Organ Specific Cytokine Therapy

Local Activation of Mononuclear Phagocytes by Delivery of an Aerosol of Recombinant Interferon-γ to the Human Lung

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Abstract

In the context of the central role of the alveolar macrophage in host defense of the respiratory epithelial surface, and the ability of IFN-γ to activate mononuclear phagocytes, we have evaluated strategies to use rIFN-γ to activate human alveolar macrophages in vivo. To accomplish this, rIFN-γ was administered to nonsmoking normals, the amounts of IFN-γ quantified in serum and respiratory epithelial lining fluid (ELF) and the status of IFN-γ related activation of blood monocytes and alveolar macrophages was evaluated by quantifying the expression of mRNA transcripts of IP-10, a gene induced specifically by IFN-γ. Systemic administration (subcutaneous) of maximally tolerated amounts of rIFN-γ (250 μg) was followed by detectable levels of IFN-γ in serum but not ELF, the expression of IP-10 transcripts in blood monocytes but not alveolar macrophages, and multiple systemic adverse effects. To circumvent the inability of systemic administration to reach respiratory ELF and activate alveolar macrophages, rIFN-γ (250–1,000 μg) was inhaled as an aerosol once daily for 3 d. Strikingly, while IFN-γ was not detected in serum it was detectable in respiratory ELF in a dose-dependent fashion. Further, alveolar macrophages, but not blood monocytes, expressed IP-10 mRNA transcripts and, importantly, inhalation of aerosolized rIFN-γ was not associated with local or systemic adverse effects. Thus, it is feasible to use rIFN-γ to activate alveolar macrophages by targeting the cytokine directly to the lung. These data suggest a potential strategy for targeted cytokine therapy, without systemic side effects, to augment respiratory tract defenses in individuals at risk for or with lung infection. (*J. Clin. Invest.* 1991. 88:297–302.) Key words: bronchoalveolar lavage • macrophage • immune defense • IP-10 • gene

Introduction

Consequent to its role as the organ for gas exchange, the lung is constantly exposed to the outside environment, and thus must have mechanisms on its epithelial surface to contend with a constant burden of inhaled pathogens (1, 2). The alveolar macrophage plays a central role in the defense of the pulmonary epithelium by virtue of its ability to phagocytize microorganisms, produce oxygen radicals, process antigens and initiate specific T-lymphocyte responses, and recruit neutrophils (3–5). As with mononuclear phagocytes elsewhere in the body, many biologic processes of alveolar macrophages associated with these host defense functions can be augmented by IFN-γ, including increased expression of IgG Fc receptors and of class II histocompatibility antigens, exaggerated release of toxic oxygen radicals, and enhanced antimicrobial activity against intracellular parasites, bacteria and mycobacteria (6–11).

In the context of these properties of IFN-γ, it is rational to consider using rIFN-γ in circumstances where activation of alveolar macrophages might augment pulmonary host defense. However, while systemic administration of rIFN-γ to humans clearly activates blood monocytes (12–16) and augments systemic host defense in chronic granulomatous disease (17–19) and visceral leishmaniasis (20), it is unclear whether systemic administration activates mononuclear phagocytes in the lung. After preliminary studies demonstrated that systemic administration of rIFN-γ does not reach the respiratory epithelial surface, we assessed the feasibility of activating alveolar macrophages on the pulmonary epithelial surface with rIFN-γ by delivering the cytokine directly by aerosolization. Capitalizing on the knowledge that droplets of 0.1–3 μm mass median diameter (50% of droplets ≤ 0.1–3 μm) inhaled as an aerosol have the potential to reach the alveolar surface (21), we administered aerosols containing rIFN-γ to normal individuals. The data document that active rIFN-γ can be safely delivered to the epithelial surface of the lower respiratory tract at concentrations well within the range necessary to activate mononuclear phagocytes and results in activation of IFN-γ specific genes in alveolar macrophages in situ. Moreover, no detectable levels of IFN-γ nor such gene activation is detectable systemically after aerosolization, demonstrating the feasibility of organ-specific cytokine therapy to the lung in which compartmentalized, local activation of mononuclear phagocytes can be achieved.

