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Interleukin 12 administration enhances Th1 activity but delays recovery from influenza A virus infection in mice[1]

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Abstract

Interleukin 12 (IL-12) directs the differentiation of undifferentiated T helper (Th0) cells to T helper type 1 (Th1) cells and induces a cell-mediated immune response. To evaluate the effect of IL-12 on the course of influenza A virus infection, BALB/c mice were administered a daily intraperitoneal dose of 1000 ng of IL-12 or saline on days -1 to $+4$ for a total of six treatments. The treatment generally enhanced Th1-mediated responses. IFNγ lung concentrations were 1193 ± 275 pg/100 μl in controls and 3693 ± 745 pg/100 μl in IL-12-treated mice at day 5. IFNγ levels were undetectable at day 13 in controls and 1335 ± 220 pg/100 μl in IL-12-treated mice. Cytokine production was also assessed at the single-cell level for mediastinal lymph nodes. IL-12 treatment increased the number of IL-2- and IFNγ-producing cells and decreased the number of IL-4- and IL-10-producing cells. IL-12 treatment decreased the anti-influenza antibody response, especially anti-influenza IgG1 antibody resulting in an increased IgG2a/IgG1 ratio. Primary pulmonary CTL activity on day 5 was low for both groups (10% specific lysis). Secondary CTL activity at day 11 was higher for control mice than for IL-12-treated mice on day 11 (44 versus 34%), but not on day 13. Despite this overall enhancement of Th1-mediated immune functions, the IL-12 treatment increased severity of the disease. Following infection, control and IL-12-treated mice decreased their body weight to $\sim 75\%$ of their initial weight. After day 5, the control mice started to recover, while IL-12-treated mice did not begin recovering until day 9. Pulmonary viral titers were 1.6 ± 0.3 $TCID_{50}$ in controls at day 5 compared to 2.4 ± 0.3 for IL-12-treated mice ($P < 0.01$). In addition, control mice had significantly less severe inflammation and damage on histologic examination. Serum TNFα concentrations, undetectable in control mice, were elevated by IL-12 treatment up to 80 pg/ml at day 5 and decreased to zero at day 13. It is concluded that IL-12 administration to influenza-infected mice induces a switch from a Th2- to a Th1-mediated response, but inhibits recovery probably through induction of TNFα.

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1. Introduction

Influenza outbreaks cause significant morbidity and mortality. In the US alone, there are 40000–80000 deaths/year and in the large pandemic of 1918–1919, over 20 million people died world-wide (Smith, 1992; Betts, 1995). Despite this severity, only two specific anti-viral drugs—amantadine and rimantadine—are available and our understanding of pathogenesis and host defense is incomplete.

Most new approaches to controlling influenza are through novel influenza vaccines. Various methods of vaccination have shown that inducing anti-influenza cell mediated immunity (CMI) in mice can have a strong beneficial effect on the recovery of mice after an influenza challenge (Kuwano et al., 1989; Ulmer et al., 1993; Bender et al., 1996). Clinical trials of CMI-based vaccines, however, have so far produced only modest results (Fries et al., 1993).

Another potential therapeutic approach to modulating the course of influenza and other viral infections is the administration of IL-12 (Gately and Mulqueen, 1996). IL-12 is primarily produced by monocyte-macrophages and other accessory cells (D'Andrea et al., 1992) as a heterodimeric protein composed of p35 and p40 subunits (Wolf et al., 1991). IL-12 mediates a broad range of effects on host immunity, including augmentation of CTL and natural killer cell activity, induction of T helper type 1 (Th1)-mediated immune responses and inhibition of Th2-mediated immune responses (Manetti et al., 1993; Trinchieri, 1993). In experimental animals, IL-12 administration promotes recovery from a variety of pathogens, including viruses (Gladue et al., 1994; Orange et al., 1994; Bi et al., 1995; Carr et al., 1997), protozoa (Heinzel et al., 1993; Urban et al., 1996), fungi (Zhou et al., 1995) and mycobacteria (Flynn et al., 1995).

Recovery from influenza infection in mice is primarily mediated by anti-influenza CD8+ CTL activity (Eichelberger et al., 1991; Bender et al., 1992); other contributing factors are interferon γ, (IFNγ), CD4+ cytolytic activity and serum neutralizing antibody (Bender et al., 1994; Graham et al., 1994; Palladino et al., 1995). As these are all presumptive Th1-mediated functions (Mosmann et al., 1986; Mosmann and Coffman, 1989), it was expected that IL-12 administration to mice would speed recovery from an influenza challenge by enhancement of Th1 activity. While IL-12 administration enhanced most measures of Th1 activity, it paradoxically delayed recovery.

2. Materials and methods

2.1. Mice, virus and IL-12

Pathogen-free female BALB/c mice 6–16 weeks of age were obtained from Charles River Laboratories (Wilmington, MA) and Department of Pathology (UF, Gainesville, FL) and housed five per cage in specific-pathogen-free conditions. Before and during the experiment they were fed and watered ad libitum.

Influenza A/Port Chambers/1/73 (H3N2) virus, grown in chicken eggs (Yetter et al., 1980), at a concentration of 1.7×10^7 50% egg infectious doses/ml, was used to infect the animals. A volume of 40 μl was given intranasally after the mice were anaesthetized (Yetter et al., 1980). Viral titers of lung samples were determined in tissue culture as previously described (Bender et al., 1992) and expressed as 50% tissue culture infective doses ($TCID_{50}$)/ml.

