Mononuclear-Cell Pulmonary Vasculitis in NZB/W Mice

I. Histopathologic Evaluation of Spontaneously Occurring Pulmonary Infiltrates

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This report describes the spontaneous occurrence of pulmonary vasculitis in NZB/W mice, a well-characterized autoimmune strain of mice. These mice develop pulmonary vasculitis in an age-related fashion. Mild perivascular and peribronchiolar lymphoid hyperplasia is first seen at 4 months of age and progresses into severe hyperplasia by 8 months. This precedes the development of angiodestructive lesions, which are first noticeable at 10 months. By 12 months of age all mice show multilobe disease characterized by transtricial infiltration of the vascular walls by plasma cells, histiocytes, and mature lymphocytes. Mitotic figures and necrosis are rare to absent. Vessel lumens are markedly narrowed and obliterated in severe cases, with focal disruption of the limiting elastic membranes. In mice older than 10 months of age, there is extension of the infiltrate into the interstitium in a manner similar to that of lymphoid interstitial pneumonia. Arteries and veins are equally affected. The cellular infiltrates and pattern of involvement bears similarity to various pulmonary vasculitides in humans. This is the first report of spontaneous pulmonary vasculitis in NZB/W mice. (Am J Pathol 1985, 120:99-105)

THERE IS A GROWING body of evidence which suggests that various forms of pulmonary vasculitis may have an autoimmune pathogenesis. Weiss et al. reported a case of pulmonary vasculitis in a patient with serum antinuclear antibodies and evidence of immune complex deposition in the lung and kidney. In lymphomatoid granulomatosis, one of the angiocentric granulomatous, positive antinuclear antibodies, rheumatoid factor, circulating immune complexes, and autoimmune hemolytic anemia have been reported. Additional pulmonary complications such as lymphoid interstitial pneumonia and rheumatoid nodules frequently accompany systemic lupus erythematosus, rheumatoid arthritis, and Sjögren's disease. To examine the role of autoimmunity in the pathogenesis of pulmonary vasculitis, we studied a known autoimmune strain of mice, the New Zealand Black × New Zealand White F1 hybrid (NZB/W). In these mice an autoimmune disease characterized by hypergammaglobulinemia, autoantibodies, circulating immune complexes, and reduced complement levels develops spontaneously. Vascular changes have been reported. Death may be due to hemolytic anemia, lymphoid malignancy, or immune complex nephritis. Both B- and T-cell populations in NZB/W mice have been reported to manifest abnormal immune functions. Lymphoproliferative disorders have been reported in these mice, such as plasma-cell lymphomas and follicular-center-cell lymphomas, as well as benign conditions such as follicular hyperplasia and pseudolymphoma.

We now report the spontaneous occurrence of pulmonary vasculitis in aging NZB/W mice. The implications of this animal model toward an understanding of human pulmonary vasculitis and speculation regarding its pathogenesis are discussed.

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Figure 1—Progression of the peribronchial and perivascular lymphoid hyperplasia with age. 

A—Four months of age. NZB/W. 
B—Six months of age. NZB/W. 
C—Eight months of age. NZB/W. 
D—Twelve-month BDF, used as a control. At 4 months of age, NZB/W mice show a mild lymphoid hyperplasia which exceeds that in a 12-month control animal. (H&E, ×85)

Materials and Methods

Animals

NZB/W mice and normal, nonautoimmune BDF, mice were obtained from the breeding colonies of National Jewish Hospital/National Asthma Center. Only female mice were examined.

Histology

Groups of 6 female NZB/W and BDF, mice at 2, 4, 6, 8, 10, 12, and 14 months of age were sacrificed, and autopsies were done. Representative sections of lung and kidney were obtained from each animal up to 10 months of age. In the 12- and 14-month-old NZB/W
animals, representative sections of heart, liver, spleen, brain, and peripheral lymph nodes were also obtained. All sections were fixed in buffered formalin. Paraffin-embedded sections were routinely stained with hematoxylin and eosin (H&E). Sections of lungs from 10, 12, and 14 month old animals were also stained with periodic acid–Schiff, Masson trichrome, Congo red, and Verhoeff-van Gieson stains. Organ cultures were not obtained.

