Gamma Interferon is Spontaneously Released by Alveolar Macrophages and Lung T Lymphocytes in Patients with Pulmonary Sarcoidosis

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Abstract

Gamma interferon (IFNγ) is a potent immune mediator that plays a central role in enhancing cellular immune processes. This study demonstrates that while lung mononuclear cells from normal individuals spontaneously release little or no interferon (<10 U/10^6 cells per 24 h), those from patients with pulmonary sarcoidosis spontaneously release considerable amounts (65±20 U/10^6 cells per 24 h, $P < 0.02$ compared to normals). Furthermore, cells from patients with active disease release far more interferon than those from patients with inactive disease (101±36 compared to 24±8 U/10^6 cells per 24 h, $P < 0.02$). Characterization of this interferon using acid sensitivity, specific antibody inhibition, and target cell specificity criteria demonstrated that it was almost entirely IFNγ. This spontaneous release of IFNγ appeared to be compartmentalized to the lung of these patients in that their blood mononuclear cells spontaneously released little or no IFNγ ($P < 0.02$, compared to sarcoidosis lung mononuclear cells) and no IFNγ was detected in their serum. Both lung T lymphocytes and alveolar macrophages contributed to the spontaneous release of IFNγ by lung mononuclear cells from sarcoid patients; purified preparations of T lymphocytes and alveolar macrophages from these patients spontaneously released similar amounts of IFNγ (56±21 and 32±11 U/10^6 cells per 24 h, respectively, $P > 0.3$). At least one role for IFNγ in the pathogenesis of sarcoidosis appeared to be related to activation of alveolar macrophages, as alveolar macrophages recovered from patients with active disease spontaneously killed [^{3}H]uridine-labeled tumor cell targets (17.7±4.5% cytotoxicity compared with 2.8±0.9% in normals, $P < 0.02$) and purified IFNγ enhanced the ability of alveolar macrophages from sarcoidosis patients with inactive disease to kill similar targets ($P < 0.001$, compared to alveolar macrophages cultured in medium alone). Treatment of sarcoid patients with corticosteroids, a therapy known to suppress the activity of the disease, caused a marked reduction in the level of spontaneous IFNγ release by lung mononuclear cells compared with untreated patients ($P < 0.02$), which suggests that the effectiveness of corticosteroid therapy in controlling active pulmonary sarcoidosis may, at least in part, be due to suppression of IFNγ release.

Introduction

Human gamma interferon (IFNγ)[1] is produced by immune effector cells in response to a variety of stimuli (1). Although IFNγ is defined as an interferon by virtue of its ability to inhibit viral growth, it is thought to play a major role in intensifying immune processes by virtue of its ability to enhance a variety of macrophage and lymphocyte actions. For example, in human mononuclear phagocytes, IFNγ induces the expression of DR-determinants (2, 3), enhances the presentation of antigens to lymphocytes (4), and activates blood mononuclear phagocytes to kill tumor cell targets (5) and intracellular parasites (6). In addition, IFNγ has been shown to enhance a variety of lymphocyte functions, including the stimulation of the expression of interleukin-2 (IL-2) receptors on T lymphocytes (7), enhancement of natural killer cell activity (8), inhibition of suppressor T lymphocyte function (9), and induction of the expression of DR-determinants on the surface of B lymphocytes (10). In view of this broad-based range of activities, it is logical to conceptualize IFNγ as a multipurpose mediator that mononuclear phagocytes and lymphocytes use to up-regulate immune and inflammatory processes.

While the availability of such a mediator provides mononuclear cells with a common means of responding to immune and inflammatory stimuli, the existence of such mediators could be dangerous if their release were exaggerated and/or uncontrolled. Since IFNγ stimulates immune and inflammatory cells, failure to regulate its release could logically result in a sustained, heightened cellular immune process that could damage normal tissues.

Sarcoidosis may be an example of such a situation. It is a multisystem disorder characterized by exuberant, apparently uncontrolled cellular immune activity in affected organs (11). In this context, in active pulmonary sarcoidosis, lung T lymphocytes are spontaneously releasing monocyte chemotactic factor (12) and IL-2 (13, 14) and provide nonspecific polyclonal help to B cells (15). Further, lung lymphocytes from patients with active sarcoidosis demonstrate exaggerated natural killer cell activity (16), an observation consistent with heightened local immune processes. Mononuclear phagocytes from the lung of sarcoid patients also appear activated, in that they demonstrate an enhanced ability to present antigen to autologous T lymphocytes (17) and are spontaneously releasing fibronectin (18) and the alveolar macrophage-derived growth

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1. *Abbreviations used in this paper:* HTLV-1, human T cell lymphoma virus; IFNα, IFNβ, and IFNγ, alpha, beta, and gamma interferon, respectively; IL-2, interleukin-2; PHA, phytohemagglutinin; SRBC, sheep erythrocyte(s).

factor (19), which are mediators that stimulate fibroblasts to replicate and thus promote the formation of fibrous tissue. Further, patients with active pulmonary sarcoidosis have positive gallium-67 scans (20), a finding thought to result, at least in part, from the enhanced uptake of gallium-67 by activated alveolar macrophages (21). Strikingly, all of these exaggerated inflammatory and immune processes in pulmonary sarcoid appear compartmentalized such that they are manifest in the lung but not in the blood.

