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*J Immunol* 2000; 165:3782-3789; doi: 10.4049/jimmunol.165.7.3782

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Type 1 IFN Maintains the Survival of Anergic CD4+ T Cells

Giovanna Lombardi,1† Pádraic J. Dunne,2* Dagmar Scheel-Toellner,3 Tina Sayal,3 Darrell Pilling,§ Leonie S. Taams,* Paul Life,§ Janet M. Lord,§ Mike Salmon,§ and Arne N. Akbar3#

Anergic T cells have immunoregulatory activity and can survive for extended periods in vivo. It is unclear how anergic T cells escape from deletion, because both anergy and apoptosis can occur after TCR ligation. Stimulation of human CD4+ T cell clones reactive to influenza hemagglutinin peptides can occur in the absence of APCs when MHC class II-expressing, activated T cell clones present peptide to each other. This T:T peptide presentation can induce CD95-mediated apoptosis, while the cells that do not die are anergic. We found that the death after peptide or anti-CD3 treatment of a panel of CD4+ T cell clones is blocked by IFN-β secreted by fibroblasts and also by IFN-α. This increases cell recovery after stimulation, which is not due to T cell proliferation. This mechanism for apoptosis inhibition rapidly stops protein kinase C-δ translocation from the cytoplasm to the nucleus, which is an early event in the death process. A central observation was that CD4+ T cells that are rescued from apoptosis after T:T presentation of peptide by IFN-αβ remain profoundly anergic to rechallenge with Ag-pulsed APCs. However, anergized cells retain the ability to respond to IL-2, showing that they are nonresponsive but functional. The prevention of peptide-induced apoptosis in activated T cells by IFN-αβ is a novel mechanism that may enable the survival and maintenance of anergic T cell populations after TCR engagement. This has important implications for the persistence of anergic T cells with the potential for immunoregulatory function in vivo. The Journal of Immunology, 2000, 165: 3782–3789.

The activation and expansion of T lymphocytes are central events in the majority of immune responses. However, excessive T cell activation must be controlled to prevent the development of nonspecific immunopathology (1). Both anergy and apoptosis can regulate T cell responsiveness and contribute to the generation of peripheral tolerance to self-Ags (2). Anergic T cells have been shown to persist for extended periods in vivo (3, 4). In addition, these cells exhibit immunoregulatory activity and can inhibit the proliferation of responsive T cells in an Ag-specific manner in humans (5), rats (6), and mice (7). Anergic T cells must therefore be able to escape apoptosis after TCR ligation during antigenic encounters, but mechanisms by which this is achieved have not been identified.

To determine the relationship between apoptosis and anergy as a consequence of TCR ligation, we added specific peptide to human CD4+ T cell clones. The resulting T:T presentation of this peptide by T cells to each other in the context of MHC class II induces apoptosis in a proportion of the responding cells, while the non-deleted cells are nonresponsive to subsequent challenge with Ag-pulsed APCs (6, 8–10). Previous studies have demonstrated that IFN-β secreted by fibroblasts and IFN-α secreted by other cell types can prevent cytokine deprivation-induced T cell apoptosis in both humans (11, 12) and mice (13), achieved in part by the up-regulation of Bel-7, but not Bel-2, expression (12, 14, 15). Fibroblast-conditioned medium (FCM)4 and IFN-αβ could also prevent the translocation of the signaling molecule protein kinase C-δ (PKC-δ) from the cytoplasm to the nucleus of IL-2-deprived T cell lines, which prevented its activation by caspase-3 (11). The active form of PKC-δ can induce apoptosis when transfected into hemopoietic cells (16, 17), and specific activation of PKC-δ by Bistratene A could lead to death (16–18). These results prompted us to investigate whether FCM and IFN-αβ can also prevent apoptosis resulting from TCR ligation during T:T presentation of Ag and whether these CD4+ T cells are responsive to subsequent challenge with Ag-pulsed APC. We demonstrate that fibroblast-secreted IFN-β inhibits the peptide-induced, CD95-mediated apoptosis of CD4+ T cell clones and that this rescue from death enables the persistence of an anergic T cell population.

