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The Role of Interleukin-10 in the Inhibition of T-Cell Proliferation and Apoptosis Mediated by Parainfluenza Virus Type 3

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We have previously demonstrated that parainfluenza virus type 3 (PIV3), a significant respiratory pathogen, can markedly inhibit T-cell function in vitro. We now report that the virus potently induces interleukin-10 (IL-10) production by peripheral blood mononuclear cells. The IL-10 produced contributes to viral inhibition of T-cell proliferation and protects T cells from PIV3-mediated apoptosis. These findings suggest that IL-10 is likely to play an important immunoregulatory role in PIV3 infections.

Interleukin-10 (IL-10), an important immunoregulatory cytokine, is produced by both monocytes (6) and lymphocytes (10, 24). The cytokine acts indirectly to depress T-cell blastogenesis and IL-2 secretion (30) by inhibiting antigen-presenting cell function (6–8, 11).

Besides affecting T-cell activation, IL-10 has been shown to protect both T and B cells from apoptotic death. IL-10 was found to inhibit spontaneous apoptotic cell death in T-cell cultures that were established from patients with acute Epstein-Barr virus (EBV) infections (29). Additionally, the cytokine enhanced T-cell viability in IL-2-starved cultures by inhibiting apoptosis (28) and prevented the spontaneous death of germinal center B cells in vitro (20).

IL-10 is postulated to have an important role in EBV infection and pathogenesis. The EBV genome encodes a biologically active homolog of IL-10 (22) which has been shown to be essential for viral transformation of B lymphocytes (21). Moreover, the capacity of viral IL-10 to inhibit T-cell effector mechanisms suggests that the viral homolog may play an additional role in viral persistence (16).

Many viruses lack the genetic flexibility to capture host genes such as IL-10 but could conceivably achieve IL-10-mediated immunoregulation by inducing endogenous production of the cytokine by host cells. For example, IL-10 production has been shown to occur in monocytes infected with human immunodeficiency virus (1, 4) and has been postulated to influence the T helper phenotype that develops with disease progression (5). Thus, IL-10 can significantly impact immune responsiveness either as an encoded product of a viral genome or as an induced product of host cells.

We have previously demonstrated that parainfluenza virus type 3 (PIV3), a negative-stranded RNA virus which can reinfect (12, 32) and persist (2, 13, 14, 23) in human hosts, is able to inhibit both T-cell proliferation (26) and cytotoxicity (27) in vitro. Exposure to PIV3 resulted in profound inhibition of T-cell proliferation even under circumstances that did not result in detectable productive infection (26). On the basis of this observation, we hypothesized that soluble factors such as cytokines might operate to mediate PIV3-induced immunoregulatory effects.

To examine the cytokine profiles in PIV3-infected cultures, we obtained peripheral blood mononuclear cells (PBMCs) from seven healthy adult donors and put these cells into culture with PIV3 grown from either CV-1 monolayers or CV-1 control supernatants. Supernatants from the PBMC cultures were harvested 24 h later and analyzed by enzyme-linked immunosorbent assay (ELISA) for the presence of cytokines. We were unable to detect either IL-4 or IL-2 by ELISAs with sensitivities of <20 and <200 pg/ml, respectively (data not shown); however, virus-induced enhancement of IL-10 secretion was observed in the PBMCs of all seven donors (Table 1). In addition, we found that virus purified from CV-1 supernatant on a sucrose gradient, as well as virus grown in K562 cells, induced IL-10 production in PBMCs (data not shown). These data demonstrate that PIV3 selectively induces high levels of IL-10 production in PBMC populations.