With this background, it is reasonable to hypothesize that lung, but not blood, mononuclear cells recovered from patients with active sarcoidosis are releasing substantial amounts of IFNγ. This would not only help to explain some of the exaggerated localized immune processes in sarcoidosis, but also identify a specific process that might be vulnerable to therapeutic attack, resulting in suppression of the disorder.

To evaluate this concept, we have evaluated IFNγ release by lung mononuclear cells from normals and sarcoidosis patients. The results demonstrate that this mediator is being spontaneously released in pulmonary sarcoidosis by both lung T lymphocytes and alveolar macrophages, suggest that IFNγ may play a role in the activation of alveolar macrophages in this disorder, and demonstrate that at least one consequence of corticosteroid therapy of sarcoidosis is the suppression of the lung mononuclear cell spontaneous release of IFNγ.

Methods

Study population. Normal volunteers (n = 10, seven males, three females, age 25±2 yr [all data is presented as mean±SEM; all statistical comparisons are made using the two-tailed t test]) had no history of pulmonary disease and had normal chest X-rays and pulmonary function tests. None were taking medications and all were nonsmokers.

Sarcoidosis patients (n = 31, 10 males, 21 females, age 37±3 yr) were diagnosed as previously described (22). Six were current or ex-smokers and 25 were nonsmokers. None was receiving therapy when initially evaluated. As a group their pulmonary function tests showed vital capacity (VC) 77±3% predicted; total lung capacity 74±3% predicted; forced expiratory volume in 1 s (FEV_1) 75±4% predicted; FEV_1/forced VC 80±2% observed; and diffusing capacity (carbon monoxide, single breath, corrected for volume and hemoglobin) 77±5% predicted. Where indicated, the sarcoidosis patients were subclassified as active (high intensity alveolitis) or inactive (low intensity alveolitis) using bronchoalveolar lavage and gallium-67 scan criteria as previously described (23).

Preparation of lung and blood effector cells. Lung mononuclear cells were obtained using bronchoalveolar lavage (24). Briefly, five 20-ml aliquots of normal saline were infused via a fiberoptic bronchoscope into each of three lobes of the lower respiratory tract and immediately removed by gentle suction. The recovered cells were washed twice in RPMI-1640 (MA Bioproducts, Walkersville, MD) and a differential count obtained using Millipore filter preparations (25). Normal lung mononuclear cell preparations contained 12±2% lymphocytes, whereas those from sarcoidosis patients contained 48±5% lymphocytes. Alveolar macrophages comprised the majority of the remainder of both cell populations with polymorphonuclear leukocytes representing ≤2% of recovered cells. In all cases, recovered cells were ≥94% viable.

Blood mononuclear cells were purified from heparinized peripheral blood obtained just before bronchoalveolar lavage by density gradient centrifugation using lymphocyte separation medium (Litton Bionetics, Kensington, MD) and washed sequentially with normal saline, Hanks' balanced salt solution (MA Bioproducts), and RPMI-1640.

Purification of lung T lymphocytes and alveolar macrophages. T lymphocytes were purified from sarcoidosis lung mononuclear cell populations using two methods. First, lung mononuclear cells were incubated with neuramididase (Sigma Chemical Co., St Louis, MO)-treated sheep erythrocytes (SRBC) for 90 min at 4°C and the rosette-forming cells removed by density gradient centrifugation through lymphocyte separation medium (15). Resultant T cell populations were routinely ≥94% pure (morphology and nonspecific esterase staining) and ≥98% viable (trypan blue exclusion). Second, lung mononuclear cells were suspended in culture medium (RPMI-1640 supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 50 mM glutamine, and 10% fetal calf serum, all were from MA Bioproducts), incubated in nylon wool columns (Associated Biomedic Systems, Buffalo, NY) for 30 min at 37°C, and then eluted at 2 ml/min using warm (37°C) medium (26). The resultant lymphocytes populations were ≥97% pure (morphology and nonspecific esterase staining) and ≥97% viable (trypan blue exclusion). Evaluation with the pan-T cell monoclonal antibody Anti-Leu-4 (Becton-Dickinson & Co., Sunnyvale, CA) demonstrated that these lymphocytes were comprised of ≥98% T lymphocytes.

Alveolar macrophages were purified from sarcoidosis lung mononuclear cell populations using two methods. First, T lymphocytes were removed by rosetting with SRBC as described above. The nonrosetting cells were then washed twice, and incubated in 24-well tissue culture plates (Falcon 3047, Becton-Dickinson & Co., Oxnard, CA) at 1.25 × 10^6 viable macrophages/well in 250 μl medium for 60 min at 37°C. The nonadherent cells were then removed and the adherent cell layer washed twice with warm (37°C) medium. The resultant alveolar macrophage populations were ≥95% pure (morphology and nonspecific esterase staining). Second, lung mononuclear cells were incubated in 24-well plates as above and the nonadherent cells removed. T lymphocytes contaminating the macrophage preparations were then lysed using complement plus monoclonal antibody (27). To accomplish this, the adherent cells were incubated in 1 ml RPMI-1640 with 1 μg/ml of the mouse anti-human T cell monoclonal antibody Lyt-3 (New England Nuclear, Boston, MA) at 4°C for 60 min with repeated agitation, gently washed twice with cold (4°C) RPMI-1640, and then incubated for a further 45 min at 37°C with 1 ml rabbit complement (Cedarlane Laboratories, Hornby, Ontario, Canada) and gently washed twice with warm (37°C) medium. No significant loss of macrophages by detachment occurred during this procedure and the resultant alveolar macrophage populations were ≥95% pure (morphology and nonspecific esterase stain).