Recombinant murine IL-12 (Roche Laboratories, Nutley, NJ) was diluted in phosphate-buffered saline (PBS)/1% bovine serum albumin (BSA) in concentrations ranging from 100 ng to 10 μg/ml. A 100 μl volume was injected intraperitoneally.

2.2. Protocol

On day 0 mice were inoculated with influenza virus. Mice were given six daily IL-12 doses start-

ing at 1 day before infection (day −1). In a preliminary experiment, mice were given varying doses of IL-12 ranging from 10–1000 ng/day. Control mice were given PBS/1%BSA injections instead of IL-12 of the same volume. In the main experiments, mice received 1000 ng/day. In one experiment, a separate group received the same injections with a 1000 ng primer dose 1 week before the first injection (day −8). As a control, some mice were given IL-12 only.

On days 5, 11 and 13 post-infection, a number of mice from all groups were sacrificed for assays described below. Body weight was measured on a daily or every other day basis.

2.3. Histology

The cranial lobe of the right lung was prepared for histologic examination as described earlier (Bender et al., 1995). A scale from 0 to 5 was used to describe the percentage of tissue which showed morphologic abnormality: 0, 0–5% of examined tissue showed the abnormality; 1, 5–20%; 2, 20–40%; 3, 40–60%; 4, 60–80%; 5, 80–100%.

2.4. CTL assay

Primary anti-influenza CTL activity was measured using freshly isolated pulmonary lymphocytes (Bender et al., 1995) and secondary CTL activity was measured using splenocytes and a secondary in vitro culture system (Bender et al., 1991).

2.5. Antibody assays

Serum samples were frozen at −20°C until used for assays. Serum neutralizing antibody titers were determined as previously described (Bender et al., 1994).

Total IgG antibody and antibody subclass detection were done by ELISA as previously described (Taylor et al., 1997). Microtiter plates were coated with 50 μl/well of antigen (A/PC/73), blocked with 1% Knox gelatin solution, washed and duplicate 2-fold serum samples (initial dilution 1:10) added. After a 1-h incubation, plates were washed with PBS/Tween and goat-derived IgG anti-mouse IgG, IgG2a or IgG1 (Sigma Immuno Chemicals, St. Louis, MO) added. The plates were washed and 50 μl of rabbit anti-goat IgG alkaline phosphatase conjugate solution per well (Sigma Immuno Chemicals, St. Louis, MO) were added. After another incubation and washing, 200 µl/well of substrate (*p*-nitrophenyl phosphate disodium, 1 mg/ml diethanolamine) were added, allowed to incubate for 30 min at room temperature and the reaction stopped with 50 μl/well NaOH. The plates were read at 405 nm for optical density and values obtained at a dilution of 1/80.

2.6. Cytokines

IFNγ concentration in lung samples and TNFα concentration in serum were measured by an ELISA. The mouse IFNγ ELISA kit was obtained from Endogen (Cambridge, MA) and the TNFα kit from Genzyme (Cambridge, MA). The lower limit of detection was 10 pg/ml.

In order to quantitate the numbers of cytokine-producing cells, mediastinal lymph nodes were harvested and an enzyme-linked spot forming (ELISPOT) assay was performed as previously described (Taylor et al., 1997). Briefly, specific cytokine capture monoclonal antibody was added to a 96 well Millipore Multiscreen-HA plate and incubated overnight. After washing, 100 μl of serially-diluted cells were added to triplicate wells and incubated in a 37°C/5% CO_2 incubator. The plate was washed and 100 μl of specific biotinylated anti-cytokine detecting monoclonal antibody were added to each well and incubated overnight at 4°C. The plate was washed and peroxidase-conjugated polyclonal goat anti-biotin antibody was added and incubated overnight at 4°C. The plate was washed and 200 μl of chromogen substrate added to each well. After 30–60 min, the number of spots was counted using a stereo microscope and the ratio of spot-forming cells to total cell population was calculated.

2.7. Statistics

Data were entered onto a Macintosh Power PC computer and analyzed using InStat 2.00 (GraphPad Software).