**Results**

Representative sections of each lobe were examined in NZB/W and BDF<sub>1</sub> mice at the varying ages. The progression of the lesions was time-related (Figure 1A–D). The first observable change was noted at 4 months of age (Figure 1A). This consisted of a mild perivascular and peribronchiolar lymphoid hyperplasia. This finding is identical to that first reported by East et al. in 1965. The lymphoid hyperplasia continued to increase with age (Figure 1B and C). By 8 months of age all mice had a moderate to severe perivascular and peribronchiolar lymphoid hyperplasia. This now occupied a significant portion of the parenchyma, often with 50% of entire lobes being obliterated. The cells consisted of small mature lymphocytes, plasma cells, plasmacytoid lymphocytes, and histiocytes. Polymorphonuclear leukocytes and eosinophils were absent. Mitotic figures were rare to absent.

The earliest vascular changes were noted at 8 months of age. In half of the 8-month-old animals there was infiltration of the tunica intima by the above-described cells. This resulted in concentric thickening of the tunica intima with narrowing of the vessel lumen (Figure 2A). Arterioles, veins, and venules were primarily affected and only occasional small muscular arteries were involved. The remaining 8-month-old animals showed only severe perivascular lymphoid hyperplasia with no evidence of infiltration of vasculitis (Figure 2B). At 10 months of age, 5 of 6 mice showed extensive disease, with multifocal lesions in all lobes. Lesions now showed transmural infiltration of vessels by the same mononuclear cells (Figure 3A). Vessel lumens were markedly narrowed and frequently obliterated. Elastic tissue stains revealed fragmentation of the limiting elastic membranes (Figure 3B). By 12 months of age, all animals showed severe multifocal angiodestructive lesions with large areas of coalescence (Figure 4). Despite the highly dense cellular infiltrate, necrosis was absent. Bronchi and bronchioles were frequently compressed by the large infiltrates into slitlike spaces. Mucosal ul-
ceration or infiltration was minimal. When present, it was secondary to involvement of an adjacent bronchial artery (Figure 5).

In all NZB/W mice 10 months of age and older, there was extension of mononuclear cells into the interstitium. This infiltrate, composed of small mature lymphocytes, plasma cells, and histiocytes, with widened alveolar septa, gave a picture similar to lymphoid interstitial pneumonia (Figure 3B). Interstitial lymphoid nodules, multinucleated giant cells, and viral inclusion bodies were not seen. Discrete sarcoid-like granulomas were also absent. Connective tissue stains failed to reveal fibrosis. Congo red stains were negative for amyloid. Occasionally, PAS-positive material was observed in the tunica media of large arteries.

Examination of other organs in 12- and 14-month animals confirmed previous reports; ie, there was severe glomerulonephritis and follicular and intrafollicular lymphoid hyperplasia of peripheral lymph nodes and spleen.\(^\text{14}\) Kidney sections revealed perivascular lymphoid hyperplasia, first noted at 6 months of age, which progressed with age. By 14 months of age the hyperplasia was severe and occupied most of the renal pelvis. Despite the pronounced perivascular lymphoid hyperplasia, angiodestructive lesions were never seen. Sections of liver showed mild portal triaditis composed mainly of lymphocytes and occasional eosinophils. Bile ducts were intact, and the parenchymal architecture was unremarkable. Myocardium revealed no infiltration by mononuclear cells or evidence of infarction. Multiple sections of brain were unremarkable.

Examination of BDF, mice at similar ages failed to reveal any evidence of lymphoid hyperplasia or vasculitis. In fact, the lymphoid hyperplasia seen in the lungs of 4-month-old NZB/W mice was greater than that observed in 12-month BDF, mice (Figure 1D). Examination of the other organs was unremarkable. Renal pelvis perivascular lymphoid hyperplasia was never seen in the nonautoimmune BDF, mice.

