

IFN- τ inhibits IgE production in a murine model of allergy and in an IgE-producing human myeloma cell line

Mustafa G. Mujtaba, PhD,^a Lorelie Villarete, PhD,^b and Howard M. Johnson, PhD^a

Gainesville, Fla, and Alameda, Calif

Background: IFN- τ , a type I IFN, is an antiviral, immunomodulating, and antiproliferative agent similar to IFN- α and IFN- β , but IFN- τ lacks the toxicity associated with high concentrations of these IFNs in tissue culture and in animal studies. We have previously shown that IFN- τ inhibits antibody production in a murine model of an autoimmune disease.

Objective: We investigate the effectiveness of ovine IFN- τ and other type I IFNs in suppressing the development of allergic sensitization in a murine model of allergy by using ovalbumin (OVA) antigen as an allergen and in suppressing IgE production by using a human IgE-producing myeloma cell line.

Methods and Results: Mice that were treated with IFN- τ in vivo before and after intraperitoneal immunization with aluminum hydroxide-precipitated OVA had significantly lower OVA-specific IgE levels than the PBS-treated group. IFN- τ -treated mice had reduced inflammatory cell infiltration into the lung tissue. Furthermore, in vitro IFN- τ treatment of splenocytes taken from OVA-immunized mice suppressed OVA-induced proliferation. Also, treatment of the IgE-producing human myeloma cell line U266BL with IFN- τ -reduced IgE production and inhibited cell proliferation compared with media controls. Similar suppression of proliferation and inhibition of IgE production was seen with other type I IFNs, as well as a humanized IFN- τ /IFN- α D chimeric that consists of residues 1 to 27 of the ovine IFN- τ and residues 28 to 166 of the human IFN- α D. The chimeric was not toxic to human peripheral white blood cells at concentrations as high as 10^5 U/mL, whereas human IFN- α D was toxic at 10^3 U/mL.

Conclusion: These data suggest that IFNs may be useful in preventing allergic sensitization by suppressing the production of allergen-specific IgE antibodies without toxic side effects. (*J Allergy Clin Immunol* 1999;104:1037-44.)

Key words: IFN, IgE, ovalbumin, mouse, human myeloma, lung, infiltration, inhibition

IFN- τ is a member of the type I IFN family, but unlike IFN- α and IFN- β , IFN- τ lacks toxicity at high concentrations in vitro and when used in vivo in animal studies.¹⁻⁶ Like the type I IFNs, IFN- τ is acid stable and

Abbreviations used

EAE: Experimental allergic encephalomyelitis
HRP: Horseradish peroxidase
OVA: Ovalbumin

exerts antiviral, antiproliferative, and immunomodulatory activities.¹⁻³ We have previously shown that IFN- τ suppresses the humoral and cellular responses in experimental allergic encephalomyelitis (EAE), an animal model for the autoimmune disease multiple sclerosis.⁷ In this study we investigate the effectiveness of type I IFNs in suppressing IgE production in vivo by using a murine model of allergy and in vitro by using a human IgE-producing myeloma cell line.

Allergic sensitization of mice by intraperitoneal injection with ovalbumin (OVA) as an allergen and aluminum hydroxide as an adjuvant is a well-characterized method of IgE production in vivo.⁸⁻¹⁰ Production of IgE is important in mediating allergic diseases, such as allergic rhinitis, atopic dermatitis, bronchial asthma, and food allergy. When OVA-immunized mice are challenged with aerosolized OVA, they show inflammatory cell infiltration in the submucosal layer of the lungs.¹¹⁻¹³ IgE can stimulate the release of certain chemotactic mediators from mast cells that can lead to active accumulation of macrophages and granulocytes at the site. Also, production of a further set of inflammatory molecules by these cells can lead to allergic asthma. Thus allergen-specific IgE production by B cells is important in the pathogenesis of allergic diseases. The U266BL cell line, an IgE-producing myeloma line that was isolated from the peripheral blood of a patient, provides a system to assess the direct effects of IFN- τ on an IgE-producing cell.¹⁴ This allows us to determine the effects of IFN- τ on B cells with ongoing IgE synthesis. Thus we investigate the effectiveness of IFN- τ in inhibiting IgE production both in vitro and in vivo, which may lead to a useful therapy for allergic diseases, such as asthma.

METHODS

IFNs

The ovine IFN- τ gene was expressed in *Pichia pastoris* by using a synthetic gene construct, which was kindly provided by Dr Gino Van Heeke (Ciba Pharmaceuticals, London, England).¹⁵ IFN- τ was secreted into the medium and was purified by successive diethylaminoethyl cellulose and hydroxylapatite chromatography to electrophoretic homogeneity as determined by SDS-PAGE and silver staining analysis. The purified protein had a specific activity of

From ^athe Department of Microbiology and Cell Science, University of Florida, Gainesville; and ^bPepgen Corp, Alameda.

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Reprint requests: Mustafa Mujtaba, BS, Department of Microbiology and Cell Science, University of Florida, Bldg 981, Room 1052, Gainesville, FL 32611.

