allowed to incubate for a specified time for each cytokine. ELISA according to the manufacturer’s protocol. Excess biotinylated Ab was removed by extensive washing, followed by the addition of a solution containing streptavidin-HRP. Excess unbound enzyme was removed by extensive washing, followed by the addition of a chromogenic enzyme substrate solution. Plates were read on a Bio-Tek plate reader model ELx800 (Bio-Tek Instruments) at 450 nm.

**Assay of cytolytic activity**

Target DC were labeled by incubation with 31Cr for 1 h, washed three times, and then mixed in 200 μl of complete medium at a 1:1 ratio with HEK 293 cells that were either mock-transfected (293) or transfected with human Fasl (293/Fasl) bearing a mutation at the membrane proximal proteolytic cleavage site, which was a gift of Dr. P. Schneider (University of Lausanne, Lausanne, Switzerland). Target cells were also treated with soluble FLAG-tagged Fasl (Apotech) that was cross-linked with anti-FLAG Ab M2 (Sigma-Aldrich), or with anti-FLAG alone. After 4 h at 37°C, 100 μl supernatant was removed and counted for gamma emission. Supernatant was analyzed using a reference-pooled sample (calibrator), which was included in each assay. Maximal release was determined by labeling targets with 1.0 N HCl. The percentage of maximal 31Cr release was calculated as (experiment cpm − spontaneous cpm)/maximal cpm − spontaneous cpm.

**Real-time quantitative PCR**

Primers for human Fasl were designed to amplify an 84-bp fragment. Primers were: forward 5′-TGCGGCAATTTACAGGCGA-3′ and reverse 5′-CCAGAGACGGCGAATTCICA-3′. The amplified fragment contained the sequence bound by the fluorochrome-labeled primer: 5′-FAM TCCAACATCAAGGTTGACAAGTTGTTAG TMARA-3′ (Biosearch Technologies). Control amplification was assessed using endogenous control 18S ribosomal RNA (PE Biosystems) labeled with a VIC reporter dye. RNA was extracted from cells using Ultraspec (Biotech Laboratories), or by guanidinium thiocyanate (Ambion), and cDNA was made using Superscript reverse transcriptase (Invitrogen Life Technologies). Quantitative PCR was performed using a 7700 Sequence Detection System (Applied Biosystems) at the Vermont Cancer Center DNA Analysis Facility (Burlington, VT). Fluorescence signal was expressed as normalized reporter (R) signal, which represents the reporter signal (FAM or VIC) divided by the fluorescence signal of a passive reference dye (ROX). Validation control experiments were performed to measure efficiency of the target (Fasl) and reference (18S) gene amplifications over a range of 3 logs of sample dilution. All assays were run in duplicate and corrected to a reference-pooled sample (calibrator), which was included in each separate run. Threshold cycle (Ct) values were expressed as 2−ΔΔCt (Ct Fasl−Ct 18 S).

Primers for human CD40L were designed to amplify a 74-bp fragment. The primers were: forward 5′-ATCCACAGTTCGCCCAAAC-3′ and reverse 5′-GACCTTGTTTGAATTCATATG-3′. The amplified fragment contained the sequence bound by the fluorochrome-labeled probe: 5′-FAM TCGGGGCAAAATCCATCTACCTTG BHQ-1-3′ (Biosearch

**T cell and DC cocultures**

Cultures of DC with V61 clone cells were done at a ratio of 1:1 (each 1 × 10⁶/ml) in complete medium. Supernatants were removed after 24 h for cytokine analysis by ELISA, and cells were stained for measurement of TCR-γ, CD25, CD40, CD40L, and HLA-DR. Inhibition studies were done using blocking Abs to Fasl (5G31; Alexis), CD40L (24-31; a gift of Dr. R. Noelle, Dartmouth Medical School, Hanover, NH), IFN-γ (MAB25718; R&D Systems), IL-12 (clone 8.6; Endogen), and TNF-α (MAB1; BD Pharmingen).

