Mechanisms of Hypergammaglobulinemia in Pulmonary Sarcoidosis

SITE OF INCREASED ANTIBODY PRODUCTION AND ROLE OF T LYMPHOCYTES

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ABSTRACT Pulmonary sarcoidosis is a disorder in which local granuloma formation is perpetuated by activated lung T lymphocytes. The present study suggests that lung T lymphocytes may also play a critical role in modulating local production of antibodies in this disorder. In untreated patients with pulmonary sarcoidosis, the numbers of IgG- and IgM-secreting cells per 10^3 lung lymphocytes are markedly increased compared with those in normal individuals ($P < 0.001$ and $P < 0.01$, respectively); the numbers of IgA-secreting cells in lavage fluid of these patients are not increased ($P > 0.2$). In contrast to lungs, the numbers of IgG-, IgM-, and IgA-secreting cells in blood of patients with this disorder are similar to those in normal individuals ($P > 0.2$, each comparison). In patients with pulmonary sarcoidosis, there is a direct correlation between the percentage of bronchoalveolar cells that are T lymphocytes and the percentage of bronchoalveolar cells that secrete IgG ($r = 0.79$; $P < 0.001$); in normal individuals there is no such relationship ($P > 0.2$). When purified sarcoid lung T cells from patients with high proportions of T lymphocytes in their lavage fluid were co-cultured with blood mononuclear cells from normal individuals (without added antigens or mitogens), the B lymphocytes in these normal mononuclear cell suspensions were induced to differentiate into immunoglobulin-secreting cells ($P < 0.01$). In contrast, blood T lymphocytes from these same patients and lung T lymphocytes from sarcoidosis patients with low proportions of T lymphocytes in their lavage fluid did not stimulate normal B cells to produce immunoglobulin ($P > 0.2$, all comparisons). These findings suggest that in pulmonary sarcoidosis (*a*) the lung is an important site of immunoglobulin production; (*b*) activated lung T lymphocytes play an important role in modulating this local production of antibody, and thus are likely to modulate the polyclonal hyperglobulinemia observed in these individuals.

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INTRODUCTION

Pulmonary sarcoidosis is a disease of unknown etiology characterized by the presence of an alveolitis and noncaseating granulomata in the lung parenchyma (1–8). In addition to these histological parameters of delayed hypersensitivity, several other lines of evidence suggest that this disease is mediated by a heightened cellular immune response within the lung: (*a*) the alveolitis of this disorder contains large numbers of activated T lymphocytes (4–9); (*b*) these activated T lymphocytes spontaneously secrete a variety of lymphokines which attract monocytes, the building blocks of granulomata, to the lung and also modulate granuloma formation by these cells (4–6, 8); (*c*) there is a significant correlation between both a histological as well as a clinical assessment of disease activity and the proportions of bronchoalveolar cells that are T lymphocytes in patients with this disorder (8).

However, in addition to this heightened local cellular immune response, patients with pulmonary sarcoidosis also appear to have a heightened humoral immune response (10–15) manifested by increased amounts of circulating immunoglobulins (10–15). Another feature of this humoral immune response is the presence of antibodies with reactivity toward multiple antigens, including self-antigens (10–15). This polyclonal response suggests that different multiple clones of B cells are continuously being stimulated to produce immuno-

globulin. It is also of interest that this hypergammaglobulinemia is present in spite of the fact that blood lymphocytes of these patients appear to secrete decreased amounts of immunoglobulins (16). These observations raise the possibility that the increased amounts of immunoglobulins present in the blood of these patients may be produced locally at sites of disease activity and that the polyclonal activation of this humoral response may be a secondary, nonspecific phenomenon related to the continuing local cellular immune response.

The purpose of this study, therefore, was to determine whether increased amounts of immunoglobulins are produced locally in the lung in patients with sarcoidosis, and to evaluate the role of activated T lymphocytes in modulating antibody production by B cells, which are present at sites of disease activity in these patients. To accomplish this, mononuclear cells were obtained from the lung (via bronchoalveolar lavage and by open-lung biopsy) and from blood of normal individuals and patients with sarcoidosis. To determine whether increased amounts of immunoglobulins are produced at sites of disease activity, the numbers of immunoglobulin-secreting cells were quantified in each of these cell suspensions using a standard reverse hemolytic plaque assay. To evaluate the interaction of T lymphocytes and antibody-producing cells in the lungs of sarcoidosis patients, the proportions of lung immunoglobulin-secreting cells were correlated with the proportions of lung T lymphocytes. In addition, purified sarcoid lung T lymphocytes were co-cultured with normal blood mononuclear cells to determine whether these activated T cells might stimulate normal cells to secrete immunoglobulin.