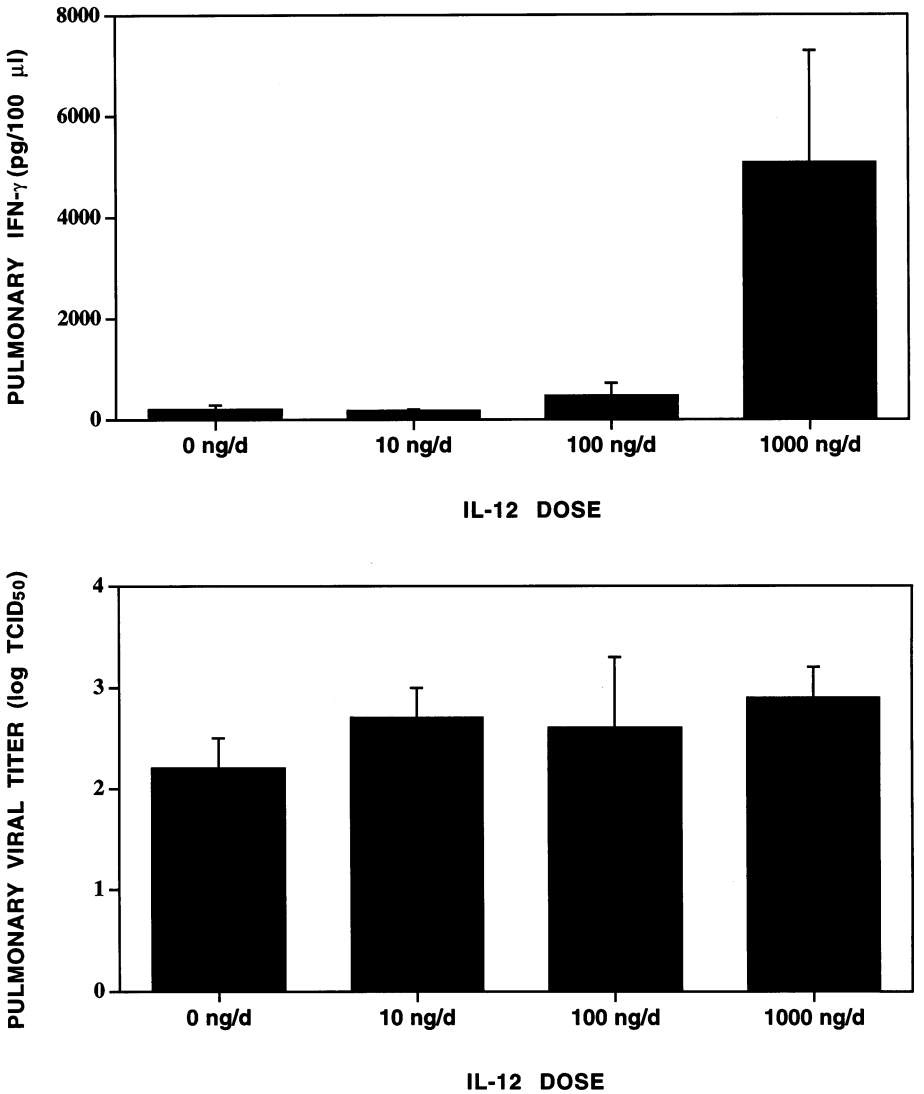

Fig. 1. Effect of increasing doses of IL-12 on influenza-infected mice. BALB/c mice were challenged intranasally with 10^5 $TCID_{50}$ of H3N2 influenza virus on day 0 and and sacrificed on day 5 for determination of pulmonary IFNγ in pulmonary supernatants by an ELISA (top) and pulmonary influenza viral titers (bottom). Mice were given varying doses of IL-12 i.p. on days -1 through $+4$. Data are mean $\pm$ S.E. of 3–5 mice/group. By one-way ANOVA, there was a significant effect of IL-12 on pulmonary IFNγ levels ($P = 0.0065$), but not viral titers.

3. Results

3.1. Cytokines

It was found that increasing daily doses of IL-12 increased pulmonary IFNγ levels (Fig. 1). Because the maximal effect seemed to be reached with 1000 ng/day, in subsequent experiments mice were treated with 1000 ng/day. It was found that IL-12 administration significantly enhanced IFNγ in the lungs of mice infected with influenza virus on days 5, 11 and 13 (Fig. 2).

Because many other cytokines are not readily detected in pulmonary supernatants (Hennet et al., 1992 and our unpublished observations), an ELISPOT assay, was performed. Fig. 3 shows

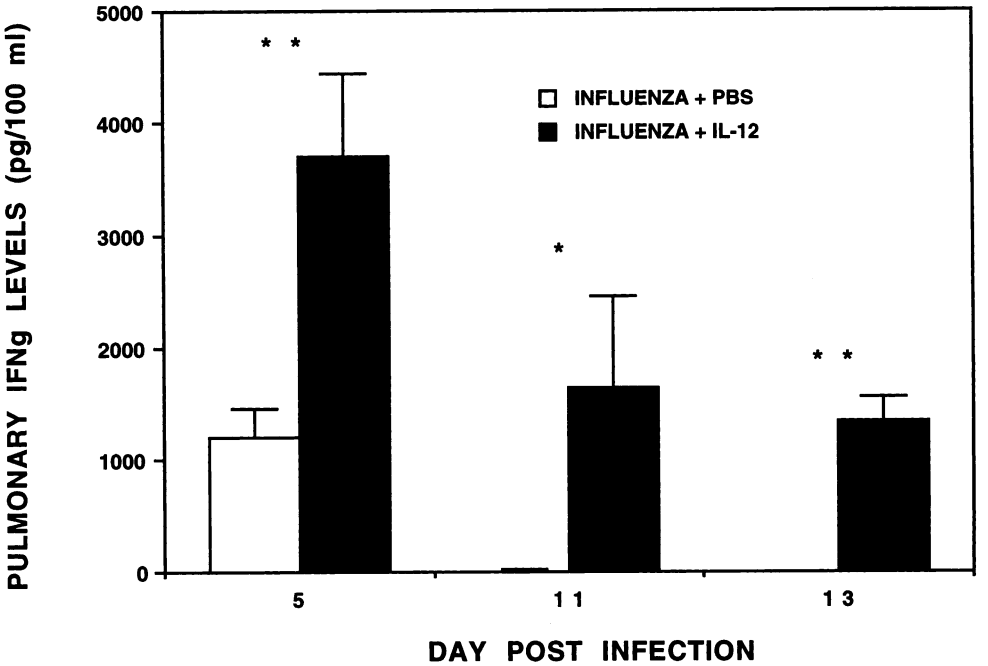

Fig. 2. Pulmonary IFNγ production. BALB/c mice were challenged intranasally with 10^5 $TCID_{50}$ of H3N2 influenza virus on day 0 and body weights were determined thereafter. Mice were also given IL-12 (1000 ng/day) i.p. on days -1 through $+4$ (■) or PBS (□). Mice were sacrificed on days 5, 11 and 13 for measurement of IFNγ in pulmonary supernatants by an ELISA. The data are the combination of four separate experiments and represent the mean $\pm$ S.E. of 15–16 mice/group per day on day 5 and 5–6 mice/group per day on days 11 and 13. By Student's two-tailed t-test: $^*P < 0.05$, $^{**}P < 0.01$, influenza + IL-12 versus influenza + saline.

that on both days 5 and 13 post-challenge, the numbers of putative Th1-like IL-2-producing and IFNγ-producing mediastinal T-cells were increased, though this effect was most pronounced on IFNγ-producing cells. To determine the effect of IL-12 administration on Th2-type cells, the numbers of IL-4- and IL-10-producing cells were measured. IL-12 administration decreased the numbers of these cells on day 5, but on day 13 there was an increased number of IL-4-producing cells and no difference in the IL-10 cells.