Gamma interferon assay. To quantify IFNγ release by lung and blood cells, the various preparations were cultured at 5 × 10^6 cells/ml medium in 24-well plates for 24 h at 37°C. As a positive control, parallel wells were incubated in the presence of phytohemagglutinin (PHA; 5 μg/ml, Wellcome, Beckenham, Kent, United Kingdom). The supernatant was then aspirated, centrifuged at 1,000 g for 6 min, and a 200-μl aliquot removed for assay.

Interferon was quantified as the reciprocal of the dilution that produced a 50% reduction in vesicular stomatitis virus-induced lysis of human amniotic WISH cells (28) as compared with a standard preparation of IFNγ of 10^2 interferon U/ml (Meloy Laboratories, Springfield, VA). The interferon was characterized by determining: (*a*) its stability at pH 2 (24 h at 4°C); (*b*) its inhibition by polyclonal antibody to IFNα (Interferon Sciences, New Brunswick, NJ), polyclonal antibody to IFNβ (Research Resources Section, National Institute of Allergy and Infectious Diseases, Bethesda, MD), and monoclonal antibody to IFNγ (Meloy Laboratories) and (*c*) its species specificity by substituting bovine fibroblasts for human WISH cells in the assay. All characterization procedures listed above were evaluated against standard preparations of IFNα (Interferon Sciences), IFNβ (HEM Research, Rockville, MD), and IFNγ (Meloy Laboratories).

Assessment of serum and epithelial lining fluid for IFNγ activity. Serum was obtained by centrifuging clotted samples of whole blood from normal volunteers and sarcoidosis patients obtained just before bronchoalveolar lavage. Alveolar epithelial lining fluid, which is diluted ~100-fold by the normal saline used for lavage (29), was concentrated 100-fold using a minicon B-15 macrosolute concentrator (membrane rejection 15,000 mol wt, Amicon Corp., Danvers, MA) so that the concentration of macromolecules of molecular weight >15,000 was

restored to its approximate in vivo value. Serum and epithelial lining fluid were then evaluated for IFNγ activity as described above. To determine if serum of epithelial lining fluid contained inhibitors of IFNγ activity, a standard preparation of IFNγ was added to these samples then assayed for IFNγ.

Evaluation of macrophage activation. Activation of alveolar macrophages from normal volunteers and nonsmoking patients with sarcoidosis was assessed by measuring their capacity to spontaneously kill labeled tumor cell targets in a 72-h assay (30). Briefly, the lung mononuclear cells were allowed to adhere in wells of 96-well flat bottom plates (Falcon 3072, Becton-Dickinson & Co.) at 2×10^5 macrophages/well at 37°C for 1 h. The nonadherent cells were then removed and the adherent cell layer washed twice with warm (37°C) medium. The cells were then cultured in medium alone at 37°C for 24 h to facilitate detachment of contaminating cells (31) and then washed twice with warm (37°C) medium. The resultant macrophage populations were routinely ≥95% pure. The target cells used were human A375 melanoma cells (kindly provided by I. J. Fidler, Frederick Cancer Research Facility, Frederick, MD) that were cultured for 24 h in medium containing 1 μCi/ml [^{3}H]uridine (30 Ci/mmol, 1 mCi/ml, Amersham Corp., Arlington Heights, IL), washed four times with Ca^{++}- and Mg^{++}-free Hanks' balanced salt solution (MA Bioproducts), and detached using one times trypsin-EDTA (0.05% trypsin, 5 mM EDTA, Gibco Laboratories, Grand Island, NY). The cells were washed twice and suspended at 10^5 cells/ml medium. A 200-μl aliquot of this suspension was added to the adherent cell layer (i.e., effector to target ratio 10:1) and the plates cultured for 72 h at 37°C. All assays were performed in quadruplicate.

To determine if IFNγ could stimulate the alveolar macrophages from sarcoidosis patients with inactive disease to express a level of activation similar to that seen in alveolar macrophages from patients with active disease, purified IFNγ (Interferon Sciences, 250 U/ml) was added to cultures of alveolar macrophages (obtained as above) from nonsmoking patients with inactive sarcoidosis. After incubation for 24 h at 37°C, the cells were washed twice and then evaluated for cytotoxicity against the A375 melanoma target over 72 h as above, and compared with the cytotoxicity seen in alveolar macrophages incubated in medium alone or in the presence of lipopolysaccharide (Difco Laboratories, Detroit, MI; 10 μg/ml), a known alveolar macrophage-activating agent (30). In all experiments, background release was determined by culturing target cells in medium alone (control wells) and was routinely ≤25% of total dpm over the 72-h period. To terminate the assay, the supernatant was aspirated and the cell layer lysed using 0.1 N NaOH. The samples were counted and a cytotoxicity index (percentage) calculated as (supernatant counts in test wells − supernatant counts in control wells) × (100/total counts per well).