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2.9 to 4.4×10^7 U/mg protein as measured by antiviral activity by using a standard viral microplaque reduction assay on Modin Darby bovine kidney cells. The recombinant human IFN- α D was purchased from Biosource International (Camarillo, Calif). The humanized IFN- τ /IFN- α D chimeric was constructed by using residues 1 to 27 of the ovine IFN- τ and residues 28 to 166 of the human IFN- α D and was expressed in *P pastoris* as previously described for ovine IFN- τ .¹⁵

IFN- τ was administered intraperitoneally at 5×10^5 U per mouse daily starting 96 hours before immunization and continuing everyday thereafter for a month. PBS was used as the vehicle for administration.

Immunization of mice

BALB/c mice were immunized intraperitoneally with 10 μ g of OVA (Sigma, St Louis, Mo) precipitated with 5 mg of aluminum hydroxide gel in a total volume of 100 μ L. Aluminum hydroxide gel was prepared as previously described.^{16,17} Mice were immunized again 7 days after the initial immunization by using the same protocol. The mice were exposed to aerosolized OVA from 1% OVA (wt/vol) in PBS on days 19 and 20 after immunization. Aerosolization was performed for 20 minutes by using the Pari Is Jet + nebulizer and compressor (Pari Respiratory Equipment, Inc, Midlothian, Va). Control groups consisted of OVA-sensitized mice that only received PBS (no IFN). Nonsensitized mice were mice that did not receive OVA, and untreated mice (naive) were mice that received neither OVA nor IFN and were used as controls for comparison with OVA-sensitized PBS- and IFN- τ -treated mice. Mice were housed and cared for at the Animal Resource Center at the University of Florida, and all experimental animal uses were approved by the Institutional Animal Care and Use Committee.

Histologic evaluation

The lungs of OVA-immunized mice that had been exposed to aerosolized OVA were intratracheally perfused with 4% paraformaldehyde solution. The lungs were fixed for 2 to 3 days in the same solution, after which lung samples taken from the hilar region of the left lung were embedded in paraffin and sectioned. Samples were then stained with hematoxylin and eosin. Also, blood smears were prepared on slides from the same mice, and slides were stained with the Leukostat staining kit (Fisher Scientific, Pittsburgh, Pa) for the determination of differential white blood cell count. A total of 150 white blood cells were evaluated.

Proliferation assay

Spleen cells taken from mice 21 days after immunization from PBS or IFN- τ -treated mice were cultured at 5×10^5 cells/well in presence of OVA for 72 to 84 hours in RPMI-1640 medium containing 10% FBS. In other assays PBS-treated mouse splenocytes were incubated at 5×10^5 cells/well in the presence of OVA and various IFNs (10,000 to 15,000 U/mL) for 72 to 84 hours. The cultures were pulsed with tritiated thymidine (1.0 μ Ci/well; Amersham, Indianapolis, Ind) and harvested 12 hours later onto filter paper discs by using a cell harvester. Cell-associated radioactivity was quantified by using a β -scintillation counter, and activity was reported in counts per minute. Proliferation assays on the U266BL myeloma B cells were also carried out by incubating the cells in RPMI-1640 medium overnight before culturing 4×10^5 cells/well with various IFNs at 10,000 to 15,000 U/mL in RPMI-1640 containing 4% FBS. Cultures were incubated for 72 to 84 hours, after which cells were pulsed with tritiated thymidine before harvest 12 hours later. Cell-associated radioactivity was quantified by using a β -scintillation counter, and activity was reported in counts per minute.

ELISA

OVA was resuspended in binding buffer (0.1 mol/L carbonate-bicarbonate, pH 9.6) and absorbed onto the flat bottoms of plastic 96-well tissue-culture wells overnight at 4°C at a concentration of 2 μ g/well and subsequently evaporated to dryness. The plates were treated with blocking buffer (5% powdered milk in PBS) for 2 hours to block nonspecific binding and then washed 3 times with PBS containing 0.05% Tween 20. Various dilutions of sera from BALB/c mice that were treated with IFN- τ or PBS or nonimmunized (naive) mice were added to the wells and incubated for 3 hours at room temperature. After extensive washing, a 1:2000 optimal dilution of rabbit anti-mouse IgE antibody (Accurate, NY) was added. Plates were washed 3 times before addition of a 1:1000 dilution of horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin (Amersham Pharmacia Biotech, Piscataway, NJ). Color development was monitored at 490 nm in an ELISA plate reader (BioRad, Richmond, Calif) after the substrate solution (0.002 mol/L o-phenylenediamine dihydrochloride, 0.012% H₂O₂, 0.05 mol/L Na Citrate, and 0.05 mol/L citrate) was added, and the reaction was terminated with 2 mol/L H₂SO₄.

For the detection of IL-4 in blood, sera samples were collected from PBS- or IFN- τ -treated mice and incubated in 96-well plates that had 1 μ g/well of rabbit polyclonal anti-mouse IL-4 antibody (Biosource Int, Camarillo, Calif) bound to it. After washing, 25 μ g/mL rat monoclonal anti-mouse IL-4 biotinylated antibody (Biosource Int) was added for 1 hour of incubation. A 1:1000 dilution of HRP-conjugated avidin was added after the incubation and washings, and substrate color development was monitored as described above. The limit of detection of the IL-4 ELISA was 7 ng/mL.

