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Ocular Fundus Lesions in Systemic Lupus Erythematosus Model Mice

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Abstract: We investigated the ocular fundus of (NZWXBXSB) F1 mice, which are considered to be a good model for systemic lupus erythematosus (SLE) with antiphospholipid syndrome. The abnormal fundus findings were documented by fundus photography, and the chorioretinal lesions were studied histopathologically. The incidence of ocular fundus abnormalities and systemic signs in these F1 mice was significantly higher in males than in females, as with the systemic symptoms. This indicated an influence of the *Yaa* (Y chromosome-linked autoimmune acceleration) gene. Lesions in the fundus appeared as white spots, which increased in number during the course of the disease and developed into retinal detachment in some animals. This developmental course resembled the course of patients with multifocal posterior pigment epitheliopathy. Dilatation of the veins and narrowing of arteries were marked. Histopathological findings included: (1) destruction of the photoreceptor cell layer, (2) degeneration and loss of the retinal pigment epithelium, (3) thickening of the wall of the choroidal arterioles, and (4) narrowing and occlusion of the choriocapillaris associated with hyaline degeneration and thrombus formation. The study of the SLE mouse model should contribute to the understanding of the abnormalities in the fundus associated with collagen diseases. **Jpn J Ophthalmol 1998;42:345–351**

Key Words: (NZWXBXSB) F1 mice, ocular fundus lesions, systemic lupus erythematosus, *Yaa* gene.

Introduction

Systemic lupus erythematosus (SLE), a type of collagen disease, has attracted considerable attention and has been investigated extensively because it impairs multiple tissues, including the skin, blood, kidney, heart, joints, serous membranes, and the central nervous system. The ocular complications of SLE are also variable and include decreased lacrimation and corneal disorders, as well as lupus retinopathy.

Lupus retinopathy in humans is characterized by cotton-wool patches, retinal hemorrhages, and retinal vasculitis.[1–6] Recently, multifocal posterior pigment epitheliopathy has been reported in SLE patients.[7–13]

Approximately 20% of SLE patients have ocular complications from this syndrome.[14] Asherson et al[15] have suggested that antiphospholipid antibodies represent a risk factor for ocular vascular diseases in patients with SLE. This syndrome is also characterized by recurrent thrombosis, abortion, and thrombocytopenia associated with antiphospholipid antibodies, such as the anticardiolipin antibodies (aCL).[16,17] These antibodies are frequently detected in SLE patients. The percentage of SLE patients with the lupus anticoagulant factors (LA) is about 34%, and the percentage with anticardiolipin antibody is about 44%.[18] It has also been suggested that the antiphospholipid antibody may induce thrombus formation, thereby causing secondary thrombocytopenia.[16,17]

To investigate the pathogenesis of the ocular fundus abnormalities associated with collagen diseases, we examined the ocular fundus lesions clinically and

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histopathologically in the SLE-prone F1 mice obtained from NZWXBXSB crossings with accompanying antiphospholipid syndrome.

Systemic lupus erythematosus mice have been used in numerous studies on SLE ever since Bielschowsky et al[19] employed the New Zealand Black (NZB) mice as a model for autoimmune diseases in 1959.[20] (NZBXNZW) F1 mice,[21–24] MRL/*lpr* mice[25,26] and BXSB mice[27] have also been considered to be typical SLE mice. The diversity of the pathological conditions of mice with SLE arises from differences in the genetic factors that influence each condition. The New Zealand mouse model has led to the understanding of the genetic basis of autoimmune diseases in which various genetic factors play crucial roles.[28] The (NZWXBXSB) F1 male mice with systemic lupus-like disease employed in this study have been shown to have thrombocytopenia, hemorrhagic lesions,[29] produce autoantibodies against cardiolipin,[30] and frequently have lupus nephritis complicated with myocardial infarction.[31,32] These mice are also attracting attention as an appropriate model for idiopathic thrombocytopenic purpura (ITP).[29]

This report describes our observation on the ocular fundus abnormalities in the SLE mice. To the best of our knowledge, the findings presented here are the first documentation of the ocular fundus lesion in the SLE mice.

Materials and Methods

Forty male and 34 female (NZWXBXSB) F1 mice were used as the SLE model mice, and 56 female B10 mice without SLE were used as controls. After instilling 0.5% tropicamide with 0.5% phenylephrine hydrochloride topically, the ocular fundus lesions were photographed with a fundus camera for small animals (Genesis, KOWA, Tokyo) under diethylether inhalation anesthesia. The urinary protein level was assessed periodically (Knight & Adams method)[33] and the animals were sacrificed by dislocation of the cervical spine when proteinuria became severe (protein level >1.0 gm/dL).