METHODS

Study population: pulmonary sarcoidosis. A diagnosis of pulmonary sarcoidosis was made in 15 untreated patients (age 37±6 yr; 5 males, 10 females)[1] using the following criteria: (*a*) a lung biopsy demonstrating noncaseating epithelioid cell granulomata in the lung parenchyma and co-existing morphologic features compatible with sarcoidosis; (*b*) no history of mycobacterial, fungal, or parasitic infection; (*c*) no history of exposure to inorganic or organic materials known to cause granulomatous lung disease; and (*d*) a positive Kveim-Siltzbach skin test. All in vitro studies were completed before placement of the Kveim skin test. As a group, patients with sarcoidosis had the following functional abnormalities: vital capacity, 73±7% predicted; total lung capacity, 72±9% predicted; single-breath diffusing capacity, 64±12% predicted; ratio of forced expiratory volume in 1 s to forced vital capacity, 101±6%; resting arterial P_{O_2}, 92±9 mm Hg; and exercise arterial P_{O_2}, 80±10 mm Hg. All 15 patients had levels of IgG and IgM in their serum which were above the normal range for these immunoglobulins.

Study population: normal controls. 10 normal, nonsmoking volunteers had a mean age of 29±9 yr; there were 5 males and 5 females. Pulmonary function tests and chest roentgenograms of all 10 individuals were within normal limits. None was receiving medication.

Evaluation of lung-inflammatory and immune effector cells obtained by bronchoalveolar lavage. Inflammatory and immune effector cells were obtained from the lung by bronchoalveolar lavage using previously described methods (4–8). All lavages used a total of 100 ml of 0.9% saline. The cells were separated from lavage fluid by centrifugation (500 *g*, 5 min) and resuspended in Hanks' balanced salt solution without Ca^{++} or Mg^{++}. The cells were then evaluated for cell number, viability, and differential count as described (4–8). Mononuclear cells that were present in lavage were then purified free of polymorphonuclear leukocytes by Hypaque-Ficoll centrifugation (17) (Pharmacia Fine Chemicals, Piscataway, N. J.). The resulting cell suspensions were >98% viable and contained, on the average, 93±4% macrophages and 7±1% lymphocytes in normals, and 60±10% macrophages and 40±8% lymphocytes in patients with sarcoidosis ($P < 0.01$, each comparison between normals and sarcoid patients). Macrophages were distinguished from lymphocytes by Wright-Giemsa stains, neutral red dye uptake, and nonspecific esterase stains (4). Following Hypaque-Ficoll centrifugation, these cell suspensions were adjusted to a concentration of 10^7/ml in RPMI 1640 medium.

Evaluation of lung-inflammatory and immune-effector cells obtained from open-lung biopsies. Inflammatory and immune effector cells were obtained directly from open-lung biopsies of the lung parenchyma from 4 of the 15 sarcoidosis patients using previously described methods (7, 18). Briefly, a portion of the lung biopsy was placed in iced heparinized (100 U/ml) Hanks' balanced salt solution and teased with a surgical scapel blade. The teased cell suspension, together with the lung tissue, was then filtered through several layers of sterile gauze to obtain a single cell suspension. These cells were evaluated for cell number, viability, and differential count; mononuclear cells were then obtained from these cell suspensions following Hypaque-Ficoll centrifugation (17). The resulting mononuclear cell suspensions were >98% viable and contained on the average 56±11% macrophages and 44±10% lymphocytes. These cell suspensions were adjusted to a concentration of 10^7/ml in RPMI 1640 medium.

Isolation of mononuclear cells from blood. Mononuclear cells were obtained from peripheral blood by Hypaque-Ficoll centrifugation (17).

Quantification of immunoglobulin-secreting cells. Rabbit IgG, specific for the heavy chains of either human IgG, IgM, or IgA, were purified by solid phase absorption until each antiserum could be shown to be monospecific by Ochterlony analysis and immunoelectrophoresis. Protein A (*Staphylococcus aureus*) (Pharmacia Fine Chemicals, Piscataway, N. J.) was coupled to sheep erythrocytes (SRBC)[2] with $CrCl_3$ as described (19). The protein A-coated SRBC were then washed four times and diluted to a 30% suspension in RPMI 1640 medium.

