JOURNAL OF VIROLOGY, Aug. 1996, p. 5230–5235 Vol. 70, No. 8
0022-538X/96/$04.00+0

Interleukin-4 Causes Delayed Virus Clearance in Influenza Virus-Infected Mice

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Received 9 February 1996/Accepted 10 May 1996

Two different subsets of T cells, Th1 and Th2 cells, have been demonstrated to secrete different profiles of cytokines and to influence various infections in different ways. Whereas cytokines secreted by Th1 cells, particularly gamma interferon, promote the generation of cell-mediated immunity, Th2 cells and their cytokines (interleukin-4 [IL-4], IL-5, IL-10, and IL-13) have been shown to function in recovery from parasitic infections and in antibody responses. In this study, we analyzed the effects of the dominant Th2 cytokine, IL-4, on immunity to virus infection. We assessed the effects of IL-4 on both secondary immune responses by an adoptive transfer assay and primary immune responses by in vivo treatment of influenza virus-infected mice with IL-4. The results demonstrated that IL-4 can function to inhibit antiviral immunity at both stages. We found that IL-4 treatment of sensitized cells during secondary stimulation in vitro had little effect on their ability to lyse virus-infected target cells in a ^{51}Cr release assay. Nevertheless, the clearance of influenza A/PR/8/34 (H1N1) virus from the lungs of infected BALB/c mice was significantly delayed after the transfer of virus-specific T cells secondarily stimulated in the presence of IL-4 in comparison to virus clearance in recipients of cells stimulated in the absence of IL-4. In contrast to the adoptive transfer results, the treatment of PR8 virus-infected mice with IL-4 during primary infection greatly suppressed the generation of cytotoxic T-cell precursors, as assessed by secondary stimulation in vitro. In addition, culture supernatants of secondarily stimulated spleen cells from IL-4-treated mice contained significantly less gamma interferon and more IL-4 than did spleen cells from controls. More importantly, the treatment of mice with IL-4 resulted in an extremely significant delay in virus clearance. Thus, IL-4 can inhibit both primary and secondary antiviral immune responses.

Considerable interest in the contributions of Th1 and Th2 subsets to resistance to and control of various infections has been generated recently. Th1 cells, which secrete interleukin-2 (IL-2), transforming growth factor β, and gamma interferon (IFN-γ) (24, 27), have been shown to be important in the control of intracellular infections, such as *Leishmania* (17), mycobacterial (32), *Listeria* (13), and fungal infections (30). On the other hand, Th2 cells secrete IL-4, IL-5, IL-6, and IL-10 (22) and have been shown to contribute to resistance to intestinal nematode infections (7, 8). Both Th1 and Th2 cells are capable of providing help to antibody-producing B cells, and the cytokines they secrete dictate the isotypes which are generated (27, 39). Furthermore, IFN-γ, IL-12, and transforming growth factor β induce the differentiation of Th1 cells, while IL-4 encourages the differentiation of Th2 cells and inhibits the development of Th1 cells (36, 38). Distinct subsets of CD8 cells have also been described (31). TC1 cells secrete IL-2 and IFN-γ, whereas TC2 cells release IL-4, IL-5, IL-6, and IL-10. Both cell types are cytotoxic.

