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Immunoglobulin and Complement Deposits in the Lungs of New Zealand Black/White Mice

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Summary. Forty-four New Zealand Black/White (NZB/W) F1 hybrid mice were studied for evidence of immune complex deposition in the lungs and kidneys. Positive immunofluorescence was seen in the lungs of 27 mice. The pattern of lung fluorescence was granular in association with capillary walls in 16 mice and intracellular in 12. The incidence of granular fluorescence was increased with age and was seen in 80% of the lungs of mice over 13 months old.

There was a correlation between capillary wall fluorescence in the lungs and the kidneys ($P < 0.001$) and between lung cellular fluorescence and renal mesangial fluorescence ($P < 0.001$). The role of immune complex deposition in human pulmonary disease is discussed.

Key words: Immune deposits – NZB/W mice – Lung immunofluorescence systemic lupus – Pulmonary fibrosis.

Introduction

An immune complex disease similar to human systemic lupus erythematosus (SLE) occurs spontaneously in NZB/W mice (Burnet and Holmes, 1965; Howie and Helyer, 1965). This is manifested by glomerulonephritis with deposits of DNA-anti DNA (Lambert and Dixon, 1968).

In human SLE, although lung involvement is seldom the dominant clinical problem it is probably very common and lung function abnormalities are present in almost all patients with the disease (Huang, Hennigar and Lyons, 1966; Gold and Jennings, 1966). There has been little work on the pathogenesis of the lung lesion in man and published reports are few. Granular deposits of complement along alveolar walls have been demonstrated by immunofluores-

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cence in one patient with SLE (Turner-Warwick, 1974) and electron dense deposits in the pulmonary capillary basement membrane were seen in a patient who subsequently developed the full clinical picture of SLE (Elliot and Kuhn, 1970; Kuhn, 1972). These observations suggest that pulmonary immune complex deposition may be important in initiating the lesions in the lungs. We have therefore examined the lungs for NZB/W mice for evidence of immune complex deposition by immunofluorescent microscopy. Preliminary results were reported by Leathem, Geddes and Corrin (1976).

Materials and Methods

Forty-four NZB/W FI hybrid mice (30 males, 14 females, bred from specific pathogen free NZB females and NZW males, were killed by exsanguination in six approximately equal groups with ages varying from 3 to 17 months.

For immunofluorescent microscopy blocks of lung and kidney were frozen in iso-pentane pre-cooled in liquid nitrogen. Cryostat sections 2–4 μm thick were cut of kidney. Lung was cut at 6 μm, or if first inflated with OCT embedding medium (Ames) at 2 μm. The sections were air-dried, fixed with acetone for 1 min and incubated with 1 in 4 dilution of FITC conjugated goat anti-mouse IgA, IgG, IgM, and C3 antisera (Meloy Laboratories, USA) for 30 min. The sections were then washed in phosphate buffered saline (pH 7.4) and mounted in barbitone buffer: Glycerol 1:9 (pH 8.6) to enhance fluorescence, before being examined with a Leitz Ortholux microscope equipped with epifluorescence HBO 50W mercury vapor illumination using filters BG38 and KP490. Photographs were taken on HP4 27DIN/400 ASA black and white film.

Sera from 26 mice were examined for anti-DNA activity by Dr. Edmonds at the Royal Post Graduate Medical School, London, using the Farr binding technique and electro-immunodiffusion.

Results

Lung

Positive immunofluorescence was seen in 27 of the 44 lungs examined (61%). Deposits were seen in a granular pattern along capillary walls in 16 (Fig. 1) and within cells in 12 (Fig. 2). In only one mouse were both granular and cellular deposits seen. The granular deposits contained mixed IgG, IgM and C3 in 13 and isolated IgG in 3. Intra-cellular deposits consisted of IgA alone in 5 mice and multiple immunoglobulins in 7, although these were not necessarily present within the same cells. Complement was not detected in association with intracellular deposits. Although it was not possible to identify the cell types accurately, some showed a diffuse intracellular fluorescence suggestive of plasma cells while others within the same section showed granular fluorescence (Fig. 2) suggestive of macrophages.