Immunoblot

Culture supernatants from both IFN- or media-treated OVA-sensitized splenocytes and U266BL myeloma B cells were loaded at 10 μ g/lane (total protein) on 15% and 10% reducing SDS-PAGE Ready Gels (BioRad), respectively, and run at 100 V. Overnight transfer onto nitrocellulose membrane was carried out, after which the membrane was blocked with 5% milk in Tris-buffered saline (pH 7.5) and 0.1% Tween 20 for 1 hour. Immunoblots were incubated with a 1:1000 dilution of either goat anti-human IgE (Biosource Int) coupled to HRP or rabbit anti-mouse IgE (Accurate, NY). The mouse IgE blot was incubated further after 3 washes with HRP-conjugated anti-rabbit Ig (Amersham Pharmacia Biotech) for 1 hour. Blots were washed and analyzed through film development.

Toxicity of IFNs

Toxicity assays with human PBMCs were carried out by culturing human PBMCs in the presence of varying concentrations of IFN- τ , IFN- α D, and chimeric IFN- τ /IFN- α D for 7 days. Metabolic activity of human PBMCs was assessed by using WST-1 (Boehringer-Mannheim), which measures cell proliferation and viability on the basis of the enzymatic activity of mitochondrial dehydrogenase in viable cells.

RESULTS

IFN- τ -inhibited production of OVA-specific IgE antibody in mice

The basic question was whether IFN- τ could inhibit production of IgE antibody specific for OVA in BALB/c mice. As shown in Fig 1, A, daily intraperitoneal injections of IFN- τ at 5×10^5 U beginning before OVA-aluminum hydroxide intraperitoneal injection blocked IgE

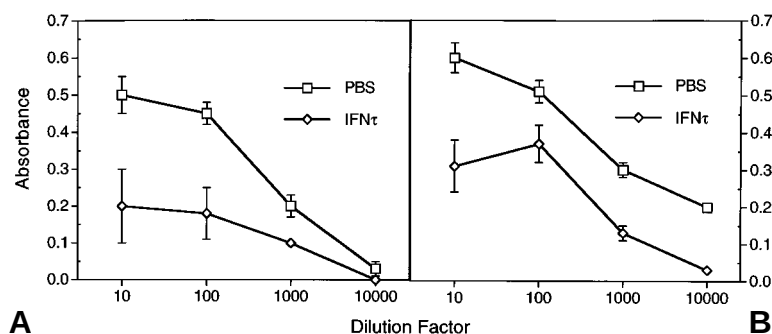


FIG 1. Inhibition of OVA-specific IgE antibody production in OVA-sensitized mice by IFN- τ treatment. BALB/c mice were immunized by intraperitoneal injection with OVA mixed with aluminum hydroxide and boosted 7 days later. The mice were exposed to aerosolized OVA (1% wt/vol) on days 19 and 20 after immunization for 20 minutes. Mice were treated daily with intraperitoneal injections of IFN- τ (5×10^5 U/d) or PBS starting 3 days before immunization. Blood was collected 24 hours before (**A**) and 24 hours after (**B**) aerosolized OVA exposure. Direct ELISA was performed to detect OVA-specific IgE levels. Two to 3 mice per group were used, and average absorbance is shown. Control absorbance with a normal mouse serum has been subtracted out from each dilution point. Statistical significance for the inhibition of OVA-specific IgE antibody production was shown by means of the Student *t* test at all dilutions (except for the 10,000 dilution) for IFN- τ treatment compared with PBS treatment ($P < .05$).

TABLE I. IFN- τ reduces OVA-induced inflammatory cell infiltration into the bronchioles

Treatment	Lung sections		Blood smears				
	Granulocyte aggregates (% airways)	Lymphocyte aggregates (% airways)	Eosinophil (%)	Basophil (%)	Neutrophil (%)	Monocyte (%)	Lymphocyte (%)
PBS	64 $\pm$ 6.0	78 $\pm$ 7.0	11 $\pm$ 5.0	6.0 $\pm$ 1.0	20 $\pm$ 0.7	10 $\pm$ 1.4	58 $\pm$ 6.0
IFN- τ	20 $\pm$ 7.0	34 $\pm$ 3.0	2.5 $\pm$ 0.7	1.5 $\pm$ 0.7	15 $\pm$ 1.4	5.0 $\pm$ 1.4	72 $\pm$ 8.0
Naive	0	0	0.2 $\pm$ 0.3	0.5 $\pm$ 0.7	19 $\pm$ 1.4	10 $\pm$ 0.7	71 $\pm$ 4.0

Mice were treated intraperitoneally with 5×10^5 U of IFN- τ daily starting 3 days before OVA immunization as described in the "Methods" section. Airways having peribronchiolar aggregates were enumerated and divided by the total number of bronchioles examined in each section. Differential white cell counts are presented as a percentage cell type. Statistical significance for the inhibition of granulocyte and lymphocyte aggregates around the bronchioles was shown by means of the χ^2 test for IFN- τ treatment compared with PBS treatment ($P < .001$). Statistical significance for the inhibition of eosinophil ($P < .05$) and basophil ($P < .1$) by IFN- τ treatment compared with PBS treatment was shown by means of the Student *t* test.

antibody production by over 50%. Furthermore, the blocking continued even when the mice were challenged with aerosolized OVA (Fig 1, B). Thus IFN- τ inhibited OVA-specific IgE antibody production under conditions in which the mice were immunized to OVA by injection and challenged by inhalation.