There are only limited data concerning the effects of Th1 and Th2 subsets and their cytokines on immunity to virus infections. Since virus clearance in many virus infections is dependent on the generation of cytotoxic T lymphocytes (CTL) and the release of cytokines, such as IFN-γ and tumor necrosis factor alpha (5), it is reasonable to hypothesize that Th1-type immune responses are more effective than are Th2 responses against virus infections. However, a number of groups have documented the presence of Th2 cytokines in the lungs of influenza virus-infected mice (4, 33) which successfully clear virus from their lungs. The evidence implicating Th1 cells in antiviral immunity is largely circumstantial. Th1 responses have been reported to be responsible for recovery from hepatitis B infection, whereas Th2 cells are associated with chronic infection (19, 20). IL-12, which is believed to induce the generation of Th1 T cells, has been shown to facilitate clearance of lymphocytic choriomeningitis virus (25). Helminth infection, a Th2 inducer, inhibited the generation of CTL and clearance of vaccinia virus in animals infected with both (1). In other experiments, adoptive transfer of T-cell clones secreting cytokines of the Th2 cell profile failed to promote virus clearance in influenza virus infections (11). Th1 and Th2 clones have different lung immunopathologies when infused into respiratory syncytial virus-infected mice (3). Possibly the strongest evidence for the role of Th1 cytokines in virus clearance comes from viruses themselves. Epstein-Barr virus produces a protein, BCRF1, which mimics the Th2 cytokine IL-10 (14). It functions to down regulate macrophages and inhibit the release of IFN-γ (22). Several poxviruses have been shown to encode soluble receptors for tumor necrosis factor and IFN-γ which function as antagonists of the respective cytokine activities (2). Many other examples of cytokine or cytokine receptor mimicry have been reported, but all have in common the fact that they inhibit or block Th1-type responses (reviewed in reference 37).

Although these results suggest a dominant role for Th1

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TABLE 1. Cytotoxic activity and IL-4 and IFN-γ production after secondary in vitro stimulation of PR8-immune spleen cells in the presence of different cytokines or anticytokines

Cytokine or anticytokine	Specific immune lysis (%)[a]	IL-4 (pg/ml)	IFN-γ (ng/ml)
None	43.7	0	17
IL-4	45	172	7.2
Anti-IL-4	48.4	94	21.3
IL-12	58	80	85.9

[a] At an E/T ratio of 20:1.

responses in antiviral immunity, the only direct attempt to divert a Th1 response to a Th2 response was performed by administering anti-IFN-γ binding antibodies to class I-deficient mice infected with influenza virus (34). This caused a delay in virus clearance but failed to switch CD4 T cells to the Th2 type. In addition, no one has directly observed the effects of IL-4 on secondary anti-influenza virus responses, although Sad and Mosmann (31) have shown that IL-4 in vitro can shut off the release of IL-2 from TC1 cells and to a lesser degree from Th1 cells. In vivo use of IL-4 has been limited, presumably because it has been difficult to observe the effects in vivo. Finkelman and colleagues, using the expression of CD23 (immunoglobulin E [IgE] receptor) as an indicator of activation, demonstrated that IL-4 was much more effective if administered as an immune complex with anti-IL-4 (10). It has been suggested that this complex in some way prolongs the half-life of IL-4 in vivo. We have taken advantage of this finding to use IL-4 in vivo and observe its effects on immunity to virus.

In these experiments, we employed an experimental model of influenza virus infection in mice to investigate whether the normal cellular immune responses which lead to the clearance of virus from the lungs could be modified by cytokines or anticytokines promoting Th1 or Th2 responses, respectively. Two models were utilized. In the first, cells from sensitized mice were secondarily stimulated in vitro in the presence of various cytokines or anticytokines and then adoptively transferred to recipient mice 1 day after aerosol infection. In the second model, infected mice were inoculated daily with IL-4. In both models, we found that IL-4 treatment promoted Th2-type cytokine responses and diminished virus clearance.

MATERIALS AND METHODS

Mice. BALB/c mice were purchased from Charles River Laboratories and used at 8 to 10 weeks of age.

Viruses and infectivity titrations. Influenza A/PR/8/34 (H1N1) virus was grown in the allantoic cavities of embryonated hen eggs and stored at −80°C. The titers of viruses were determined by infection of Madin-Darby canine kidney cells, as previously described (23). Briefly, flat-bottom 96-well plates were seeded with 3×10^4 cells per well, and 24 h later, the serum-containing medium was washed away and 25 μl of seed virus or lung homogenate, diluted from 10^{-1} to 10^{-8} was inoculated into triplicate wells. After a 45-min incubation, 175 μl of Dulbecco modified Eagle medium–1% bovine serum albumin (BSA), sodium bicarbonate, antibiotics, and 2.5 μg of trypsin per ml (maintenance medium) were added to each well. After a 48-h incubation at 37°C, the presence or absence of virus was determined by hemagglutination of chicken erythrocytes. The virus titers were determined by the interpolation of the dilution endpoint that infected 50% of wells by the method of Reed and Muench (29).