Materials and Methods

Monoclonal Abs and cytokines

IFN-α and IFN-β were obtained from PeproTech (London, U.K.) and BioSource Europe (Nivelles, Belgium). IL-2 was obtained from R&D Systems (Abingdon, U.K.) and Roche (Mannheim, Germany). Sheep anti-human IFN-β and anti-hepatocyte growth factor (anti-HGF) Abs were obtained from Sigma (Poole, U.K.). Anti-CD95 blocking Abs were a gift from Immunex Research and Development Corp. (Seattle, WA). Anti-CD-95-inducing Ab, which triggers death, was obtained from Upstate Biotechnology (Lake Placid, NY). The MR6 (IgG1) Ab was used as a control. Neutralizing sheep anti-human IFN-β Ab and a control sheep anti-human HGF Ab were obtained from Serotec (Oxford, U.K.). Polyclonal Abs specific for STAT-1, -3, or -5 and affinity-purified rabbit anti-human PKC-δ Ab were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Abbreviations used in this paper: FCM, fibroblast-conditioned medium; PKC-δ, protein kinase C-δ; HGF, hepatocyte growth factor; HA, hemagglutinin; B-LCL, B lymphoblastoid cells; AICD, activation-induced cell death.

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Received for publication May 3, 2000. Accepted for publication July 13, 2000.

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This work was supported by a Medical Research Council/Rhone-Poulenc Rorer studentship (to P.J.D.), The Sir Jules Thorne Charitable Trust (to L.T.), and a Glaxo-Wellcome/Arthritis and Research Campaign studentship (to M.S., D.P., and S.T.). The Sir Jules Thorne Charitable Trust (to L.T.), and a Glaxo-Wellcome/Arthritis and Research Campaign studentship (to M.S., D.P., and S.T.). The Sir Jules Thorne Charitable Trust (to L.T.), and a Glaxo-Wellcome/Arthritis and Research Campaign studentship (to M.S., D.P., and S.T.).

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Induction of energy in CD4+ T cell clones

The stimulation and maintenance of three human CD4+ clones, 7P.24, 7P.41, and 7P.61, which are specific for influenza hemagglutinin (HA$_{307-319}$), restricted by HLA-DRB1*0701, and derived from an HLA-DRB1*0701 DRB1*1001 individual have been described in detail previously (10). Additional human CD4+ T cell clones used in this study include NF4, which is specific for HA$_{307-319}$ and restricted by DRB1*0101, and HC3, which is specific for HA$_{90-112}$ restricted by DRB1*1010, and obtained from a DRB*0101, DRB1*0403 individual. Polyclonal CD4+ T cell lines were generated as described previously (19). A murine IL-2-dependent cytotoxic T cell line (CTLL) was used to determine IL-2 secretion of peptide-stimulated clones as described previously (10). To induce apoptosis and anergy, 7P.24 and 7P.41 T cell clones were cultured with 1 μg/ml of peptide in the absence of IL-2 or accessory cells for 18–24 h (10). To test for anergy, the cells that were preincubated with peptide were washed and recultured with irradiated B lymphoblastoid cells (B-LCL), which were pulsed with different concentrations of peptide. Proliferation was measured by [3H]thymidine uptake after 72 h of culture (5, 10). Apoptosis was also induced in T cell clones by stimulation with anti-CD3 Abs that were immobilized on plastic in the absence of costimulation as described previously (20).