3.2. Anti-influenza CTL activity

IL-12 administration had a minimal effect on anti-influenza CTL activity (Fig. 4). Primary pulmonary CTL activity was low for both control ($10 \pm 2\%$ specific lysis) and IL-12-treated mice ($12 \pm 4\%$). Secondary splenic CTL activity was significantly lower in IL-12-treated mice on day 11 post-infection (44 ± 2 versus $34 \pm 2\%$, $P < 0.05$), but this difference was lost by day 13 (60 ± 4 versus $61 \pm 3\%$ specific lysis).

3.3. Anti-influenza antibody response

Serum samples collected at days 5, 11 and 13 were tested for anti-influenza virus neutralizing antibodies. On day 5 no neutralizing antibodies were detected in either IL-12 treated or PBS treated mice. On days 11 and 13, the geometric mean neutralizing titer was 1/36 and 1/22 for control mice, while IL-12-treated mice were still $< 1/10$ ($P < 0.01$, Wilcoxon, Table 1).

IL-12 administration can profoundly effect the immunoglobulin isotype produced to various antigens (Morris et al., 1994; Germann et al., 1995; Van Cleave et al., 1995; Gracie and Bradley, 1996); serum anti-influenza IgG, IgG2a and IgG1 levels were therefore determined on days 11 and

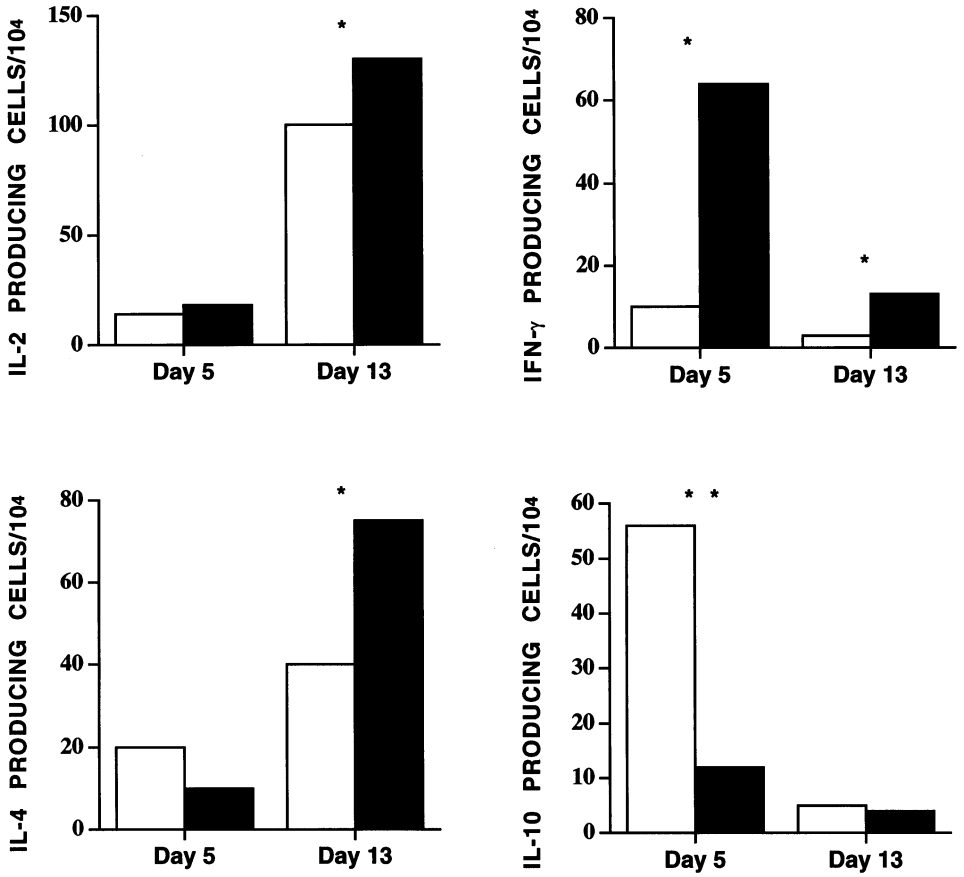

Fig. 3. Frequency of IL-2-, IL-4-, IL-10- and IFNγ-producing mediastinal T-cells. Mice were challenged with influenza and given IL-12 (■) or saline (□) as in Fig. 2 legend and sacrificed on days 5 and 13. Mediastinal lymph nodes were harvested, T-cells separated using a nylon wool column and an ELISPOT assay performed as detailed in Section 2. The frequency (geometric mean titer) of Th1-type (IL-2 and IFNγ, top) and Th2-type (IL-4 and IL-10, bottom) cells/10^4 T-cells from 6 mice/group are shown. By Mann–Whitney (non-parametric) two-tailed test: $^*P < 0.05$, $^{**}P < 0.01$, influenza + IL-12 versus influenza + saline.