Infection procedure. Eight- to 10-week-old BALB/c mice were infected in an aerosol chamber as previously described (35). Briefly, a 1:1,000 dilution of PR8 seed virus was diluted in normal saline and added to a glass nebulizer (Tri-R Instruments) connected to an aerosol chamber. Ten milliliters of the virus mixture was added to the nebulizer, and the mice were left in the chamber for 30 min. Each mouse is calculated to have been exposed to 7,500 50% tissue culture infective doses during the 30-min exposure.

Antibodies. Monoclonal anti-IFN-γ (XMG1.2) and anti-IL-4 (BVD4-1D11.2 and BVD6-24G2.3) were kindly provided by John Abrams (DNAX, Palo Alto, Calif.), and rat anti-mouse IFN-γ was purchased from Upstate Biotechnology Inc., Lake Placid, N.Y. XMG1.2, BVD4-1D11.2, and BVD6-24G2.3 were purified by passing hybridoma supernatants through an anti-rat IgG–agarose column (Sigma Immunochemicals). After a wash with phosphate-buffered saline (PBS; pH 7), the bound antibody was eluted with glycine-HCl (pH 2.5) and neutralized immediately. Peak fractions were dialyzed with PBS (pH 7) for 24 h.

Antibody detection. Hemagglutination-inhibiting antibody was determined as described previously (26). Briefly, receptor-destroying enzyme-treated serum samples were diluted in $\log_2$ increments. Standardized virus antigen and chicken erythrocytes (0.5%) were added to each well. The inhibition of agglutination was read after 1 h at 4°C.

The detection of total PR8 virus-binding antibody was determined by enzyme-linked immunosorbent assay (ELISA). Four Dynatech Immulon plastic microtiter plates were coated with 5 μg of sucrose-purified PR8 influenza virus per ml. After blocking with PBS–1% BSA, dilutions of serum samples were added and incubated for 1 h, and bound antibody was detected by the addition of horseradish peroxidase-linked rabbit anti-mouse IgG (whole molecule). The wells were washed; substrate was added; and after a 30-min incubation, the plates were read on an ELISA reader at 405 nm. Influenza virus-specific IgG1 and IgG2a were detected similarly with horseradish peroxidase-linked goat anti-mouse IgG1 or IgG2a (Boehringer Mannheim, Indianapolis, Ind.). Substrate was added, and the plates were read at 405 nm.

Total serum IgE was determined by ELISA (all reagents kindly provided by Seiji Haba and Alfred Nisonoff, Brandeis University). AE19, an A/J anti-mouse IgE, was used to coat ELISA plates, and dilutions of serum samples were added to blocked wells. After a 2-h incubation, the plates were washed and bound IgE was identified by the addition of biotin-labeled rabbit anti-mouse IgE and avidin-alkaline phosphatase. Lastly, *p*-nitrophenyl phosphate was added and the reaction was allowed to proceed for 30 min, after which plates were read on an ELISA reader at 405 nm.

Generation of IL-4 immune complexes. IL-4 immune complexes were prepared by incubating IL-4 and anti-IL-4 (BVD4-1D11.2) (1:5) in a small tube for 1 min. Then the mixture was diluted with normal saline, and 2.5 μg of IL-4 and 12.5 μg of anti-IL-4 were injected into each animal. The animals were injected intravenously for the first 3 days and intraperitoneally (i.p.) for subsequent injections.

In vivo treatment with IL-4 immune complexes. Eight-week-old BALB/c mice were infected with aerosolized PR8 virus and, beginning 24 h later, were injected daily with immune-complexed IL-4. Groups of five animals were sacrificed on days 5, 7, 8, and 10, and the pulmonary virus titers and lung lesions were determined. Spleen cells removed from animals on day 7 were restimulated in vitro with PR8 virus-infected irradiated syngeneic spleen cells and cultured for 6 days. Cultured cells then were tested in a cytotoxicity assay, and the supernatants were collected and tested for cytokine content.

