Pathogenesis of Lupus Dermatoses in Autoimmune Mice

IV. Association Between Cutaneous Immunoglobulin Deposition and Anti-Single-Stranded DNA Antibodies in Sera

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Summary. The skin of New Zealand, MRL and BXSB mice was immunohistopathologically examined in order to study the appearance of skin immunoglobulin (Ig) deposition and its correlation with the occurrence of anti-single-stranded (ss) DNA antibodies in sera. Our studies revealed Ig deposition at the dermo-epidermal junction (DEJ) in non-lesional skin and a significant age-related correlation between skin Ig deposition and serum anti-ssDNA antibodies. However, immunofluorescent study of autoimmune mice using anti-ultraviolet-irradiated DNA antiserum failed to demonstrate DNA antigens at the DEJ.

Key words: New Zealand mice — MRL mice — BXSB mice — Lupus-band test — Anti-ssDNA antibodies

Skin lesion is one of the main symptoms of systemic lupus erythematosus (SLE) [4]. In order to clarify the pathogenesis of these lesions, autoimmune mice have been used for dermatopathological analysis. The hybrid of New Zealand Black and New Zealand White mice (B/W F1), MRL/Mp-lpr/lpr (MRL/l) mice and BXSB/Mp (BXSB) mice spontaneously develop a disease which is remarkably similar to human SLE. The immunoglobulin (Ig) deposition at the dermo-epidermal junction (DEJ) in B/W F1 mice has been described previously [5, 10, 21]. Recently, we have found that MRL/l mice also show a positive deposition of Ig at the DEJ [5, 7].

As an extension to these studies, we immunohistopathologically examined several autoimmune mice, including New Zealand Black (NZB), B/W F1, MRL/l and BXSB mice, in order to clarify the common features. We studied the appearance of Ig deposition at the DEJ and the age-related correlation between skin Ig deposition and anti-single-stranded (ss) DNA antibodies in sera. Also, an attempt was made to demonstrate DNA antigens at the DEJ in autoimmune mice.

Materials and Methods

Mice

MRL/Mp-lpr/lpr (MRL/l), MRL/Mp-++/+ (MRL/n) and BXSB/Mp (BXSB) mice were purchased from the Jackson Laboratory (Bar Harbor) and maintained under specific pathogen-free conditions in the Experimental Animal Centre (Faculty of Medicine, Kyoto University). New Zealand Black (NZB), New Zealand White (NZW), the hybrid of NZB and NZW (B/W F1), BALB/c and C57BL/6J mice were obtained from our colony. All of the mice were female except for the BXSB mice, because the autoimmune disease in this strain is greatly accelerated in males [19]. Sera were obtained bimonthly or monthly for MRL/l BXSB and B/W F1 mice, and sera from 1-year-old BALB/c mice were used as the control. These sera were stored at −80°C until use.

Light-Microscopical Observation

Skin specimens were obtained from the dorsal region of MRL/l and MRL/n mice, and the tail of B/W F1, NZB, NZW, BXSB, BALB/c and C57BL/6J mice, because MRL/l mice show skin lesion on the back [7, 19], and the tail of B/W F1 mice shows the highest incidence of skin Ig deposition [5]. One-half of each specimen was fixed in 10% formalin and stained with haematoxylin and eosin (H & E) solution and periodic acid-Schiff(PAS) solution. Five serial sections from each mouse were investigated.

Skin Tissues for Immunofluorescent Studies

The immunofluorescent (IF) studies were carried out according to methods which have recently been reported [5, 7]. The application of anti-ultraviolet-irradiated (UV) DNA antisera for IF studies was performed according to the method of Van Joost [26] with a recently described modification [15]. Briefly, the skin sections were placed in phosphate-buffered saline (PBS) and irradiated by UV light (Toshiba Electric, Tokyo; 15 W) from a distance of 10 cm for 15 min. The control sections were not irradiated. All of the sections were reacted with diluted (1:20) rabbit anti-UV-DNA antisera at room temperature (RT)
for 30 min. After they had been washed three times in PBS, the sections were treated with fluorescein isothiocyanate (FITC)-labelled goat anti-rabbit Ig antisera.