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**Abs and flow cytometry**

Abs were to the determinants CD4 (S1.5; Caltag Laboratories), γδ TCR (5A6E9; Caltag Laboratories), human Fasl (DX2; BD Pharmingen), CD25 (2D3-5G10; Caltag Laboratories), CD80 (MEM-233; Caltag Laboratories), CD86 (BU63; Caltag Laboratories), CD40 (14G7; Caltag Laboratories), HLA-DR (TDR31.1; Ancell), and human Fasl (monoclonal ALF2.1a; Ancell, or monoclonal NOK-1; BD Pharmingen). Surface Fasl was analyzed using the catalyzed reporter deposition system of enzymatic amplification staining (EAS kit; Flow-Amp Systems) (47). Cells were stained as previously described (44). Samples were analyzed on an upper Coulter Elite (Coulter), or BD LSR II flow cytometer (BD Biosciences). At least 2 × 10⁶ events were accumulated for analysis.
CD40L is a principal molecule by which CD4 levels of CD40L. As shown in Fig. 2, dorferi CD25 expression on the V1 subset of the presence of B. burgdorferi and exogenous IL-2 (46). Although the determinant recognized by these V61 cells is not known, a strong induction of CD25 expression on the V61 cells was apparent after 24 h of stimulation by DC that were pulsed for 16 h with a sonicate of B. burgdorferi (Fig. 1A). Fixation of DC with EDCI following an overnight incubation with B. burgdorferi reduced the ability of DC to activate V61 cells to either up-regulate CD25 (Fig. 1A) or to produce IFN-γ (data not shown). This suggested that full activation of V61 cells required metabolically active DC. In addition to CD25 expression, the production of IFN-γ by the V61 cells, and production of bioactive IL-12 (p70) by DC were both considerably augmented when the V61 cells and DC were cocultured in the presence of B. burgdorferi (Fig. 1, B and C). Culture supernatants from B. burgdorferi-pulsed DC were insufficient to activate V61 cells, and vice versa, demonstrating that cell contact was necessary (data not shown).

Expression of low levels of CD40L and high levels of FasL by V61 cells
CD40L is a principal molecule by which CD4+ αβ T cells activate DC (48). Whereas B. burgdorferi-reactive CD4+ αβ T cell clones expressed high levels of surface CD40L upon activation, the synovial-derived V61 cell clones expressed very low to negligible levels of CD40L. As shown in Fig. 2, A and B, following B. burgdorferi stimulation, the CD4+ αβ T cell clones expressed high levels of surface CD40L for at least 3 days before declining to baseline levels. By contrast, following B. burgdorferi stimulation, V61 cells expressed nearly undetectable levels of surface CD40L over the same period of time (Fig. 2, A and B). This finding was also true of fresh γδ T cells in Lyme synovial fluid (Fig. 2, A and B). These findings were similar at the message level over an 18-day period using quantitative PCR (Fig. 2C).

The opposite pattern of expression was observed for FasL (Fig. 3). Although both αβ and γδ B. burgdorferi-reactive T cells quickly increased surface FasL following stimulation, the αβ T cells rapidly down-regulated surface FasL, whereas the V61 cells maintained FasL expression for at least 16 days (Fig. 3B), and in further studies for as long as 30 days (data not shown). This was also apparent at the mRNA level (Fig. 3C). The findings are also consistent with our previous observations of intense cytolytic activity by synovial V61 cells (49).

DC are resistant to Fas-mediated death due to high level expression of c-FLIP
Although V61 cells are highly cytolytic to several cell types, they did not lyse immature myeloid DC (data not shown). As Fas expression was very high on DC (Fig. 4A), this suggested an internal mechanism of resistance. c-FLIP is the natural inhibitor of Fas-induced cell death as it competes with caspase-8 for recruitment to DED.