Methods

Study population. Twenty nonsmoking normals were evaluated, including 18 males and 2 females (age 26.9±3.7 yr [all data are presented as mean±standard error of the mean; all statistical comparisons were made using the two tailed Student's *t* test]). None had a history of ongoing illness, and all had normal physical examinations, screening blood studies, arterial blood gases, chest roentgenograms, pulmonary function testing, and fiberoptic bronchoscopy with bronchoalveolar lavage (22).

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Recombinant IFN-γ preparation. The rIFN-γ (Genentech, Inc., S. San Francisco, CA) was produced by *Escherichia coli* transformed with an expressing plasmid containing a human cDNA encoding the mature human IFN-γ protein (23). The rIFN-γ has a molecular mass of 17 kD and is identical to human IFN-γ except for the absence of four terminal carboxyl residues, the addition of an NH_2-terminal methionine, and the lack of glycosylation. It has a specific activity of 2.7×10^7 U/mg (corrected to the World Health Organization reference standard Gg 23-901-530) and functions in the same manner as human IFN-γ as an inducer of IFN-γ-specific genes as well as an activator of mononuclear phagocytes (9, 24). The rIFN-γ was formulated in an excipient composed of sodium succinate, mannitol, and polysorbate 20.

Measurement of levels of IFN-γ in biologic fluids. To quantify the amounts of IFN-γ in respiratory epithelial lining fluid (ELF),[1] the lavage fluid was separated into cells and supernatant by centrifugation (500 *g*, 15 min, 23°C). The fluid was assessed for IFN-γ using a bioELISA specific for IFN-γ (25). A bioELISA was chosen over conventional radioimmunoassay for the ELF IFN-γ measurements because of its capacity to measure functional IFN-γ (via the induction of a phenotypic change in a viable population of cells, as described below) versus total IFN-γ (active + inactive) measured by conventional radioimmunoassay techniques. The bioELISA uses the characteristic of the COLO 205 cell line (ATCC CCL 222; American Type Culture Collection, Bethesda, MD), to induce the expression of the major histocompatability antigen DR on its cell surface after exposure to functionally active IFN-γ. Briefly, serial dilutions of lavage fluid were incubated with monolayers of COLO 205 cells, 10^5/well, in 96-well tissue culture plates (Becton Dickinson & Co., Mountain View, CA) for 40 h, 37°C. The cells were then washed with 4°C PBS, pH 7.4 (Whittaker M. A. Bioproducts, Walkersville, MD), and fixed to the plate with 100% ethanol. Monolayers were incubated with a mouse anti-HLA-DR antibody (No. 7360; Becton Dickinson & Co.) for 1 h, washed, and reincubated with a goat anti-mouse IgG horseradish peroxidase conjugate (No. 6450; Tago Inc., Burlingame, CA) for an additional 1 h. After washing, *o*-phenylene-diamine (Zymed Laboratories, San Francisco, CA) was added as a substrate and the colorimetric reaction allowed to proceed for 15 min. The reaction was terminated with 5N sulfuric acid and the optical densities of each well determined in an ELISA plate reader (Biomek 1000; Beckman Instruments, Inc., Berkeley, CA) at both 490 and 405 nanometers. Serial dilutions of purified rIFN-γ were used as standard curves. All lavage aliquots were run in duplicate serial dilutions as well as on duplicate plates (four replicates total). ELISA curves were analyzed by Immunofit 2.0 (Beckman Instruments, Inc.). A four parameter logistic fit was applied to the OD_{490}-OD_{405} values versus the log concentration expected from the standard curve. Validity of curve fit and coefficients of variation were confirmed, and the lavage aliquot IFN-γ concentrations were determined by extrapolation against the known standards. In the presence of normal ELF, the bioELISA was sensitive to IFN-γ levels ≥ 2 U/ml. The epithelial lining fluid volume was determined by the urea method (26).

