Spontaneous Autoimmune Skin Lesions of MRL/n Mice: Autoimmune Disease-Prone Genetic Background in Relation to Fas-Defect MRL/lpr Mice

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The autoimmune-prone MRL/Mp-*lpr/lpr* (MRL/lpr) mouse is characterized by the *lpr* mutation, which is a defect in the Fas antigen. Since Fas mediates apoptosis, this defect results in $CD4^-CD8^-$ double negative T-cell proliferation, lupus nephritis, and macroscopic lupus erythematosus-like skin lesions. The control counterpart of MRL/lpr mouse is the MRL/Mp-+/+ (MRL/n) mouse, which lacks the *lpr* mutation and is almost normal during the first 6 mo of life. The *lpr* mutation, however, accelerates autoimmune phenomena in MRL/lpr mice. Thus, it is important to investigate autoimmune diseases like systemic lupus erythematosis in relation to the autoimmune disease-prone genetic background of MRL/n mice.

We found that skin lesions in aged MRL/n mice had unique characteristics. The first characteristic is spontaneous occurrence, and the second is epidermal cell nuclear immunostaining with IgGs by direct immunofluorescence. The skin lesions in aged MRL/n mice showed milder inflammation than in MRL/lpr mice. A homogeneous pattern of epidermal cell nuclear staining was always associated with nuclear staining in kidney cells and also correlated with the *in vitro* binding of sera to keratinocytes cultured from newborn MRL/n mice. These results suggest that the skin lesions of aged MRL/n mice are a good model for certain types of cutaneous lupus erythematosus and also can provide new insights into the long-standing controversy whether epidermal cell nuclear staining occurs *in vivo*.Key words: lupus erythematosus/antinuclear antibody/mixed connective tissue disease. J Invest Dermatol 107:95–100, 1996*

MRL mice were originally developed by Murphy and Roths at The Jackson Laboratories (Murphy and Roths, 1978). Two strains of MRL mice were developed: the MRL/Mp-*lpr/lpr* (MRL/lpr) mouse and the MRL/Mp-+/+ (MRL/n) mouse. The autoimmune-prone MRL/lpr mouse carries the autosomal recessive *lpr* (lymphoproliferation) gene and is characterized by massive $CD3^+CD4^-CD8^-$ cell (double negative T cell) proliferation, lupus nephritis, and spontaneous lupus erythematosus (LE)-like skin lesions beginning at the age of 3 mo even under pathogen-free conditions. The congenic MRL/n mice are 99.6% homozygous to MRL/lpr mice but lack the *lpr* mutation and are almost normal during the first 6 mo of life (Murphy and Roths, 1978).

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Abbreviations: LE, lupus erythematosus; SLE, systemic lupus erythematosus; MCTD, mixed connective tissue disease; ANA, antinuclear antibody; DEJ, dermoepidermal junction; MRL/lpr, MRL/Mp-*lpr/lpr*; MRL/n, MRL/Mp-+/+.

The MRL/lpr mouse is a good model for the spontaneous development of skin lesions similar to those in human LE (Murphy and Roths, 1978; Furukawa *et al*, 1984; Horiguchi *et al*, 1986; Kanauchi *et al*, 1991). Macroscopically, these skin lesions have been described as showing "alopecia and scab formation" by Murphy and Roths (1978). We have been studying the light microscopic, ultrastructural, and immunohistologic characteristics of these skin lesions in MRL mice and have noted liquefaction-like changes in the basal keratinocytes, dermal T-cell infiltration, and vasodilatation (Furukawa *et al*, 1984) as well as ultrastructural changes similar to those observed in human LE skin lesions (Horiguchi *et al*, 1986). Furthermore, we have also demonstrated pathogenic roles for epidermal Langerhans cells, $CD4^+$ cells, and $CD8^+$ cells (Kanauchi *et al*, 1991). Finally, there is information that strongly suggests that this mouse develops cutaneous lesions that are pathologically similar to those of human cutaneous LE (Provost and Watson, 1993).

The most recent finding for MRL/lpr mice was the striking proliferation of $CD4^-CD8^-$ double negative cells caused by a defect in the Fas antigen, which has been reported to mediate apoptosis (Watanabe-Fukunaga *et al*, 1992). Fas is expressed in actively proliferating cells in the thymus, skin, liver, ovary, and gut epithelium as well as in activated peripheral T and B cells. After interaction with its ligand, it transduces signals that lead to apoptotic death. In systemic and/or organ specific autoimmune diseases, the Fas antigen plays an important role in the negative selection of autoreactive T cells in the thymus (Watanabe-Fukunaga *et al*,

1992). The Fas defect, which induces the massive double negative T-cell proliferation, is believed to accelerate autoimmunity in MRL/lpr mice (Murphy and Roths, 1978; Theofilopoulos and Dixon, 1981; Theofilopoulos, 1995). Thus, to investigate these autoimmune phenomena in MRL/lpr mice, it is important to fully understand the autoimmune disease-prone genetic background of MRL mice. Detailed studies on the skin lesions of MRL/n mice have not been performed because most MRL/n mice were examined while under the age of 6 mo as controls for MRL/lpr mice, which died of lupus nephritis or other autoimmune diseases by 6 mo of age (Furukawa *et al*, 1984; Furukawa *et al*, 1993b). In this study, we performed an immunohistologic and serologic analysis of aged MRL/n mice to elucidate their autoimmune disease-prone genetic background.