The mononuclear cells from lavage fluid, lung biopsies, and blood were first incubated at 37°C for 1 h to elute off any adherent immunoglobulin or immune complexes. The cells were then washed four times and resuspended in RPMI 1640 medium at a concentration of 10^7/ml. To 10 × 75-mm glass culture tubes, preheated to 43°C, was added 0.84 ml of 0.8% agarose (Indubiose A 37; L'Industrie Biologique Francaise S. A., Gennevilliers, France) in RPMI 1640 medium, 0.1 ml of the mononuclear cell suspensions, and 0.6 ml of the protein A-coated SRBC. The contents of the tube were then mixed and transferred to 60 × 15-mm petri dishes precoated

[1] All data are expressed as the mean±SEM.

[2] *Abbreviation used in this paper*: SRBC, sheep erythrocytes.

with 4 ml of 0.8% agarose in RPMI 1640 medium. The petri dishes were quickly swirled so that the cell-agarose mixture was uniformly distributed into a monolayer. The agarose was allowed to solidify and the petri dishes were placed in a humidified 5% CO_2 incubator at 37°C for 2 h. Following this incubation, 1 ml of appropriately diluted antiserum, or medium as a control, was added to each dish. The dishes were incubated for an additional 1 h, and the antiserum was removed by aspiration and replaced with 1 ml of a 1:10 dilution of SRBC-absorbed guinea pig complement. The dishes were then incubated for an additional 1 h, and the complement was removed by aspiration. The numbers of plaques on duplicate dishes were determined for each class of antiserum, as well as for the control dishes which had not been exposed to antiserum. The numbers of plaques in the control dishes never exceeded three per plate. No plaques were present on the dishes when the mononuclear cells were cultured in the plaque assay with antiserum in the presence of puromycin (25 μg/ml) or cycloheximide (10 μg/ml). The data were adjusted to the percentages of lymphocytes in the mononuclear cell suspensions and expressed as immunoglobulin-secreting cells per 10^3 or 10^6 lymphocytes.

Identification and isolation of T lymphocytes. T lymphocytes were identified in the mononuclear cell suspensions from lavage fluid by their ability to form rosettes with neuraminidase-treated SRBC at 4°C (5–6, 20). The data for T lymphocytes are expressed as the percentage of total bronchoalveolar cells (including both macrophages and lymphocytes) that were T lymphocytes. Purified T lymphocyte populations were obtained from lavage fluid and blood by rosetting the mononuclear cell suspensions twice with neuraminidase-treated SRBC at 4°C followed by Hypaque-Ficoll centrifugation as previously described (5–6).

T and B lymphocyte interactions in lung and blood of patients with sarcoidosis. To determine whether T lymphocytes from lung or blood of patients with sarcoidosis might directly stimulate normal B lymphocytes to secrete immunoglobulin, highly purified lung and blood T lymphocytes (>98%, obtained as described above) from five of the sarcoidosis patients with >40% T lymphocytes in their lavage fluid and from three of the sarcoidosis patients with 20–40% T lymphocytes in their lavage fluid were co-cultured with equal numbers of blood mononuclear cells (monocytes and lymphocytes) from normal individuals. Unfractionated blood mononuclear cells from normal individuals rather than from patients with sarcoidosis were used for these studies because previous studies have demonstrated that it is difficult to stimulate blood mononuclear cells from sarcoidosis patients in vitro to secrete immunoglobulin. This is probably because of the presence of "suppressor monocytes" in these cell suspensions (16, 21).

As a control for each experiment, blood T lymphocytes from normal individuals were co-cultured with unfractionated mononuclear cells from other (allogenic) normal individuals. Because of the small numbers of T cells present, it was not possible to obtain enough purified lung T lymphocytes from either normal individuals or sarcoidosis patients with <20% T lymphocytes in their lavage fluid.

For these studies, 2.5×10^6 T lymphocytes and 2.5×10^6 unfractionated normal mononuclear cells were co-cultured without additional stimulation in sterile 1 × 7.5-cm plastic tubes containing 2 ml of RPMI 1640 media with 10% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). The cells were incubated on a rocker platform (7 cycles/min) at 37°C in 5% CO_2 in air at 100% humidity. After 7 d in culture, the cells were harvested and the numbers of IgM-secreting cells were enumerated as described above. Only IgM-secreting cells were counted, since this type of co-culture resulted in a primary immune response characterized by the selective secretion of IgM rather than a secondary immune response characterized by secretion of multiple types of antibodies. The numbers of IgM-secreting cells that were present in these co-cultures of sarcoid T cells and normal mononuclear cells were expressed as a percentage of the number of IgM-secreting cells present in co-cultures of normal T cells with normal mononuclear cells.