Serum levels of IFN-γ were quantified by radioimmunoassay (Centocor Inc., Malvern, PA) (27). (The bioELISA was not used to evaluate IFN-γ in serum because of the interference of serum with the stable growth of the indicator, COLO 205, cell line [25].) Similar to the bioELISA, the radioimmunoassay was sensitive to IFN-γ levels of ≥ 2 U/ml when standardized against the reference-specific activity of 2.7×10^7 U/mg, noted above.

IFN-γ-specific activation of mononuclear phagocytes. The IFN-γ-specific activation of blood monocytes and alveolar macrophages was evaluated by quantifying mRNA transcripts for the IP-10 gene, a gene coding for a 1.6-kb mRNA and 10 kD protein of unknown function but which is expressed in mononuclear phagocytes specifically in response to IFN-γ (28–31). As a control, mRNA transcripts for γ-actin were evaluated. To demonstrate that IP-10 transcripts are upregulated by rIFN-γ in alveolar macrophages (as has been demonstrated in blood monocytes and keratinocytes [28–30]), alveolar macrophages and blood monocytes from normal individuals ($n = 4$) were cultured with or without 250 U/ml rIFN-γ for 4 h. RNA was then extracted and evaluated by Northern analysis with ^{32}P-labeled IP-10 cDNA probe (pIFN-γ-31.7; courtesy of J. V. Ravetch, Memorial Sloan-Kettering Cancer Center, New York [28]) and γ-actin cDNA probe (pHγA-1; courtesy of P. Gunning and L. Kedes, Stanford University [32]). To quantify IP-10 and γ-actin transcripts in blood monocytes and alveolar macrophages of individuals receiving systemic or aerosol rIFN-γ, RNA was extracted from the cells after recovery and evaluated by slot blot analysis using 10-fold serial dilutions of total RNA and the ^{32}P-labeled IP-10 and γ-actin probes.

Systemic administration of rIFN-γ. rIFN-γ (250 μg) was injected subcutaneously in the upper deltoid ($n = 6$). Each individual was evaluated before and at intervals after the administration of rIFN-γ for serum and ELF IFN-γ levels and the expression of IP-10 mRNA transcripts in blood monocytes and alveolar macrophages. Safety evaluations were carried out before and after IFN-γ administration (see below).

Aerosol administration of rIFN-γ. rIFN-γ was aerosolized using a compressed air driven nebulizer (Ultravent; Mallinckrodt Inc., St. Louis, MO) selected on the basis of its ability to generate an aerosol with droplets in a size range (0.2–3 μm mass median aerodynamic diameter) allowing for deposition in the lower respiratory tract (21). Preliminary in vitro testing demonstrated that rIFN-γ aerosolized in this fashion retains its structure and function as an inducer of γ-IFN-specific genes and as an activator of mononuclear phagocytes. Using a solution of rIFN-γ at 1.0 mg/ml, the Ultravent nebulizer generated an aerosol containing 20 μg rIFN-γ/liter air. Approximately 60% of the aerosol droplets produced were ≤ 3 μm mass median aerodynamic diameter.

Before receiving aerosol administration, each study participant underwent lung function testing to quantify minute ventilation under conditions similar to that under which they would receive the IFN-γ aerosol (i.e., with nose clips in place and with counseling in maintaining an even and constant minute ventilation). The total amount of rIFN-γ administered was regulated by defining, for each participant, the amount of time the rIFN-γ aerosol was inhaled from the nebulizer.

An aerosol of 250, 500, or 1,000 μg rIFN-γ was administered daily for 3 consecutive days to 11 individuals (250 μg, $n = 3$; 500 μg, $n = 3$; 1,000 μg, $n = 5$). Placebo aerosols of excipient equivalent to the 1,000-μg dose were administered daily for 3 consecutive days to 3 additional individuals. Each individual was evaluated by bronchoalveolar lavage (22) before the first aerosol, 1 h after the first aerosol, and 24 h after the third aerosol.