The incidence of granular capillary wall deposits increased with age, reaching 80% in mice older than 13 months (Table 1). There was no relationship between age and the findings of intracellular deposits. Both sexes were affected equally although there was some tendency for the changes to be more florid and to occur earlier in females.

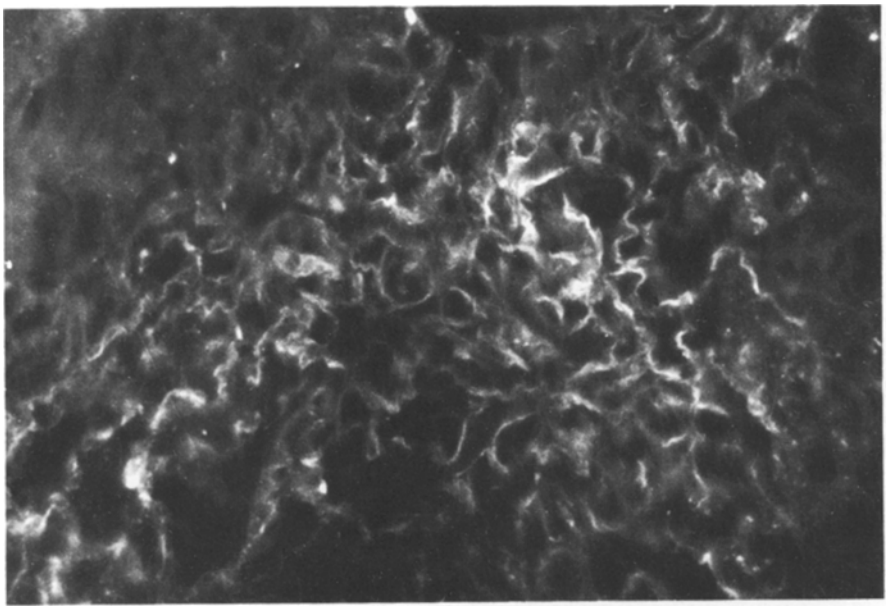

Fig. 1. Mouse 342 lung. Cryostat section stained with FITC anti-mouse IgG showing numerous finely granular deposits, probably along the alveolar walls. ×400

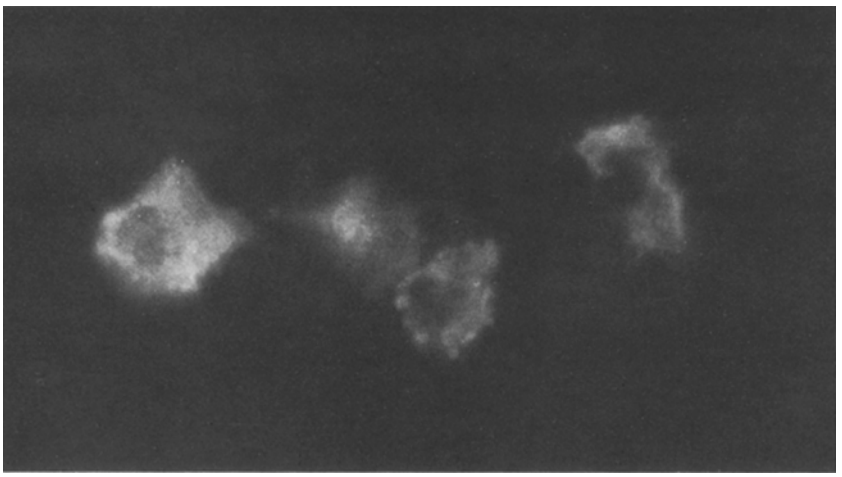

Fig. 2. Mouse 340 lung. Cryostat section stained with FITC anti-mouse IgA showing intracellular deposits. ×400

Kidney

Immunoglobulin deposition was seen in 38 of the 44 mouse kidneys. Two patterns of deposition were noted, namely granular deposits associated with the glomerular capillary walls and mesangial deposits. These patterns were al-