MATERIALS AND METHODS

Mice MRL/lpr and MRL/n mice were purchased from The Jackson Laboratories (Bar Harbor, ME) and were maintained under standard conditions at the Animal Center (Faculty of Medicine, Kyoto University). BALB/c mice and C57BL/6J mice were purchased from Shimizu Animal Laboratories (Kyoto, Japan). All of the mice examined in this study were free from Sendai virus, mycoplasma pulmonis virus, and mouse hepatitis virus.

F1 and F2 hybrids from MRL/lpr crossed with MRL/n mice were raised, and only virgin female mice were used in this study.

The mice were checked weekly in order to calculate the survival rate and to determine the incidence of skin lesions. When a mouse was sacrificed, its skin and internal organs were prepared for the histopathologic study. Blood serum samples also were obtained and kept at −80°C until used.

Light Microscopic Observations Skin specimens were taken from the upper back region because of the appearance of spontaneous LE-like skin lesions in MRL/lpr mice in this region (Murphy and Roths, 1978; Furukawa *et al*, 1984). Internal organs including the kidney were also obtained when the mice were sacrificed. The specimens were fixed in 10% formalin and stained with hematoxylin and eosin and periodic acid-Schiff solutions. Five serial sections from each mouse were prepared and investigated.

Immunohistochemical Observations The specimens were frozen immediately in a bath of dry ice and acetone. Cryostat sections (6 μm thick) were prepared and were stained with fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG $F(ab')_2$ antisera which were purchased from three manufacturers including Jackson ImmunoResearch (West Grove, PA), Cappel (West Chester, PA), and Caltag (South San Francisco, CA), and FITC-conjugated anti-mouse C3 (Cappel) according to methods previously described (Kanauchi *et al*, 1991; Furukawa *et al*, 1993b). FITC-conjugated antiserum against mouse albumin (Bethyl Laboratories, Montgomery, TX) was used as the control.

Nick Labeling of Apoptotic DNA Apoptotic cells were detected with an *in situ* hybridization kit (Oncor, Gaithersburg, MD) according to the manufacturer's instructions. This method is based on the specific labeling of nicked ends of fragmented DNA (Gavrieli *et al*, 1992). Briefly, tissue cryosections were fixed in 10% neutral buffered formalin and post-fixed in ethanol:acetic acid (2:1). After incubation with terminal deoxynucleotidyl transferase, anti-digoxigenin-peroxidase was applied. Then, a fresh mixture of 0.05% diaminobenzidine and 0.02% hydrogen peroxide was used for color development. Specimens were counterstained with methyl green solution.

Measurement of Anti-Nuclear Antibodies The anti-nuclear antibody titer in the serum samples was determined with HEp-2 cultured cells (Japan Hoechst, Tokyo). Serial diluted serum samples were applied to the substrate, and then FITC-conjugated anti-mouse IgG was reacted with the samples.

Anti-DNA antibodies were determined by an enzyme linked immunosorbent assay according to our previous report (Furukawa *et al*, 1993b). Anti-U_1RNP antibodies in the serum samples were measured based on the methods of Billings *et al* (1982). Anti-histone antibodies were examined by an enzyme-linked immunosorbent assay according to the methods of Sato *et al* (1993).

***In Vitro* Binding of Mouse Sera to Cultured Keratinocytes** Cultured keratinocytes from newborn MRL/n mice were prepared according to our previously reported method (Furukawa *et al*, 1989). Briefly, skin samples were incubated with trypsin at 4°C for 18 h. The epidermal sheets were agitated gently, centrifuged, and suspended in culture medium containing 10% fetal calf serum. The epidermal cell suspensions were then cultured in plastic flasks. Next, first passage cells were cultured in keratinocyte growth medium (Clonetics, San Diego, CA) without bovine pituitary extracts on a Lab-Tek chamber slide. When the cells had grown to 60–70% confluency, they were incubated with either mouse serum diluted with keratinocyte growth medium (final dilution, 1:400) or with a purified IgG fraction for 30 min at 37°C under a humidified atmosphere containing 5% CO_2. The serum samples were decomplemented (56°C for 30 min) and the cells fixed in cold acetone for 30 s and dried. After washing in phosphate-buffered saline, the cells were incubated with FITC-conjugated anti-mouse IgG.

Histone (Type II-S; Sigma, St. Louis, MO) and lyophilized DNA (Sigma), which are derived from calf thymus, were used for the absorption assay. Single-stranded DNA was prepared by heating calf thymus DNA at 100°C for 10 min and then immediately cooling it in an ice bath (Furukawa *et al*, 1984). Rabbit thymus extracts were used as the source of RNP antigens (Norris *et al*, 1984). Diluted sera (1:200) were incubated with the equal volume phosphate-buffered saline containing appropriate histone, single-stranded DNA, or rabbit thymus extract at 37°C for 30 min and then at 4°C for 1 h. After centrifugation, absorbed serum samples (final dilution 1: 400) were applied for the *in vitro* binding assay. The titers of anti-U_1RNP antibody and anti-single-stranded DNA antibody of these absorbed serum samples were reduced to the levels of control BALB/c or C57BL/6J mice by using assay systems described above.