Dual chamber coculture of T cells and fibroblasts

T cells and fibroblasts were separated by a semipermeable 0.22-μm pore size Transwell culture insert (Costar, High Wycombe, U.K.) in 24-well plates (14, 15). The T cell clone was placed into the insert above the fibroblasts. FCCM was also added directly to T cells with identical results. The T cells were preincubated with the fibroblasts for 1 h before peptide was added and were then harvested at different times for characterization of anergy or apoptosis. We also used a control fibroblast cell line derived from a patient with a self-limiting viral arthritis (NR), which is the only one we have derived from any tissue used to date that does not rescue cytokine-deprived T cells from apoptosis due to defective IFN-β secretion (12). Student’s t test was used to analyze the results.

Enumeration of viable, apoptotic, and cycling T cells

Viable cells were distinguished by their forward angle scatter and 90° side scatter profiles and were counted using a Cytoron Absolute flow cytometer (Ortho Diagnostics, High Wycombe, U.K.) and also by trypan blue exclusion as described previously (14). A apoptosis and cell cycling were measured at a particular time point by flow cytometry using propidium iodide as the DNA-specific label (12). Apoptosis was also measured by counting cytospin centrifuge slides stained with May-Grunewald-Giemsa (14). We also measured the cumulative proliferation of T cells in culture after various treatments by the sequential addition of [3H]thymidine followed by harvest of radiolabeled cells 18–24 h later.

Analysis of STAT proteins

Activated T cells were cultured in the presence of cytokines for 15 min and washed in ice-cold PBS before the extraction of nuclear proteins (12, 21). EMSA was performed using the 32P-labeled FcγRII DNA probe (GTATT TCCCAGAAAAAGGAC and its complementary sequence) was added, and the sample was incubated for an additional 20 min. Loading dye was added, and the mixture was loaded onto a 6% nondenaturing 30/1 acrylamide/bisacrylamide gel made with 0.25× TBE. After electrophoresis, the gel was dried without fixation and exposed at −70°C to x-ray film. To identify the specificity of STAT protein activation, nuclear protein extracts were preincubated with polyclonal Abs specific for STAT-1, -3, or -5 as described previously (12).

PKC-δ staining

T cell clones that had been cultured under different conditions were spun onto slides using a Cytospin centrifuge (Shandon, Pittsburgh, PA). Slides were air-dried, fixed in acetone for 10 min at room temperature, and stained in an indirect immunofluorescence assay using rabbit anti-human PKC-δ antisera (Santa Cruz Biotechnology). Biotinylated goat anti-rabbit IgG antisera (Southern Biotechnology, Birmingham AL) was used as the secondary Ab, which was developed with FITC-conjugated streptavidin (Life Technologies, Grand Island, NY). The slides were then counterstained with 5 μg/ml propidium iodide in PBS and analyzed by laser scanning confocal microscopy using a MRC 500 confocal microscope (Bio-Rad, Hercules, CA). All experiments included slides stained with species- and isotype-matched irrelevant antisera as negative controls, which showed no or very faint staining as described previously (11).

Results

Prevention of peptide-induced death by fibroblasts

We incubated 7P.24 CD4+ T cells either in medium alone or in the presence of fibroblasts in dual chamber cultures (Fig. 1A). The addition of specific peptide to the medium in the absence of fibroblasts led to a significant reduction in viable cell recovery compared with the controls over the 3-day culture period (p < 0.001). This peptide-induced death was CD95 mediated, because the addition of a blocking Ab, but not an isotype-specific control Ab, could prevent this apoptosis (Table I) (10). When the T cells were cocultured with fibroblasts and peptide, peptide-induced death was significantly reduced after 1 (p < 0.005), 2 (p < 0.001), and 3 (p < 0.001) days of culture, respectively (Fig. 1A). We obtained identical results if we used 50% FCM to rescue the 7P.24 cells from peptide-induced death (not shown). We were also able to...
prevent peptide-induced apoptosis in the 7P.41 clone by coculturing these cells with fibroblasts (Fig. 1B; p < 0.01) or in the 7P.61 clone by culture with 50% FCM (Fig. 1C; p < 0.01). We could also protect three different CD4^+ T cell clones from activation-induced cell death (AICD) with FCM upon stimulation with anti-CD3 Ab in the absence of costimulation (Table II; p < 0.01 in all cases). In addition, FCM, but not IL-2, could inhibit anti-CD3-induced death in a polyclonal CD4^+ T cell line that was tested at the same time (Table II; p < 0.05).