The eyes were enucleated, fixed in 10% formalin and embedded in paraffin for histopathological examinations. The major organs, including the kidney, were also removed and fixed in formalin for histopathological study. The sections were stained with hematoxylin-eosin and periodic acid Schiff (PAS).

Results

Among the 40 male F1 mice, ocular fundus abnormalities were observed in 6 at 4 months, 10 at 5 months, 13 at 6 months, and 15 at 7 months. Thus, 15/40 (37.5%) of the male mice with SLE symptoms had fundus abnormalities. Among the 34 female F1 mice with mild SLE symptoms, ocular fundus abnormalities were noted in only one animal at the age of 4 months. In contrast, none of the 56 female B10 control mice showed any fundus abnormalities, even at the age of 10 months. A chi-square test showed that the higher incidence of ocular fundus abnormalities in the F1 males than in the F1 females at the age 6 months and older was statistically significant ($\chi^2 = 12.95$; $P < 0.001$; Figure 1).

Funduscopic examination revealed marked dilatation of the retinal veins and narrowing of arteries in the F1 males when compared with the fundus of the female B10 mice (Figure 2A). White spots were seen subretinally in the posterior pole (Figure 2B). The spots increased in number, size, and distribution with the passage of time (Figures 2C,D). These lesions developed into exudative retinal detachment in some of the animals (Figure 4A).

Histopathological observations of the chorioretina showed disturbances in the alignment of the outer nuclear layer as well as the distortion of the inner and outer segments of the photorecepters. In some, the retina was totally detached with marked subretinal retention of exudate. In some animals, the detached retina was adherent to the posterior surface of the lens (Figure 4A). There was vacuolization and a partial degeneration of the retinal pigment epithelial (RPE) cells. In the choroid, there was narrowing or occlusion of the choriocapillaris, cellular infiltra-

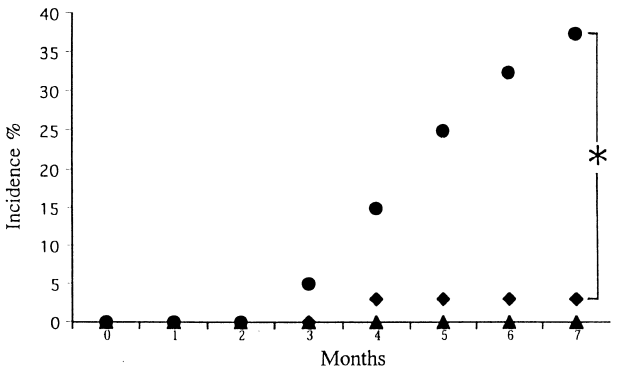

Figure 1. Incidence and statistical significance of ocular lesions. *Chi-square test revealed significantly higher incidences of ocular fundus abnormalities in F1 males than in F1 females at age of 6 months and older ($\chi^2 = 12.5$, $P < 0.0001$). ●: (NZWXBXSB) F1 male (40 mice), ■: (NZWXBXSB) F1 female (34 mice), ▲: B10 (56 mice).