The same *in vitro* binding assays were performed using cultured keratinocytes from newborn MRL/lpr mice and two transformed keratinocyte cell lines such as PAM 212 cells established from BALB/c mouse and HSC-1 cells originally derived from human squamous cell carcinoma (Kondo and Aso, 1981). These cell lines were generous gifts from Dr. Kouichi Ikai (Faculty of Medicine, Kyoto University). Cell viability was determined by the trypan blue dye exclusion and acridine orange/ethidium bromide methods (Parks *et al*, 1979).

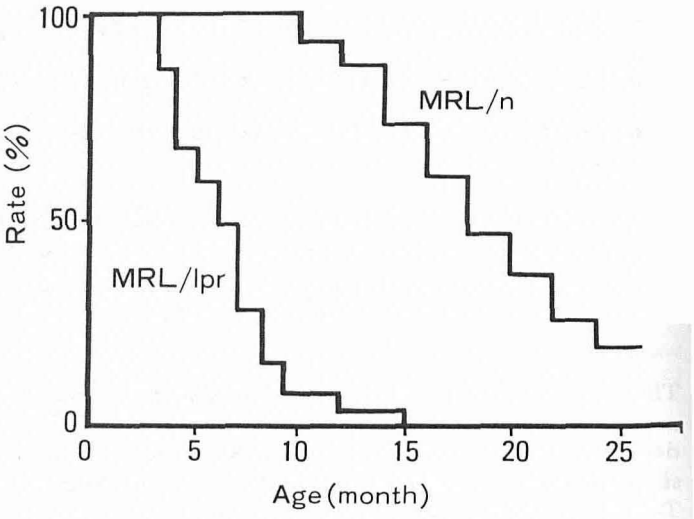

Figure 1. Survival rates of virgin female MRL/lpr and MRL/n mice. Fifty mice in each strain were monitored weekly in order to check the mortality and the incidence of skin lesions.

RESULTS

High Survival Rate in MRL/n Mice Thirty mice of each strain were monitored. As shown in **Fig 1**, virgin female MRL/lpr mice had a 50% mortality rate at 6 mo of age, whereas virgin female MRL/n mice had a 50% mortality rate at 18 mo of age. These results are quite compatible with a previous report by Theofilopoulos and Dixon (1981).

Spontaneous Skin Manifestation of Alopecia and Erythematosus Lesions in Aged MRL/n Mice Skin manifestations were found on the upper dorsal sites, and a few mice also showed necrotic skin lesions on the ears. The cumulative incidence, however, was calculated based solely on the appearance of upper dorsal skin lesions. One-third of the MRL/lpr mice had spontaneous skin manifestations of alopecia and erythematosus lesions at 3 mo of age. At 5 mo of age the incidence rose to 80%. In the MRL/n mice, at 12 mo of age, almost half had skin lesions similar to those

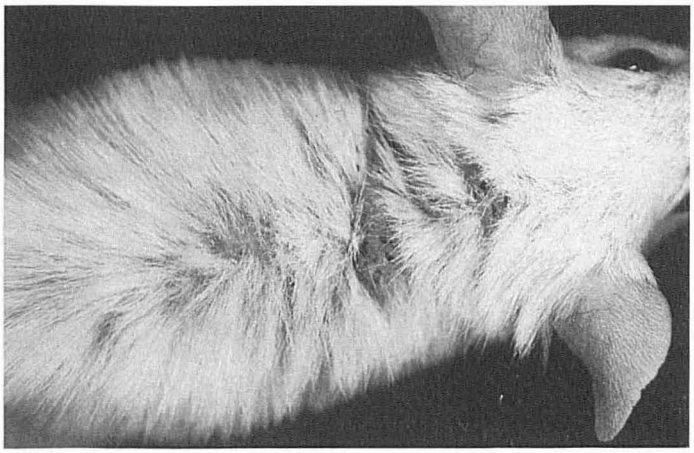

Figure 2. Skin lesions on the back region of aged MRL/n mice. Alopecia and erythematosus lesions on upper dorsal region of 12–mo-old MRL/n mice.

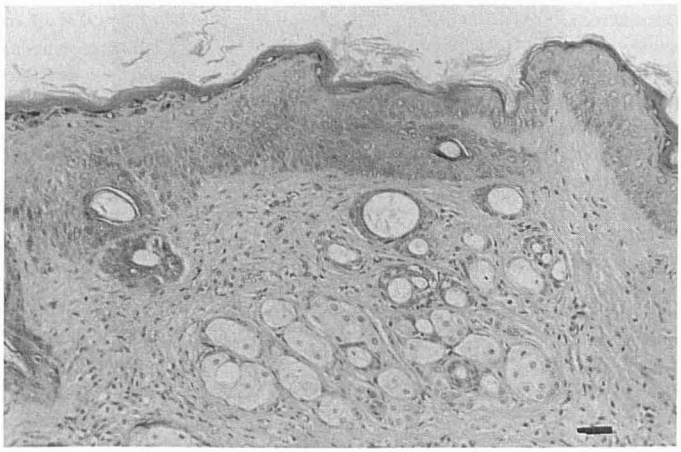

Figure 4. Light microscopic findings from skin lesions on the back region of aged MRL/n mice. Skin lesions in **Figure 2** were prepared for hematoxylin and eosin staining. (*Bar*, 20 μm)