**FIGURE 1.** Mutual activation of V61 cells and DC in the presence of B. burgdorferi. Synovial V61 clone Bb03 and immature myeloid DC were either cultured separately or cocultured at a 1:1 ratio (each 1 × 10^5 cells/ml) overnight in the absence or presence of 10 μg/ml sonicate of B. burgdorferi as indicated (+). Cells were then stained for expression (A) of CD25 on the V61-gated subset, or supernatants examined for production of IFN-γ (B) by V61 clones or IL-12 (C) by DC. In some cultures in A, DC were first cultured overnight with B. burgdorferi sonicate alone, then washed and left either unfixed (un) or fixed (fx) with EDCI. Shown are the findings from one of five similar experiments. Statistically significant increase (+) in CD25 (p = 0.0006), IFN-γ (p = 0.0132), and IL-12 (p = 0.0025) for cultures of DC plus Bb03 in the presence of B. burgdorferi compared with its absence.
FADD in the death-inducing signal complex (38). Levels of c-FLIP were thus examined by immunoblot from lysates of the precursor CD14+/H11001 monocytes and the resulting immature DC following 7 days culture with GM-CSF and IL-4. As shown in Fig. 4B, the levels of c-FLIP were nearly undetectable in peripheral blood CD14+/H11001 monocytes but were greatly induced in the resulting DC. Consistent with the high levels of c-FLIP expression, DC were also extremely resistant to Fas-mediated cell death. As a positive control, Jurkat T cells that expressed high levels of surface Fas were very sensitive to cell death using either soluble FasL or 293 cells stably transfected with 293hFasL (Fig. 4C). In contrast, Jurkat cells stably transfected with c-FLIP were highly resistant to Fas-induced death (data not shown). In a similar manner, day 7 immature DC resembled the c-FLIP-transfected Jurkat cells in that they were also almost completely resistant to Fas-mediated cell death by either means of FasL exposure (Fig. 4C).

Fas activates ERK and NF-κB in DC

We have previously established in cell lines that c-FLIP can promote the activation of the MAPK, ERK, through its association with Raf-1 (39). Similarly c-FLIP can also augment NF-κB activity through association with TRAF1, TRAF2, and RIP1 (39). We therefore considered that ligation of Fas on DC might result in increased effector function, rather than cell death. Consistent with this view, stimulation of DC by soluble cross-linked FasL for 30 min resulted in rapid phosphorylation of ERK as well as the NF-κB inhibitor, IκB (Fig. 5), which primes IκB for ubiquitination and degradation.

To more effectively define whether Fas ligation on DC resulted in alterations in function, Fas was ligated on DC by three different methods: soluble cross-linked FasL, agonistic IgM anti-Fas Ab, and 293hFasL cells. Immature DC spontaneously produced very little IL-12, but following stimulation with soluble cross-linked FasL or agonistic anti-Fas Ab there was a significant increase in IL-12 production (Fig. 6A). A particularly dramatic induction of IL-12 by DC was observed using the 293hFasL cell line, whereas the mock-transfected 293 cells did not augment IL-12 production (Fig. 6B). The 293hFasL cells were not the source of IL-12 as none was detected in culture supernatants of 293hFasL cells or in intracellular staining of these same cells (data not shown). The findings were consistent in three separate experiments.

Alterations in the surface phenotype of DC was also apparent after Fas stimulation. Following a 24 h stimulation by soluble FasL, the surface expression increased for both CD40 and CD86 (Fig. 7). A similar change of phenotype was also apparent using anti-Fas Ab (data not shown).
FIGURE 3. Vβ1 cells express high and sustained FasL. The same clones and synovial fluid lymphocytes as in Fig. 2 were analyzed in a similar manner for expression of FasL following stimulation with B. burgdorferi. A, FACS profiles of one of five similar experiments for surface FasL on day 10 after activation. B, Temporal expression of surface FasL over 15 days. C, Representative quantitative PCR for FasL on day 13 after activation.

FIGURE 4. Immature myeloid DC express high levels of c-FLIP and are resistant to Fas-induced cell death. A, Expression of surface Fas by immature DC. B, Immunoblot of c-FLIP and control actin for cell lysates from Jurkat T cells stably transfected with c-FLIP, fresh CD14⁺ monocytes from peripheral blood, and immature myeloid DC derived from the monocytes after 7 days culture in GM-CSF and IL-4. C, Cytotoxicity measured by ⁵¹Cr release from labeled DC or Jurkat T cells not overexpressing c-FLIP, in the presence for 4 h of 293 mock-transfected cells, 293hFasL-expressing cells, anti-FLAG Ab alone, and soluble FLAG-tagged FasL (sFasL) oligomerized with anti-FLAG.
Mutual activation of V\textit{B}1 T cells and DC via surface FasL.