Safety evaluation. For the individuals receiving systemic rIFN-γ, before and at various intervals after administration, there was surveillance for local and systemic signs and symptoms as well as blood sampling for routine hematologic, chemical and coagulation parameters, anti-IFN-γ antibodies, and urinalysis. Followup safety evaluations with similar parameters were carried out 1 and 4 wk after administration. For the individuals receiving aerosolized rIFN-γ, similar evaluations were carried out, but, in addition, pulmonary function testing was assessed before and after each aerosol and bronchoalveolar lavage parameters were evaluated for the presence of inflammation. Followup safety evaluations with similar parameters were carried out 1 and 4 wk after the last aerosol.

Results

In vitro induction of IFN-γ-specific genes in mononuclear phagocytes. Evaluation of mRNA transcripts from alveolar macrophages exposed to rIFN-γ in vitro demonstrated that rIFN-γ induced the expression of IP-10 transcripts (Fig. 1). In blood monocytes and alveolar macrophages, no IP-10 transcripts were observed in cells cultured without rIFN-γ (lanes *1*

1. *Abbreviations used in this paper:* ELF, epithelial lining fluid.

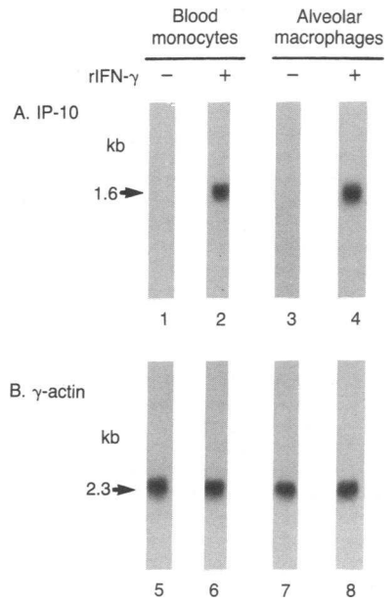

Figure 1. Induction of the expression of the IP-10 gene in mononuclear phagocytes in vitro. Shown are Northern analyses of total cellular RNA extracted from normal alveolar macrophages and blood monocytes 4 h after in vitro culture alone or with rIFN-γ (250 μg/ml). (*A*) IP-10 mRNA transcripts. (Lane *1*) blood monocytes incubated without ("–") rIFN-γ. (Lane *2*) Same as lane *1* but with ("+") rIFN-γ. (Lane *3*) Alveolar macrophages incubated without rIFN-γ. (Lane *4*) Same as lane *3* but with rIFN-γ. (*B*) γ-Actin mRNA transcripts. Lanes *5–8* are identical to lanes *1–4*, respectively, but evaluated with a γ-actin probe. The 1.6-kb IP-10 transcripts and 2.3-kb γ-actin transcripts are indicated.

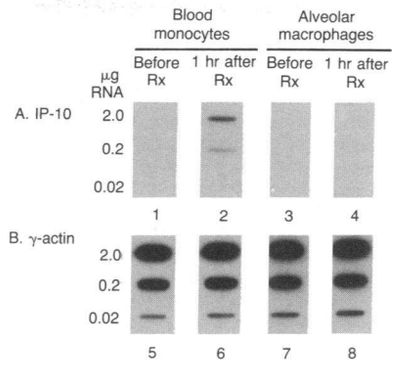

Figure 3. Evaluation of IP-10 mRNA transcripts in blood monocytes and alveolar macrophages before and after systemic administration of rIFN-γ. rIFN-γ (250 μg) was administered subcutaneously to normals, and total cellular RNA was extracted from blood monocytes and alveolar macrophages before and 1 h after administration of rIFN-γ. Shown are slot-blot analyses using the ^{32}P-labeled probes for IP-10 and γ-actin, with each slot containing a 10-fold serial dilution of total RNA as indicated. (*A*) IP-10 mRNA transcripts. (Lane *1*) Blood monocytes before rIFN-γ. (Lane *2*) Same as lane *1*, but 1 h after rIFN-γ. (Lane *3*) Alveolar macrophages before rIFN-γ. (Lane *4*) Same as lane *3*, but 1 h after rIFN-γ. (*B*) γ-Actin mRNA transcripts. Lanes *5–8* are identical to lanes *1–4*, respectively, but evaluated with a γ-actin probe.