in the MRL/lpr mice **(Fig 2)**, and 75% of the 21-mo-old MRL/n mice had such skin lesions **(Fig 3)**. The incidence of these lesions was not influenced by the number of mice per cage. No microorganisms were demonstrated by the culture of biopsy samples or by light microscopy.

Changes in Histopathology of Skin Lesions in Aged MRL/n Mice Light microscopic observation of the skin lesions in MRL/lpr mice revealed hyperkeratosis, acanthosis, liquefaction, vasodilatation, and mononuclear cell infiltration in the dermis; this was compatible with our previous report (Furukawa *et al*, 1984). In contrast, no vasodilatation, bleeding, or liquefaction were observed in MRL/n mice despite hyperkeratosis, acanthosis, mononuclear cell infiltration, an increased number of collagen bundles, and fibrosis **(Fig 4)**. The mononuclear cell infiltration in the MRL/n mice was milder than in the MRL/lpr mice.

Epidermal Nuclear Deposition of IgG in Aged MRL/n Mice IgG deposition at the dermoepidermal junction (DEJ) was observed in the MRL/lpr mice. The incidence of IgG deposits at the DEJ was 16.6%, 43.3%, and 66.7% at the ages of 2, 3, and 4 mo, respectively. The incidence was over 80% after the age of 5 mo **(Fig 5)**.

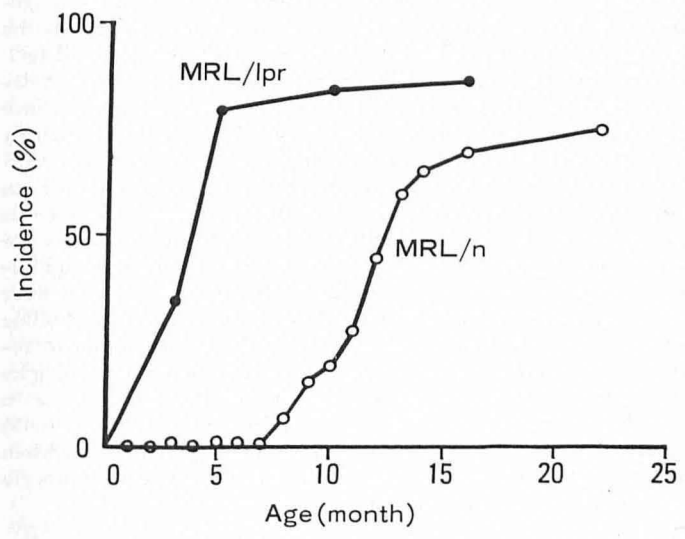

Figure 3. Cumulative incidence of macroscopic skin lesions in virgin female MRL/lpr and MRL/n mice. Results are based on the weekly observation.

In MRL/n mice, IgG deposits at the DEJ were also found in aged mice, but the incidence was very low compared to that in MRL/lpr mice **(Fig 5)**. A more characteristic finding in aged MRL/n mice by direct immunofluorescence was epidermal cell nuclear staining **(Fig 6)**. At the age of 21 mo, 50% of the MRL/n mice showed epidermal cell nuclear staining, a finding that was not observed in any of the MRL/lpr mice **(Fig 5)**. The staining pattern was homogeneous in most of the mice and was not influenced by using FITC-conjugated antisera from three different manufacturers. Albumin deposits in a pattern like the epidermal cell nuclear staining were not demonstrated in any of the mice examined, and the epidermal cell nuclear staining was associated with nuclear staining in the kidney in all of the mice examined. Similar nuclear staining was also observed in lung and liver of aged MRL/n mice, which showed positive epidermal cell nuclear staining. The other organs were not examined immunohistochemically. Epidermal cell nuclear staining was