**IFN-β is the main anti-apoptotic mediator in FCM**

To identify the anti-apoptotic mediators in FCM, cultures were treated with anti-IFN-β Ab (Fig. 2A). There was a significant reduction in the ability of FCM to prevent peptide-induced apoptosis at the two concentrations of Ab used (p < 0.01; Fig. 2A). The control HGF Ab had no effect. Although anti-IFN-β treatment abrogated most of the survival-promoting activity of FCM, a small amount of activity always remained regardless of the amount of Ab used (Fig. 2A). This suggests that additional fibroblast-derived factors may have a role in preventing peptide-induced death.

To confirm that IFN-β was the main anti-apoptotic mediator in FCM, we compared the ability of these agents to induce specific STAT-signaling molecules. Cytokine-deprived activated CD4^+ T cell lines were treated with either IFN-β or FCM for 15 min before extraction of nuclear proteins and analysis of STAT:DNA binding. There was a strong single band induced by both these agents that was shown to be mainly STAT-1 by supershift assays (Fig. 2B). This confirms previous studies showing that the PKC-δ protein tyrosine kinase is the principal mediator secreted by fibroblasts that prevents peptide/CD95-induced death in specific T cells after T:T presentation of Ag. IFN-β also protect three different CD4^+ T cell lines from peptide-induced death as previously described (23). IFN-αβ and FCM also blocked cell death and increased cell recovery of 7P.24 cells induced by the direct addition of an agonistic CD95-Ab rather than by peptide (Fig. 3B). Collectively, these results indicate that IFN-β is the principal mediator secreted by fibroblasts that prevents peptide/CD95-induced death in specific T cells after T:T presentation of Ag.

**Characteristics of cells that are rescued from death by FCM and/or IFN-β**

We found that the greater cell recovery induced by either FCM or IFN-αβ upon culture of peptide-stimulated T cells was not due to induction of proliferation. We first investigated the ability of T cells that were rescued from peptide-induced death after T:T presentation to incorporate [3H]thymidine over a 3-day period compared with that of control cells. In these experiments [3H]thymidine was added to parallel cultures 18–24 h before harvest, and the uptake of this label at 1, 2, and 3 days reflects the cumulative proliferation during this period (Fig. 4). While cells that were cultured with peptide-pulsed APCs or IL-2 showed increased proliferation after 3 days, peptide-cultured cells that had been rescued from death by FCM or cells cultured in peptide alone did not proliferate (Fig. 4).

We showed previously that the prevention of cytokine deprivation- or CD95 ligation-induced death in activated T cells by IFN-αβ worked in part by the ability of these cytokines to prevent the translocation of PKC-δ from the cytoplasm to the nucleus of these cells (11). These studies showed that the PKC-δ translocation could be quantitated by either Western blot analysis or confocal microscopy.

Table II. The effect of FCM and IL-2 on AICD induced by anti-CD3 stimulation

<table>
<thead>
<tr>
<th>Clone</th>
<th>Control</th>
<th>FCM</th>
<th>IL-2</th>
<th>Control</th>
<th>FCM</th>
<th>IL-2</th>
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<tr>
<td>7P.24</td>
<td>81 ± 12</td>
<td>90 ± 6</td>
<td>124 ± 9</td>
<td>39 ± 8</td>
<td>62 ± 7</td>
<td>34 ± 10</td>
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<tr>
<td>7P.41</td>
<td>89 ± 7</td>
<td>93 ± 5</td>
<td>147 ± 10</td>
<td>33 ± 9</td>
<td>66 ± 11</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>HC3</td>
<td>89 ± 8</td>
<td>95 ± 4</td>
<td>138 ± 13</td>
<td>35 ± 5</td>
<td>60 ± 5</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>CD4+ T cell line</td>
<td>68 ± 11</td>
<td>74 ± 9</td>
<td>109 ± 4</td>
<td>40 ± 6</td>
<td>53 ± 2</td>
<td>37 ± 4</td>
</tr>
</tbody>
</table>