RESULTS

Proportions of immunoglobulin-secreting cells in blood and lung of patients with sarcoidosis. There were no significant differences in the proportions of IgG-, IgM-, or IgA-secreting cells per 10^3 lymphocytes in blood of patients with sarcoidosis compared to blood of normal individuals ($P > 0.2$, each comparison). In marked contrast, the proportions of IgG- and IgM-secreting cells per 10^3 lymphocytes in lavage fluid of patients with sarcoidosis were significantly increased compared to those present in lavage fluid of normal individuals (Fig. 1) (IgG 2,420±210 vs. 192±40, respectively, $P < 0.001$; IgM 578±42 vs. 75±24, respectively, $P < 0.01$). In sarcoidosis patients, the proportions of IgG- and IgM-secreting cells were increased approximately eight- and fivefold, respectively, in lavage fluid compared to their own peripheral blood ($P < 0.01$, each comparison). These findings contrast sharply with the fact that, in normal individuals, there are similar proportions of IgG- and IgM-secreting cells per 10^3 lymphocytes in blood and in lavage fluid (4).

Although IgG and IgM production is increased in the sarcoid lung, there were no significant differences in the proportions of IgA-secreting cells in lavage fluid of patients with sarcoidosis compared with normal indi-

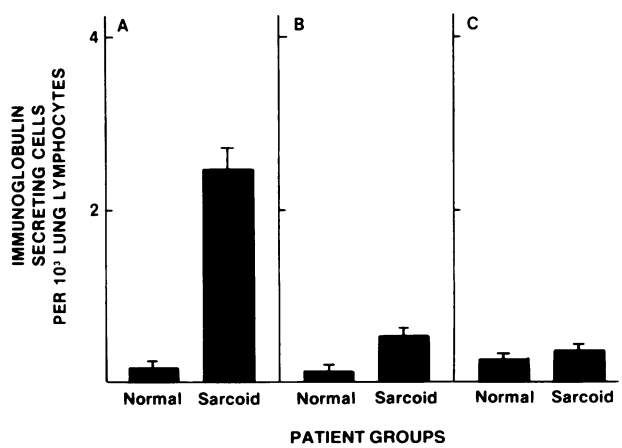

FIGURE 1 Comparison of the proportions of immunoglobulin-secreting cells in bronchoalveolar-lavage fluid of normal individuals (n = 10) and patients with pulmonary sarcoidosis (n = 15). (A) IgG-secreting cells; (B) IgM-secreting cells; and (C) IgA-secreting cells. The proportions of immunoglobulin-secreting cells are quantified on the ordinate as immunoglobulin-secreting cells per 10^3 lung lymphocytes.

viduals (380±36 vs. 256±41, respectively, $P > 0.2$). There were more IgA-secreting cells in lavage fluid compared with blood of patients with sarcoidosis, but these differences were small and not statistically significant ($P > 0.2$). This is in contrast to that expected for a secretory immunoglobulin, as normal individuals have more IgA-secreting cells per 10^3 lymphocytes in lavage fluid than in peripheral blood (4).

In patients with sarcoidosis, the proportions of immunoglobulin-secreting cells in lavage fluid clearly reflected the proportions of immunoglobulin-secreting cells that were present in the lung parenchyma. When the proportions of IgG-, IgM-, and IgA-secreting cells recovered by lavage were compared with the immunoglobulin-secreting cells isolated directly from lung biopsies, no significant differences were found ($P > 0.2$, each comparison).

Correlation of the proportions of immunoglobulin-secreting cells with the proportions of T lymphocytes in the lungs of patients with sarcoidosis. In normal individuals, the proportions of bronchoalveolar cells that were T lymphocytes ranged from 2 to 8% (lymphocytes, 2–10%; percent lymphocytes that were T lymphocytes, 65–80%). In patients with sarcoidosis, the proportions of bronchoalveolar cells that were T lymphocytes ranged from 8 to 60% (lymphocytes, 10–67%; percent lymphocytes that were T lymphocytes, 80%–95%). These alterations in the bronchoalveolar cell populations of patients with sarcoidosis are similar to those that have previously been reported in patients with this disorder (5–8).