Effect of corticosteroid therapy on lung mononuclear cell release of gamma interferon. To evaluate the effect of corticosteroid therapy on the spontaneous release of IFNγ by lung mononuclear cells from patients with sarcoidosis, IFNγ release was evaluated before therapy was begun and then reassessed 2.3±0.7 mo after the commencement of therapy. A standard therapy protocol was used (prednisone, 1 mg/kg per d for 1 mo followed by a 5-mg reduction in the daily dose every 10 d), so that at the time of follow-up testing all patients were receiving at least 30 mg prednisone daily. As a control, spontaneous IFNγ release in untreated sarcoidosis patients was measured at two intervals separated by 2.9±0.4 mo. The decision as to whether the patients were to be treated was based on clinical criteria of disease activity without knowledge of the initial IFNγ production level.

Results

Release of IFNγ by normal lung and blood mononuclear cells. Lung mononuclear cells from normal individuals did not spontaneously release detectable interferon (Fig. 1 *A*). However, in the presence of PHA the lung mononuclear cells released 156±60 U interferon/10^6 cells per 24 h ($P < 0.05$, compared

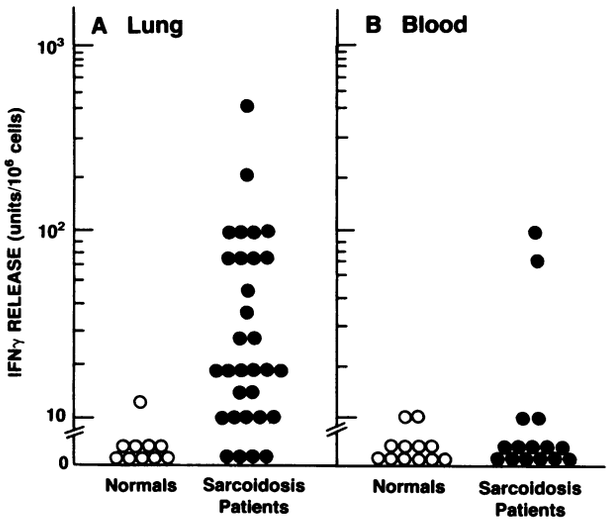

Figure 1. Spontaneous release of IFNγ by lung and blood mononuclear cells from normals and patients with sarcoidosis. (*A*) Lung cells; (*B*) blood cells. Lung mononuclear cells were obtained by bronchoalveolar lavage; blood mononuclear cells were separated from heparinized peripheral blood by hypaque-ficoll centrifugation. All cells were cultured at 5×10^6/ml medium for 24 h at 37°C and the supernatant assayed for IFNγ. Data is expressed as a single point for each individual.

to unstimulated cells), which suggested that the cells were capable of releasing interferon and that there were no inhibitors present that prevented its detection.

Similar to the lung mononuclear cells from normals, blood mononuclear cells of normals were releasing little, if any, detectable interferon (Fig. 1 *B*). However, when stimulated with PHA, these cells released interferon (194±38 U/10^6 cells per 24 h). With equivalent amounts of mitogen, the absolute level (per 10^6 mononuclear cells) of interferon release by lung and blood mononuclear cells were similar ($P > 0.2$).

Characterization of the interferon released by the PHA-stimulated normal lung mononuclear cells demonstrated that: (1) it did not inhibit viral lysis of bovine cells but did inhibit viral lysis of human WISH cells; (2) it lost activity by incubation at pH 2; and (3) it was inhibited by an antibody to IFNγ, but not to alpha or beta interferon (Table I). Together, these data strongly suggested that almost all of the interferon released by lung mononuclear cells was IFNγ.

Spontaneous release of IFNγ by lung mononuclear cells from patients with pulmonary sarcoidosis. In contrast to normal lung mononuclear cells, the mononuclear cells recovered from the lungs of patients with pulmonary sarcoidosis were spontaneously releasing considerable amounts of interferon (Fig. 1 *A*). Like the interferon released by normal lung mononuclear cells, the interferon released by sarcoid cells was also predominantly IFNγ (Table I). Interestingly, the lung mononuclear cells of sarcoid patients characterized as having active disease were releasing far more IFNγ (101±36 U/10^6 cells per 24 h) than those with inactive disease (24±8 U/10^6 cells per 24 h) ($P < 0.02$). However, when the lung cells of those with inactive disease were stimulated with PHA, the IFNγ production rose considerably and was at least as great as that of individuals with active disease ($P > 0.3$; data not shown). Importantly, time course studies with PHA-stimulated normal lung and

Table I. Characterization of Interferon Released by Lung Mononuclear Cells from Normals and Patients with Sarcoidosis