* Three different CD4^+ T cell clones and a CD4^+ T cell line were deprived of IL-2 and either cultured alone (control) or recultured with IL-2 or FCM. Parallel cultures were activated by immobilized anti-CD3 Abs under the same culture conditions. T cells were harvested 24 h later and viable cell recovery (%), relative to initial cell number, was determined using trypan blue exclusion. The results represent the mean ± SEM of triplicate determinations.
We now show, in agreement with our previous observations, that IFN-\(\beta\) also prevents PKC-\(\delta\) translocation to the nucleus during rescue of 7P.24 T cells from peptide-induced death as assessed by confocal microscopy (Fig. 5). This suggests that this translocation may be a common process that occurs when apoptosis is induced by different routes, and that part of the broad range of anti-apoptotic activity mediated by IFN-\(\alpha\) is related to their ability to block this translocation regardless of the apoptosis induction process.

**FIGURE 2.** IFN-\(\beta\) is the active anti-apoptotic agent in FCM. The FCM was incubated with a 1/100 (\(d\)) or a 1/200 (\(o\)) dilution of neutralizing anti-IFN-\(\beta\) Ab in the pretitrated optimal range for 1 h (A). The treated or untreated FCM was then added to IL-2-deprived 7P.24 T cells for an additional 1 h before the addition of 1 \(\mu\)g/ml of specific peptide. Viable cell recovery was determined after 24 h. Anti-HGF Ab was used as a control. Identical results were obtained in a second separate experiment. B, A CD4\(^+\) T cell line was deprived of IL-2 and cultured for 15 min in the presence of 10 ng/ml of IFN-\(\beta\), FCM (50\%), or FCM from the only fibroblast tested to date that does not rescue these cells from cytokine deprivation-induced death (NR-FCM). Nuclear extracts were analyzed for the presence of STAT-1, -3, and -5 by adding polyclonal Abs to these proteins in supershift assays (B). C, Treatment of FCM with polyclonal Abs to IFN-\(\beta\) for 60 min inhibits its ability to induce STAT proteins. The Ab-treated FCM was added to a CD4\(^+\) T cell line for 15 min, and nuclear extracts were then tested for the presence of STAT proteins. Lane a, Control, where FCM was not added to the cells. FCM was added to the cells in lanes b-f: lane b, no Ab; lane c, with Abs to both IFN-\(\alpha\) and IFN-\(\beta\); lane d, with Ab to IFN-\(\beta\) alone; lane e, with Ab to IFN-\(\alpha\) alone; lane f, with Ab to HGF. Fibroblasts with deficient capacity to secrete IFN-\(\beta\) cannot rescue T cells from peptide-induced death (D). 7P-24 T cells were deprived of IL-2 and cultured in medium or FCM derived from a fibroblast line that was able to secrete IFN-\(\beta\) (FCM) and one that has deficient capacity to secrete this cytokine (NR-FCM). Viable cell recovery was determined after 24 h and is expressed as a percentage of the original cell input. The results shown represent the mean and SEM of triplicate determinations of one of two experiments, which show identical results.
IFN- α or IFN-β rescued T cells remain anergic to peptide-pulsed APCs