Table 1. Immunofluorescence findings in lungs of NZB/W mice

Age (months)	Number of mice	Number of mice with lung deposits	Type of Deposit	
			Granular	Cellular
<6	6	0	0 (0%)	0
6–7	8	5	1 (13%)	4
8–9	8	5	3 (38%)	2
10–11	9	5	4 (44%)	2
12–13	8	8	4 (50%)	4
>13	5	4	4 (80%)	0
	44	27	16	12

Correlation: $r=0.98$; $m=0.15$; $b=4.18$

most (90%) mutually exclusive. Granular capillary wall deposits containing IgG and IgM were seen in 20 mice and IgG alone in 8.

Mesangial deposits containing mixed immunoglobulins were seen in 22 mice and IgA was present in 13 of these.

Serum

Of the 26 mouse sera tested 13 showed greater than 30% binding of DNA, the upper limit of normal. This increased with age but no significant correlation with lung deposits could be demonstrated. Electro-immunodiffusion failed to demonstrate precipitating antibody to DNA in any of the sera tested.

Correlative Studies

The capillary wall deposits seen in the lung correlated with the presence of capillary wall deposits in the kidney ($P<0.01$), while cellular deposits in the lung correlated with mesangial deposits in the kidney ($P<0.001$). The lung cellular and granular deposits were negatively correlated ($P<0.02$).

Discussion

These results show that deposition of host immunoglobulins and complement occurs in the lungs of mice with spontaneous immune complex disease. By immunofluorescence alone Eisenberg and his colleagues (1976) have identified immunoglobulins but not complement in the lungs in 19 of NZB/W mice. In both studies the deposits were patchy in distribution. We were unable to elute them for further characterization but the renal deposits are believed to contain DNA-anti DNA complexes (Lambert and Dixon, 1968) and it is likely that the lung

deposits are similar. Immune complex deposition in the pulmonary capillaries with subsequent complement fixation and inflammation has been proposed as one of the mechanisms of production of pulmonary diffuse interstitial fibrosis in man (Mackay and Ritchie, 1965; Nagaya et al., 1969) but demonstration of such immune complex deposition has been difficult. In a series of 64 patients with interstitial fibrosis, only 6 showed capillary wall fluorescence (Turner-Warwick et al., 1971). This low incidence may be due to the heterogenity of any group of patients with interstitial fibrosis, but such patients are also generally studied after symptoms have developed and pulmonary fibrosis is well advanced when the initiating factors may no longer be demonstrated. Immune complexes may also be overlooked if their distribution is patchy. There have been very few studies directed towards the detection of immune complexes in the lungs of patients with SLE but electron dense deposits have been reported in one patient (Elliott and Kuhn, 1970; Kuhn, 1972) and complement deposition along alveolar walls has been demonstrated in another (Turner-Warwick, 1974). More recently granular deposits of C_3 and IgG were found in both the kidneys and lungs of another patient with SLE (Rodriguez-Iturbe et al.). In the NZB/W mice the lungs can studied as the disease progresses and we have demonstrated an increasing incidence of immune complex deposition in the lungs. The 80% incidence of lung involvement in the older mice matches the high incidence of lung function abnormality in human SLE. This evidence suggests that immune complex deposition may be an important pathogenetic mechanism in the initiation of pulmonary damage in the NZB/W mouse and this appears to be a useful animal model for the further study of the process in man.

The finding of two mutally exclusive forms of immunofluorescence in the lung is of interest. Furthermore there was a high incidence of IgA (75%) in the intracellular pulmonary deposits but none in the extracellular deposits and a similar pattern was seen in the kidney where IgA was identified in the mesangium but not in the capillary walls. This suggests that IgA deposits are more readily cleared into the mesangium of the kidney and the macrophages of the lung. In both organs intracellular IgA was associated with less evidence of granular capillar wall deposition of other immunoglobulins or complement and it may be noted that in the IgA mesangial nephropathy of man the disease appears to run a generally benign course.

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