In normal individuals there was no correlation between the percent bronchoalveolar cells that were T lymphocytes and the percent bronchoalveolar cells that secreted either IgG, IgM, or IgA (data not shown) ($P > 0.2$, each comparison). In contrast, in patients with sarcoidosis, there was a significant correlation between the percent bronchoalveolar cells that were T lymphocytes and the percent bronchoalveolar cells that were secreting IgG (Fig. 2) ($r = 0.79$, $P < 0.001$). By comparison, there was no correlation between the percent bronchoalveolar cells that were T lymphocytes and the percent bronchoalveolar cells that were secreting either IgM ($r = 0.12$, $P > 0.5$) or IgA ($r = -0.11$, $P > 0.5$) in lavage fluid of sarcoidosis patients (data not shown).

Capacity of T lymphocytes from lung and blood of patients with sarcoidosis to stimulate normal B lymphocytes to secrete immunoglobulin. After 7 d in culture, blood mononuclear cells from normal individuals co-cultured with equal numbers of highly purified (>98%) blood T lymphocytes from another (allogenic) normal individual contained 255±56 IgM-secreting cells per 10^6 lymphocytes (data not shown). When blood T lymphocytes (>98%) from sarcoidosis patients with 20–40% T cells in their lavage fluid were

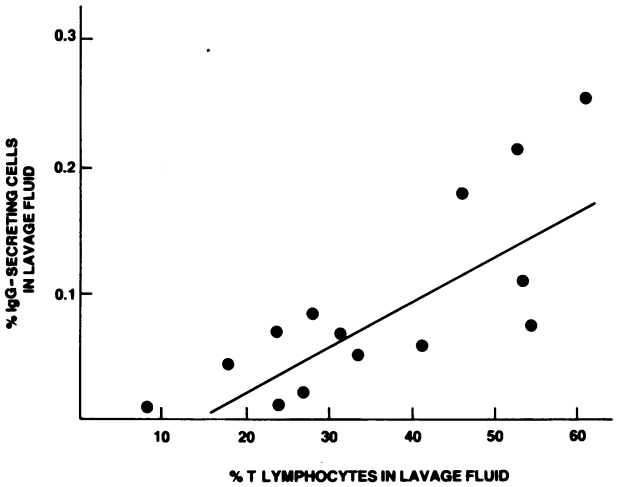

FIGURE 2 Correlation of the proportions of IgG-secreting cells in lavage fluid with the proportions of T lymphocytes in lavage fluid of patients with sarcoidosis ($n = 15$). The proportions of bronchoalveolar cells that are IgG-secreting cells are in the ordinate and the proportions of bronchoalveolar cells that are T lymphocytes are on the abscissa ($r = 0.79$, $P < 0.001$). In normal individuals ($n = 10$), there was no correlation between the proportions of IgG-secreting cells and the proportions of T lymphocytes in bronchoalveolar-lavage fluid ($r = 0.13$, $P > 0.2$).

cultured with normal mononuclear cells, there were no significant increases in the proportions of IgM-secreting cells compared with the control cultures (Fig. 3) ($P > 0.2$). Similarly, when lung T lymphocytes (>98%) from these same individuals were cultured with normal mononuclear cells, there were no significant increases in the proportions of IgM-secreting cells compared with the control co-cultures ($P > 0.2$). In addition, when blood T lymphocytes (>98%) from sarcoidosis patients with >40% T cells in their lavage fluid were cultured with normal mononuclear cells, the proportions of IgM-secreting cells in these co-cultures were also not significantly increased compared with control co-cultures ($P > 0.2$). However, when lung T lymphocytes from these same individuals were cultured with normal mononuclear cells, the proportions of IgM-secreting cells in these co-cultures were significantly increased compared to control co-cultures ($P < 0.01$) and compared with co-cultures which included blood T cells from these same patients ($P < 0.01$).