IFN- τ -treated mice have reduced inflammatory cell infiltration

OVA-immunized mice were challenged with aerosolized OVA after treatment with IFN- τ to determine where the IFN treatment inhibited inflammatory cell infiltration into the lungs. IFN- τ inhibition of cellular infiltration is shown in Fig 2, where lung sections of naive (Fig 2, A), PBS-treated (Fig 2, B), and IFN- τ -treated (Fig 2, C) mice are compared. Note the destruction of the integrity of the epithelial tissue lining the bronchiole of the PBS-treated mice (Fig 2, B) versus the protection in IFN- τ -treated mice (Fig 2, C). Eosinophil, basophil, and lymphocytic infiltration was assessed around bronchioles and blood vessels of IFN- τ - and PBS-treated (control) mice. Significantly fewer bronchioles had peribronchiolar aggregates of granulo-

cytes with IFN- τ treatment (20%) compared with PBS treatment (64%; Table I). Also, 78% of lung bronchioles in PBS-treated mice had lymphocytic infiltration compared with 34% of lung bronchioles in IFN- τ -treated mice. In addition, differential counts on the blood of mice showed that IFN- τ -treated mice had lower levels of eosinophils and basophils compared with those of PBS-treated groups, whereas neutrophil, monocyte, and lymphocyte levels were not significantly different from either the PBS-treated or naive (nonimmunized) mice. Thus the data show that IFN- τ treatment inhibits inflammatory cell infiltration into the lungs of OVA-sensitized mice when exposed to aerosolized OVA allergen.

IFN- τ -treated mice have lower IL-4 levels than control mice

IL-4 levels in sera of mice treated with PBS or IFN- τ or nonimmunized (naive) mice were measured after aerosolized OVA exposure, which was given 20 days after immunization. It has been shown previously that IL-4 may be necessary for inducing the IgE isotype class switch in B cells.¹⁸⁻²¹ As shown in Fig 3, IL-4 levels in

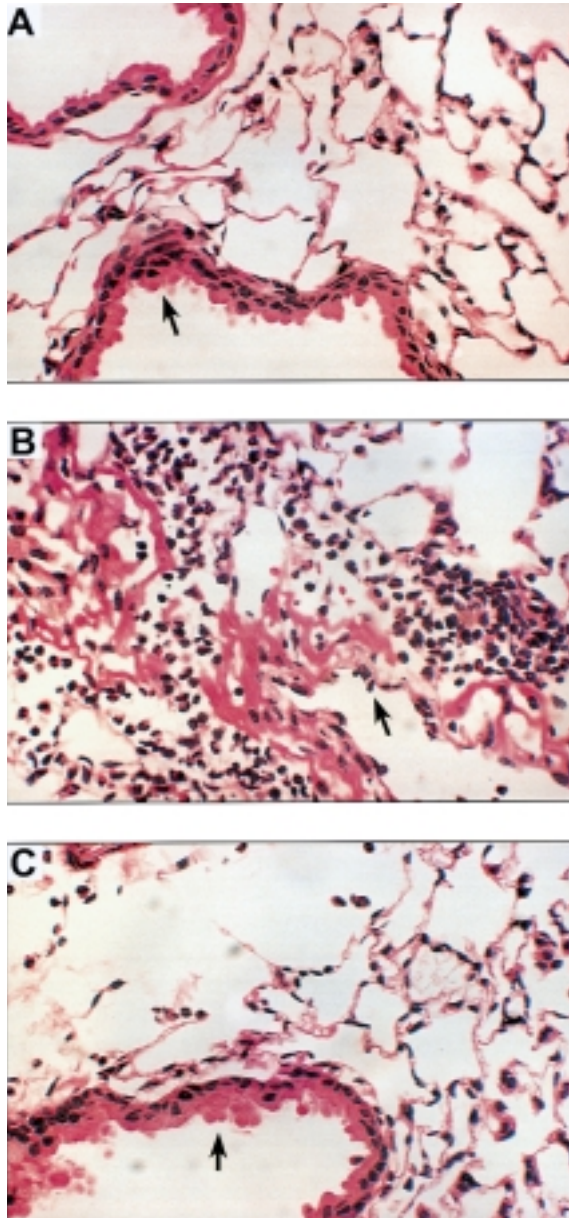


FIG 2. Histologic evaluation of OVA-immunized mice after treatment with PBS or IFN- τ . BALB/c mice were immunized with OVA and exposed to aerosolized OVA on day 20 after immunization and treated with PBS or IFN- τ , as previously described in the Fig 1 legend. Twenty-four hours after aerosolized OVA treatment, lungs from nonimmunized (**A**), PBS-treated (**B**), and IFN- τ -treated (**C**) mice were extracted, fixed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for inflammatory cells. Arrows indicate the epithelium of the bronchiole.

the IFN- τ -treated group were less than half of those of the PBS-treated group, suggesting that suppression of IL-4 production by IFN- τ may be important in the inhibition of IgE production.