Cytokine determinations. Microtiter plates were coated with anti-cytokine XMG1.2 (IFN-γ) or BVD4-1D11.2 (IL-4) antibody at 1 μg/ml and incubated overnight at 4°C. Plates were washed and blocked with PBS–1% BSA. Control IL-4 or recombinant IFN-γ was added at different concentrations. Test samples were diluted, added to plates, and incubated for 90 min at room temperature. BVD6-24G2.3, anti-IL-4, and rat anti-mouse IFN-γ monoclonal antibody (Upstate Biotechnology Inc.) were ^{125}I labeled and used for detection at 50,000 cpm in 50 μl. After the addition of these reagents, the plates were incubated for 90 min more, washed, cut up, and counted in a gamma counter. A dose-response curve with purified IL-4 or IFN-γ was determined in each assay, and the results were used to calculate the absolute concentration of cytokines by using samples diluted to fall into the linear portion of our dose-response curve.

Generation of T-cell cultures and adoptive transfers. Eight-week-old BALB/c mice were injected i.p. with 200 μl of a 1:4 dilution of PR8 seed virus, and 1 week later, their spleens were removed and restimulated with PR8 virus-infected irradiated syngeneic spleen cells and with IL-4 (0.5 ng/ml), IL-12 (0.5 ng/ml), anti-IL-4 (7.5 μg/ml), or nothing in Iscoves medium–10% fetal calf serum–sodium pyruvate–L-glutamine–2-mercaptoethanol–gentamicin for 6 days. Then cells were tested for cytotoxicity, supernatants were analyzed for cytokine con-

TABLE 2. Virus titers in the lungs of PR8 virus-infected recipient mice after adoptive transfer of spleen cells secondarily stimulated in vitro in the presence of various cytokines or anticytokines

Cells stimulated with:	Virus titer[a]	
	Day 5	Day 7
Nothing	5.2 ± 0.2 (5/5)	3.6 ± 0.5 (5/5)
Virus only	2.8 ± 0.8 (3/5)	<1.4 ± 0.5 (1/5)
Virus and IL-4	3.9 ± 0.4 (5/5)	<2.6 ± 0.8 (3/5)
Virus and anti-IL-4	<1.9 ± 0.3 (2/5)	<0.9 ± 0 (0/5)
Virus and IL-12	<2.5 ± 0.6 (3/5)	<0.9 ± 0 (0/5)

[a] Data are mean $\log_{10}$ 50% tissue culture infective doses (per 20 μl) ± standard errors. Parenthetical data are ratios of the number of mice with detectable virus to the number of mice tested.

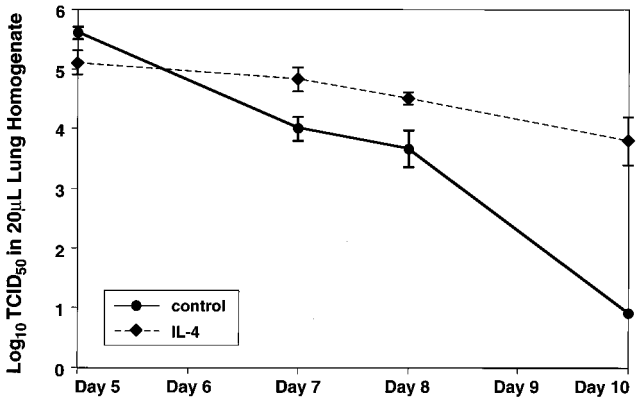

FIG. 1. Virus titers in the lungs of PR8 virus-infected mice treated with daily injections of IL-4 or untreated after infection (control). IL-4-treated animals were significantly different from control animals on days 7, 8, and 10 ($P = 0.002$, 0.01, and 0.001, respectively). The day 5 results were not significantly different. Vertical bars show standard errors. $TCID_{50}$, 50% tissue culture infective dose.

tent, and cells were infused into animals (3×10^7 cells per recipient) infected 1 day earlier with aerosolized PR8 virus. Groups of animals were sacrificed on days 5 and 7, lung virus titers were determined, and lesions were quantified. Some animals were infected but received no adoptive transfer as a virus control.