and *3*) although γ-actin transcripts were present (lanes *5* and *7*). In contrast, incubation with 250 U/ml of rIFN-γ for 4 h resulted in the expression of the 1.6-kb IP-10 mRNA (lanes *2* and *4*), while γ-actin mRNA remained relatively constant (lanes *6* and *8*). As an additional control, the nonspecific inflammatory stimulus, lipopolysaccharide (10 μg/ml), added to parallel cultures, did not induce IP-10 mRNA transcripts in alveolar macrophages (data not shown).

Systemic administration of rIFN-γ. IFN-γ levels were beneath threshold levels of detectability in all individuals tested before receiving subcutaneous rIFN-γ (Fig. 2). After administration, serum levels were detectable at 4 h, but returned to subthreshold levels by 24 h. In marked contrast, no detectable IFN-γ activity could be found in respiratory ELF after systemic administration.

Consistent with the presence of IFN-γ in serum after systemic administration, IP-10 mRNA transcripts were present in blood monocytes recovered 1 h after systemic administration (Fig. 3) and in some individuals were still faintly visible at 4 h (data not shown). Alveolar macrophages, however, showed no IP-10 mRNA transcripts, at any of the time points evaluated, from 1 to 24 h after systemic administration. Control γ-actin mRNA transcript levels remained constant before and after systemic administration in both blood monocytes and alveolar macrophages.

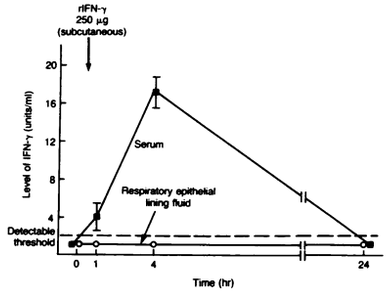

Figure 2. IFN-γ levels in serum and respiratory ELF after systemic administration of rIFN-γ. rIFN-γ (250 μg) was administered subcutaneously to normals (n = 6) and IFN-γ levels were quantified in serum (■) by radioimmunoassay and in epithelial lining fluid (○) by bioELISA before, and at various time intervals after, administration of rIFN-γ. The detectable threshold (2 U/ml) of the IFN-γ assays is indicated by the dashed line.

Participants receiving subcutaneous rIFN-γ experienced a spectrum of adverse effects lasting from hours to days that included fever, nausea, vomiting, headache, malaise and local pain, induration, and erythema. A quantitation toxicity scale ranging I–IV: minimal, moderate, severe, intolerable, respectively, was used throughout the study. The range of adverse effects was large; although all individuals experienced fever, it ranged from a single documented elevation of 1°F (grade I) to a maximum of 104°F with elevations above baseline for 3 d (grade III). Similarly, nausea ranged from mild and transient in three of six (grade I) to recurrent emesis despite anti-emetic therapy over 2 d in one individual (grade IV). Other noted adverse effects had similarly broad ranges. Mild transient leukopenia and thrombocytopenia (grade I) were noted in one individual.

Aerosol administration of rIFN-γ. Before aerosol administration no detectable IFN-γ activity was measurable in ELF or serum (Fig. 4). However, 1 h after a single aerosolization of rIFN-γ, IFN-γ activity was detectable in ELF with the amounts dependent on the amount administered (250 vs. 1,000 μg aerosol, $P < 0.05$). IFN-γ activity returned to below detectable threshold 24 h after all aerosols. At no time after aerosol administration of rIFN-γ was IFN-γ detectable in serum. Individuals receiving excipient alone, as a placebo aerosol, had no detectable IFN-γ in ELF or serum at any time point (not shown).