We have previously reported that PIV3 inhibits proliferation in anti-CD3-activated PBMC cultures (26). Because it seemed possible that the production of IL-10 could play a role in this immunoregulation, we assessed the levels of IL-10 production in anti-CD3-activated, PIV3-exposed PBMCs. Anti-CD3 stimulation of infected PBMCs resulted in a marked enhancement of IL-10 production beyond that detected in uninfected controls (Fig. 1). Furthermore, we found that PIV3-mediated inhibition of T-cell proliferation was abrogated in a dose-dependent manner by addition of neutralizing anti-IL-10 antibody to anti-CD3 antibody-stimulated, PIV3-infected cells (Fig. 2). In contrast, the addition of immunoglobulin G1 (IgG1) isotype control antibody had little effect regardless of the concentration. The addition of anti-IL-10 antibody to uninfected cultures did not enhance proliferation in comparison with that of cultures which received IgG1 control antibody, thereby demonstrating that the effects of IL-10 neutralization were specifically associated with PIV3 infection. These findings indicate that endogenously produced IL-10 contributes to the inhibition of proliferation seen in PIV3-infected cultures.

In addition to its immunoregulatory activity, IL-10 has been shown to protect both T and B cells from apoptotic death (20, 28, 29). To examine the relationship between IL-10 and apoptosis in PIV3-exposed cells, we added anti-IL-10 antibody or IgG1 isotype control antibody to infected and uninfected cultures that were either stimulated with anti-CD3 antibody or

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TABLE 1. IL-10 production by PBMCs

Donor	Stimulus (pg/ml)[a]		Fold enhancement[b]
	Control supernatant	PIV3	
1	<20	2,010	>100
2	149	798	5.4
3	33	651	19.7
4	193	1,155	6.0
5	96	2,433	25.3
6	46	592	12.9
7	<20	1,324	>66

[a] PBMCs were isolated as previously described (27), resuspended in RPMI medium supplemented with 10% fetal calf serum and put into culture at 2×10^6 cells per well in 48-well plates with 3 PFU of either PIV3 (NIH strain catalog 47884; obtained from Mark Galinski, Cleveland Clinic Foundation) or CV-1 control supernatant per cell. Experimental supernatant was harvested 24 h later and frozen at −70°C until analyzed for IL-10 content by ELISA.

[b] Fold enhancement was obtained by dividing the PIV3-stimulated response by the respective background response observed in CV-1-stimulated cultures. Monoclonal antibodies specific for IL-10 (Pharmingen, San Diego, Calif.) and polyclonal anti-IL-10 antibodies (Pharmingen) were used for the ELISAs.

left unstimulated. After 3 to 7 days of incubation, cells were analyzed for apoptosis by flow cytometry. Anti-CD3 antibody-stimulated cells that were exposed to PIV3 and additionally treated with anti-IL-10 antibody demonstrated marked levels of apoptosis on the sixth day of incubation compared with cells treated with isotype control antibody (Fig. 3). This effect was first detectable within 4 to 5 days after infection and activation. Moreover, by double staining with propidium iodide and anti-CD3 antibody, we found that cell death was predominantly restricted to T lymphocytes in these cultures (data not shown). Thus, activated T cells are particularly susceptible to PIV3-mediated apoptosis under conditions in which IL-10 is neutralized.

The number of infected cells in the anti-CD3 antibody-stimulated cultures was assessed by immunohistology as previously described (26). For donor 2, anti-IL-10 antibody enhanced apoptosis from 13 to 43% (Fig. 3), and the percentage of infected cells was enhanced from 23% to 30%. For donor 7, anti-IL-10 antibody enhanced apoptosis from 1.4% to 28% (Fig. 3), while the percentage of infected cells was enhanced from 10% to 23%. Consequently, the enhancement in infection with anti-IL-10 antibody treatment does not appear to totally account for the enhancement in apoptosis, although there may be some effect.

In the absence of infection, activation did not induce enhanced levels of apoptosis regardless of IL-10 neutralization. Moreover, anti-IL-10 antibody did not alter apoptotic profiles in unstimulated, infected cultures (data not shown), suggesting that the effects of IL-10 neutralization were dependent on both exposure to PIV3 and cellular activation.