Condition	Interferon standards			Source of lung cells*	
	IFNα	IFNβ	IFNγ	Normal‡	Sarcoidosis§
Alone (WISH)‖	190±35	280±70	320±80	200±10	110±36
+Anti-IFNα¶	<10**	286±60‡‡	412±10‡‡	280±80‡‡	140±80‡‡
+Anti-IFNβ¶	150±29‡‡	<10**	300±85‡‡	200±10‡‡	90±12‡‡
+Anti-IFNγ¶	205±37‡‡	280±80‡‡	16±4**	16±4**	10±2**
+pH 2§§	200±10‡‡	320±92‡‡	165±40**	20±5**	24±16**
Alone (Bovine)‖ ‖	250±20‡‡	280±40‡‡	<10**	<10**	10±6**

* Cells were cultured for 24 h at 37°C at 5×10^6 cells/ml and the supernatant assayed for interferon activity using human WISH cells as described in Methods. Data is represented as interferon units released/10^6 cells/24 h. ‡ Lung mononuclear cells obtained from normal volunteers using bronchoalveolar lavage and stimulated with PHA (5 μg/ml). § Lung mononuclear cells obtained from sarcoidosis patients using bronchoalveolar lavage; the cells were not stimulated. ‖ Interferon quantified by determining the dilution of the sample that produced a 50% reduction in vesicular stomatitis virus-induced lysis of human amniotic WISH cells. Each data point represents four separate experiments and is recorded as mean±SE of the mean. ¶ Specific antibodies to IFNα, IFNβ, and IFNγ added to test samples just before assay. ** Significant reduction in interferon level compared with level obtained with WISH cells alone ($P < 0.05$). ‡‡ No significant reduction in interferon level compared with level obtained with WISH cells alone ($P > 0.5$). §§ Supernatants reduced to pH 2 by the addition of 1 N HCl, held at this pH for 24 h at 4°C, then restored to pH 7.4 by the addition of 1 N NaOH. ‖ ‖ Bovine cells substituted for human WISH cells in interferon assay.

sarcoid lung mononuclear cells demonstrated that IFNγ levels were higher for both patient populations at 48–72 h. In contrast, nonstimulated lung cells from sarcoid patients were shown to exhibit maximum IFNγ release after 24 h in culture and demonstrated a decline in activity when evaluated at longer (48 and 72 h) time intervals (data not shown). In contrast to the lung mononuclear cells of sarcoid patients, but similar to that of blood mononuclear cells of normals, the blood mononuclear cells from almost all sarcoid patients were releasing little, if any, detectable IFNγ (Fig. 1 *B*). However, like normal blood mononuclear cells, when stimulated with PHA to do so, the sarcoid blood cells released amounts of IFNγ that were similar to that of normal cells stimulated with equivalent amounts of PHA (250±93 U/10^6 cells per 24 h; $P > 0.2$, sarcoid blood cells compared to normal blood cells). Further, as with the lung mononuclear cells, time course studies with PHA-stimulated sarcoid and normal blood mononuclear cells demonstrated IFNγ release and peaked for both at 72–96 h (data not shown). Although, on the average, the sarcoid blood mononuclear cells released very little IFNγ spontaneously, of the 15 patients so evaluated, blood mononuclear cells of two individuals did spontaneously release detectable amounts of IFNγ (Fig. 1 *B*). Interestingly, while most of the patients studied had sarcoidosis confined to the lung, these two individuals also had active sarcoidosis manifest in several organs including the eyes, skin, liver, spleen, and central nervous system.

The IFNγ spontaneously released by lung mononuclear cells of sarcoid patients was being released by both T lymphocytes and alveolar macrophages (Fig. 2). The conclusion that sarcoid lung T cells, as well as alveolar macrophages, spontaneously released IFNγ was supported by the observation that T cell populations purified by positive selection with rosetting methods and by negative selection by nylon wool both spontaneously released this mediator. The amount of IFNγ spontaneously released by alveolar macrophages was, on a per cell basis, similar to the amounts spontaneously released by the lung T cells ($P > 0.3$).

None of the sarcoidosis patients or normal volunteers had detectable IFNγ in their serum or epithelial lining fluid of the lower respiratory tract (data not shown). The inability to detect IFNγ in these fluids was not due to the presence of inhibitors that interfered with the IFNγ assay, since a standard IFNγ preparation added to serum or epithelial lining fluid retained full activity ($P > 0.2$, compared to the standard IFNγ preparation assayed alone).

Macrophage activation in normals and sarcoidosis patients. Alveolar macrophages from normals expressed a low level of spontaneous tumor cytotoxicity (Fig. 3). In contrast, alveolar macrophages from patients with sarcoidosis appeared to be activated in that they demonstrated enhanced spontaneous tumor cytotoxicity. This was true for sarcoid patients with inactive disease ($P < 0.05$, sarcoid compared to normals) and sarcoid patients with active disease ($P < 0.02$, sarcoid compared to normals). Further, the level of spontaneous tumor cytotoxicity was greater for the sarcoid patients with active disease

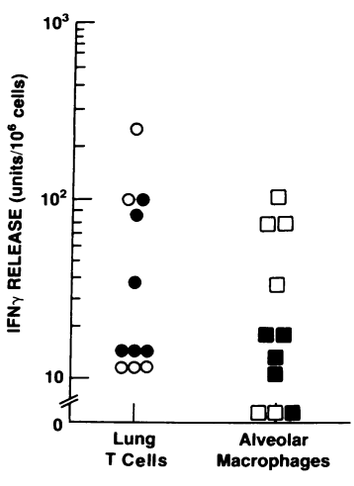

Figure 2. IFNγ release by lung T cells and alveolar macrophages from sarcoidosis patients. T cells were purified from lung mononuclear cells by rosette formation with SRBC followed by hypaque-ficoll centrifugation (●) or by passage through nylon wool columns (○). Alveolar macrophages were purified from lung mononuclear cells by depletion of T cells by SRBC rosetting as above, followed by adherence to plastic and removal of nonadherent cells (■), or by adherence to plastic followed by removal of nonadherent cells and lysis of adherent T cells using antibody (Lyt-3) plus complement (□). Cells were cultured and assayed as in Fig. 1.