To demonstrate that the increased proportions of IgM-secreting cells present in the co-cultures of lung T cells and unfractionated normal blood mononuclear cells were derived from the unfractionated normal mononuclear cell populations, rather than from B lymphocyte contamination of the purified lung T lymphocyte suspensions, we performed the following controls. First, each of the purified T-lymphocyte suspensions from normal blood and from the lung and

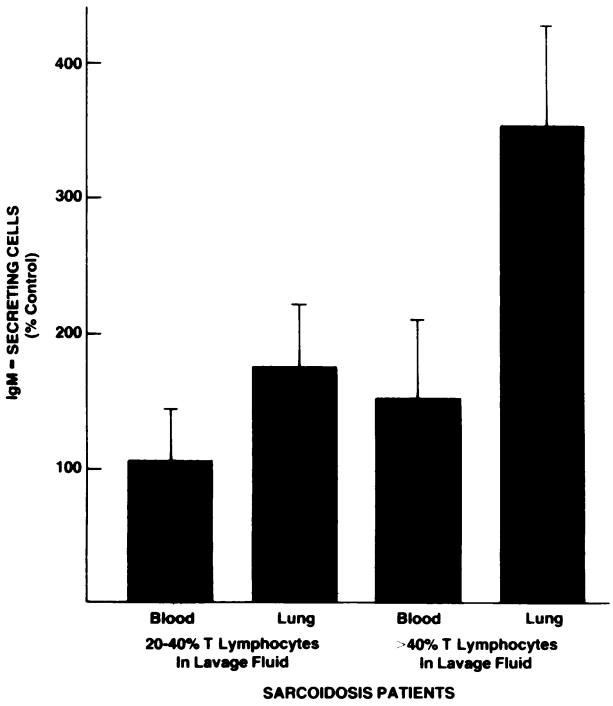

FIGURE 3 Capacity of lung and blood T lymphocytes from patients with pulmonary sarcoidosis to stimulate B lymphocytes from blood of normal individuals to secrete immunoglobulin. Highly purified lung or blood T lymphocytes (>98%) from patients with pulmonary sarcoidosis were co-cultured for 7 d with equal numbers of blood mononuclear cells from normal individuals. After 7 d in culture, the numbers of IgM-secreting cells in these co-cultures were determined. As controls for these experiments, highly purified blood T lymphocytes (>98%) from normal individuals were co-cultured with mononuclear cells from other (allogenic) normal individuals. The data for the induction of IgM-secreting cells in the co-cultures of sarcoid T cells + normal mononuclear cells is expressed on the ordinate as a percent control (number of IgM-secreting cells per 10^6 lymphocytes)/(number of IgM-secreting cells per 10^6 lymphocytes in control co-cultures). Normal mononuclear cells were co-cultured with four different groups of sarcoid T cells: (*a*) blood T cells from patients with 20–40% of their bronchoalveolar cells as T lymphocytes (n = 3); (*b*) lung T cells from patients with 20–40% of their bronchoalveolar cells as T lymphocytes (n = 3); (*c*) blood T cells from patients with >40% of their bronchoalveolar cells as T lymphocytes (n = 5); and (*d*) lung T cells from patients with >40% of their bronchoalveolar cells as T lymphocytes (n = 5). The proportions of IgM-secreting cells were increased in *d* compared with the other three groups ($P < 0.01$, all comparisons); there were no significant differences among groups *a*, *b*, or *c* ($P > 0.2$, all comparisons).

blood of sarcoidosis patients were cultured for 7 d separately (without added normal mononuclear cells); the proportions of IgM-secreting cells in these T cell suspensions were quantitated. In all instances there were <20 IgM-secreting cells per 10^6 lymphocytes. Second, pretreatment of the purified lung T lymphocyte suspensions with mitomycin C (40 μg/ml for 30 min at 37°, which would prevent the development of new immunoglobulin-secreting cells in these cell suspensions) did not decrease the proportions of IgM-secreting cells which were present in the cocultures of sarcoid lung T cells and unfractionated normal blood mononuclear cells (data not shown).

To demonstrate that the increased proportions of IgM-secreting cells in the co-cultures of sarcoid lung T cells and unfractionated normal blood mononuclear cells were not the result of the presence of small numbers (<2%) of sarcoid alveolar macrophages in these T cell suspensions, alveolar macrophages from patients with sarcoidosis were added to a final concentration of 2% to the T lymphocyte suspensions from blood of normals and patients with sarcoidosis. Addition of alveolar macrophages to these blood T cell suspensions did not increase the proportions of IgM-secreting cells in the co-cultures containing these cells and unfractionated normal blood mononuclear cells.