In vivo IFN- τ treatment inhibits OVA-specific splenocyte proliferation

Spleens from nonimmunized (naive) mice and PBS- or IFN- τ -treated OVA-immunized mice were removed 20

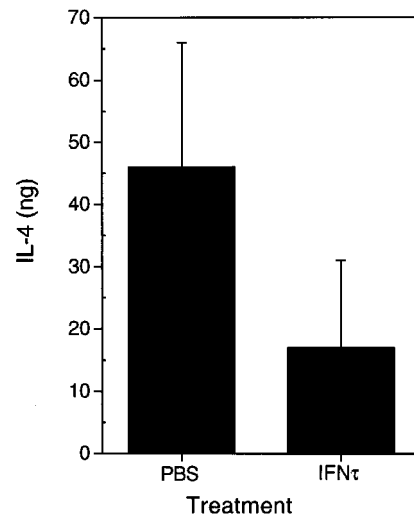


FIG 3. IL-4 levels in sera of OVA-immunized mice treated with PBS or IFN- τ . BALB/c mice were immunized with OVA and exposed to aerosolized OVA and treated with PBS or IFN- τ , as previously described in the Fig 1 legend. Blood was collected 24 hours after aerosolized OVA exposure, and a sandwich ELISA for IL-4 was performed. Two to 3 mice per group were used, and an average amount (in nanograms) of IL-4 is shown. Control level from naive mouse serum was subtracted from the PBS and IFN- τ levels.

days after OVA immunization to determine the inhibitory effect of IFN- τ treatment on OVA-induced proliferation. Previously, we showed that IFN- τ suppresses the responses of lymphocytes to mitogens, such as concanavalin A, and superantigens, such as staphylococcal enterotoxin A and staphylococcal enterotoxin B.^{3,4,6} Splenocytes were incubated in the presence of OVA for 84 hours, after which proliferation was assessed. As shown in Fig 4, significantly reduced proliferation in response to OVA was observed in splenocytes from IFN- τ -treated mice compared with PBS-treated control mice. This proliferative activity was specific for OVA because BSA and myelin basic protein did not activate splenocytes (Fig 4, *inset*). Thus in vivo IFN- τ treatment of allergen-primed mice inhibited cellular proliferation in response to allergen.

In vitro IFN- τ treatment of OVA-sensitized splenocytes inhibits OVA-specific splenocyte proliferation

OVA-sensitized splenocytes were treated in vitro with various IFNs to determine their effect on previously sensitized cells. Spleens were removed 20 days after OVA immunization and after aerosolized OVA treatment and cultured with various type I IFNs for 84 hours, after which proliferation was assessed. In addition to ovine IFN- τ treatment, human IFN- α D and a chimeric of IFN- τ and IFN- α D (IFN- τ /IFN- α D) were also tested for their effects on OVA-induced proliferation. The chimeric was tested as a potential for humanized IFN- τ for possible human therapy. As shown in Fig 5, both IFN- τ and IFN-

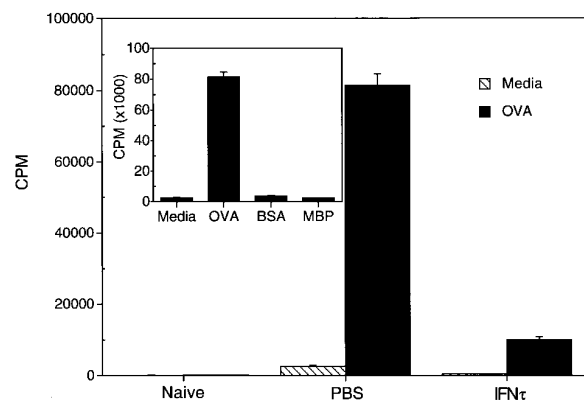


FIG 4. In vivo treatment of OVA-immunized mice with IFN- τ reduces OVA stimulation of splenocytes. BALB/c mice were immunized with OVA and exposed to aerosolized OVA and treated with PBS or IFN- τ , as previously described in the Fig 1 legend. Spleen cells (5×10^5 cells/well) were cultured with OVA at 100 μ g/mL for 84 hours, after which the cultures were pulsed with tritiated thymidine. PBS-treated splenocytes were also incubated with BSA and myelin basic protein (MBP) at 100 μ g/mL (inset). Cell-associated radioactivity was quantified 12 hours later by using a β -scintillation counter, and data from one of 3 experiments are presented as mean counts per minute (CPM) of quadruplicate wells $\pm$ SD. Statistical significance for the inhibition of OVA-induced cell proliferation by IFN- τ treatment compared with PBS treatment was shown by using the Student *t* test ($P < .001$).