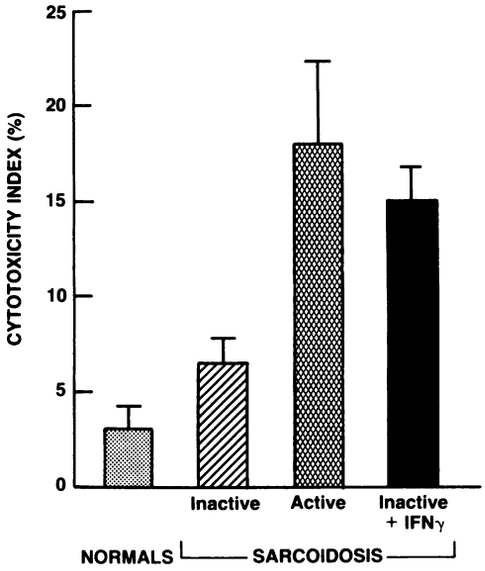

Figure 3. Assessment of activation of alveolar macrophages from normals and sarcoidosis patients as manifested by spontaneous macrophage-mediated cytotoxicity of [^{3}H]uridine-labeled A375 melanoma cells. Alveolar macrophages from nonsmoking normals (n = 10) and nonsmoking patients with inactive sarcoid (n = 13) and active sarcoid (n = 4) were adhered for 24 h, then cultured with the target cells for 72 h and a cytotoxicity index determined. The capacity of purified IFNγ to activate alveolar macrophages from patients with inactive sarcoid was assessed by culturing their macrophages in the presence of IFNγ (250 U/ml) for 24 h before assay.

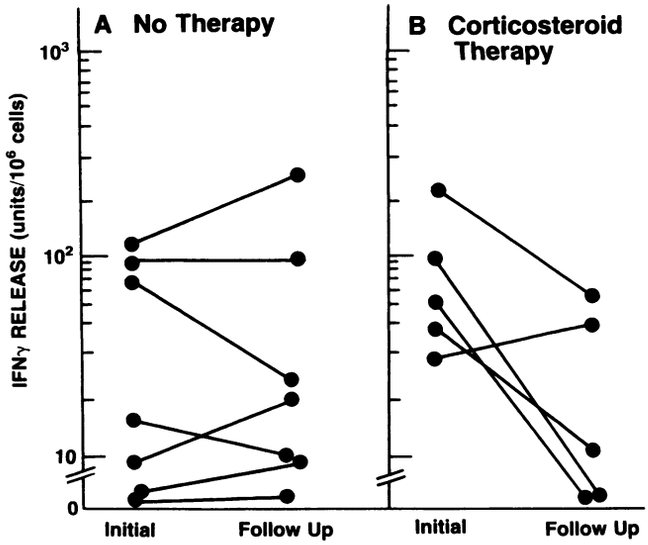

Figure 4. Effect of corticosteroid therapy on the spontaneous release of IFNγ by lung mononuclear cells from sarcoidosis patients. None of the patients was receiving therapy when initially evaluated. Follow-up studies were performed 2.9±0.4 mo after initial evaluation in (*A*) patients who were not started on therapy and after 2.3±0.7 mo in (*B*) those who were started on corticosteroids (prednisone 1/mg per kg/d for 1 mo tapered by 5 mg daily every 10 d thereafter).

than those with inactive disease ($P < 0.01$). However, when alveolar macrophages from sarcoidosis patients with inactive disease were incubated with IFNγ before addition of target cells, enhanced tumor cytotoxicity was observed ($P < 0.001$, compared to alveolar macrophages cultured in medium alone before the addition of target cells). The resultant level of activation produced by the addition of IFNγ to the cultures was similar to that seen in sarcoidosis patients with active disease ($P > 0.1$). Similar enhancement of alveolar macrophage activity in these patients was observed after culture with lipopolysaccharide ($P < 0.001$, data not shown).

Effect of corticosteroid therapy on spontaneous IFNγ release by sarcoidosis lung mononuclear cells. Sequential studies of sarcoidosis patients who received no therapy demonstrated no significant alteration in the level of IFNγ spontaneously released by their lung mononuclear cells ($P > 0.4$, Fig. 4 *A*). In contrast, almost all patients treated with corticosteroids exhibited a marked decrease in spontaneous IFNγ production ($P < 0.02$, Fig. 4 *B*).

