Localization of Immunoglobulin and Complement in Pulmonary Sarcoid Granulomas


Immunohistochemical examination of pulmonary sarcoid lesions from three patients revealed in all the presence of clumps of immunoglobulin and complement in those granulomas which were not completely fibrosed. Similar examination of lung biopsies from 36 patients suffering from a variety of chronic lung diseases but not sarcoidosis showed clumps of immunoglobulin and complement in and around the lesions of only one patient suffering from allergic pulmonary alveolitis. This observation supports the contention that the lesions of sarcoidosis might have an immunologic basis.

Though a wide variety of hypotheses have been advanced about the etiology and pathogenesis of sarcoidosis, there is no conclusive evidence favouring any one of them. We report here the results of immunohistologic examination of biopsied lung tissue from three patients suffering from pulmonary sarcoidosis. In all three patients deposits of immunoglobulin and C3 (B1C/B1A) were found in and around active sarcoïd lesions. This observation supports the postulation that lesions of sarcoïd type are the "result of immunologic reactions."

CASE REPORTS

Case 1

A 24-year-old white man, a student, was exposed to a tuberculosis patient in 1967. A skiagram of his chest taken in April, 1970, showed a reticulonodular pattern in the middle portion of both the lungs and bilateral hilar lymph-node enlargement.

Case 2

This 26-year-old white man, a carpet layer, was admitted to the Halifax Infirmary with the complaint of pain in the right flank. No abnormality was detected in his urine, but his chest x-ray film at this time revealed diffusely scattered areas of normal density and bilateral hilar lymph-node enlargement.

Case 3

This white woman, 43 years old, an upholsterer, was admitted to hospital for investigation of shortness of breath and recurrent respiratory tract infections for nine months prior to admission. Pulmonary function studies revealed some restriction of lung volumes, associated with mild reversible obstruction and mild compensated metabolic acidosis. An x-ray picture at this time showed "fluffy infiltrate" in her lower lobes and bilateral hilar lymph-node enlargement.

Apart from being involved in an auto accident in January 1975, in which she received a whiplash injury, she had no other serious illnesses.

The diagnosis of pulmonary sarcoidosis was accepted in all these three cases on the basis of: (1) x-ray findings, ie, diffuse pulmonary infiltration along with bilateral hilar lymph-node enlargement; (2) histology of the lesions, especially the lack of necrosis in any of the granulomas; (3) negative bacteriologic findings in smears and cultures of sputum and biopsied lung tissues; (4) negative tuberculin reaction with five tuberculin units of PPD (Connaught Laboratories, Toronto), and (5) lack of detectable serum precipitins against several commercial Aspergillus derived antigens (Bencard).

Kveim test was not done on any of these patients.

MATERIALS AND METHODS

One half of each piece of fresh biopsied lung tissue, containing the granulomas, was fixed in 10 percent buffered formalin and about 2 mm3 blocks from the other half were snap frozen in a slurry of liquid nitrogen and isopentane (−160°C) and stored at −70°C. Air-dried unfixed 5 micron cryostat sections of lung tissue as well as parallel sections fixed in 5 percent glacial acetic acid in ethanol at −20°C for 15 minutes were washed three times in phosphate buffered saline (PBS, 0.1M, pH 7.1) and stained by the direct immunofluorescence method using fluorescein-isothiocyanate conjugated monospecific rabbit antisera against human IgG, IgM, IgA, C3, (B1C/B1A), fibrinogen and albumin. All antisera were obtained from Hyland Laboratories (California) and were conjugated with crystalline fluorescein isothiocyanate isomer I (Baltimore Biological Laboratory, Baltimore, Maryland) by a method adapted from Clark and Shepard.7 Conjugates were freed of overlabelled globulin molecules by passing them through a column of DEAE cellulose. Only those conjugates having dye protein ratios varying from 1.3 to 4.5 were used in this study.

All antisera gave a single line on immunoelectrophoresis against normal human serum both before and after labelling. Specificity of immunofluorescence was established by: (1) the absence of staining of cryostat sections of normal human lung tissue by these conjugated antisera; (2) absence of staining of sections of sarcoïd lung tissues after absorption of the fluoresceinated antisera as appropriate with the relevant immunoglobulin fraction, fresh normal human serum or human plasma fractions I (fibrinogen) and V (albumin); (3) absence of staining of sarcoïd lung tissue with fluoresceinated rabbit antioag or rabbit antihorse globulin; (4) absence or...
inhibition of staining after "blocking" of specific staining by prior exposure of sections to nonlabelled antibodies;9 (5) the "sandwich" method, i.e., treating sections first with unconjugated reagents and then as appropriate with fluoresceinated goat antirabbit and rabbit antigoat globulins;10-18 (6) staining sections of lung granulomas with Research Standard A for fluoresceinated antibody to human immunoglobulins supplied by the World Health Organization (W. H. O. Ref. No. 68/45); (7) staining of renal biopsies from poststreptococcal and lupus nephritis patients known to contain glomerular deposits predominantly either of IgG or IgM and C3; and (8) staining of rat liver sections with the fluoresceinated reagents after exposure of sections to antinuclear sera known to have their antinuclear activity only either in the IgG or IgM fraction, and for the anti-C3 reagent after exposure of sections to complement fixing antinuclear antibodies and fresh AB group normal human serum as the source of complement.14