We next tested the ability of T cells that had been protected from peptide-induced death by FCM to respond to challenge with Ag-pulsed APC (Fig. 6). Cells cultured with FCM alone, without peptide, responded to Ag-pulsed APCs when tested after 1, 2, or 5 days after culture (Fig. 6A). Cells exposed to 1 μg/ml of peptide alone or peptide together with FCM in the first culture were completely anergic and unresponsive to further challenge (Fig. 6A). This nonresponsiveness was observed over a wide concentration range of peptide on APCs (Fig. 6B). There was significantly greater cell recovery of peptide-stimulated T cells when cultured in the presence of FCM (see above). In an additional set of experiments we showed that CD4+ T cells that were rescued from peptide-mediated death upon direct addition of IFN-α or IFN-β instead of FCM were also anergic to challenge with Ag-pulsed APC (Fig. 6C). The presence of IFN-αβ in the control T cell cultures, which were incubated without peptide, did not affect proliferation or IL-2 secretion of the CD4+ T cells in response to Ag-APC in the second culture (Fig. 6C). These results were completely reproducible in the 7P.41 T cell clone tested (see Fig. 7) and also in the NF-4 clone (not shown). Collectively, these results suggest that IFN-β secreted by fibroblasts can maintain the survival of anergic CD4+ T cells after Ag-specific activation.

Responsiveness of FCM-rescued, anergic T cells to IL-2

We next investigated whether T cell clones rescued from peptide-induced apoptosis were an end-stage nonfunctional population or retained some functional capacity (5, 24). We incubated two different CD4+ T cell clones with peptide in the presence or the absence of FCM (Fig. 7, A and B). The peptide-induced apoptosis was significantly diminished in the presence of FCM in both clones tested (see Fig. 1). However, the rescued T cells were anergic to rechallenge with peptide-pulsed APCs as assessed by their IL-2 secretion. We next investigated the ability of the anergized cells to respond to IL-2. We cultured 7P.41 (Fig. 7C) and 7P.24 (Fig. 7D) T cells in medium alone, with peptide, or with FCM or FCM together with peptide for 24 h as in Fig. 7, A and B. The cells were then washed and cultured with exogenous IL-2 for a further 48 h before assessment of [3H]thymidine incorporation. In clone 7P.41, the addition of IL-2 to the peptide-energized T cells, regardless of whether they had been rescued with FCM, significantly enhanced their ability to proliferate to IL-2 compared with control cells cultured in medium or FCM alone (Fig. 7C). This shows that the rescued anergic cells are nonresponsive to peptide-pulsed APCs but remain functional. In clone 7P.24, the cells that were anergized in the absence of FCM showed an apparently diminished capacity to respond to IL-2 (Fig. 7D). This reflects the fact that the 7P.24 cells are extremely sensitive to apoptosis induced by peptide compared with the 7P.41 clone (10). Most of the recovered 7P.24 cells that are used in the IL-2-responsive assay are therefore already dead or are in the early stages of apoptosis. Nevertheless, the observation that induction of anergy in the presence of FCM, which prevents this death, significantly increases the responsiveness of 7P.24 cells to IL-2 compared with that of cells cultured with peptide alone further strengthens the idea that FCM rescue enables the persistence of nonresponsive, but functional, T cells (Fig. 7D).

Discussion

Various mechanisms are involved in peripheral tolerance, including deletion, induction of anergy, ignorance, and active suppression of responding T cells by immunoregulatory cells (2). In this study we have investigated the relationship between the induction
of apoptosis and anergy after TCR ligation of Ag-specific CD4⁺ T cells to understand how anergic T cells with putative immunoregulatory function may be maintained after stimulation. We found that IFN-1 can inhibit apoptosis resulting from direct CD95 ligation and also CD95-mediated AICD induced by peptide or anti-CD3 Ab. This was a reproducible observation in a panel of human CD4⁺ T cell clones tested. We also showed that the AICD of CD4⁺ T cell lines that were induced by anti-CD3 Ab could be blocked by FCM, but to a lesser extent than that observed in the clones.