Cytotoxicity assay. In this assay, effectors from secondary in vitro cultures were mixed in round-bottom 96-well plates with ^{51}Cr-labeled P815 target cells. One million cells were infected with PR8 virus by incubation with 50 μl of allantoic fluid virus (2.8×10^9 50% tissue culture infective doses per ml) diluted 1:2 with Dulbecco modified Eagle medium–1% BSA. Cells were incubated for 45 min in a 37°C water bath, washed, and resuspended in 100 μCi of sodium chromate (NEN) for 1 h. Cells were washed and added to wells. T cells were added at various effector/target (E/T) ratios (20:1, 40:1, and 80:1), after which plates were incubated for 4 h, harvested, and counted. In all cytotoxicity assays, 5×10^3 target cells were added to each well. Maximum release was measured from wells incubated with 0.5% Nonidet P-40, and spontaneous release was measured from wells with medium alone. The percent cytotoxicity was calculated by using the following formula: % cytotoxicity = [(test release − spontaneous release)/(maximum release − spontaneous release)] × 100. In all cases, uninfected P815 cells were ^{51}Cr labeled and incubated with effector cells. Cytotoxicity was routinely below 10% and was subtracted from the cytotoxicity observed with infected targets. Here this is reported as specific immune lysis.

Statistical analysis. Statistical analysis was performed by using a *t* test for paired means. Differences were considered significant if $P \leq 0.05$.

RESULTS

Adoptive transfer of secondary immune cells restimulated in the presence of virus and various cytokines or anticytokines. It has repeatedly been demonstrated that adoptive transfer of secondary immune cells to influenza virus-infected mice is associated with more rapid clearance of virus from the lungs, at least in part, because of the activity of cytolytic T cells in the transferred population (16, 18, 41, 42). We investigated whether cytokines added during secondary in vitro stimulation with virus would enhance or eliminate this effect. Spleen cells taken from animals immunized 10 days previously with PR8 virus were incubated in the presence of PR8 virus alone or PR8 virus with IL-4, anti-IL-4, or IL-12. After 6 days of incubation, supernatant fluids were tested for concentrations of IL-4 and IFN-γ and cells were tested for cytolytic activity against PR8 virus-infected cells. In addition, 30 million cells were transferred intravenously to mice infected 1 day previously with PR8 virus. As shown in Table 1, the supernatants of cells incubated in the presence of IL-4 (five individual cultures per group) had higher concentrations of IL-4 and lower IFN-γ levels than did the supernatants of other groups. It should be noted that the increased level of IL-4 in the IL-4-treated culture was undoubtedly due in part to exogenously added IL-4. Conversely, the supernatants of cells incubated in the presence of IL-12 had the highest levels of IFN-γ. At an E/T ratio of 20:1, we were unable to observe differences in cytotoxicity between groups treated with different cytokines or anticytokines. Similar results were obtained at higher E/T ratios (data not shown). In subsequent experiments, we added higher concentrations of IL-4 and analyzed lower E/T ratios and still were unable to demonstrate a difference between IL-4-treated and control cultures in ^{51}Cr release assays (data not shown). After adoptive transfer of the various spleen cell populations to infected syngeneic mice, the lungs of recipient mice were removed for the measurement of virus titers. As shown in Table 2, adoptive transfer of cells restimulated with virus alone resulted in more rapid clearance of virus than that observed in control mice which did not receive 2^0 immune cells ($P = 0.04$). Animals which received spleen cells stimulated in the presence of virus and anti-IL-4 or IL-12 also cleared virus more rapidly than did controls ($P = 0.007$). However, animals receiving cells restimulated in the presence of IL-4 did not clear virus significantly better than did controls receiving no cells at all ($P = 0.18$). Thus, all of the animals receiving IL-4-stimulated cells had detectable virus upon sacrifice at 5 days after infection and three of five mice sacrificed on day 7 after infection still had measurable virus. No significant differences in the extent of lung lesions were noted (data not shown). Thus, restimulation in the presence of IL-4 had a negative effect on the ability of cells to promote recovery from virus infection.