Discussion

Gamma interferon is a mediator that lymphocytes and mononuclear phagocytes utilize to up-regulate immune and inflammatory processes. The present study demonstrates that in active pulmonary sarcoidosis, a disorder characterized by a variety of exaggerated immune and inflammatory processes localized to the lung, lung T lymphocytes and alveolar macrophages are spontaneously releasing large amounts of this mediator. When these patients are treated with corticosteroids, a therapy known to suppress the disease process, IFNγ release by lung mononuclear cells is also suppressed. At least one role for IFNγ in this disorder may be to activate alveolar macrophages, since the alveolar macrophages from sarcoid patients with active disease exhibit a high level of spontaneous tumoricidal activity, and IFNγ enhances the tumoricidal activity of alveolar macrophages of patients with inactive disease.

Release of IFNγ by lung mononuclear cells. Consistent with the concept that the human lower respiratory tract possesses a competent immune system, lung mononuclear cells have the capacity to release interferon when triggered with an appropriate stimulus. As with blood mononuclear cells, this interferon is primarily IFNγ. These observations are consistent with the knowledge that, although these lung cells can mount an appropriate response when stimulated, they are normally relatively quiescent even though the lung must handle a relatively large burden of immune and inflammatory stimuli in its role as the organ of gas exchange (11).

Release of IFNγ by sarcoidosis lung mononuclear cells. In contrast to lung mononuclear cells from normals, those from sarcoidosis patients spontaneously released considerable amounts of IFNγ. Furthermore, this process was compartmentalized in that it was observed in almost all cases in lung, but not blood, mononuclear cells. Further, IFNγ could not be detected in either plasma or the epithelial lining fluid of the lower respiratory tract, which suggests: it is not being released systemically in large quantities; and that even at the site of its release (i.e., the lower respiratory tract) it is being used on a local level in the immediate milieu of the cells releasing it.

While the majority of patients with sarcoid had lung mononuclear cells that were releasing IFNγ, those from patients with active disease released approximately fourfold more than those from patients with inactive disease. Because the quantity of cells obtained by bronchoalveolar lavage are limited, only a single time point (24 h) was routinely employed to measure

lung mononuclear cell IFNγ activity. However, a time course for IFNγ release in nonstimulated lung cells from sarcoid patients demonstrated that maximum levels were observed with 24 h left in culture. Thus, in patients with sarcoidosis, a disease of enhanced cell-mediated immune activity at sites of disease involvement, IFNγ, an enhancer of cell-mediated immune processes, is being spontaneously released by cells from the organ involved in the disease process, and its level of production corresponds to the level of disease activity, which suggests a role for IFNγ in the pathogenesis of this disease.

Interestingly, the IFNγ spontaneously released by sarcoid lung mononuclear cells is produced both by lung T cells and alveolar macrophages. These observations are consistent with the knowledge that blood T cells are capable of releasing IFNγ and that, of the subclasses of blood T lymphocytes, the major IFNγ producer is the OKT4/Leu3$^+$ helper cell (32, 33), the same lymphocyte phenotype that comprises the vast majority of the lymphocyte population in the sarcoid lung (15). It has also been shown that human blood monocytes, the precursors of alveolar macrophages, can release IFNγ (34), that murine spleen and bone marrow macrophages release IFNγ after activation by lymphokines (35), and that murine peritoneal macrophages release IFNγ when co-cultured with tumor cells (31).

Spontaneous IFNγ release has also been noted for blood mononuclear cells from patients with Behcets disease (36) and one patient with chronic lymphocytic leukemia (37), and for T cells from one patient with a T cell lymphoma (38). In contrast, human disorders have also been described in which there has been defective IFNγ production by blood mononuclear cells after stimulation, including systemic lupus erythematosis (39), leprosy (40), acquired immune deficiency syndrome (41), IgA deficiency (42), and retinitis pigmentosa (43). These observations are consistent with the concept that the release of IFNγ by mononuclear cells is a monitor of the status of cell-mediated immune function in humans (44).

Role of IFNγ in the pathogenesis of sarcoidosis. The cause of sarcoidosis is unknown, and thus the stimulus to IFNγ release by sarcoid lung mononuclear cells is unknown. However, the fact that T lymphocytes and alveolar macrophages of patients with sarcoidosis are releasing large amounts of this mediator leads to some interesting speculations concerning the pathogenesis of this disease.

First, it is consistent with the hypothesis that sarcoidosis is caused by a viral infection. In this context, it is known that viral infection can lead to IFNγ release. For example, blood mononuclear cells from patients with recurrent herpes labialis spontaneously release IFNγ for up to 6 wk after the outbreak of the disease (45). Furthermore, in vitro viral infection of blood and lung mononuclear cells induces interferon release (46). Against this hypothesis is the knowledge that viral infections are often associated with detectable interferon in serum (47, 48), whereas no interferon can be detected in the serum of sarcoid patients. Further, viral infection of cells generally induces the release of nongamma interferons (1), whereas sarcoid lung mononuclear cells release almost exclusively IFNγ.