Given the high and sustained levels of surface FasL expressed by the synovial V\textit{B}1 clones, combined with their very low levels of CD40L expression, it was of interest to assess to what extent, if any, these γδ T cells would stimulate DC via FasL, and whether this would render the DC competent to activate the V\textit{B}1 clones. For these studies we chose two representative V\textit{B}1 clones, one that expresses very high levels of FasL (Bb01) and a second that expresses moderate and sustained levels of FasL (Bb03) (see Fig. 3C). Both clones express negligible surface CD40L (Fig. 2C).

Each clone was mixed with an equal number of DC in the presence of \textit{B. burgdorferi} sonicate for 18 h and then supernatants were assayed for IL-12 and IFN-γ secretion, followed by surface staining of CD86 on DC and CD25 up-regulation on the V\textit{B}1 clones.

As shown in Fig. 8A, DC production of IL-12 was greatly augmented by the FasL\textit{high} V\textit{B}1 clone, Bb01, and to a lesser extent by the FasL\textit{moderate} V\textit{B}1 clone, Bb03. The ability to induce IL-12 production by DC was extensively extended by blocking anti-FasL Ab in a dose-dependent manner, though not by isotype control IgG. The degree of inhibition by anti-FasL was also proportional to the level of FasL expressed by the V\textit{B}1 clone. Thus, stimulation of DC IL-12 by Bb01 was blocked nearly 75% with anti-FasL at 20 μg/ml, whereas the same concentration of anti-FasL blocked IL-12 induced by Bb03 × 50%. The effectiveness of the blocking anti-FasL Ab was demonstrated by its ability to completely inhibit cytokysis of Jurkat T cells by the 293hFasL cells (data not shown). These findings were consistent in five experiments. In addition, IFN-γ is a known stimulatory cytokine for IL-12 production by DC (50). Given that the synovial V\textit{B}1 clones produce IFN-γ, it was not surprising that inhibition of IFN-γ also partly blocked IL-12 production by the DC (Fig. 8A). In a similar manner, CD86 expression by DC, which was induced by the V\textit{B}1 clones, was blocked by anti-FasL though not by anti-IFN-γ or control IgG (Fig. 8B). Consistent with the low level CD40L expression by the V\textit{B}1 clones, blocking anti-CD40L had no effect on either CD86 expression (Fig. 8C) or IL-12 production (data not shown) in this system. These findings were consistent in three separate studies.

As noted earlier, DC cultured in the presence of \textit{B. burgdorferi} can induce the V\textit{B}1 clones to up-regulate surface CD25 and to secrete IFN-γ. Expression of CD25 by V\textit{B}1 cells was greatly reduced in the presence of anti-FasL compared with control IgG (Fig. 9A). In a manner similar to the stimulation of DC, the inhibition of CD25 expression by anti-FasL was proportional to the expression of FasL by the V\textit{B}1 clone. Thus, induction of surface CD25 on FasL\textit{high} Bb01 was more effectively blocked by anti-FasL than was CD25 expression on FasL\textit{moderate} Bb03. In contrast to anti-FasL, neither anti-IFN-γ (Fig. 9A) nor anti-IL-12 (Fig. 9B) had any effect on CD25 expression by the V\textit{B}1 clones in three separate experiments, whereas anti-IL-12 very efficiently inhibited production of IFN-γ (Fig. 9C). Thus, despite the absence of significant CD40L expression by synovial V\textit{B}1 clones, there is nonetheless a strong mutual stimulation between DC and V\textit{B}1 cells that is driven largely by FasL. This extends the number of cell types that, in the presence of high levels of c-FLIP, can divert Fas-mediated signals from a death pathway toward activation and effector function.