Because the neutralization of IL-10 enhanced apoptosis in infected cells, we hypothesized that the addition of recombinant IL-10 (rIL-10) to cultures at the time of infection might promote cell viability. To test this possibility, we added either rIL-10 or phosphate-buffered saline (PBS) to infected and uninfected cells that were stimulated with anti-CD3 antibody. At various time points, cells were taken from the cultures and assessed for their ability to exclude trypan blue. As can be seen in Fig. 4A, the addition of rIL-10 at the beginning of the culture period significantly prolonged the viability in virus-exposed cells; however, by performing IL-2-driven prolifera-

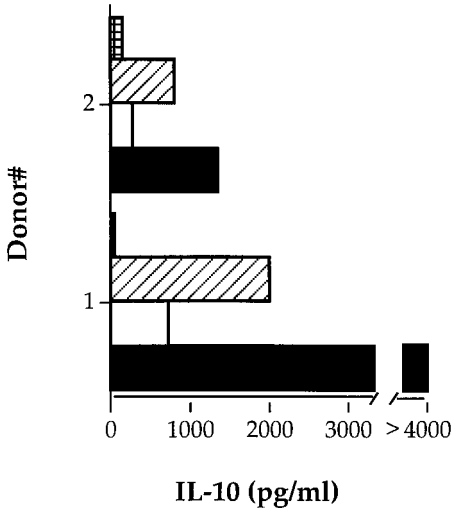

FIG. 1. PIV3 enhances IL-10 production in PBMCs that are stimulated with anti-CD3 antibody. PBMCs from donors 1 and 2 were put into culture at 2×10^6 cells per well in RPMI medium supplemented with 10% fetal calf serum. PIV3 (3 PFU per cell), or CV-1 control supernatant was added, and the cells were either stimulated with anti-CD3 antibody (2 ng/ml) or left unstimulated. After a 24-h incubation, experimental supernatant was collected and frozen at −70°C until tested by ELISA for IL-10. ⊞, uninfected, ▨, infected; □, uninfected anti-CD3 antibody stimulated; ■, infected anti-CD3 antibody stimulated.

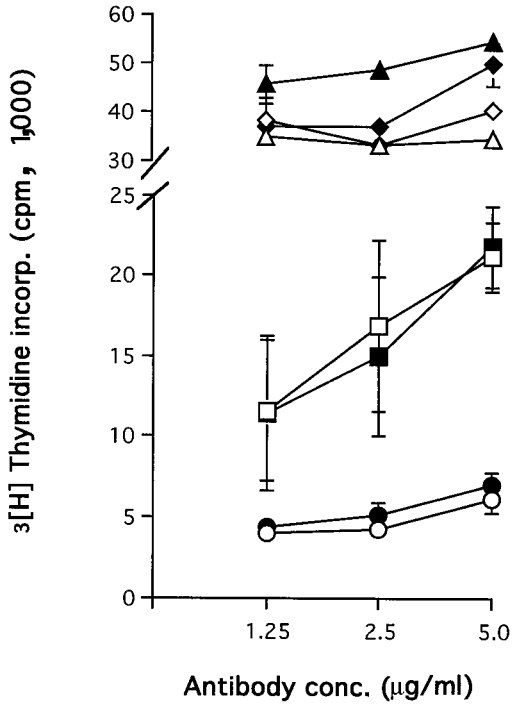

FIG. 2. IL-10 contributes to PIV3-mediated inhibition of anti-CD3-driven proliferation. PBMCs from either donor 2 (open symbols) or donor 7 (solid symbols) were put into culture at 10^5 cells per well. PIV3 was added at 3 PFU per cell along with either anti-IL-10 antibody or IgG1 isotype control antibody at concentrations of 5, 2.5, or 1.25 μg/ml. OKT3 (anti-CD3; Ortho, Raritan, N.J.) antibody was added to cultures at 2 ng/ml to induce T-cell proliferation. After 4 days in culture, the cells were pulsed with tritiated thymidine at 0.5 μCi per well and harvested 18 h later onto glass fiber filters with an automated harvester. Counts per minute were obtained on a liquid scintillation counter. Each datum point represents the average counts per minute ± standard error for triplicate wells. Similar results were found with cells from donor 1 (data not shown). ◇ and ◆, uninfected IgG1-treated; △ and ▲, uninfected anti-IL-10 antibody treated; □ and ■, infected anti-IL-10 antibody treated; ○ and ●, infected IgG1 treated.