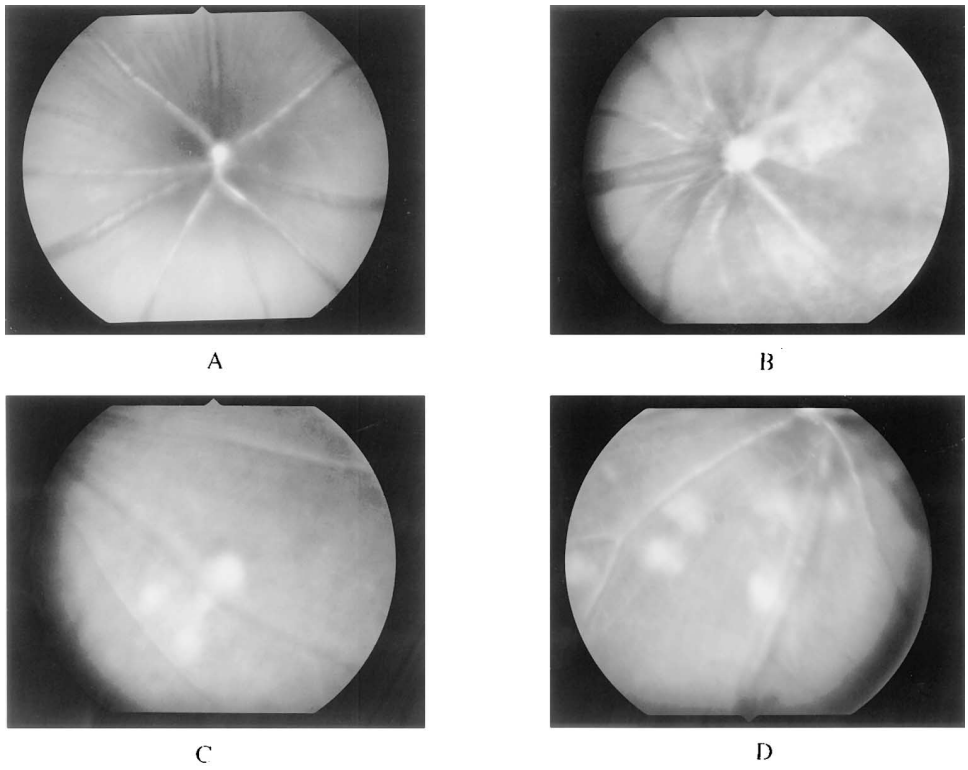

Figure 2. Clinical findings (Funduscopic photographs). (**A**) B10 mouse, 10 months; no abnormal findings existed. (**B**) (NZWXBXSB) F1 male mouse, 4 months; dilatations of veins and narrowing of arteries were marked. (**C**) (NZWXBXSB) F1 male mouse, 4 months; white spots are present in posterior pole. (**D**) (NZWXBXSB) F1 male mouse, 5 months; white spots have increased in number, size, and distribution.

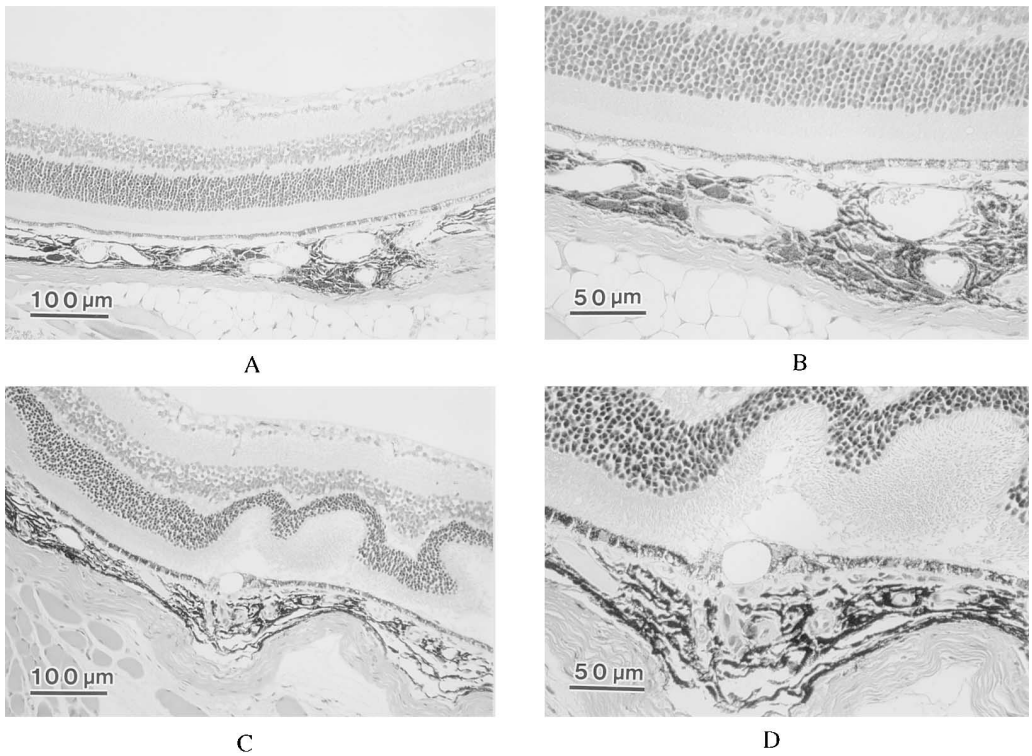

Figure 3. Histopathological findings. (**A, B**) B10 mouse, 10 months; no abnormal findings existed (hematoxylin and eosin staining). (**C, D**) (NZWXBXSB) F1 male mouse, 4 months. Structural destruction of photoreceptors, and degeneration and loss of retinal pigment epithelial cells occur in early stage (hematoxylin and eosin staining).