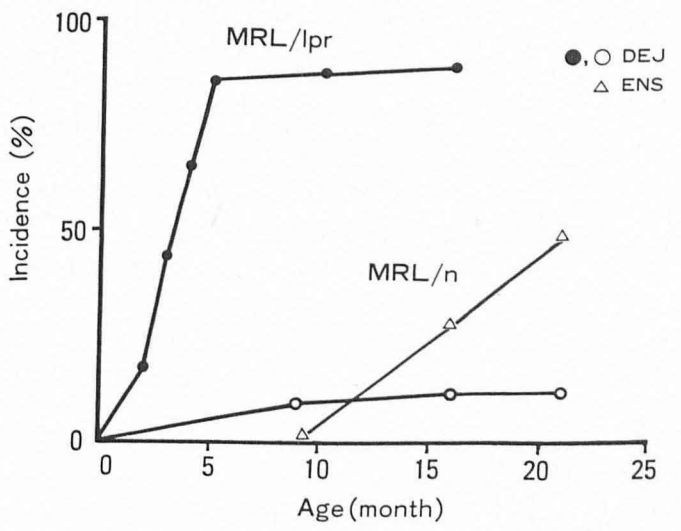

Figure 5. Cumulative incidence of IgG deposition visualized by direct immunofluorescence in skin lesions from virgin female MRL/lpr and MRL/n mice. More than 30 MRL/lpr mice were studied at the examined ages. The incidences of IgG deposition at the DEJ of MRL/n mice were 9.3% (4/43), 9.4% (3/32), and 10.7% (3/28) at the age of 9, 16, and 21 mo, respectively. Epidermal cell nuclear staining of IgG were found in 0% (0/43), 34.3% (11/32), and 50% (14/28) of MRL/n mice examined at the age of 9, 16, and 21 mo, respectively. *ENS*, epidermal cell nuclear staining.

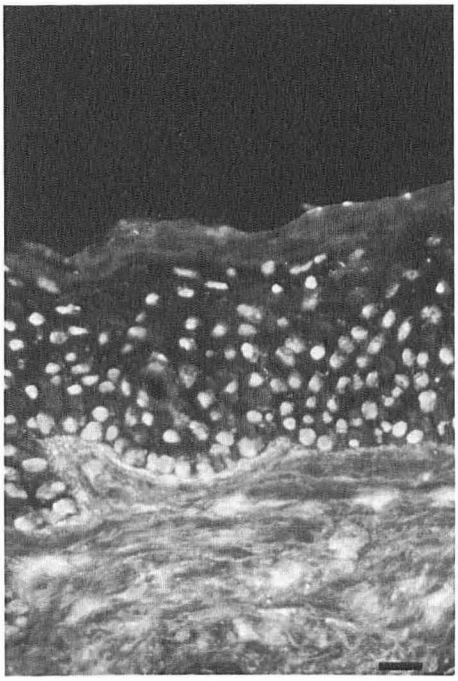

Figure 6. Direct immunofluorescence photomicrograph of IgG deposition in epidermal cell nuclei and partial staining at the DEJ in skin lesions from aged MRL/n mice. Direct immunofluorescence was performed by standard methods as described in *Materials and Methods.* (*Scale bar*, 20 μm)

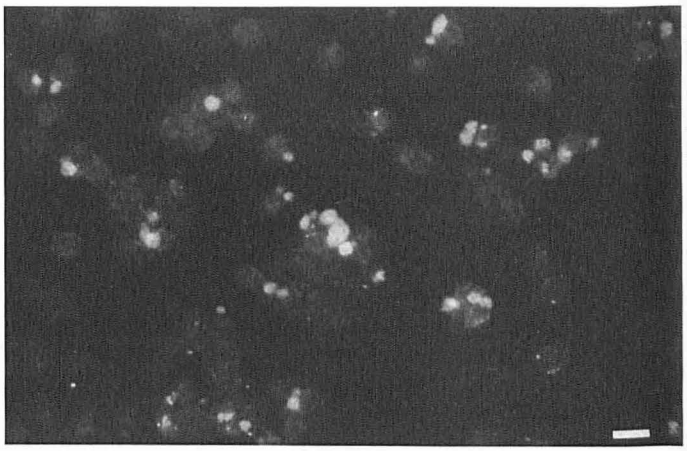

Figure 8. *In vitro* binding of MRL/n mouse sera to the nuclei of keratinocytes cultured from newborn MRL/n mice. Cultured cells were incubated with a purified IgG fraction of examined mice for 30 min at 37°C under a humidified atmosphere containing 5% CO_2. Then, cells were fixed in cold acetone and dried. After washing in phosphate-buffered saline, cells were incubated with fluorescein isothiocyanate-labeled anti-mouse IgG. (*Scale bar*, 20 μm)

also observed in both lesional skin and nonlesional skin; no significant correlation was found between the appearance of these skin lesions and epidermal cell nuclear staining ($\chi^2 = 0.63$). Such Ig deposits at the DEJ or in epidermal cell nuclei were not observed in any of the aged BALB/c or C57BL/6J mice.

The presence or absence of epidermal cell nuclear staining of MRL/n mice at 16 mo of age did not affect the survival rate of MRL/n mice at more than 21 mo of age.

The incidence of apoptotic cells as determined by the *in situ* method did not differ among MRL/n, MRL/lpr, BALB/c, or C57BL/6J mouse strains. Positive cells were observed on the nuclei of the hair follicles but not in the epidermis, which is almost comparable to the results in human skin reported by Tamada *et al* (1994).