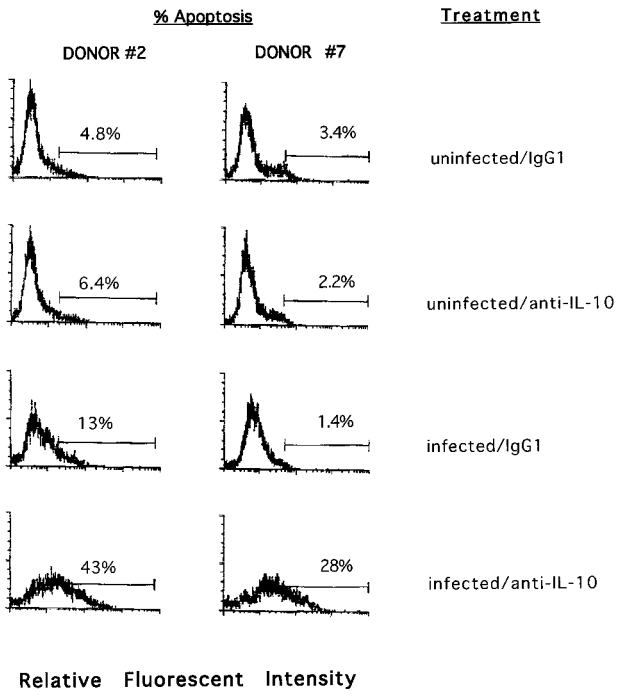

FIG. 3. Anti-IL-10 antibody treatment of infected, stimulated cells results in enhanced apoptosis. PBMCs were put into culture at 10^6 cells per ml in 24-well plates and exposed to either PIV3 at 3 PFU per cell or CV-1 control supernatant. Cells were stimulated with anti-CD3 antibody (2 ng/ml) and additionally received either anti-IL-10 antibody or IgG1 isotype control antibody at 10 μg/ml. After 6 days of incubation, cells were analyzed for apoptosis as outlined in the Apoptag kit manual (Oncor, Inc., Bethesda, Md.). Briefly, cells were fixed in 1% paraformaldehyde, washed three times with PBS and then washed again in equilibration buffer. Cells were then treated with terminal deoxynucleotide transferase enzyme (30 min at 37°C) to allow fragmented DNA to be labeled with digoxigenin residues. The reaction was stopped by washing the cells twice in stop-wash buffer. Cells were then resuspended in fluorescein isothiocyanate-conjugated anti-digoxigenin antibody and incubated at room temperature for 30 min. After this incubation period, the cells were washed with PBS and analyzed on the flow cytometer. Gates are based on an infected, anti-IL-10 antibody-treated control population that was not incubated with terminal deoxynucleotide transferase enzyme prior to staining with fluorescein isothiocyanate-conjugated anti-digoxigenin antibody. PBMCs from a third donor and a repeat experiment with cells from donor 2 demonstrated results similar to those shown (data not shown).

