Wegener's granulomatosis is a syndrome characterized by necrotizing granulomas involving upper and lower respiratory tracts, generalized angitis, and glomerulonephritis. To date, there have been over 200 cases of Wegener's granulomatosis reported in the literature, but the pathogenesis of the disease remains unclear. Immunoglobulins, complement, and subendothelial electron-dense deposits have been seen in glomeruli in some cases of Wegener's granulomatosis suggesting an immune-complex pathogenesis for the renal disease. These observations have not been confirmed in other studies. Only one case report of the ultrastructural appearance and another of the immunofluorescent findings in pulmonary Wegener's granulomatosis have been published. They provide no support for an immune-complex pathogenesis for the lung lesions. We present a combined immunofluorescence and electron-microscopic study of respiratory lesions in a case of Wegener's granulomatosis. Our findings suggest the possibility of immune-complex mediation of respiratory disease in Wegener's granulomatosis.

CASE REPORT

A 59-year-old white man, a school teacher, was admitted to the LAC-USC Medical Center with a one-month history of impaired hearing in the right ear and a 25 pound weight loss. Physical examination showed a perforated right tympanic membrane, but was otherwise unremarkable.

X-ray films of the chest, maxillary and ethmoid sinuses showed a 3 cm non-cavitated nodule in the lower lobe of right lung and bilateral opacification of the sinuses. Skin tests for tuberculosis, coccidioidomycosis, mumps and Candida were negative, while there was a positive response to trichophyton.

Initial laboratory studies revealed: hemoglobin of 12.6 gm/dl; erythrocyte sedimentation rate, 51 mm per hour; serum IgE, 24 mg/dl (normal < 13 mg/dl); and serum cryoglobulins, 10.5 mg/dl (normal < 2 mg/dl). Serum chemistry panel, urinalysis, sputum cultures for acid-fast bacilli, antinuclear antibody, serum electrophoresis, and complement studies for C3, C4, and CH50 were normal or negative.

Within one month, the patient developed microscopic hematuria, and proteinuria of 1.5 gm/24 hours. The serum creatinine rose to 1.8 mg/dl. The Clq binding test rose from 3.7 percent to 14.6 percent (normal < 10 percent). Simultaneously, the pulmonary nodule cavitated and an indurated 2 x 3 cm lesion developed in the soft palate. Open lung and right maxillary sinus biopsies were performed and confirmed the diagnosis of Wegener's granulomatosis.

MATERIALS AND METHODS

Tissue from biopsies of lung, maxillary sinus and soft palate for light microscopy were fixed in 10 percent buffered formalin. Sections from paraffin blocks were stained with hematoxylin and eosin, Verhoeff elastic, periodic acid-Schiff and Ziehl-Neelsen stains.

For electron microscopy, tissues from the lung and maxillary sinus were sliced into 1 mm to 3 mm cubes. The samples were fixed, embedded in Epon 812 and sectioned and stained by methods previously described.

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Biopsy specimens from the lung and maxillary sinus were studied by direct immunofluorescence for human IgG, IgM, IgA, IgE, C3, Clq and fibrinogen. Unlabelled monospecific antiserum were used to demonstrate specificity according to methods previously described.10

Elution of immune complexes from tissue sections was carried out according to methods described.10 Control preparations used phosphate-buffered saline 0.01M pH 7.2 as incubation medium. The intensity of the fluorescent staining was graded from 0 to 4+. A reduction in the intensity was taken to indicate elution of the immune deposits.

Cryoproteins were isolated and analyzed using methods previously described.11 Their compositions were analyzed by double diffusion in agarose using monospecific antisera.

Circulating immune complexes were measured by a modified Clq binding assay using unheated sera12 (normal < 10 percent binding). Sera from patients with systemic lupus erythematosus, rheumatoid arthritis and healthy adults were used as positive and negative controls respectively.

RESULTS

Light Microscopy

The lung biopsy showed confluent granulomas surrounding zones of coagulative and leukocytoclastic necrosis (Fig 1). Small pulmonary arteries and veins distant from the necrosis demonstrated granulomatous vasculitis.

Sinus and soft palate biopsies also showed granulomas with central foci of necrosis. In the palate biopsy, granulomas were found in the interstitial tissue between acini of the minor salivary glands. Vasculitis was not seen. Ziehl-Neelsen and PAS stains for acid-fast bacilli and fungi were negative in these biopsies.

Electron Microscopy

Findings from the electron-microscopic examinations of the lung and maxillary sinus were similar. The sections showed extensive areas of necrosis. One residual

Figure 1. Section of lung shows area of confluent granulomas with surrounding zones of leukocytoclastic necrosis.

Figure 2. Low magnification electron micrograph shows a necrotic area infiltrated by macrophages, plasma cells and giant cells.

Figure 3. Lumen of a small pulmonary artery is filled with fibrin, degenerated lymphocytes and platelets. The endothelial cell is swollen.
Immunofluorescence microscopy

Finely granular deposits of IgG and to a lesser extent, IgM were found along some alveolar walls (Fig 4) in the lung. Complement and other immunoglobulins were not seen as extracellular deposits nor were deposits of immunoglobulins or complement found in the walls of large pulmonary arteries or veins. Some lymphoid cells surrounding the granulomas showed cytoplasmic staining for IgM and Clq. Fibrin was also found within alveolar lumina and along alveolar walls, especially within areas of necrosis and inflammation.