Anti-DNA and Anti-U_1RNP Antibodies in the Sera of Aged MRL/n Mice The presence of anti-single-stranded DNA and anti-U1RNP antibodies was determined by an enzyme-linked immunosorbent assay method. As shown in **Fig 7**, MRL/lpr mice

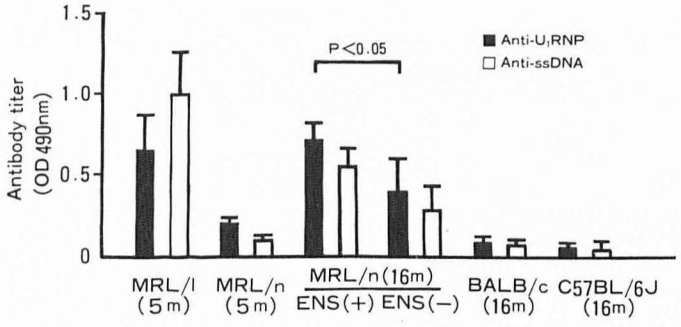

Figure 7. Elevated levels of anti-DNA and anti-U_1RNP antibodies in serum samples from MRL and control mice. Each bar represents mean ± SD of 5 to 8 mice examined.

at 5 mo of age had higher serum levels of both antibodies than MRL/n mice at the same age. By the age of 16 mo, MRL/n mice showed significantly higher levels of both antibodies than control BALB/c mice or C57BL/6J mice. MRL/n mice with epidermal cell nuclear staining also had slightly but significantly higher levels of anti-U_1RNP antibodies than those without epidermal cell nuclear staining ($p < 0.05$). High levels of anti-histone antibodies (more than the mean plus 2 SD values of normal BALB/c and C57BL/6J mice) were detected in 60% of MRL/lpr mice at 5 mo of age but not in aged MRL/n mice. In addition, the mean levels of the antibody titer were not significantly different between 5-mo-old MRL/n mice and 16-mo-old MRL/n mice (data not shown).

***In Vitro* Binding of Mouse Sera to Cultured Keratinocytes** Twenty-eight serum samples of MRL/n mice were examined. All of these samples had high titers of antinuclear antibodies (ANA) (> 1280-fold in dilution) and were also positive for anti-DNA and anti-U_1RNP antibodies. Eleven out of the 28 samples showed binding to the nuclei of over 30% of keratinocytes cultured from newborn MRL/n mice **(Fig 8)**. The binding pattern was homogeneous in most of the mice examined. Immunoreactivity in the diluted sera was equal to the immunoreactivity for purified IgG. When absorbed serum samples were used in this assay, single-stranded DNA showed a marked reduction in immunofluorescence intensity compared to histone or thymus extract, but the intensity was not completely lost. Similar findings were observed when PAM 212 cells, HSC-1 cells, or cultured keratinocytes from newborn MRL/lpr mice were used as substrates (data not shown). There was no difference in the titers of ANA, anti-DNA antibodies, or anti-U_1RNP antibodies between nuclear binding-positive and nuclear binding-negative serum samples. Over 95% of the cells were viable as determined by the trypan blue dye exclusion and acridine orange/ethidium bromide methods. A slight but significant correlation was demonstrated between epidermal cell nuclear staining by direct immunofluorescence and the *in vitro* binding of sera to keratinocytes from newborn MRL/n mice ($0.02 < p < 0.05$) **(Table I)**. Such statistical differences were not demonstrated when keratinocytes from newborn MRL/lpr mice and two keratinocyte cell lines were used as substrates.

Association Among Subepidermal IgG Deposits, the Appearance of Spontaneous Skin Lesions, and *lpr* Mutation This study was designed to elucidate the role of *lpr* mutations in the development of LE-like skin lesions in MRL mice. The basic

Table I. Correlation Between Epidermal Cell Nuclear Staining and *In Vitro* Binding to Keratinocytes Cultured from Newborn MRL/n Mice[a]

Epidermal cell nuclear staining	*In vitro* binding		Total number
	Positive	Negative	
Positive	8	4	12
Negative	3	13	16
Total	11	17	28

[a] $X^2 = 4.74$ (with Yates correction)

analysis was conducted according to our previous genetic studies in New Zealand mice (Maruyama *et al*, 1983; Furukawa *et al*, 1985; Shirai *et al*, 1986). In the F1 hybrids (MRL/lpr × MRL/n), all of the mice were expected to be lpr/+. In the F2 hybrids (F1 × F1), the ratio of lpr/lpr, lpr/+, and +/+ mice was expected to be 1:2:1. Since lymphoproliferation in MRL/lpr mice manifests as an autosomal recessive trait, the expected frequency of lymphoproliferation was 1.00 for MRL/lpr mice (N = 224), zero for MRL/n mice (N = 132) and the F1 hybrid mice (N = 124), and 0.25 for the F2 hybrid mice (N = 187). The observed frequency of lymphoproliferation was identical to the expected ratio.

Subepidermal IgG deposits were found in 85% of the MRL/lpr mice, none of the MRL/n mice and F1 hybrid mice, and 21% of the F2 hybrid mice. If the rate of IgG deposition in MRL/lpr mice is scaled to 100%, then the rate in F2 hybrid mice is almost equal to the expected frequency.

Skin lesions were observed in 78% of the MRL/lpr mice, none of the MRL/n mice and F1 hybrid mice, and 15% of the F2 hybrid mice. If the rate of skin lesions in MRL/lpr mice is scaled to 100%, then the rate of lesions in F2 hybrid mice is 19%. This corrected frequency is much lower than the frequency expected for a recessive gene (25%) and is equal to the frequency expected from a recessive gene and a dominant gene.