Antinuclear, antithyroid, antigastric parietal cell, antimitochondrial, antismooth muscle and antilung activities of the sera from these patients were assessed also by the "sandwich" method.12-14

To eliminate the possibility of the reaction of the antoglobulin reagents with dried-up serum or exudate trapped in tissue spaces, all sections were washed for 15 minutes in three changes of PBS prior to exposure to the reagents. In addition, parallel sections from lesions showing immunoglobulin deposits were washed overnight at 4°C either with PBS or acid citrate buffer (0.02 M, pH 3.2) to elute out globulin.18

Sections washed in the acid buffer were rewashed in PBS for 30 minutes to adjust the pH before staining for immunoglobulin. All sections washed with PBS retained immunoglobulin whereas those washed with citrate buffer lost most of their immunoglobulin content.

The formalin-fixed tissues were embedded in paraffin and sections were stained with hematoxylin and eosin, Masson's trichrome (for collagen), elastic Van Gieson (for elastic fibers), Congo red (for amyloid) and periodic acid Schiff (for polysaccharides). After immunofluorescence examination, all sections were fixed in 10 percent formalin and stained with eosin and hematoxylin.

RESULTS

On open lung biopsy, all these patients revealed variable numbers of small, grayish firm nodules scattered throughout the lung parenchyma. Histologically, these nodules consisted of discrete and conglomerated noncaseating granulomas containing whorls of epithelioid cells, giant cells, lymphocytes, occasional plasma cells and bundles of collagen in the periphery (Fig 1, 4, 6). None of the granulomas showed necrosis or any birefringent material.

Immunohistochemical examination of the lesions from all the three biopsies revealed clumps of extracellular and extravascular immunoglobulins (predominantly IgG and a small amount of IgM), complement and small amounts of fibrin in these granulomas, but neither in the unaffected areas of the lung nor in the areas of dense fibrosis. Immunoglobulins and complement deposits were more common in the periphery of the granulomas which mainly contained lymphocytes and plasma cells than in the central part of the granulomas where epithelioid and giant cells predominated (Fig 1, 2, 3, 5, 6, 7). In the more cellular granulomas, the distribution of immunoglobulins and complement was more uniform throughout the lesions (Fig 4, 5). In the ethanol-acetic acid-fixed sections of the granulomas, a few IgG, IgM and IgA containing cells could be seen in the periphery of the lesions. An occasional cell had distinct eccentric nucleus and the typical morphology of plasma cells (Fig 3).

Only the serum from the second patient had very mild antinuclear activity (homogenous nuclear staining up to a titer of 1/16). None of the patients had antithyroid, antigastric parietal cell, antimitochondrial, antismooth muscle or antilung activity in their serum.

DISCUSSION

Similar intracellular and extracellular deposits of human immunoglobulin16 and more specifically of IgG, IgM, IgA, and C3,17 have been detected in the periphery of sarcoid granulomas in lymph nodes by immunohistologic methods. This confirmed an earlier postulation based on morphologic studies that sarcoid granulomas are the result of "an immunity reaction."4 Though similar localization of immunoglobulin has been noted in the subcutaneous nodules of rheumatoid arthritis and in the lesions of rheumatoid carditis, lupus erythematosus18 and several other chronic pulmonary diseases19,20 immunohistochemical study of pulmonary sarcoid lesions has not yet been reported.31 After immunohistologic examination of biopsied lung tissue from 36 lung patients
suffering from a variety of lung diseases (primary carcinoma of lung, 16; Hodgkin’s disease, 1; benign cyst, 1; lung abscess, 2; bronchiectasis, 4; extrinsic allergic alveolitis, 5; possible old healed tuberculosis, 2; diffuse interstitial pulmonary fibrosis, 4; and emphysema, 1), we found extracellular deposits of immunoglobulin and C3 in the lung of only one suffering from allergic alveolitis.