Discussion

Although a parallel to human pulmonary disease poses obvious difficulties, the NZB/W disease described is best understood when referenced to known human disease models. Lymphoreticular tumors and infiltrates of the lung in humans are relatively uncommon, which lends difficulty to their diagnosis and classification. The lymphangitic pattern is the hallmark of pulmonary lymphoreticular infiltrates and allows
separation into classification by pattern of involvement. In our mice, the pleomorphic infiltrate, which are bilateral and diffuse, the absence of necrosis, cellular atypia, extrapulmonary involvement, and mitosis point to a benign process. In addition, the infiltrates were polyclonal by heavy- and light-chain analysis (data not presented). This allows us to pursue a differential diagnosis of benign lymphoreticular pulmonary infiltrates in a lymphangitic pattern which includes the following: lymphoid hyperplasia, angioimmunoblastic lymphadenopathy (AILD), benign lymphocytic angiitis and granulomatosis (BLAG), pseudolymphoma, and lymphoid interstitial pneumonia (LIP).\(^1\)

Lymphoid hyperplasia is normally prominent in children and infants but rarely seen in adults. In most cases the cause of the hyperplastic lymphoid tissue is apparent: bronchiectasis, chronic infection, or focal scarring. In florid cases, germinal centers may be present. The lymphoid infiltrates described in the NZB/W mice at an early age could be considered analogous to lymphoid hyperplasia in young adults and infants. However, these infiltrates increase with age, are not solely limited to airways, and later demonstrate vascular involvement. These features, along with diffuse bilateral involvement, point to a more diffuse, insidious process, perhaps LIP. The possibility that LIP starts as lymphoid hyperplasia cannot be ruled out in its pathogenesis.

AILD is a systemic disease with specific lymph node
findings, and in a small number of cases there is pulmonary involvement. This includes a polymorphous infiltrate following lymphatic routes with perilymphatic nodules and secondary vascular infiltration. The infiltrate is composed of immunoblasts, plasma cells, lymphocytes, and histiocytes. When large numbers of immunoblasts are present, it may resemble malignant lymphoma. Immunoblasts were rare to absent in the NZB/W mice. More importantly, the characteristic morphologic findings of AILD, such as arborizing vessels, PAS-positive material, and immunoblasts were never seen in these animals.

BLAG characteristically features an angiocentric, high cellular, benign infiltrate of lymphocytes, plasmacytoid cells, histiocytes, and occasional fibroblasts. Fibrinoid necrosis of vessel walls and sarcoid like granulomas are usually absent. Much debate exists in the literature as to the exact classification of BLAG. Saldana, in a review of 62 cases of pulmonary angiitis, describes it as a separate entity with a good prognosis. Others believe that BLAG is a premalignant lesion best typified as lymphomatoid granulomatosis. In support of this, there are case reports describing the transition of BLAG to lymphomatoid granulomatosis. Until further work is done, lymphomatoid granulomatosis and BLAG are best considered as lymphoproliferative disorders. Our results do show histologic characteristics similar to those described for BLAG, though clearly there are no features of lymphomatoid granulomatosis or malignant lymphoma.

Pseudolymphoma appears to be a hyperplastic inflammatory process. As such, it has features common to reactive processes: a mixed inflammatory infiltrate and variable fibrosis. There may be zonal scattering, germinal centers, and granulomas. Key features of pseudolymphomas are an overall heterogeneity and a clear tendency toward scattering. LIP and pseudolymphoma are histologically similar; however, pseudolymphoma is a localized lesion, and LIP is a bilateral diffuse process. Since fibrosis was never seen in the NZB/W mice, nor were there germinal centers or granulomas, pseudolymphoma appears unlikely.

LIP is usually a chronic bilateral interstitial pneumonia in which the pulmonary parenchyma is diffusely infiltrated by a dense lymphoid infiltrate. This infiltrate is pleomorphic and includes lymphocytes, plasma cells, histiocytes, and epithelioid histiocytes which may coalesce to form random granulomas. Giant cells may or may not be present and need not be associated with granulomas. Lymphoid follicles may impart a nodular appearance. Massive peribronchiolar and perivenous accumulations of lymphoid tissue may be seen. A significant number of patients have an associated collagen vascular disease, most notably Sjögren’s syndrome and rheumatoid arthritis. Polyclonal serum immunoglobulin elevations have also been reported.