13 post-infection (Table 1). Control mice had more total anti-influenza IgG than IL-12 treated mice and antibody titers increased from day 11 to day 13 in both groups. Anti-influenza IgG1 was significantly inhibited by IL-12 treatment, but not IgG2a antibody, resulting in an increased IgG2a/IgG1 ratio.

3.4. Body weight

As a general measure of the severity of illness the mice were weighed daily. As shown in Fig. 5, IL-12 administration alone caused a weight loss of less than 5%. After influenza challenge, the body weight of mice given PBS decreased to 78% of their initial weight by day 4. Recovery began at day 5 and the mice reached 93% of their initial weight by day 13, when the experiment was terminated. IL-12 administration caused influenza-infected mice to continue to lose weight until day 9, when its nadir was 72%, after which recovery began. At day 13, the IL-12-treated mice were back to approximately 80% of their initial weight. The difference in body weight between control and IL-12 treated mice was significant at day 8 through day 13 ($P < 0.05$).

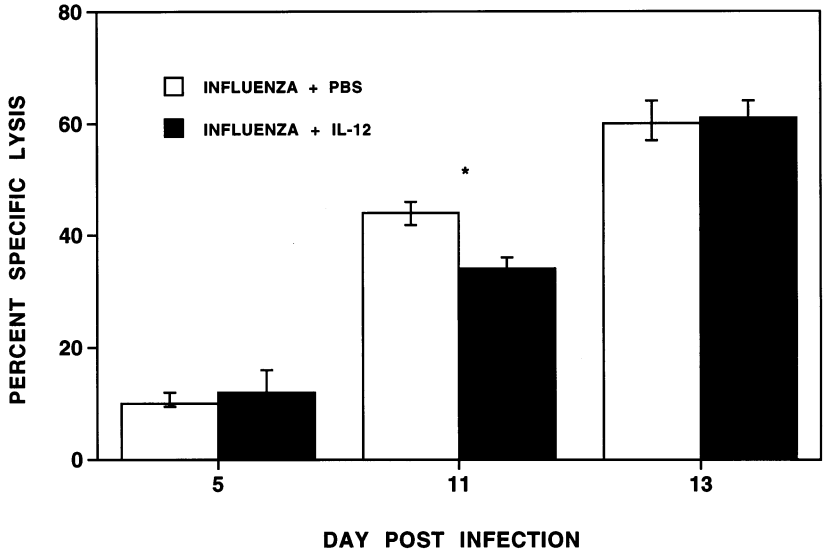

Fig. 4. Effect of IL-12 administration on primary pulmonary and secondary splenic anti-influenza CTL activity. Mice were challenged with influenza and given saline or IL-12 as in Fig. 2 legend and sacrificed for primary pulmonary CTL activity (Bender et al., 1995) on day 5 or secondary splenic CTL activity (Bender et al., 1991) on days 11 and 13. Data are the mean ± S.E. of ^{51}Cr released from P815 cells sensitized with H3N2 virus of 8–12 mice/group per day. $*P < 0.05$, Student's two-tailed t-test.

3.5. Viral titers

Following influenza challenge, pulmonary viral titers of naive mice peak at day 1–3 post-infection and begin to drop by day 5, with enhanced clearance being noted in mice with heterotypic immunity induced by prior infection with a shifted virus or vaccination (Yetter et al., 1980; Bender et al., 1992, 1996; Bender and Small, 1993; Meitin et al., 1994). In a small, preliminary experiment (Fig. 1), it was found that IL-12 administration slightly, but not significantly, increased pulmonary influenza virus titers 5 days post-challenge. This experiment was repeated three times with a total of ten control and 11 IL-12-treated mice; Table 2 shows that control mice shed 1.6 ± 0.3 $TCID_{50}$ of influenza virus compared to mice given IL-12 which shed 2.4 ± 0.3 $TCID_{50}$ ($P < 0.01$). By days 11 and 13, when all mice were recovering, the virus was cleared from the lungs (Table 2).

3.6. Histologic examination

To determine whether the delay in viral clearance was also reflected in histopathology, pulmonary tissue samples were evaluated by an experienced pulmonary pathologist who was blinded to the identity of the samples. It was found (Table 2) that IL-12 administration had a modest, but statistically significant, effect on enhancing pulmonary necrosis and inflammation on day 11 post-challenge, with non-significant effects on either days 5 or 13.

3.7. Induction of TNFα

As the main biological effect of IL-12 administration appeared to be inducing more weight loss, serum levels of TNFα, a cytokine known to induce this effect, were measured (Fong et al., 1989). It was found that either influenza infection or IL-12 administration alone induces small amounts of TNFα (2 ± 1 and 9 ± 3 pg/ml, respectively) while IL-12 administration to influenza-infected mice significantly raised serum TNFα levels (80 ± 10 pg/ml, $P < 0.05$, Fig. 6). By day 13 post-infection, TNFα was no longer detectable in the serum, except for one mouse in the influenza only group where it was 2 pg/ml (data not shown).

TNFα also appears in the broncho-alveolar washings of influenza-infected mice (Vacheron et al., 1990; Hennet et al., 1992) where it may exert some antiviral effects (Van Campen, 1994). Contrary to the above data, it was found that IL-12 administration to influenza-infected mice inhibited pulmonary TNFα production (Fig. 6).