Jpn J Ophthalmol
Vol 42: 345–351, 1998

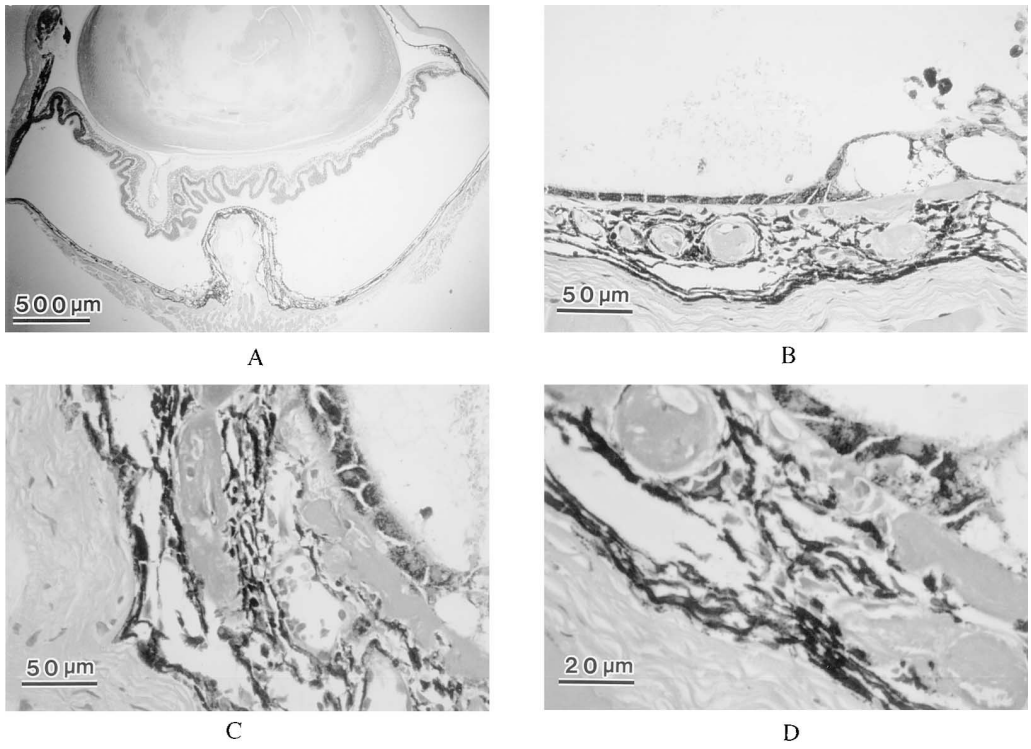

Figure 4. Histopathological findings of retinal detachment in (NZWXBXSB) F1 male mouse at 5 months. (**A**) Detached retina is adherent to posterior surface of lens (hematoxylin and eosin staining). (**B**) Narrowing of choriocapillaris and thickening of choroidal arteriolar wall with hyaline degeneration and thrombus formation (hematoxylin and eosin staining). (**C**) High power view of Figure 4B (PAS staining). (**D**) Occlusion of choriocapillaris, narrowing of lumen of arterioles (periodic acid Schiff staining).

tion into the surrounding tissues, and thickening of the choroidal arteriolar wall (Figures 3C,D).

Degeneration and detachment of some of the RPE cells and occlusion of the choriocapillaris immediately below these cells, as well as the narrowing of the adjacent choroidal arterioles, were also observed (Figure 4B). Periodic acid Schiff staining of these sites revealed an occlusion of the choriocapillaris and marked narrowing of the lumen of the arterioles with hyaline degeneration and thrombus formation. Alterations of the endothelial cells were also observed in some areas (Figures 4C,D).

We also examined histopathologically 6 F1 mice (3 males and 3 females) with fundi free of lesions. Histopathological abnormalities were not found in the 3 female mice at the age of 7 months, but 2 of the 3 male F1 mice showed slight thickening of choroidal arteriolar walls at 4 months of age. However, there were no abnormalities in the RPE cells and photoreceptors.

Proteinuria developed at an earlier age and the lesions in the kidneys were more advanced in males than in females. Wire-loop lesions, hypertrophy of the glomerular basement membrane and proliferative changes were observed in the glomerulus. In the males, the incidence of proteinuria was 75% (30/40) and 90% (36/40) at the age of 4 and 6 months, respectively. In the females, the incidence of proteinuria was 0% (0/34) and 8.3% (3/34) at 4 and 6 months, respectively. Among the males with ocular fundus abnormalities, proteinuria was noted in 81.7% (5/6) at the age of 4 months and 100% (13/13) at the age of 6 months. In the females, proteinuria was not detected at 4 and 6 months of age (Table 1).