T cell clones both phenotypically and functionally resemble highly differentiated CD4⁺ T cells that are found in the blood and other tissues of normal individuals, because they are CD45RO<sup>high</sup>, CD45RB<sup>low</sup> and have reduced capacity to secrete IL-2 (25, 26). In addition, clones that have been maintained in culture have experienced more rounds of proliferation and have shorter telomeres than recently established T cell lines, further indicating that they are an older and more differentiated population (27). The decreased capacity of CD4⁺ T cell clones to secrete IL-2 (25) may make them more sensitive to the survival-promoting effects of type 1 IFN. Hence, the impact of type 1 rescue of T cells from peptide-induced death may have greater bearing on the function of highly differentiated CD4⁺ T cells, with important consequences for immune function in older individuals.

In previous studies we have shown that a wide range of fibroblasts can prevent the apoptosis of optimally stimulated T cells (15), and in this situation the prevention of activated T cell death may enable the persisting of responding cells and contribute to the maintenance of immune memory (28). We now show that when T cells are stimulated to specifically induce anergy, IFN-β and IFN-α can prevent the CD95-mediated apoptosis of these cells (5–7). These results suggest that IFN-αβ may have a general role in regulating the survival of T cells after TCR ligation. However, the context of T cell activation, depending on whether there is complete or partial TCR signaling (10) or whether costimulatory signals are present or absent (1), may determine whether the rescued cells retain responsiveness or are anergic.

It has been shown that IFN-1 may have anti-proliferative activity for activated T cells (29) and also that these cytokines may induce, rather than prevent, apoptosis under certain conditions (30). Both these studies were performed on T cells that were activated from an initially resting state, while we investigated the effects of IFN-1 on T cells that were already activated. This indicates that apart from age and/or state of differentiation, the activation status of the T cell may also determine the type of effect that IFN-1 can induce. This suggests that the timing of IFN-1 secretion during an immune response is a crucial factor in regulating the extent of T cell death and proliferation (31). While anti-proliferative effects of these cytokines may manifest themselves during the induction of stimulation, the anti-apoptotic effects may have a role once T cell activation has taken place (31).

It is unclear whether the signaling pathways for the anti-proliferative and anti-apoptotic actions of IFN-1 are interlinked. IFN-1 has been shown to induce Bcl-x<sub>L</sub> expression (12, 15), inhibit caspase-3 activation, and prevent PKC-δ translocation and activation (11) and can therefore interfere with the induction, commitment, and execution phases of apoptosis (31). The present study suggests that the ability of IFN-1 to prevent the death of anergic cells, which can have inhibitory activity on some immune responses, may be another way in which the immune system may return to a quiescent state after activation. It would be important to clarify whether the efficacy of IFN-1 treatment in various diseases is related to the anti-viral, anti-proliferative, or anti-apoptotic activity of these cytokines (31).

Many recent studies support the possibility that T:T presentation of Ag occurs in vivo. Inflammatory processes may generate degraded peptides from the Ag by enzymatic cleavage (9). These
peptides could then bind to the HLA-DR of the activated T cells, in analogy to the addition of peptide to specific T cells in vitro (6, 8, 9). In addition, activated T cells can actively acquire MHC/peptide complexes from APCs, which can then be presented to other specific T cells (32, 33), and this may be associated with the induction of tolerance (33). Furthermore, T cells themselves may process and present Ag under certain conditions (34). Alternatively, activated T cells can internalize their TCR and process and present fragments of these molecules in the context of class II on their surfaces to other T cells, leading to the induction of anergic immunoregulatory cells (35). In support of this latter possibility, TCR peptide-specific T cells that recognize processed TCR components exist in vivo and are able to play a regulatory role in autoimmunity (36).

The results presented here have several important implications. The ability of IFN-αβ to promote the survival of anergic T cells may not only be restricted to situations of T:T presentation of Ag. Anergy can also be induced by presentation of Ag to T cells by endothelial and also epithelial cells in the context of MHC class II, which is induced on these cells during inflammatory responses (37). The secretion of IFN-αβ by these cells themselves or by neighboring cells may also promote anergic T cell survival in the tissues in these situations. Furthermore, during viral infections the secretion of IFN-αβ can be up-regulated considerably, especially by certain specialized cells, such as type 2 dendritic cells and their

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