4. Discussion

T helper cells exist in two subsets that are distinguished by their effects on the immune response. Th1 cells produce IFNγ, IL-2 and other cytokines which evoke a CMI response and Th2 cells produce such cytokines as IL-4, IL-5, IL-6 and IL-10 and induce a humoral response (Mosmann et al., 1986; Mosmann and Coffman, 1989). The two cell subsets differ in effectiveness to control different pathogens. Leishmaniasis, for example, is best controlled by activation of Th1 cells (Scott et al., 1988; Heinzel et al., 1989), while borreliosis requires Th2-mediated cytokines (Keane-Myers et al., 1996). It is therefore important for an organism to control the differentiation of uncommitted T-cells (Th0) into the most appropriate T helper type to cure an infection (Scott, 1993; Trinchieri and Scott, 1994).

Table 1
Effect of IL-12 administration on development of anti-influenza serum antibody

Antibody class	Group	Day post-infection		
		5	11	13
Neutralizing	Control	<1/10	1/36	1/22
	IL-12	<1/10	<1/10	<1/10
	P value		<0.001	<0.001
IgG	Control	n.d.	780 ± 40	1410 ± 30
	IL-12	n.d.	300 ± 70	980 ± 90
	P value		<0.05	<0.05
IgG2a	Control	n.d.	370 ± 70	830 ± 70
	IL-12	n.d.	260 ± 50	730 ± 120
	P value		N.S.	N.S.
IgG1	Control	n.d.	360 ± 40	680 ± 60
	IL-12	n.d.	50 ± 11	60 ± 40
	P value		<0.01	<0.01
IgG2a/IgG1	Control	n.d.	1.6 ± 0.3	1.8 ± 1.3
	IL-12	n.d.	35 ± 13	15 ± 0
	P value		<0.001	<0.01

Mice were challenged with influenza and given saline or IL-12 as in Fig. 2 legend and anti-influenza serum antibody titers were determined by viral neutralization and subclass determination was done by ELISA.
ELISA values are optical density at 405 nm of a 1/80 serum dilution.
Data are geometric mean titers for neutralizing antibody and mean ± S.E. for IgG subclass for 5–6 mice/day per group.
N.d., not determined.
N.S., not significant.

IL-12 and IL-4 compete in directing the response to primarily CMI or humoral immunity, respectively. In experiments with *Leishmania* infection, anti-IL-12 antibody administration exacerbated the disease and either IL-12 or anti-IL-4 antibody administration helped the mice to recover (Sadick et al., 1990; Heinzel et al., 1993; Hsieh et al., 1993; Sypek et al., 1993). This effect was correlated with a shift from Th2 to Th1 cells. Comparable studies with other pathogens in mice examined the effect of exogenous IL-12 on resistance, including treatment for viral infections such as lymphocytic choriomeningitis virus, cytomegalovirus and murine retrovirus (Gazzinelli et al., 1994; Gladue et al., 1994; Orange et al., 1994; Hendrzak and Brunda, 1995). Further, Moran et al. showed that IL-4 administration to influenza infected mice delayed viral clearance, probably via reduction in anti-influenza CTL activity and IFNγ production (Moran et al., 1996).

It was therefore determined whether IL-12 administration would be beneficial in mice infected with influenza A virus. Similar to results from experiments with other pathogens (Gladue et al., 1994; Ozmen et al., 1995), it was found that the most striking immunological consequence of IL-12 administration was a dose-dependent induction of IFNγ. Pulmonary IFNγ is induced by influenza infection; it is also induced by IL-12 and appears to assist in the recovery from influenza (Carding et al., 1993; Sarawar et al., 1993) and other infections (Gladue et al., 1994; Orange et al., 1994; Flynn et al., 1995; Ozmen et al., 1995; Zhou et al., 1995; Urban et al., 1996). Pulmonary IFNγ levels were higher on day 5 post-challenge in IL-12-treated mice, when pulmonary levels are usually