Table 1. Relationship Between Proteinuria and Fundus Lesions

(NZW × BXSB)F1	Incidence of Proteinuria (+)	Incidence of Proteinuria (+) in Fundus Lesions (+)
4 months		
Male	75% (30/40)	81.7% (5/6)
Female	0% (0/36)	0%
6 Months		
Male	90% (36/40)	100% (13/13)
Female	8.3% (3/36)	0%

Discussion

The present study demonstrated that ocular fundus lesions developed in the SLE model mice (15/40, 37.5%) in the same way as in SLE patients. The time of appearance of the ocular fundus lesions coincided with the presence of proteinuria. The fundus lesions were seen as white spots mainly in the posterior pole, which gradually increased in number, size, and distribution with the passage of time. These lesions developed into exudative retinal detachment.

Histopatholgoically, the lesions were seen as a narrowing and occlusion of the choriocapillaris and choroidal vessels. Disturbances of the alignment of the photoreceptors and degeneration and loss of RPE cells were also noted. The site of degeneration and loss of RPE cells tended to coincide with the site of severe narrowing and occlusion of the capillary vessels. These findings suggest that the vascular occlusion and local hypoxia induced the impairment of the underlying RPE cells. Because deposition of the immune complex has been demonstrated at the site of nephritis or vasculitis in the kidney,[34] it will be of interest to examine the site of narrowing and occlusion of the choroidal vessels immunohistologically. It will also be important to examine the hypertensive changes that cause fibrinoid degeneration.

The results of genetic analysis of mouse models of SLE have revealed that multiple, independent genetic factors determine the onset of SLE, and differences in the combinations of these genetic factors lead to the diversity of the symptoms of SLE. The higher incidence of fundus lesions and systemic symptoms in the male (NZWXBXSB) F1 mice suggested that the disease was greatly influenced by the Y-chromosome–linked autoimmune acceleration (*Yaa*) gene[35–38] and the major histocompatiblity complex genes.[39–42] It is characteristic of the autoimmune promoting gene, the *Yaa* gene on the Y chromosome of BXSB male mice, that more advanced SLE symptoms develop at an earlier age in males than in females.[35–38]

In recent years, studies on experimental autoimmune uveitis have contributed significantly to the field of ophthalmology. While these animal models have lesions induced experimentally, the SLE model mouse is extremely valuable because the lesions develop spontaneously.

Human SLE retinopathy, characterized by cotton-wool patches, cytoid bodies and retinal hemorrhages, is widely known as the SLE-associated ocular fundus lesions.[1–9] These lesions are presumed to be due to the abnormalities in the retinal vessels, including the capillaries supplying the nerve fiber layer of the retina. The white lesions in our mice, on the other hand, were associated with degeneration of the RPE and choroid. Thus, these findings are different from those in human SLE retinopathy. Recently, however, multifocal posterior pigment epitheliopathy (MPPE) has been reported to be due to an extensive retinal pigment epithelium (RPE) disorder in SLE patients.[7–13] The mechanism for the pathology in MPPE is assumed to be the direct impairment of the RPE by autoantibody or is secondary to the occlusion of the choriocapillaris.[7] In such cases, an increase in the blood viscosity due to an increase in pathogenic proteins, such as antinuclear antibody and antigen-antibody complex appear to promote the RPE disorder. The increase in nitrogen compounds due to renal failure, hypertension and steroids, and the thrombus formation secondary to the accelerated coagulation also contribute to the RPE disorder.

As stated, the lesions in the SLE mice are very similar to those in human MPPE which appear first in the subretina in the posterior pole and then develop into exudative retinal detachment. Our histopathological observations showed that there was damage to the RPE cells but these MPPE-like lesions were not due to direct impairment of the RPE but were more likely secondary to a choroidal circulatory disorder of the arterioles. One possible cause of the choroidal disorder is the deposition of immunoglobulin or antigen-antibody complexes on the choroidal vascular membrane because of the vasculitis and thrombus formation associated with the antiphospholipid syndrome. While only a small number of patients with MPPE and SLE have been examined for the presence of antiphospholipid antibody, in recent years the onset of occlusive chorioretinal vascular lesions has been often reported in SLE patients with antiphospholipid syndrome.[15,43–45] Because there have not been any previous reports of ocular fundus lesions in SLE model mice, we believe that our observations in SLE mouse will contribute to the understanding of the relationship between ocular fundus abnormalities and collagen disease.

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Jpn J Ophthalmol
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