It is unlikely that the localization of immunoglobulins in these pulmonary sarcoid lesions resulted from exudation of plasma proteins due to increased capillary permeability in the inflamed tissue because (i) there was no detectable amount of albumin in the sections examined and (ii) the extracellular deposits of immunoglobulin could be washed off only with citrate buffer (pH 3.2) which dissociates antigen-antibody complexes, but not with PBS (pH 7.1). The localization of C3 in those areas where extracellular deposits of immunoglobulin could be detected suggest that antibody which participated in this reaction is a complement fixing antibody. However, C3 could be bound by any immunoglobulin aggregate. The precise mechanism of localization of extracellular immunoglobulin and C3 in the pulmonary sarcoid lesions has not been elucidated by our study. This localization could have resulted from an immunologic reaction of the host with an autologous or extrinsic antigen. However, no lung localizing antibody could be detected in the serum of these patients when cryostat sections of nongranulomatous areas of their own lung tissue or other normal human lung tissues were exposed to these sera for indirect immunofluorescence. Hence, if lung tissue was damaged by an antigen-antibody reaction, the antigen is likely to be unrelated to lung tissue. The possible mechanism of production of granulomatous lesions in lungs by
such antigen-antibody complexes and complement has recently been discussed. The granulomatous component of the reaction has been attributed to "low solubility and an unmetabolizable" nature of the antigens. Localization of IgG, IgM and complement in the periphery of active silicotic granulomas in lungs has also been reported; however, there was no demonstrable birefringent or foreign material in the lung tissues examined by us.

ACKNOWLEDGMENT: We wish to thank Dr. M. T. Casey, Halifax Infirmary, for referring to us lung biopsy specimens for immunohistochemical assessment; and Miss V. Cantelope for technical help.

REFERENCES

1 Siltzbach LE: Etiology of sarcoidosis, Practitioner 202: 613, 1969
2 Grant RP, Van Ordstrand HS: Sarcoidosis—Recent progress in etiology and pathogenesis. Cleveland Clin 34:205, 1967
5 Waksman BH: Auto-immunization and the lesions of auto-Immune, Medicine (Baltimore) 41:93, 1962
17 Wanstirnl PJ, Elling F: Immunohistochemistry of sar-
Kartagener's syndrome is one of the pleiotropic anomalies, with pathologic manifestations in multiple organ systems: situs inversus, or dextrocardia, paranasal sinusitis, possible agenesis or hypoplasia of the paranasal sinuses, occasional nasal polyps, and bronchiectasis. It is considered a result of autosomal genetic trait with recessive mode of inheritance. It is not related to sex, blood groups or allergic predisposition but uncommon in the negro race. It has been observed in identical twins and in offsprings of consanguineous marriages. It is more common in young persons; it had been reported in a five-day-old infant. At the other extreme, its occurrence in an 88-year-old white man was recorded by Amjad, H et al (JAMA 227:1422, 1974). Crawitz is quoted as one of the early observers of the association of dextrocardia and bronchiectasis, by Marland, P et al (Le Poumon et Coeur 22:41, 1967). Siewert, A R (Berl Klin Wchnschr 41:139, 1904) suggested the congenital origin of this condition. Even so, Kartagener is duly credited with establishing the essential characteristics of the disease which bears his name as an eponym. (Beitr z Klin d Tuberk 83:489, 1933; 87:331, 1935). Some pertinent data are of interest. The estimated incidence of dextrocardia with situs inversus is 1:29,000, and that of bronchiectasis in the general population is 0.1 to 0.5 percent. According to Olsen, A M (Am Rev Tuberc 47:435, 1943) bronchiectasis may be found in 12-13 percent of persons with situs inversus. Sharma, O P found no immunologic deficiency in two siblings with Kartagener's syndrome (New York State J Med 72:1057, 1972), while other observers noted transient immunoglobulin deficiency in about 15 percent of their cases. Heucken-kamp, P U et al (Klin Wchnschr 50:789, 1972) found increased serum alpha antitrypsin, possibly due to chronic inflammatory process of the respiratory tract. It is thought that the underlying pathologic factors are: structurally and functionally impaired mucosal lining of the entire respiratory tract, together with weakness of the bronchial wall, adversely affected cellular and glandular secretory activity and decreased ciliary motion on the bronchial mucosal surface, dysfunction of the autonomic nervous system, with lessened bronchial peristalsis. In the great majority of over 400 reported cases, manifestations of the disease appeared prior to 14 years of age, including some of the following: rhinitis with nasal discharge (mucoid or purulent), nasal speech and stuffiness, mouth breathing, chronic bronchitis with periods of wheezing (the latter appears sometimes upon recumbency or exertion), nonproductive or productive cough (the sputum may be foul-smelling), periodic slight hemoptysis (pulmonary hemorrhage as the cause of death has also been reported), episodes of pneumonia, recurrent fever, clubbing of the digits. The condition does not necessarily imply invalidism. Some of these patients may reach old age without disability. X-ray of the chest and paranasal sinuses, bronchoscopy, bronchograms, and ECG (indicative of dextrocardia), together with medical history, physical and laboratory findings establish the diagnosis. Treatment is based on bacteriologic findings, microbial sensitivity tests and it should follow an individualized design for respective ailments of the upper and lower respiratory tracts.

Andrew L. Banyai, M.D.