DISCUSSION

This study demonstrates that skin lesions in aged MRL/n mice have several unique characteristics. The first is their spontaneous appearance on the upper back region of aged mice. These skin lesions were similar to those in MRL/lpr mice over 3 mo of age, but the histologic features were slightly different from those of MRL/lpr mice. Light microscopic observations revealed mononuclear cell infiltration and an increase in collagen and fibroblasts in the dermis. Liquefaction-like changes, bleeding, and vasodilatation were not observed in the skin lesions of MRL/n mice, although such pathologic changes are characteristic of the spontaneous LE-like skin lesions in MRL/lpr mice (Furukawa *et al*, 1984). The second characteristic is epidermal cell nuclear staining by direct immunofluorescence methods. IgG deposition in the skin lesions of MRL/n mice was observed in 50% of the mice at 21 mo of age. This IgG epidermal cell nuclear staining was always associated with IgG deposition in the nuclei of kidney cells.

IgG epidermal cell nuclear staining has been demonstrated in the skin lesions of most patients with mixed connective tissue disease (MCTD) and in one-third to one-half of the patients with systemic lupus erythematosus (SLE) (Velthuis *et al*, 1990; Burrows *et al*, 1993). Specific anti-SS-A/Ro binding occurred in the nucleus as well as the cytoplasm of suction-blister skin specimens (Furukawa *et al*, 1990), and particulate epidermal IgG deposition was frequently observed in both lesional skin and nonlesional skin specimens from patients with subacute cutaneous LE (David-Bajar *et al*, 1992). MCTD was described by Sharp *et al* (1971) and differs significantly from SLE in both clinical and laboratory features. The typical clinical pattern consists of Raynaud's phenomenon as well as swelling of the fingers leading to a sausage-like appearance, sclerodactyly, and other visceral organic abnormalities. There are two decisive laboratory features, the presence of very high titers of serum antibodies to U_1RNP and epidermal cell nuclear staining by direct immunofluorescence (Gilliam and Prystowsky, 1977). In most cases, the epidermal cell nuclear staining is speckled (Gilliam and Prystowsky, 1977). The aged MRL/n mouse, however, has a significant but low titer of anti-U_1RNP antibodies with a predominance of anti-DNA antibodies. Furthermore, the epidermal cell nuclear staining pattern was homogeneous in most of the mice. Thus this mouse does not represent a complete model of human MCTD.

In SLE, nuclear IgG deposits in the skin have been reported by several investigators (Baart de la Faille-Kuyper, 1974; Shu *et al*, 1977). Recently, a homogeneous pattern as well as a speckled/thready pattern of epidermal cell nuclear IgG deposition was reported in skin samples from LE patients, including systemic, discoid, and subacute cutaneous LE (Velthuis *et al*, 1990). The most recent report from Burrows *et al* (1993) showed a relatively high incidence of epidermal cell nuclear staining in LE patients and mentioned that the thready and homogeneous pattern occurs specifically in SLE. Based on these findings, it is likely that the skin lesions of aged MRL/n mice are an adequate model for certain types of cutaneous LE. Furthermore, absorption studies in this model mouse revealed that DNA components may play more important role in ANA binding to the nucleus than other nuclear components including histone and RNP.

It is still controversial whether epidermal cell nuclear staining actually occurs *in vivo*. Tan and Kunkel (1966) suggested that *in vivo* epidermal cell permeability factors may play a role in immunoglobulin deposition in lupus patients. A few investigators have also discussed the possibility that this *in vivo* binding is a technical artifact (Shu *et al*, 1977; Burrows *et al*, 1993) because of the lack of nuclear deposition of complement components[1]. In contrast, Tuffanelli (1975) argued that the *in vivo* binding was not an artifact, because in their experience the same patients showed the same phenomenon repeatedly. Cellular penetration of anti-RNP IgGs has also been demonstrated by Alarcon-Segovia *et al* (1978), who showed that the antibody penetrated live human mononuclear cells through Fc receptors. Galoppin and Saurat (1981) studied the *in vivo* binding of anti-RNP and anti-DNA antibodies to a cell suspension of live keratinocytes. They found that 70% of the cells had speckled nuclear staining with anti-RNP antibodies, and 10% of the cells had homogeneous staining with anti-DNA antibodies. Recent immunohistologic studies have also suggested the possibility of *in vivo* binding (Velthuis *et al*, 1990; Burrows *et al*, 1993); murine monoclonal anti-DNA antibodies interacted with living mononuclear cells and penetrated the T cells, thus resulting in perinuclear staining or intranuclear globular staining by a peroxidase immunohistochemical technique (Okudaira *et al*, 1987).