Slot-blot analysis of RNA from alveolar macrophages recovered by lavage confirmed, in each study participant, no IP-10 mRNA transcripts before aerosolization. However, alveolar macrophages recovered 1 h after a single aerosolization of rIFN-γ clearly showed specific IP-10 mRNA transcripts (Fig. 5). Transcript levels were undetectable by 24 h after administration. Participants receiving excipient alone did not show induction of IP-10 mRNA transcripts. Alveolar macrophage γ-actin

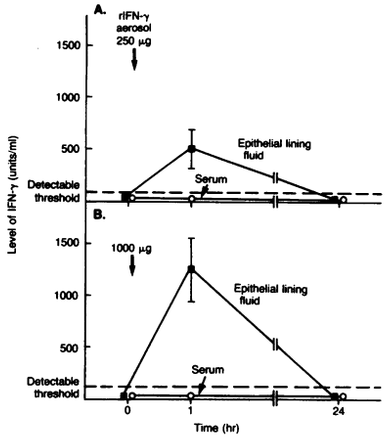

Figure 4. IFN-γ levels in respiratory ELF and serum before, 1 h after a single aerosolization, and 24 h after the final aerosolization of rIFN-γ. rIFN-γ was administered by aerosol to normals and IFN-γ levels quantified in serum (○) by radioimmunoassay and in epithelial lining fluid (■) by bioELISA before administration and at various time intervals afterwards. The detectable threshold (2 U/ml) of the IFN-γ assays is indicated by the dashed line. (*A*) Aerosolization of 250 μg rIFN-γ (n = 3). (*B*) Aerosolization of 1,000 μg rIFN-γ (n = 5).

mRNA transcripts, as control, remained constant at all time points before and after rIFN-γ (or excipient alone) aerosol administration. Interestingly, no induction of IP-10 mRNA transcripts was observed in blood monocytes at any time after the aerosol administration of rIFN-γ, although stable levels of γ-actin mRNA transcripts were present.

Monitoring for local and systemic effects revealed no evidence of any decrements in respiratory function nor clinical signs or symptoms of adverse systemic effects associated with the aerosol delivery of the rIFN-γ. No hematologic, chemical, coagulation, radiographic, or renal abnormalities were detected. No differences were observed in the cellular components of lavage fluid that could be attributable to rIFN-γ. In this regard, $51\pm7 \times 10^6$ total cells were recovered 1 h after one aerosol administration of rIFN-γ (all doses combined) compared with $41\pm2 \times 10^6$ in the control group receiving the aerosol without rIFN-γ ($P > 0.5$), and $63\pm9 \times 10^6$ total cells were recovered 24 h after three aerosol administrations of rIFN-γ (all doses combined) compared with $51\pm11 \times 10^6$ in the control group receiving the aerosol without rIFN-γ ($P > 0.4$). Evaluation of the cell differentials showed an elevation of neutrophils 1 h after one aerosol administration of rIFN-γ (16±6%, all doses combined) and 24 h after three aerosol administrations of rIFN-γ (7±2%, all doses combined), but this was no different than that observed in the control group receiving the aerosol without rIFN-γ (1 h after one aerosol 22±21%, $P > 0.6$ compared with rIFN-γ group; 24 h after three aerosols 4±1%, $P > 0.4$ compared with rIFN-γ group).