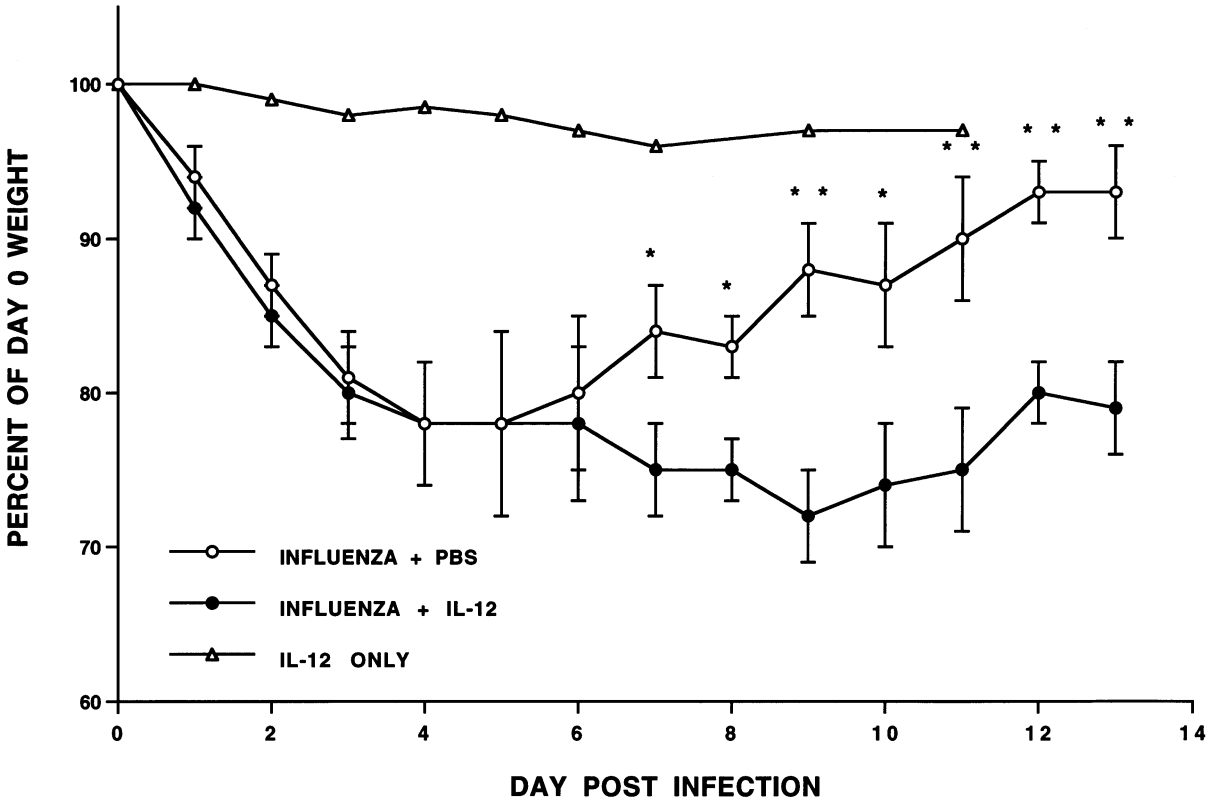

Fig. 5. IL-12 administration delays recovery from an influenza challenge. BALB/c mice were challenged intranasally with 10^5 $TCID_{50}$ of H3N2 influenza virus on day 0 and body weights were determined daily or every other day thereafter. Mice were also given IL-12 (1000 ng/day) i.p. on days -1 through $+4$ (●) or PBS (○). A separate group of three mice was also given IL-12 alone on days -1 through $+4$ (△). Standard error bars are shown for the influenza infected animals only. The data are a combination of four experiments with a total of 12 and 16 animals for the influenza + IL-12 and influenza + PBS control groups, respectively. $^*P < 0.05$, $^{**}P < 0.01$, difference in weights between influenza + IL-12 and influenza + PBS groups.

at or near their peak (Hennet et al., 1992; Sarawar et al., 1993; Conn et al., 1995) and pulmonary IFNγ was still present on days 11 and 13, when it was undetectable in control mice (Figs. 1 and 2). This correlated with an increase in the number of IFNγ-producing T-cells (Fig. 3).

Our other immune evaluations were also concordant with reported observations on the effects of IL-12 administration (Manetti et al., 1993; Trinchieri, 1993). It increased the number of IL-2-producing mediastinal T-cells and decreased the number of IL-4- and IL-10-producing mediastinal T-cells (Fig. 4). It decreased total anti-influenza antibody production, especially anti-influenza IgG1, while increasing the anti-influenza IgG2a/IgG1 ratio (Table 1). Perhaps the only unexpected finding was that it had little effect on anti-influenza CTL activity and on the only day a statistically significant effect was seen (day 11), it actually lowered this response (Fig. 4). This contrasts with previous reports that IL-12 is a potent stimulator of cytotoxicity against herpesvirus-infected cells (Chehimi et al., 1993).

Despite this expected enhancement of of Th1-mediated activities, it was found that IL-12 administration generally had an adverse effect on the clinical course of the influenza infection. The IL-12-treated mice had more pulmonary histopathology, especially on day 11 (Table 1), did not regain weight as quickly (Fig. 5) and had higher levels of virus in their lungs on day 5 (Fig. 1 and Table 2). Gladue et al. in a small study found that

Table 2
Effect of IL-12 administration on pulmonary viral titers and histology of influenza infected mice

	Group	Days		
		5	11	13
Viral titers (log $TCID_{50}$)	Control	1.6 ± 0.3	0	0
	IL-12	2.4 ± 0.3	0	0
	P value	<0.01	NS	NS
Necrosis	Control	1.4 ± 0.3	2.8 ± 0.5	1.0 ± 1.0
	IL-12	2.5 ± 0.4	4.4 ± 0.5	2.0 ± 0.3
	P value	NS	<0.05	NS
Inflammation	Control	1.3 ± 0.2	3.8 ± 0.2	3.0 ± 0.3
	IL-12	1.6 ± 0.3	4.6 ± 0.2	2.7 ± 1.3
	P value	NS	<0.05	NS

Mice were challenged with influenza and given saline or IL-12 as in Fig. 2 legend and sacrificed on days 5, 11 and 13 for viral titers and histological examination.
The data are mean $\pm$ S.E. of 5–11 mice/day per group for the viral titers and 4–6 mice/day per group for histology.

low dose treatment with IL-12 of influenza-infected mice also increased IFNγ levels, but did not affect pulmonary viral titers (Gladue et al., 1994). It was also found that treatment of mice with lower doses of IL-12 also increased pulmonary IFNγ levels, but without significantly changing viral titers (Fig. 1). It was tried giving the mice a primer dose of 1 μg of IL-12 one week prior to influenza challenge and IL-12 administration; this resulted in finding similar to our data on IL-12 treatment—it increased pulmonary IFNγ levels while delaying recovery from infection (data not shown).

Another concern was whether 1000 ng/day of IL-12 was too high of a dose. It was therefore compared the effects of six daily doses of IL-12 at 100 ng/day and 500 ng/day. These doses also enhanced pulmonary IFNγ levels on day 5 while delaying recovery of body weight (data not shown).