Discussion

The current findings extend the potential functions of the subset of γδ T cells expressing the V\textit{B}1 TCR through its high and sustained expression of FasL. Immature myeloid DC are resistant to Fas-mediated cell death due to their up-regulated expression of c-FLIP during differentiation from peripheral blood monocytes in the presence of GM-CSF and IL-4. c-FLIP prevents Fas signaling from propagating down the caspase cascade, and diverts it toward activation of ERK and NF-κB. This results in the secretion of IL-12 by DC and up-regulation of surface markers such as CD86 that are important to activation of naïve T cells in the adaptive immune response. This renders DC capable of promoting CD25 up-regulation on the V\textit{B}1 cells in the presence of \textit{B. burgdorferi}. A mutual
costimulation thus results between Vδ1 cells and DC, which involves to a large extent interactions of Fas/FasL rather than CD40/CD40L.

A growing number of cell types have been shown to respond to Fas signaling with increased proliferation or differentiation rather than cell death. This includes fibroblast growth (42), cardiac myocyte hypertrophy (51), neurite outgrowth (41), hepatocyte regeneration (43), and growth of certain tumors (52). However, the signaling pathway leading to this unexpected effect was not defined. We have previously observed that increased expression of the death receptor inhibitor c-FLIP is able to block activation of the caspase cascade and hence cell death by Fas, and simultaneously divert signals toward the NF-κB and ERK MAPK pathways (39).

c-FLIP is homologous to caspase-8 in containing two N-terminal death effector domains, but lacks the critical C-terminal cysteine at the caspase enzymatic pocket, rendering c-FLIP enzymatically inert. As such, c-FLIP functions as a competitive inhibitor of caspase-8 recruitment to FADD following Fas ligation (38). The ability of c-FLIP to activate the NF-κB and ERK pathways derives from the ability of c-FLIP to bind to RIP1, TRAF1, TRAF2, and Raf1 (39). The model of Fas signaling through c-FLIP thus may not require caspase activation. This idea is consistent with our finding that blocking caspase activity did not alter Fas-induced production of IL-12 or IL-12 up-regulation of CD86 (data not shown). Based on these qualities c-FLIP is an ideal candidate for switching Fas signals from the caspase cascade toward those associated with growth or differentiation. This would also be consistent with the finding that freshly isolated peripheral blood CD14+ monocytes express very low levels of c-FLIP and are very sensitive to Fas-induced death. This is consistent with recent reports that T cells can kill activated macrophages via Fas-FasL interactions (53). By contrast, the immature DC derived from monocytes have high levels of c-FLIP and are extremely resistant to Fas-induced death.

Our findings are consistent with those of Rescigno et al. (40) who also observed that Fasl stimulation of human or mouse DC increased production of IL-12 and up-regulated MHC class II, CD40, and CD86. However, those studies did not suggest a signaling pathway nor did they show any interaction of DC with Vδ1 T cells. Because both CD4+ and CD8+ T cells express Fasl transiently upon activation, this mechanism of DC activation may be more widely applied than to just Vδ T cells. Lack of Fas expression by DC may contribute in part to the susceptibility of Fas-deficient lpr mice to certain infections such as with Toxoplasma gondii (54, 55).