DISCUSSION

Pulmonary sarcoidosis is a chronic disorder characterized by the presence of noncaseating epithelioid cell granulomata in the lung parenchyma and a polyclonal hypergammaglobulinemia (1–15). Granulomata formation in this disorder is mediated by an alveolitis comprised of large numbers of activated T lymphocytes (4–9). In the present study, we have demonstrated that although antibody production is not elevated in peripheral blood of patients with sarcoidosis, antibody secretion is markedly increased at sites of disease activity. In addition, there is a significant correlation between the intensity of the T lymphocyte component of the alveolitis of sarcoidosis and the numbers of bronchoalveolar cells that are secreting immunoglobulin. One explanation for this association of activated T lymphocytes and antibody formation in the sarcoid lung is that sarcoid lung T lymphocytes are capable of directly stimulating B lymphocytes to differentiate into immunoglobulin-secreting cells. In this regard, lung T lymphocytes from patients that have intense cellular immune responses in their lungs provide a greater stimulus for this B cell differentiation than do blood T cells from these same patients or lung T cells from sarcoidosis patients who have less intense cellular immune responses in their lungs.

The present study resolves at least in part two conflicting observations in patients with sarcoidosis, i.e., there are increased amounts of circulating immunoglobulins present in blood of these patients (10–15), yet blood lymphocytes of these patients do not secrete increased amounts of immunoglobulin (16). In this regard, sarcoid blood lymphocytes, compared with normal blood lymphocytes, exhibit a decreased capacity to secrete immunoglobulin following in vitro stimulation

with antigens or mitogens. This in vitro defect in immunoglobulin secretion has been attributed to the presence of circulating "suppressor" monocytes (16, 21). The observations, however, that the sarcoid lung contained increased numbers of immunoglobulin-secreting cells (as well as increased numbers of T lymphocytes which augment B cell differentiation into immunoglobulin-secreting cells) suggest that, while suppressor monocytes may play a role in the system anergy of this disorder, these cells play little role "in vivo" in decreasing immunoglobulin production at sites of disease activity and that the lung, and not blood, is the major site of immunoglobin production in this disorder.

The hypergammaglobulinemia of sarcoidosis is characterized by antibodies with reactivity toward multiple antigens, including self-antigens, rather than to a single antigen (10–15). It is possible that this heightened humoral immune response results from a continual abnormal exposure in these patients to a wide variety of antigens. It is, however, more likely that this response represents an abnormal stimulation of multiple clones of B lymphocytes to produce antibody as a result of the intense cellular immune response that is ongoing at sites of disease activity. One mechanism by which activated T lymphocytes might stimulate an overproduction of immunoglobulins with this disorder is by prolonging the abnormal humoral response that occurs following exposure to various types of common antigens. Alternatively, as suggested in this study, activated T lymphocytes might directly activate certain clones of B cells to produce immunoglobulins without associated antigen stimulation. The precise in vivo mechanism(s), however, by which activated T lymphocytes that are present at sites of disease activity stimulate production of increased amounts of antibody, remain to be defined.

What role, if any, the increased levels of immunoglobulins play in the pathogenesis of granuloma formation in patients with sarcoidosis is unclear. One possibility is that a portion of the immunoglobulins produced at sites of disease activity might directly participate in granuloma formation by combining with antigen to form immune complexes. In this context, it is known that immune complexes are present in some patients with sarcoidosis (10, 11, 14) and that, under certain conditions, the deposition of immune complexes in various tissues can result in the formation of granulomata (22). Alternatively, the hypergammaglobulinemia may simply be a "by-product" of the presence of activated T lymphocytes at the site of disease activity and play no role in the pathogenesis of this disorder. At this point, we do not have enough information to resolve this question.

Although it is clear that the total population of lung T cells present at sites of disease activity in sarcoidosis augments rather than suppresses local antibody formation, the precise alterations in the subsets of T cells that mediate this response are unclear. It is known, however, that various subsets of T cells are proportionately increased at sites of disease activity in patients with sarcoidosis. These T cell subsets include: (*a*) T lymphocytes with Fc receptors for IgG (4, 6, 8), (*b*) T lymphocytes that form rosettes with SRBC at 37°C (4, 6–9), and (*c*) T lymphocytes that are spontaneously secreting various lymphokines (4–6, 8). In addition, these patients also have anti-T cell antibodies, which might preferentially deplete certain subsets of T cells (15). Although it is not clear how these alterations in T cell populations result in an increased local production of immunoglobulins, it is clear that increased amounts of antibodies are produced at sites of disease activity in this disorder and thus offer a rational explanation for the hypergammaglobulinemia of sarcoidosis.

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