α D inhibited OVA-specific splenocyte proliferation. Furthermore, the IFN- τ /IFN- α D chimeric, which contained amino acid residues 1 to 27 of ovine IFN- τ and residues 28 to 166 of human IFN- α D, also had an inhibitory effect. Thus treatment of OVA-sensitized splenocytes in vitro with type I IFNs suppressed cell proliferation.

Type I IFNs inhibit mouse and human IgE production

Immunoblots for the detection of IgE antibodies were performed on culture supernatants taken from the proliferation assay experiments performed in Figs 4 and 5. As shown in Fig 6, A, IgE was detected in cultures containing PBS-treated splenocytes that were incubated in the presence of OVA. There was little or no IgE in supernatants from splenocytes of IFN- τ -treated mice. Immunoblots for detection of IgE in culture supernatants from in vitro IFN treatment of OVA-sensitized mouse splenocytes showed an inhibition of IgE production by all of the type I IFNs compared with the media control (Fig 6, B). The chimeric IFN- τ /IFN- α D and IFN- α D were better inhibitors than was IFN- τ , however. Also, the U266BL human myeloma cell line, which produces IgE antibodies constitutively, was incubated with the type I IFNs to determine whether they had a direct effect on the human IgE-producing B cells. Cells were starved overnight before treatment with various type I IFNs, including the IFN- τ /IFN- α D chimeric. After incubation with the IFNs for 96 hours, supernatants were collected, and IgE levels were detected by immunoblot. As shown in Fig 6, C, IgE levels were lower in the IFN-

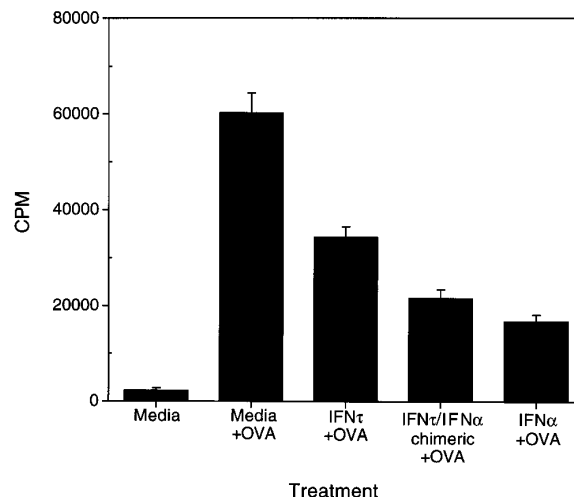


FIG 5. In vitro treatment of splenocytes with type I IFNs inhibits OVA-specific proliferation. BALB/c mice were immunized by intraperitoneal injection of OVA mixed with aluminum hydroxide and boosted 7 days later. The mice were exposed to aerosolized OVA (1% wt/vol) on day 19 and 20 for 20 minutes. Spleen cells (5×10^5 cells/well) were cultured with 15,000 U/mL of various IFNs and media in the presence or absence of 100 μ g/mL OVA for 84 hours, after which the cultures were pulsed with tritiated thymidine. Cell-associated radioactivity was quantified 12 hours later by using a β -scintillation counter, and data from one of 3 experiments are presented as mean counts per minute (CPM) of quadruplicate wells $\pm$ SD. Inhibition of OVA-specific splenocyte proliferation by all the IFNs was statistically significant compared with OVA-specific splenocyte proliferation of OVA-sensitized medium-treated cells as shown by means of the Student *t* test ($P < .001$).

treated groups compared with the media control. Thus type I IFNs inhibit U266BL human myeloma cells and OVA-specific mouse B cells from producing IgE antibodies.

IFN inhibition of proliferation of the human IgE-producing myeloma cells

The U266BL myeloma cells were cultured in the presence of 10,000 U/mL IFNs for 72 hours, after which proliferation was measured. All IFNs inhibited proliferation of the cells by about 50% or more, with IFN- α D being the most effective and IFN- τ being the least effective (Fig 7). Viabilities were determined and showed that IFN- α D was the most toxic (67% viability) compared with IFN- τ (80% viability) and the IFN- τ /IFN- α D chimeric (75% viability). The IFN- τ /IFN- α D chimeric suppressed proliferation more effectively than IFN- τ but not as effectively as IFN- α D. Thus type I IFNs, with various toxicity levels, inhibit the cell proliferation of the human IgE-producing cell line U266BL.