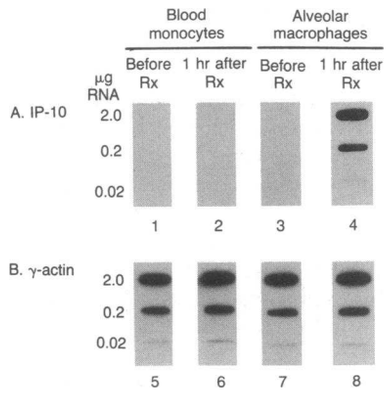

Figure 5. Evaluation of IP-10 mRNA transcripts in blood monocytes and alveolar macrophages before and after a single aerosol administration of rIFN-γ. rIFN-γ (250 μg) was administered by aerosol to normals (n = 3), and total cellular RNA was extracted from blood monocytes and alveolar macrophages before and 1 h after administration of rIFN-γ. Shown are slot-blot analyses using ^{32}P-labeled probes for IP-10 and γ-actin with each slot containing a 10-fold serial dilution of total RNA as indicated. (*A*) IP-10 mRNA transcripts. (Lane *1*) Blood monocytes before rIFN-γ. (Lane *2*) Same as lane *1*, but 1 h after rIFN-γ. (Lane *3*) Alveolar macrophages before rIFN-γ. (Lane *4*) Same as lane *3*, but 1 h after rIFN-γ. (*B*) γ-actin mRNA transcripts. Lanes *5–8* are identical to lanes *1–4*, respectively, but evaluated with a γ-actin probe.

Discussion

The largest interface between the human host and the environment occurs on the respiratory tract epithelium. The alveolar macrophage, the respiratory representative of the mononuclear phagocyte family, plays a major role in the defense of the respiratory epithelium against a continual barrage of pathogens (1–5). As for mononuclear phagocytes in general, alveolar macrophages have baseline levels of defense capabilities that can be enhanced by IFN-γ, a cytokine that augments a number of mononuclear phagocyte functions including those involved in host defense (6–12). This study demonstrates that a single systemic administration of rIFN-γ raises serum, but not lung levels of IFN-γ and concomitantly activates blood monocytes, but not alveolar macrophages. While it is conceivable that blood monocytes activated after systemic administration might ultimately migrate to and remain activated within the lung, no evidence of this was found at any of the time points evaluated by bronchoalveolar lavage after systemic administration. In addition, the rate of this process is unclear and even if the monocytes remained activated over the time course, no benefit to the already resident alveolar macrophages would be realized. Importantly, not only does a single systemic administration not activate mononuclear phagocytes on the respiratory epithelial surface, but dosing is attended by the development of systemic adverse effects. Lower dosages might allow repeated systemic administration with tolerable adverse effects, but the lack of documented IP-10 mRNA transcripts after the dosages used makes the success of lower dosages, even if repeated, speculative. Moreover, we have devised a strategy to overcome these issues by targeting the rIFN-γ directly to the lower respiratory tract by delivery of the cytokine in an aerosol that is inhaled. Strikingly, aerosol administration at these dosages raised respiratory epithelial lining fluid, but not serum, levels of IFN-γ and, importantly, activated alveolar macrophages, but not blood monocytes. Further, aerosol administration achieved this local activation of alveolar macrophages without systemic or local adverse effects. In this regard, no general blood or urine safety parameters demonstrated abnormalities, nor were there changes observed in extensive daily lung function testing, all carried out in an inpatient controlled setting. Further, while the total numbers and proportions of neutrophils present were higher than generally observed in normals, no differences were observed among cells recovered by lavage of those receiving rIFN-γ and those receiving the control aerosol without rIFN-γ. In this context, it will be important to follow such parameters in long term safety studies to determine if aerosols per se (e.g., without the active agent) directed toward the lower respiratory tract have any adverse effects or whether the increased cells result from the repeated use of lavage to recover the cells, as has been observed in experimental animals undergoing repeated bronchoalveolar lavage (33).

These observations suggest that systemic administration of IFN-γ may have limited usefulness in activating alveolar macrophages for augmented respiratory host defense. Aerosol delivery (in this dosage range) may be a direct strategy to selectively activate alveolar macrophages, thus compartmentalizing the action of this potent cytokine while circumventing problems with systemic toxicity.