FIGURE 8. Vδ1 clones stimulate DC via FasL. Fasl

A

B

C

Vδ1 clones stimulate DC via FasL. Fasl

\[ \text{Vδ1 clones Bb01 and Fasl moderate Vδ1 clone Bb03 were cultured for 16 h in the presence of immature DC, B. burgdorferi, and either murine IgG (mIgG), blocking anti-FasL at the indicated concentrations, or blocking IFN-γ, CD40L, or TNF-α (each 20 μg/ml) and supernatants assessed for IL-12 production (A) by Vδ1 clones using ELISA, or up-regulation of surface CD86 expression by (B and C) DC as assessed by FACS. The findings are representative of three experiments. Shown are the mean ± SD for DC for IL-12 production. Statistically significant decrease (\*) in IL-12 production between control murine IgG vs anti-Fasl at 20 μg/ml (Bb01 α = 0.0077 and Bb03 α = 0.067), 10 μg/ml (Bb01 α = 0.0110 and Bb03 α = 0.0129), and anti-IFN-γ at 20 μg/ml (Bb01 α = 0.0098 and Bb03 α = 0.0051).} \]
Cell-to-cell contact between synovial V81 cells and DC was necessary to produce maximal activation of each cell type. However, soluble factors subsequently produced by both cells were instrumental in achieving full activation. Thus, IFN-γ production by V81 cells contributed considerably to IL-12 production but not CD86 up-regulation by DC. Conversely, IL-12 production by DC promoted secretion of IFN-γ but not CD25 expression by the V81 cells. This cytokine interplay may be partly responsible for the previously reported ability of V81 cells to promote a Th1 cytokine environment in a Fas-dependent manner during the immune response to Coxsackievirus (56). Furthermore, in contrast to activated αβ T cells, CD40L had little influence in the interaction between V81 cells and DC as the V81 cells expressed negligible levels of CD40L and blocking this had no effect of the ability of the V81 cells to activate immature DC.

The transgenic expression of FasL by different tissues was initially considered as a potentially effective method to deplete the immune system of T cells reactive with that tissue. In many of these cases the opposite result was observed, with augmented tissue injury. Tumor cells expressing FasL were rejected even faster than the parent clone, due in part to activation of macrophages via FasL to secrete chemokines that recruited polymorphonuclear neutrophils (57). A related study of NOD mice observed that islet cells transgenically expressing FasL were more sensitive to diabetogenic T cells in a FasL-dependent manner (58). These FasL+ β islet cells also resulted in more rapid rejection of pancreases transplanted under the kidney capsule of allogeneic mice. Thus, FasL expression, either normally by activated T cells, or ectopically in target tissues, can lead to enhanced activation of responding T cells, possibly through stimulation of DC and subsequent recruitment of other inflammatory cells.

γδ T cells likely make important contributions in the response to various infections as well as in autoimmune conditions. These include protective roles in infectious models of tuberculosis (22), listeriosis (20), malaria (23), leishmaniasis (21), and salmonellosis (24). In humans the Vγ2Vδ2 subset of γδ T cells increases substantially in response to infection with Mycobacterium tuberculosis, Brucella melitensis, Listeria monocytogenes, and Ehrlichia chaffeensis (59), and has been shown to respond to small non-peptide Ags that include alkylphosphate (16, 60), alkylamine (17), and bisphosphonate (17). Whereas Vγ2Vδ2 T cells represent the major γδ cells in peripheral blood (61), those expressing the V81 TCR are distributed largely in tissues, including intestine, spleen, and inflamed joints (62). V81 cells are also increased during infection with HIV (63, 64) and Plasmodium (65). In the latter case this may also represent depletion or anergy of Vδ2 cells that were activated by the parasite’s alternative pathway of isoprenoid synthesis. It is not currently known how synovial V81 cells maintain high levels of

![Figure 9](https://example.com/figure9.png)

**FIGURE 9.** DC activate V81 cells via Fas signaling and IL-12 production. FasL<sup>high</sup> V81 clone Bb01 and FasL<sup>moderate</sup> V81 clone Bb03 were activated with DC pulsed with *B. burgdorferi* in the presence of control murine IgG (mIgG), or blocking Abs to human FasL, IFN-γ, or IL-12. After 16 h culture, the V81 clones were examined for surface CD25 expression (A and B) and IFN-γ production (C) in supernatants using ELISA. Statistically significant decreases (*) in CD25 expression by V81 clone plus DC plus *B. burgdorferi* in the presence of blocking anti-FasL at 20 µg/ml (Bb01 p = 0.0008 and Bb03 p = 0.0010), 10 µg/ml (Bb01 p = 0.0010 and Bb03 p = 0.0019), 5 µg/ml (Bb01 p = 0.0061 and Bb03 p = 0.0041). Differences with blocking anti-IFN-γ or anti-IL-12 were not significant. Statistically significant inhibition (+) of anti-IFN-γ production by anti-IL-12 (p = 0.0247).
of Fas-L. This T cell subset nonetheless represents an important link between the innate and adaptive immune responses through Fas stimulation of DC. 

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References