Second, IFNγ is released spontaneously by human bone marrow T cells infected with the human T cell lymphoma virus (HTLV-1) and T cells from patients with T cell lymphomas thought to be caused by HTLV-1 (49). Since HTLV-1 is a retrovirus in which DNA complementary to the viral genome is inserted into the human genome, these observations lead to the obvious speculation that sarcoid is associated with HTLV-1 infection. However, direct evaluation of lung T cells and serum from patients with active sarcoid has not demonstrated any signs of active or prior HTLV-1 infection (50, 51).

Third, while the extent of T cell proliferation accompanying antigen presentation by normal alveolar macrophages to autologous T lymphocytes is relatively limited, in sarcoid, T cell proliferation induced by antigen-presenting alveolar macrophages is enhanced approximately twofold (17). In this context, the primary abnormality in sarcoid may be a loss of control of immune responses, such that with any given antigenic stimulus the immune response is greatly exaggerated and/or uncontrolled. If this were true, the exaggerated IFNγ release demonstrated by sarcoid alveolar macrophages and lung T cells may reflect such a loss of control. Little is known of the in vivo regulatory factors that switch off IFNγ production, but it likely involves removal of the initial stimulus, such as eradication of the antigenic signal as well as suppression of IFNγ production. In this latter context, it is known that E-series prostaglandins and other inducers of intracellular cyclic AMP suppress IFNγ production (52), as do suppressor T lymphocytes (53) and corticotropin (ACTH) (54). Interestingly, sarcoidosis alveolar macrophages demonstrate reduced release of E-series prostaglandins (55) and epithelial lining fluid from sarcoidosis patients has lower concentrations of these prostaglandins than does epithelial lining fluid from normals (56).

Fourth, recent studies have suggested that IFNγ is similar, if not identical, to the T cell product referred to as macrophage activating factor (57), a mediator that activates animal alveolar macrophages (58) and human blood monocytes (5). In this regard, the fact that: (*a*) sarcoid lung T cells spontaneously release IFNγ; (*b*) sarcoid alveolar macrophages are activated as manifested by their ability to spontaneously kill a human tumor cell target; and (*c*) IFNγ enhances the ability of alveolar macrophages to kill tumor cell targets, all argue that at least one role for IFNγ in the pathogenesis of sarcoid is to activate the alveolar macrophage. Consistent with such a role, it is known that IFNγ also activates T lymphocytes, cells known to be activated in the lung of patients with active sarcoid. Thus, IFNγ may be a central messenger in T cell-T cell, T cell-alveolar macrophage, and alveolar macrophage-alveolar macrophage interactions in this disease.

An alternative to these hypotheses is that the release of IFNγ plays no role in the pathogenesis of pulmonary sarcoid, and that it is just a marker of the presence of activated lung T lymphocytes and/or alveolar macrophages. For example, it is the OKT4/Leu 3$^+$ helper T cell population, particularly those that are DR$^+$ and/or Tac (IL-2 receptor)$^+$, that produces most of the IFNγ (32, 33). Since this population of cells is increased in the lungs of patients with active sarcoid (14), the enhanced release of IFNγ by the lung T lymphocytes may simply reflect a shift in the normal population of lung T cells toward a population that releases more IFNγ for any given stimulus. Further, IL-2 is spontaneously released by lung T lymphocytes in sarcoidosis patients (13, 14) and IL-2 can stimulate IFNγ production by activated lymphocytes (59, 60). Also, IFNγ in turn increases IL-2 effectiveness by increasing the expression of IL-2 receptors (7). Thus, these two mediators work closely together to enhance the immune response. In this context, the stimuli required for their production, the appear-

ance of their respective mRNAs in the cell cytoplasm, and the kinetics of their release are almost identical (61). In addition, lung lymphocyte populations from patients with active sarcoidosis are relatively deficient in the OKT8/Leu 2^+ suppressor cell subpopulation, cells which can suppress IFNγ production (53). Thus, the cell population of the sarcoidosis lung may represent a milieu that encourages augmented IFNγ production without IFNγ actually playing a role in the pathogenesis of the disease. However, even if this is the case, the release of IFNγ by lung mononuclear cells would still be important from a clinical point of view, since its release likely represents the overall state of activation by the lung cells. In this regard, measurement of its release may be useful as a sensitive monitor of disease activity.

Role of corticosteroid therapy in treating pulmonary sarcoidosis. Although the long-term effectiveness of corticosteroids in the therapy of pulmonary sarcoidosis is controversial, there is no question that in the short term, corticosteroids clearly suppress the disease (11). Consistent with this knowledge, and with the concept that IFNγ plays a fundamental role in the pathogenesis of this disorder, treatment of sarcoid patients with corticosteroids caused a marked suppression of the spontaneous release of IFNγ by lung mononuclear cells. These observations are also consistent with in vitro studies, which demonstrates that corticosteroids suppress the release of IFNγ by mononuclear cells (62), and with the in vitro studies that corticosteroids suppress the expression of IFNγ mRNA by mitogen-stimulated mononuclear cells (63).

Together, these studies suggest that release of IFNγ by lung mononuclear cells may be a step in the pathogenic process of pulmonary sarcoid that is vulnerable to therapeutic attack. In this context, the release of IFNγ by lung mononuclear cells may not only be a useful monitor of disease activity, but also a useful monitor of the minimal dose of corticosteroids necessary to suppress the exaggerated inflammatory and immune process that characterizes this disorder.

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