Effects of in vivo injections of IL-4 in virus-infected mice. PR8 virus-infected mice were injected intravenously (days 1

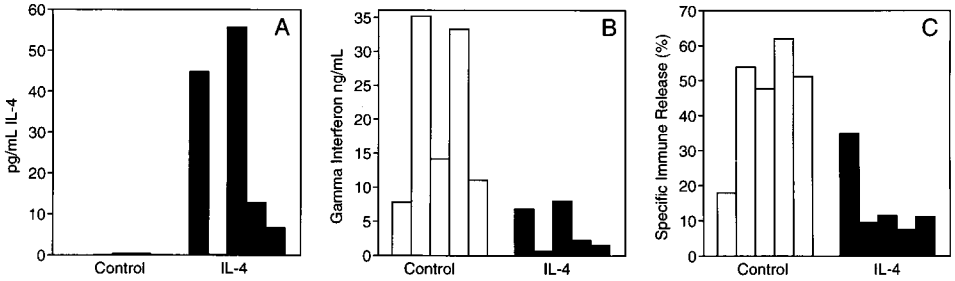

FIG. 2. IL-4 (A) and IFN-γ (B) levels in individual supernatants of secondary stimulated spleen cells from IL-4-treated and untreated (control) PR8 virus-infected mice; (C) cytolytic activities in secondary stimulated spleen cells from IL-4-treated and untreated (control) mice. Cells were obtained 7 days after infection and were cultured in vitro for 6 days in the presence of PR8 virus-infected irradiated syngeneic cells. Supernatants were collected for cytokine assay, and spleen cells were tested for cytotoxic activity against ^{51}Cr-labeled PR8 virus-infected P815 cells at an E/T of 40:1. In panel A, the means ± standard errors of the means for control and IL-4-treated mice were 0.04 ± 0.02 and 24.22 ± 11 pg/ml, respectively ($P = 0.05$). In panel B, the means ± standard errors of the means for control and IL-4-treated mice were 19.6 ± 5.8 and 3.8 ± 1.2 ng/ml, respectively ($P = 0.02$). In panel C, secondary spleen cells from the control group had a mean release ± standard error of 46.2% ± 8% while secondary spleen cells from IL-4-treated animals had a mean release ± standard error of 14.9% ± 5% ($P = 0.03$).

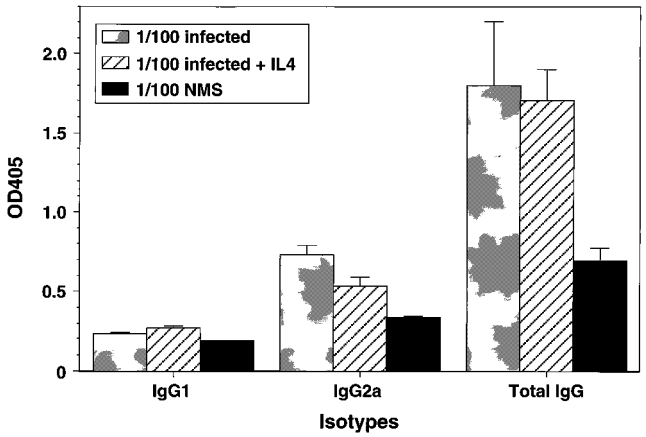

FIG. 3. Sera from BALB/c mice 8 days after infection were tested for binding to ELISA plates coated with sucrose-purified PR8 influenza virus (5 μg/ml). After a 1-h incubation, bound antibodies were detected by the addition of horseradish peroxidase-linked rabbit anti-IgG, anti-IgG1, or anti-IgG2a to triplicate wells. Substrate was added, and plates were read on an ELISA reader at 405 nm. Vertical bars show standard errors. OD_{405}, optical density at 405 nm.

through 3) or i.p. (days 4 through 9) daily with a mixture containing 2.5 μg of IL-4 and 12.5 μg of anti-IL-4 (10). Anti-IL-4 alone at this concentration had no effect on immune responses (data not shown) (10). Lungs were removed for the determination of virus titers on days 5, 7, 8, and 10 after infection. In addition, serum specimens were obtained on days 8 and 10 for antibody determinations. Spleen cells obtained 7 days after infection were restimulated in vitro by exposure to PR8 virus-infected irradiated syngeneic spleen cells in the absence of any cytokines. As seen in Fig. 1, IL-4-injected mice had significantly more virus when sacrificed at 7, 8, or 10 days postinfection. The differences were significant, with P values of 0.002, 0.01, and 0.001 at these three time points, respectively. On day 10, five of five mice treated with IL-4 still had high levels of virus while virus was not detectable in the lungs of any control mice. Again, no differences in lung lesions were detected (data not shown). This experiment was performed twice with essentially similar results.