**Antisera**

Several kinds of anti-mouse Ig, C3 and the viral antigen (gp70) antisera were used; their origin and specificity have been described in our earlier studies [5, 6, 7, 9, 18, 23]. Anti-UV-DNA antisera were prepared according to the methods of Tan and Stoughton [22]. Anti-UV-DNA antibodies are known to recognise the thymic dimer in ss or double-stranded (ds) DNA after UV-light irradiation [17]. In the Ouchterlony test, the anti-UV-DNA antisera formed precipitation lines with both UV-DNA and ssDNA. This antiserum was used after absorption with ssDNA solution and mouse-liver powder in order to inhibit non-specific binding.

**Measurement of Anti-ssDNA Antibodies**

Enzyme-linked immunosorbent assays for antibodies to ssDNA were carried out according to the method of Kavai et al. [14] with a minor modification [8]. Briefly, the wells of flat-bottomed MicroELISA plates (Dynatech, FRG) were coated with 200 µl methylated bovine serum albumin (mBSA) solution at a concentration of 1 mg/ml in distilled water and left overnight at 4°C. After washing with PBS, 100 µl ssDNA (50 µg/ml; for isolation procedures and specificity, see [7, 8]) was added to the wells. As a control 100 µl PBS-1 mM ethylenediaminetetraacetate (EDTA) - 4 Na was added. After incubation at RT for 3 h and washing, 100 µl serum samples, which had been diluted with 1% BSA-PBS-EDTA-4Na/0.05% Tween 20, was added to the wells. The plates were then incubated at RT for 1 h and washed with PBS. Protein A which had been conjugated with alkalinephosphatase (Sigma, USA) was used at a dilution of 1:400. The wells were incubated with 100 µl at RT for 1 h. After washing, 200 µl p-nitrophenylphosphate (0.4 mg/ml in diethanolamine buffer, pH 9.8) was added to the wells. The colour was measured in a Titertek Multiscan (Flow Laboratories, USA) at 405 nm. The level of anti-ssDNA antibodies was determined as follows:

The level of anti-ssDNA antibodies = (titre of DNA-coated wells) - (titre of DNA-uncoated wells).

Levels of anti-ssDNA antibody which were greater than the mean + 2 SD of the normal control (BALB/c mice) were considered to be positive. Between 7 and 20 mice of each strain were examined at the indicated ages.

**Results**

**Immunohistological Findings**

Figure 1 shows the age-related incidence of skin Ig deposition at the DEJ of MRL/l, B/W F1, NZB and BXSB mice after the use of FITC-labelled rabbit anti-mouse Ig antiserum. At 2 months, some of the MRL/l mice showed a positive Ig deposition at the DEJ, and the incidence progressively increased with age. In MRL/l mice at 5 months the Ig deposition at the DEJ was 100% positive. At 6 months, about 10% of the B/W F1 mice showed Ig deposition. The incidence of positive staining gradually increased between 8 and 12 months; all were positive by 12 months. In NZB mice, the incidence of Ig deposition was 13%, 33% and 60% at 8, 10 and 12 months, respectively, whereas in male BXSB mice, it was 20%, 40%, 50% and 73% at 4, 6, 8 and 10 months, respectively.

The patterns of Ig deposition were granular and confluent in NZ and BXSB mice (Fig. 2), and linear and confluent with varying intensity in MRL/l mice. As a control, 15 MRL/n mice at 6 months, 10 NZW mice at 12 months, 10 female BXSB mice at 10 months and 10 C57BL/6J mice at 12 months were investigated. NZW mice showed no deposition at the DEJ. Two MRL/n mice, one C57BL/6J mouse, one BALB/c