Lack of toxicity of the IFN- τ /IFN- α D chimeric on human PBMCs

Because the IFN- τ /IFN- α D chimeric is ultimately to be used in treating multiple sclerosis in human subjects, we compared it with recombinant ovine IFN- τ and recombinant human IFN- α D for toxicity on human

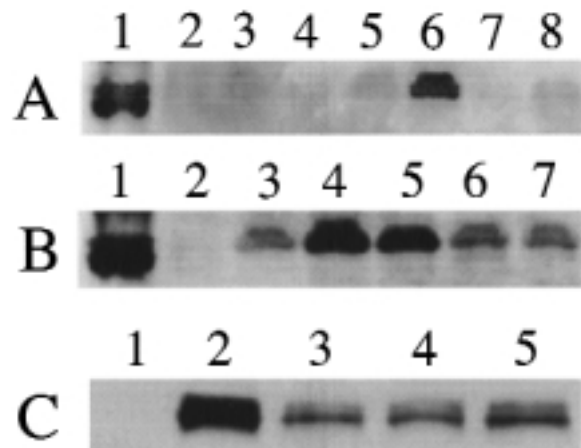


FIG 6. Immunoblot for the detection of mouse and human IgE in culture supernatants taken from OVA-sensitized mouse splenocytes or human myeloma B cells treated with various IFNs or media. **A:** Lane 1, control mouse IgE; lane 2, RPMI-1640 supplemented with 10% FBS; lanes 3 and 4, 84-hour supernatants from naive mouse splenocytes cultured in the absence or presence of OVA, respectively; lanes 5 and 6, 84-hour supernatants from PBS-treated OVA-sensitized mouse splenocytes cultured in the absence or presence of OVA, respectively; lanes 7 and 8, 84-hour supernatant from IFN- τ -treated OVA-sensitized mouse splenocytes in absence or presence of OVA, respectively. **B:** Lane 1, control mouse IgE; lane 2, RPMI-1640 medium only; lane 3, 84-hour splenocyte culture in the absence of OVA; lanes 4, 5, 6, and 7, 84-hour splenocyte (5×10^5 cells/well) cultures with OVA in presence of media, IFN- τ , IFN- τ /IFN- α chimeric, and IFN- α D, respectively. **C:** Lane 1, RPMI-1640 medium only; lanes 2, 3, 4, and 5, IgE-producing U266BL cells that were starved overnight and incubated at 2×10^5 cells/well for 96 hours in the presence of media, 1.0×10^4 U/mL IFN- α D, IFN- τ /IFN- α D chimeric, and IFN- τ , respectively.

PBMCs. After 7 days of treatment of the human PBMCs with various concentrations of IFNs (250 to 100,000 U/mL), toxicities were measured on the basis of the enzymatic activity of the mitochondrial dehydrogenase in viable cells. As shown in Fig 8, human IFN- α D was toxic at concentrations of 1000 to 100,000 U/mL compared with ovine IFN- τ and IFN- τ /IFN- α D chimeric, which did not show toxicity at any concentration. Thus the IFN- τ /IFN- α D chimeric, like ovine IFN- τ , lacked the toxicity associated with human IFN- α D. This is a first step to humanizing ovine IFN- τ with respect to lack of toxicity.

DISCUSSION

The cytokine IL-4 plays a central role in isotype switching of the B cells to IgE production.¹⁸⁻²¹ It is estimated that 40% of the population has atopic allergies as a result of this. We have shown here that the effector arm of these events, the production of IgE, is blocked by a naturally occurring cytokine, ovine IFN- τ , and its chimeric, ovine IFN- τ /human IFN- α D. Although IFN- τ is a type I IFN, it was originally identified as a pregnancy recognition hormone produced by trophoblast cells of the placenta of ruminants, such as sheep and cows.²²⁻²⁵ It

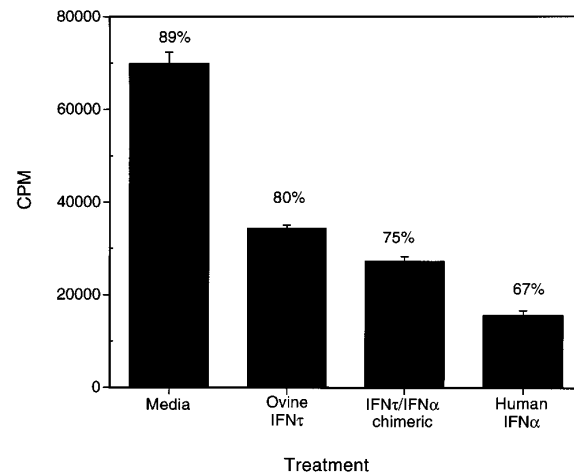


FIG 7. Inhibition of proliferation of the IgE-producing human myeloma B-cell line U266 by type I IFNs. The IgE-producing U266BL cells, which were starved overnight, were incubated at 2×10^5 cells/well in the presence of 1.0×10^4 U/mL of IFN- τ , IFN- τ /IFN- α D chimeric, IFN- α D, and media for 72 hours. Cultures were pulsed with tritiated thymidine, and cell-associated radioactivity was quantified 12 hours later by using a β -scintillation counter. Data from one of 3 experiments are presented as mean counts per minute (CPM) of quadruplicate wells $\pm$ SD. Percentage cell viability, as measured by trypan blue exclusion test, is presented above each bar. Statistical significance for the inhibition of cell proliferation was shown by means of the Student *t* test for all the IFN treatments compared with the media treatment ($P < .001$).