Measurements of cytokine levels from supernatants of spleen cells removed from animals sacrificed on day 7 and secondarily stimulated with influenza virus demonstrated higher levels of IL-4 ($P = 0.05$) from infected mice injected with IL-4 than those found in the supernatants of spleen cells from control mice not treated with IL-4 (Fig. 2A). Conversely, the IFN-γ levels in the supernatants of spleen cells from control mice were higher ($P = 0.02$) (Fig. 2B). Measurements of the cytotoxic activities in secondary spleen cells from IL-4-treated mice demonstrated significantly ($P = 0.03$) less cytotoxicity than in spleen cells obtained from control mice (Fig. 2C). Furthermore, analysis of the total IgG, IgG1, and IgG2a antibody titers in serum samples of mice 8 days after infection demonstrated a reduction in IgG2a antibodies and a very slight increase in IgG1 antibodies in the sera of mice treated with IL-4 (Fig. 3). The total antibodies in treated and untreated mice were approximately equal. The results shown in Fig. 3 are those for a 1/100 dilution of serum. At a dilution of 1/1000, the binding was lower but identical for these two groups, supporting the finding of equal total antibody production in treated and untreated mice. IL-4-treated mice had a mean of 42 ng of total IgE per ml in their sera, while controls had a mean of 12 ng/ml (data not shown).

DISCUSSION

The possibility of experimentally manipulating immune responses by using various cytokines has been demonstrated in other systems. Thus, it has been shown that it is possible to prevent or reverse signs of experimental autoimmune encephalitis with either IL-4 or anti-IL-12 (15, 28). Similarly, the inoculation of mice with schistosoma eggs has been shown to induce Th2 cytokines; when followed by vaccinia virus challenge, this results in impaired virus clearance (1). Adoptive transfer of Th2 clones specific for influenza virus failed to clear virus compared with the transfer of Th1 clones (11). Unfortunately, the effects of these clones on the generation of CD8 T cells could not be determined in these experiments and the mechanism by which major histocompatibility complex class II-restricted T cells clear virus from infected cells which don't express class II has not been fully elucidated (40).

In these experiments, we directly investigated the effects of treatments with selected cytokines on the course of influenza virus infection in mice. In the adoptive transfer experiment, we found that the treatment of sensitized cells with IL-4 during secondary in vitro stimulation resulted in lower concentrations of IFN-γ in supernatant fluids (Table 1); after adoptive transfer to PR8 virus-infected mice, IL-4 treatment of cells was associated with the loss of the virus clearing activity seen after adoptive transfer of cells restimulated in the absence of cytokines (Table 1). Related results were obtained in experiments in which virus-infected mice were treated with IL-4 in vivo after infection. Such treatment resulted in delayed virus clearance without a significant change in the amount of virus-specific antibody responses when compared with those of control mice (Fig. 1 and 3). Moreover, in vitro stimulation of spleen cells from PR8 virus-infected, IL-4-treated mice resulted in significantly lower class I-restricted CTL activity, less IFN-γ, and more IL-4 than was seen after in vitro stimulation of spleen cells from infected untreated mice (Fig. 2). Taken together, these observations point to an inhibitory effect of IL-4 treatment on virus clearance mechanisms.