Delivery of biologically active rIFN-γ by aerosol. Two lines of evidence have demonstrated that rIFN-γ can be aerosolized and inhaled and still retain its biologic activity after reaching the lower respiratory tract. First, the method of detection of the IFN-γ in ELF was based on a bioELISA, an assay that depends on IFN-γ induction of HLA-DR expression on the COLO 205 cells (25), i.e., the cytokine must have biologic activity to be detected by this assay. Second, IP-10 transcripts were detected in alveolar macrophages recovered from these individuals, suggesting that functional IFN-γ interacted with IFN-γ receptors on the macrophages, and subsequently transduced the signal to activate the IP-10 gene transcription.

Importantly, the levels of IFN-γ achieved by aerosolization were well in excess of those needed to activate mononuclear phagocytes in vitro (6–12, 28, 34–36). Although the limitation in performing repeated lavage procedures precluded detailed characterization of the time course of alveolar macrophage activation after rIFN-γ aerosolization, the effect appears to last for at least 1 h after in vivo administration. However, in the context that aerosol administration of rIFN-γ to experimental animals is associated with upregulation of class II histocompatibility molecules on the surface of alveolar macrophages for at least 24 h, it is reasonable to hypothesize that at least some effects on augmenting host defense may be long lasting (37).

The specificity of the alveolar macrophage response to the aerosolized rIFN-γ was suggested by expression of IP-10 transcripts after aerosolization of the rIFN-γ but not its excipient. In this regard, a variety of studies have demonstrated that IFN-γ induces mononuclear phagocytes to express genes for a specific group of proteins (28–31, 38, 39). Of these proteins some are inducible by multiple activation signals while others, most notably IP-10, are specific for the cellular activation associated exclusively with IFN-γ (28–31, 40). In this context, there are no known examples in vitro or in vivo in which IP-10 is induced in the absence of IFN-γ, i.e., the presence of transcripts of the gene coding for IP-10 in the target cell is a sensitive and specific marker for biologically active IFN-γ interacting with the target cell. A similar strategy has been used to document the presence of IFN-γ in the cutaneous reactions to exogenously-administered rIFN-γ and purified protein derivative (30) as well as to implicate IFN-γ in the pathophysiology of psoriatic skin lesions (41).

Augmentation of respiratory host defenses with rIFN-γ. Despite the evidence that IFN-γ plays a significant role in normal host defense, and the in vivo evidence in animals and humans that systemic administration of rIFN-γ will augment systemic host defense (6, 8, 12–20, 42–44), there is no evidence that systemic administration of IFN-γ to humans is capable of augmenting respiratory host defense. The evidence in the present study suggests that a single systemic administration of rIFN-γ does not result in detectable levels of IFN-γ in respiratory ELF nor the activation of alveolar macrophages recovered from the respiratory epithelial surface. However, in individuals with lung disease, altered permeabilities to both cells and molecules might enhance or decrease the effects.

In contrast, the clear demonstration that aerosolized rIFN-γ will augment respiratory ELF levels of functional IFN-γ sufficient to activate alveolar macrophages in vitro (6–12, 28, 34–36), and the evidence that the alveolar macrophages recovered after aerosolization are in fact activated, strongly suggests this should be an effective way of safely activating alveolar macrophages for augmented respiratory host defense. Although this will require controlled trials in individuals at risk for, or with, respiratory infection, the fact that IFN-γ upregulates the expression of mononuclear phagocyte surface molecules relevant to host defense (6, 13–15, 45–49), enhances oxidant production by blood monocytes as well as tissue macrophages such as alveolar macrophages (6, 7, 9–12, 16, 34–36, 43, 44, 50), markedly reduces the incidence of infection in individuals with chronic granulomatous disease (17–19), induces enhanced phagocytosis and intracellular killing of multiple pathogens (6, 20, 35, 36, 42–44, 51), and has antiviral effects (52–54) suggests that it may be effective.

Acknowledgments

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