has been reported that a human IFN- τ exists,²⁶ but this observation has not been confirmed. Thus it is currently unknown whether there is a human IFN- τ . IFN- τ is of particular interest because its antiviral and cell inhibitory properties are very similar to those of IFN- α and IFN- β .¹⁻³ However, IFN- τ lacks the cellular toxicity associated with high concentrations of IFN- α and IFN- β .^{1,2} Furthermore, the weight loss and bone marrow suppression that is associated with administering high doses of IFN- α and IFN- β to individuals is absent with IFN- τ in animal systems.³⁻⁵ Thus we have included ovine IFN- τ (residues 1-27) and human IFN- α D (residues 28-166) in the present study, with a view toward humanizing IFN- τ for human use. The chimeric lacks the toxicity associated with human IFN- α D, while differing from it by only 15 residues. The chimeric was constructed on the basis of our studies showing that the N-terminus of type I IFNs played a central role as to their toxicity or lack thereof.^{27,28}

Our data suggest that IFN- τ inhibition of IgE production probably occurred at several levels. The fact that the IFN inhibited IgE production by the human IgE myeloma cell line is evidence that it was capable of directly blocking B-cell secretion. Thus one mechanism of IFN inhibition of OVA-specific IgE production by mice and their splenocytes most probably occurred by a direct effect on the B cells. We have previously shown that type I IFNs inhibit the progression of Daudi B cells through the G1 phase of the cell cycle.²⁹ Thus the inhibition of the cell cycle of cells in G1 may be a mechanism by

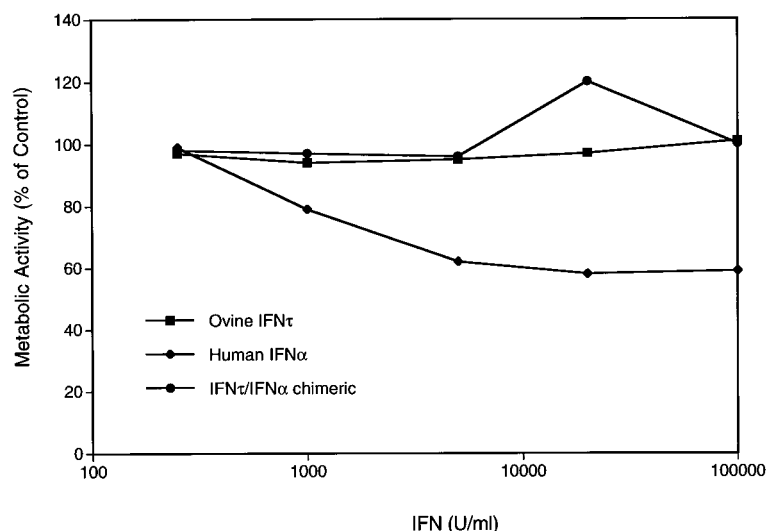


FIG 8. Metabolic activity of human PBMCs after treatment with IFNs. Human PBMCs were cultured in the presence of varying concentrations (250 to 100,000 U/mL) of IFN- τ , IFN- α D, and IFN- τ /IFN- α D chimeric for 7 days. Metabolic activity of human PBMCs was assessed by measuring cell proliferation and viability as described in the "Methods" section and reported here as a percentage of the untreated control. Values for human PBMCs treated with ovine IFN- τ and IFN- τ /IFN- α chimeric were not significantly different from those of the untreated control, whereas values for human PBMCs treated with human IFN- α were significantly different ($P < .05$) from those of the untreated controls as determined by using the Wilcoxon signed-rank test.

which IFN- τ could inhibit IgE production directly. One can draw similar conclusions with respect to B-cell proliferation. IFN- τ inhibited the myeloma proliferation and similarly inhibited splenocyte proliferation. Additionally, we have previously shown that IFN- τ could block antigen-specific B-cell proliferation and antibody production in EAE, a mouse model for multiple sclerosis.⁷ IFN- τ thus had a direct effect on B-cell secretion and proliferation.

Inhibition of IL-4 production is evidence of IFN- τ effect on CD4 T_H2 and other IL-4-secreting cells. It is quite possible that this effect is also direct because we have previously shown, again in the EAE model, that IFN- τ and other type I IFNs can induce IL-10 and tumor growth factor β , but not IL-4, production by cells that have already been activated by antigen-presenting cells.^{5,7,30} The greater than 90% inhibition of OVA-specific induction of proliferation of mouse splenocytes, as per Fig 4, suggests that T-cell proliferation, as well as B-cell proliferation, was inhibited by IFN- τ . Thus IFN- τ directly inhibited both T-cell proliferation and IL-4 production.

It is of particular significance that IFN- τ treatment of the mice inhibited inflammatory cell infiltration of lung bronchioles in mice challenged with aerosolized OVA. This protected the epithelial tissue lining the bronchioles from destruction as a result of OVA challenge. This is of potential clinical importance in the use of ovine IFN- τ and its humanized chimerics in protection of human subjects against tissue damage as a result of atopic allergies. Also, other studies with IFN- α and IFN- γ have shown similar suppression of IgE production in allergic diseases.³¹⁻³⁶ We have thus shown here that IFN- τ and its

derivatives may have potential significance as therapeutics for atopic allergies.

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