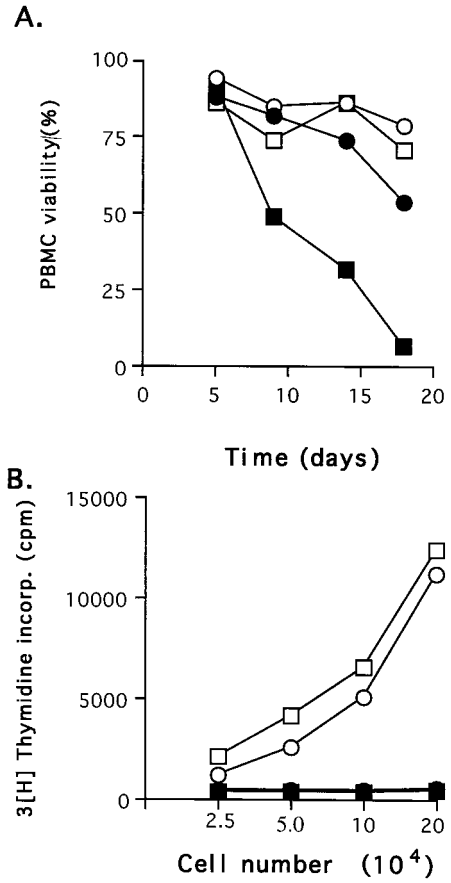

FIG. 4. IL-10 enhances viability but not function in PBMCs infected with PIV3. (A) PBMCs from donor 2 were given PIV3 at 3 PFU per cell or control CV-1 supernatant. Either rIL-10 (50 ng/ml) or PBS was added at the beginning of the culture period, and the cells were stimulated with anti-CD3 antibody at 2 ng/ml. Seven days after the initial stimulation, the cells were resuspended in fresh media containing 500 U of rIL-2 per ml, and this step was repeated thereafter every 4 days until the infected cultures died out. Cell viability was assessed at various time points by trypan blue dye exclusion. (B) In a separate experiment, the ability of cells to proliferate in response to rIL-2 was tested 8 days after the initial stimulation. Cells were recovered, washed, and placed into 96-well plates at various concentrations, and rIL-2 was added at 40 U/ml. Cells were pulsed with tritiated thymidine (0.5 μCi/well) 18 h later and harvested after an additional 4 h. Although this experiment was distinct from that shown in panel A, the differences in viability between the cell populations were similar (viability of 82 and 60% for infected cells that received rIL-10 on the first day of culture and infected cells that received PBS, respectively). Standard deviations were less than 10% of the mean counts per minute shown. ○, uninfected rIL-10 treated; □, uninfected PBS treated; ●, infected rIL-10 treated; ■, infected PBS treated.

tion assays on the eighth day of culture, we found that infected cells, unlike uninfected cells, were essentially unable to proliferate in response to IL-2 regardless of the presence of exogenous rIL-10 (Fig. 4B). Thus, although rIL-10 enhanced viability in PIV3-infected cultures, it did not improve the functional capacity of these cells to respond to rIL-2. These results are consistent with the IL-10 neutralization experiments, demonstrating that IL-10 enhances viability but adversely affects T-cell function in PIV3-exposed cells.

PIV3 induces PBMCs to produce large amounts of IL-10, and the secretion of this cytokine contributes to the inhibition of T-cell proliferation mediated by the virus. It seems likely that the PIV3-induced production of IL-10 contributes to enhanced viral survival in the host by inhibiting T-cell function. A similar role has been postulated for the IL-10 homolog encoded by EBV. In contrast to EBV, PIV3 is a small virus with limited capacity to increase its genomic size. Instead of capturing the IL-10 gene as EBV has, PIV3 potently induces IL-10 production by PBMCs.

Virus-mediated apoptosis of infected cells has been reported to occur with human immunodeficiency virus (31), influenza A and B viruses (15), Sindbis virus (19), chicken anemia virus (17), feline leukemia virus (25), and measles virus (9). We have demonstrated for the first time that PIV3 induces apoptosis in T lymphocytes and that IL-10 protects the cells from this effect. Our findings are similar to those reported by Taga et al. (29), who showed that T cells recovered from patients infected with EBV undergo apoptosis in vitro and that protection from apoptosis can be conferred by the addition of exogenous IL-10. In contrast to the EBV studies in which IL-10 was supplied exogenously, we show that endogenously synthesized IL-10 protects cells from apoptosis in PIV3-infected cultures.

PIV3 has been shown to have the potential to cause persistent infections (2, 13, 14, 23). Moreover, the virus readily reinfects a host even in the face of neutralizing antibodies (3, 18). We have hypothesized that the immunoregulatory properties of the virus are important under these clinical circum-

stances (26, 27). Our demonstration that the virus potently induces the production of the immunoregulatory cytokine IL-10 supports this contention and suggests that the cytokine may play an important role in viral reinfection and persistence.

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