In the maxillary sinus, deposits of IgM were found within the walls of arteries. The inflammatory cell infiltrate within the mucosa showed staining for IgG along cell surfaces. There was no staining for other immunoreactants.

Immunoglobulin deposits in alveolar walls and maxillary sinus vessels were partially eluted when the tissue sections were incubated with citrate buffer prior to reaction with fluorescein-labelled anti-human IgG. Incubation with phosphate-buffered saline solution did not alter the fluorescent staining compared to unwashed sections.

The cryoprotein isolated from the serum of the patient was found to be a "mixed" type of cryoglobulin containing immunoglobulins (IgG and IgM) and complement components (Clq and C3).

Discussion

Circulating immune complexes have been demonstrated in the blood of some patients with Wegener's granulomatosis. Immunoglobulins, complement and electron-dense deposits have been found in glomeruli of other patients with Wegener's granulomatosis, suggesting that circulating immune complexes may deposit in the kidney and cause glomerulonephritis. Our patient had circulating immune complexes as shown by elevated cryoglobulin levels initially and by the Clq binding test later in his course. It is now well accepted that the mixed type of cryoglobulin found in our patient represents a subpopulation of circulating immune complexes. A pathogenetic relation between these circulating immune complexes and the vasculitis in the respiratory tract is raised by our finding of IgG and IgM in pulmonary alveolar walls and of IgM in maxillary sinus arteries. The partial elution of these tissue immunoglobulins with acid buffer suggests that they are immune complexes.

Several caveats to an immune-complex pathogenesis may be raised. The only previous immunofluorescence microscopy study of lung in Wegener's granulomatosis showed fibrinogen, rather than immunoglobulins or complement, in pulmonary vessels and granulomas. Our study also failed to demonstrate immune deposits in pulmonary arteries or veins, the typical sites of vasculitis in Wegener's granulomatosis. It is known, however, that the age of a vasculitic lesion plays a determining role in the ability to demonstrate immune deposits in it. This explanation might also account for the absence of complement components in areas of immunoglobulin localization.

The discrepancy between the immunofluorescence and electron-microscopic observations also requires comment. We do not believe that the absence of electron-dense deposits in lung capillaries militates against our immunofluorescence findings. The discordance between the studies may reflect problems of sampling inherent in the small amounts of tissue taken for electron microscopy and the focal distribution of the deposits seen by immunofluorescence microscopy. Furthermore, electron microscopy is a less sensitive technique for the demonstration of immune deposits than immunofluorescence microscopy.

We did find intravascular coagulation in small pulmonary vessels by electron microscopy. Donald et al made similar ultrastructural observations and speculated that intravascular clotting was the initial step in the production of the infarctlike necrosis seen in Wegener's granulomatosis. If immune deposits play an important role in the pathogenesis of Wegener's granulomatos
role in the pathogenesis of Wegener’s granulomatosis, they may do so in part by initiation of the coagulation “cascade.” 17 We cannot, however, exclude the alternative possibility that intravascular clotting follows tissue necrosis.

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Pulsus Alternans*

Echocardiographic Evidence of Reduced Venous Return and Alternating End-diastolic Fiber Length as Causative Factors

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The echocardiographic findings are described in a patient with left heart failure both before and after development of severe pulsus alternans (PA). Right ventricular dimension (RVD) before and after PA was 2.7 and 1.0 cm, respectively. Left atrial dimension (LAD) before and after PA was 4.8 cm and 2.7 cm, respectively. Left ventricular end-diastolic dimension (LVDd) without PA was 6.7 cm; with PA was 6.0 cm before the strong beat and 5.7 cm before the weak beat. End-diastolic thickness of the left ventricular wall (LVW) was 0.8 cm before the strong beat and 1.1 cm before the weak beat. Thus, the weak beat was initiated from a shorter end-diastolic fiber length than the strong beat. The weak beat had no aortic valve opening, and the accompanying phonocardiogram revealed absence of the early ejection sound and aortic component of the second heart sound. Decreased blood volume owing to diuretic therapy most likely contributed to the development of PA, since the RVD, LAD, and LVDd became smaller, the heart rate did not change, and the rhythm was regular.

Pulsus alternans (PA) refers to the characteristic pulse pattern in which strong and weak pulses alternate at regular intervals. Ever since this phenomenon was first described by Traube in 1872, many investigators have attempted to explain its mechanism. Wenckebach postulated that extracardiac factors influencing the degree of ventricular filling, end-diastolic pressure, and volume were the major determinants of PA. Staub believed that the weak beat (WB) was initiated from a smaller volume but at a high pressure and attributed this to incomplete metabolic recovery from the previous strong beats (SB). Another popular view is the presence of alternate failure of contraction of certain myocardial segments. Recently, Mitchell and associates demonstrated evidence of alternating end-diastolic fiber length as a causative factor of PA in experimental animals.

We describe the echocardiographic findings in a patient who had severe PA and in whom the echo revealed failure of the aortic valve to open in every other beat (total alternans).

CASE REPORT

A 24-year-old man was admitted on Aug 9, 1979, after being struck on the head and becoming unconscious during

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