We postulate that in the present situation, influenza virus infection of the respiratory tracts of BALB/c mice stimulates an immune response with a strong Th1 cell component (40). In turn, this promotes virus clearance either as a consequence of the ability of Th1 cells to facilitate the generation of cytotoxic T cells or possibly in part because of the cytokines secreted by Th1 cells. In any case, the present data document that it is possible (with the use of IL-4) to deviate the response from one in which Th1 cells are dominant to a Th2 cell-dominated response, with accompanying delays in viral clearance. Whether it is possible to convert a Th2 cell-dominant response to one in which Th1 cells predominate remains to be seen. Observations by others suggest that it is difficult to convert a Th2 response to a Th1 response once it has been initiated (9, 21). The profound inhibition of antiviral immunity in vivo by small amounts of IL-4 may be significant in situations in which excess IL-4 might be present (pregnancy, parasitic infection, and allergic response). Its presence might inhibit an individual's ability to recover from an ongoing virus infection.

The in vivo effects of IL-4 seen in these experiments are not limited to BALB/c mice. In other experiments, we have observed similar effects in C57BL/6 mice infected with adenovirus (data not shown).

The data of Sad and Mosmann (31) demonstrating that IL-4 may inhibit ongoing Th1 responses by extinguishing the production of IL-2 are intriguing. In their report, TC1 cells continued to function in a cytotoxicity assay but failed to continue to produce IL-2. They speculated that this may be a mecha-

nism for controlling activated Th1 and TC1 activity. This may explain the failure of adoptive transferred T cells incubated in IL-4 to efficiently clear virus from the lungs of infected animals. The lack of IL-2 production may have inhibited their ability to proliferate after being injected into infected mice. We are currently testing this hypothesis by the administration of exogenous IL-2 in culture and in vivo.

In vivo treatment of mice with IL-4 probably functions to prevent the generation of CTL and Th1 helper cells. This is demonstrated by the failure of treated mice to generate efficient CTL responses and Th1 cytokines when restimulated in vitro with viral antigens in the absence of added cytokines. Our results show that neither cytotoxicity nor Th1 cytokine secretion is completely inhibited, but this may result from difficulties in systemic administration of IL-4, particularly when given i.p. Whether interference with antiviral immunity results from diminished CTL activity or a reduction in Th1 cytokine production will have to be determined in further studies.

One issue which must be addressed is the difference in the effects of IL-4 on cytotoxicity when added during secondary stimulation in vitro or given in vivo during primary response. When IL-4 was given to animals after infection, secondary stimulation of spleen cells taken 7 days after infection generated less cytotoxic activity than that seen in secondary spleen cells from control infected animals. In contrast, IL-4 added during secondary in vitro stimulation had no effect on the generation of cytotoxic activity. One explanation for this seeming discrepancy may be that Th1 and CTL precursors generated during primary immunization are not inhibited with respect to the maturation of cytotoxic T-cell activity during secondary culture even if IL-4 is present. On the other hand, IL-4 present during the primary response in vivo might block the expansion of Th1 or CTL precursors. In any case, IL-4 added to secondary cultures prior to adoptive transfer to infected mice was associated with less rapid clearance of virus than that seen in recipients of secondary immune cells obtained in the absence of IL-4, even though the cytotoxic activities in the two cultures were equivalent. IL-4 administered in vivo to infected mice was also associated with delayed virus clearance (in this case in a context of diminished cytotoxic activity). These observations raise the possibility that the effects of IL-4 on virus clearance are mediated by cytokine release rather than by effects on cytotoxicity.

Currently, we are testing the hypothesis that a Th2 cell-dominant response may be generated by immunization with inactivated virus. If so, we may be able to convert it to a Th1 response by the inclusion of IL-12 in the immunization. Furthermore, the present data may bear indirectly on the ongoing controversy (6, 12) over whether the onset of AIDS in human immunodeficiency virus-infected individuals is associated with a gradual shift from a Th1-dominated response to a Th2-dominated one. Evidence obtained in the influenza virus system clearly indicates that a cytokine (IL-4)-mediated effect seen both in vivo and in adoptive transfer experiments is associated with defects in virus clearance.

ACKNOWLEDGMENTS

We appreciate the technical assistance of Caroline Koeppel, Helen Huh, and Saori Isobe. We thank Alfred Nisonoff and Seiji Haba for the kind gift of reagents for the IgE assay, Maurice Gately (Hoffmann-La Roche) for IL-12, and Peter Palese for critical reading of the manuscript.

This work was supported by grant 1RO1A132081 from the National Institute of Allergy and Infectious Diseases.

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