Infection of mice or humans with influenza virus leads to a distinct histopathological picture characterized by an accumulation of inflammatory cells and destruction of respiratory epithelium (Straub, 1937; Wyde et al., 1978; Bender et al., 1995; Baumgarth and Kelso, 1996). The precise messages that recruit cells to the respiratory tract following influenza infection are not known, but Baumgarth and Kelso recently showed that administration of an anti-IFNγ antibody to influenza-infected mice lead to a marked reduction of pulmonary cellular infiltrate (Baumgarth and Kelso, 1996). It therefore seems likely that the higher amounts of inflammation seen in our IL-12-treated mice was due to the higher amounts of induced pulmonary IFNγ.

It is believed that the excess weight loss was due to enhanced induction of TNFα, a cytokine well known to induce this effect (Fong et al., 1989). IL-12 or influenza infection alone induced small amounts of serum TNFα, while together they induced a significant increase in serum TNFα levels (Fig. 6). Orange et al. demonstrated that IL-12 administration to mice infected with lymphocytic choriomemingitis virus (LCMV) also caused significant weight loss (Orange et al., 1995); this was accompanied by increased expression of TNFα mRNA expression. They also found that treatment of mice with anti-TNFα antibody protected against IL-12 mediated toxicity and that the induced TNFα contributed to lower CD8 + T-cell responses. Clinical studies are now underway to determine whether the administration of anti-TNFα antibody can improve survival in gram-negative bacterial sepsis (Abraham et al., 1995) and it is possible that a similar approach may be of benefit in severe viral infections. For example, the mechanism for the in-

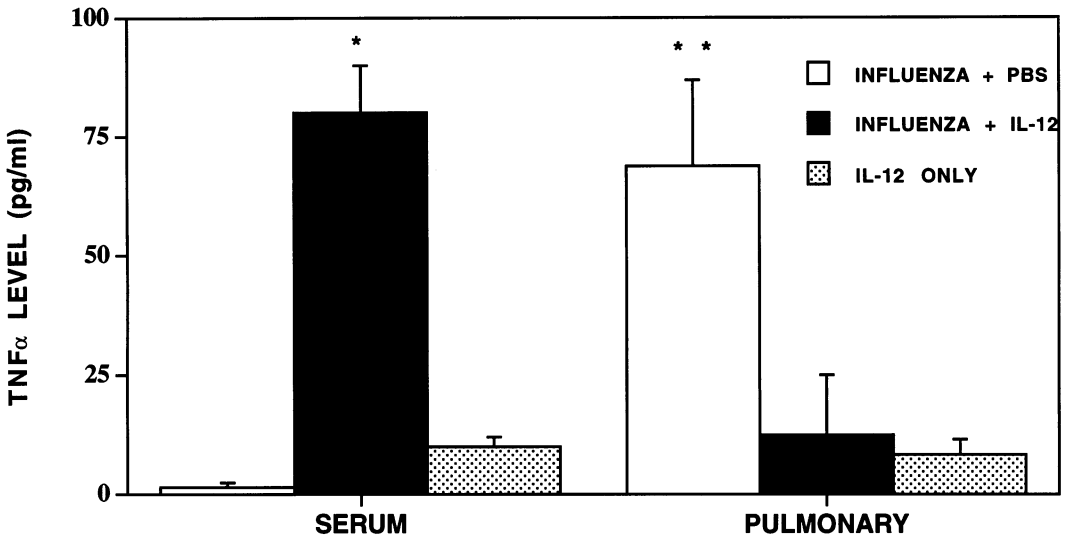

Fig. 6. Effect of IL-12 administration on induction of serum and pulmonary TNFα. Mice were challenged with influenza and given saline or IL-12 as in Fig. 1 legend and sacrificed on day 5 for determination of serum and pulmonary TNFα by an ELISA. Data are mean ± S.E. for 5 mice/group for mice given influenza and saline (□) influenza and IL-12 (■) and three mice for IL-12 only (□). No TNFα was found in the sera or lungs of naive mice. By one-way ANOVA with post-hoc Dunnet's test: $*P < 0.05$, influenza + IL-12 versus influenza + PBS and IL-12 only; $**P < 0.05$, influenza + PBS versus influenza + IL-12 and IL-12 only.

creased mortality, especially of young adults, in the severe influenza pandemic of 1918–1919 is not known. Could this increased mortality be due to enhanced induction of serum TNFα? It was noted that in our experiments, the only animals that died were influenza-infected mice given IL-12; overall, 3/22 mice given IL-12 died as compared to 0/25 mice given saline.

The other clear adverse effect of IL-12 administration was the delayed viral clearance. This was probably due to a number of factors. The primary viral clearance mechanism is anti-influenza CD8+ CTL activity (Eichelberger et al., 1991; Bender et al., 1992) and a small, but statistically significant, lower anti-influenza CTL activity in the IL-12-treated mice, was found (Fig. 4). Another viral clearance mechanism that was lowered by IL-12 administration was serum neutralizing antibody (Table 1). It needs to be pointed out that both of these effects were only seen on later days, while the significantly different viral titers were found early in the course of infection (day 5). Influenza virus replication can be inhibited in vitro by addition of TNFα to cell cultures (Van Campen, 1994) and it was found that contrary to its systemic effects, IL-12 administration actually inhibited pulmonary TNFα production (Fig. 6). Thus, as with other cytokines, local production of TNFα is independent of its serum levels.

Our report clearly demonstrates the potential adverse consequences of administering IL-12. Though potentially beneficial in other infections (Gazzinelli et al., 1994; Gladue et al., 1994; Orange et al., 1994; Hendrzak and Brunda, 1995; Carr et al., 1997), there would appear to be no overall net benefit from treating influenza infections with IL-12. It might still be useful, however, in enhancing cellular responses to novel influenza vaccines (Wynn et al., 1995; Marinaro et al., 1997).

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