The disease found in the NZB/W mice shows many similarities to LIP. NZB/W mice also have clinical and serologic features similar to those of humans with LIP, namely, a Sjögren’s-like salivary gland infiltrate, polyclonal hypergammaglobulinemia, positive antinuclear antibodies, circulating immune complexes, and reduced complement levels. However, the vasculitis seen in the NZB/W mice is not usually seen in LIP.

A spontaneously occurring animal model for a pulmonary lymphoreticular vasculitis may have important implications for the characterization of human lymphoreticular diseases of the lung. For instance, although BLAG is a poorly understood disease entity, the NZB/W mouse shows histologic changes similar to those reported in BLAG in humans. In the human disease, some believe BLAG and lymphomatoid granulomatosis are part of a spectrum of lymphoproliferative disease with a final progression to lymphoma. In addition, three case reports exist of patients who presented with BLAG and in whom lymphomatoid granulomatosis subsequently developed. The NZB/W mice die at an average of 8–10 months with massive lymphoreticular infiltrates but no evidence of neoplasia. If their life span could be prolonged, would this hyperplasia progress to lymphomatoid granulomatosis, and what would “trigger” this transformation? Thus, the NZB/W mouse could provide a convenient model for malignant lymphoid transformation. This model may also prove useful for defining the temporal sequence of pulmonary vasculitis, something as yet not fully delineated. For example, in lymphomatoid granulomatosis the vascular involvement is felt to precede the parenchymal cellular infiltrate. In contrast, we found that the vasculitis in the NZB/W mouse was secondary to the advanced lymphoid hyperplasia. If the various forms of pulmonary vasculitis have a common mechanism, then a rethinking is in order regarding their pathogenesis.

The association of vasculitis with disorders of immunoregulation is not without precedent. Two distinctive forms of vasculitis occur in various strains of mice with systemic lupus erythematosus. Seventy-five percent of MRL/1 mice have a necrotizing polyarteritis involving mostly medium-sized arteries of the kidney, genital organs, and heart; 80% of (NZB × BXSB) F1 (WBF1) males develop a degenerative vascular disease confined predominantly to the coronary artery system, often associated with myocardial infarction. Berden et al reported that MRL/1 males with necrotizing polyarteritis have higher levels of autoantibodies and circulating immune complexes than those without vascul-
litis, and that WBF, males with degenerative vascular disease have significantly higher levels of immune complexes than those without vasculitis.

This is the first description of pulmonary vasculitis arising spontaneously in NZB/W mice. These mice, including the mice from our colony, routinely have hypergammaglobulinemia, circulating immune complexes, and immune complex deposition in the kidneys. 

The spontaneous occurrence of pulmonary vasculitis in a strain of mice with documented disorders of immunoregulation provides further evidence that an autoimmune pathogenesis may be involved in pulmonary involvement of collagen vascular diseases.

It is interesting to speculate on the mechanism of disease pathogenesis, because several genetic strains of mice develop different clinical manifestations of vasculitis. It is important to summarize two findings of pulmonary vasculitis in NZB/W mice. First, lymphoid hyperplasia precedes the development of pulmonary vasculitis, and second, the vasculitis was never seen in the absence of lymphoid hyperplasia. We propose that the lymphoid hyperplasia may be due to chronic antigenic stimulation in the context of disordered immunoregulation with the recruitment of polyclonally activated B cells. Once the infiltrates reach a critical cellular mass and level of activation, exposure to an offending antigen, either endogenous or exogenous, could result in in situ immune complex formation. In support of this, immune complexes have been reported to be present in the lungs of NZB/W mice. Additionally, we have partially characterized the nature of the perivascular lung infiltrates in NZB/W mice and found a high number of polyclonally activated B cells. Large numbers of Ia-positive cells and helper T cells were present, whereas suppressor T cells were rare or absent. Thus, pulmonary vasculitis may represent another clinical manifestation of a disorder in immunoregulation because of an inability to locally down-regulate a chronic inflammatory response.

References

21. Harbeck R, Launder T, Staszak C: Mononuclear cell pulmonary vasculitis in NZB/W mice: II. Immunohistochemical characterization of the pulmonary infiltrates. (Manuscript Submitted)

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