There are many strains of autoimmune-prone mice, including New Zealand mice, BXSB mice, and MRL/lpr mice (Theofilopoulos and Dixon, 1981). Although these autoimmune mouse strains show subepidermal IgG deposits at the DEJ, epidermal cell nuclear staining using direct immunofluorescence was rarely found (Furukawa *et al*, 1986). Thus, the aged MRL/n mouse is a better model for the investigation of epidermal cell nuclear staining. In half of the aged MRL/n mice, epidermal cell nuclear staining was demonstrated, as were nuclear IgG deposits in the kidney. These findings correlated significantly with the *in vitro* binding of mouse sera or purified IgG to keratinocytes cultured from newborn MRL/n mice. Based on these findings and recently published data (Galoppin and Saurat, 1981; Velthuis *et al*, 1990; Burrows *et al*, 1993), it is not likely that epidermal cell nuclear binding occurs simply as a result of contamination by diffusable ANA in the dermis that gain access to the epidermal cell nuclei during the processing of the specimens, irrespective of whether a speckled or a homogeneous staining pattern was observed.

It is believed that the *lpr* mutation causes the acceleration of subclinical autoimmune phenomena in MRL/n mice (Theofilopou-

[1] Gilliam JN, Smiley JD, Ziff M: Association of mixed connective tissue disease (MCTD) with immunoglobulin localization in epidermal nuclei of biopsies from areas of normal skin. *Clin Res* 23:229A, 1975 (abstr.).

los and Dixon, 1981; Theofilopoulos, 1995). Although the *lpr* mutation has also been reproduced in several other mouse strains, such as the C3H, C57BL/6J, and AKR mice, these strains did not show severe lupus nephritis, vasculitis, or arthritis as did the MRL/lpr mouse, except for the appearance of lymphoproliferation, the presence of rheumatoid factor and a decrease in IL-2 production (Takahashi *et al*, 1991; Berney *et al*, 1992). Thus, in order to investigate autoimmune phenomena in MRL/lpr mice, it is important to examine the autoimmune disease-prone genetic background of the MRL mice. From this study, we can conclude that the *lpr* mutation accelerates the progression of a mild type of systemic and cutaneous connective tissue disease into a more severe type, especially in the MRL mouse strains.

Next, the mechanism of action of the *lpr* mutation was analyzed. The results of F1 and F2 hybrid mice indicated that the *lpr* mutation regulates lymphoproliferation and subepidermal IgG deposition at the DEJ, which is a characteristic of MRL/lpr mice (Furukawa *et al*, 1984). Interestingly, the appearance of macroscopic skin lesions was not regulated by the *lpr* mutation. The appearance of macroscopic skin lesions was therefore thought to be due to the *lpr* mutation plus an additional factor, which probably affected the induction of these macroscopic skin lesions in an autosomal dominant fashion. The candidates for such an additional factor may include environmental stimuli such as changes in temperature, ultraviolet light, and biologic stress (Horiguchi *et al*, 1987; Furukawa *et al*, 1993a).

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REFERENCES

Alarcon-Segovia D, Ruiz-Arguelles A, Fishbein E: Antibody to nuclear ribonucleoprotein penetrates live human mononuclear cells through Fc receptors. *Nature (London)* 271:67–69, 1978

Baart de la Faille-Kuyper EH: In-vivo nuclear localization of immunoglobulins in clinically normal skin in systemic and procainamide induced lupus erythematosus. *Neth J Med* 17:58–65, 1974

Berney T, Fulpius T, Shibata T, Reininger L, van Snick J, Weigert M, Marshak-Rothstein A, Izui S: Selective pathogenicity of murine rheumatoid factors of the cryoprecipitable IgG3 subclass. *Int Immunol* 4:93–99, 1992

Billings PB, Allen RW, Jensen FC, Hoch SO: Anti-RNP monoclonal antibodies derived from a mouse strain with lupus-like autoimmunity. *J Immunol* 128:1176–1180, 1982

Burrows NP, Bhogal BS, Jones RR, Black MM: Clinicopathologic significance of cutaneous epidermal nuclear staining by direct immunofluorescence. *J Cutaneous Pathol* 20:159–162, 1993

David-Bajar KM, Bennion SD, DeSpain JD, Golitz LE, Lee LA: The clinical, histologic, and immunofluorescent distinctions between subacute cutaneous lupus erythematosus and discoid lupus erythematosus. *J Invest Dermatol* 99:251–257, 1992

Furukawa F, Ikai K, Matsuyoshi N, Shimizu K, Imamura S: Relationship between heat shock protein induction and the binding of antibodies to the extractable nuclear antigens on cultured human keratinocytes. *J Invest Dermatol* 101:191–195, 1993a

Furukawa F, Kashihara-Sawami M, Lyons MB, Norris DA: Binding of antibodies to the extractable nuclear antigens SS-A/Ro and SS-B/La is induced on the surface of human keratinocytes by ultraviolet light (UVL): implications for the pathogenesis of photosensitive cutaneous lupus. *J Invest Dermatol* 94:77–85, 1990

Furukawa F, Lyon MB, Norris DA: Susceptible cytotoxicity to ultraviolet B light in fibroblasts and keratinocytes cultured from autoimmune-prone MRL/Mp-lpr/lpr mice. *Clin Immunol Immunopathol* 52:460–472, 1989

Furukawa F, Maruyama N, Yoshida H, Hamashima Y, Hirose S, Shirai T: Genetic studies on the skin lupus band test in New Zealand mice. *Clin Exp Immunol* 59:146–152, 1985

Furukawa F, Ohshio G, Imamura S: Pathogenesis of lupus dermatoses in autoimmune mice. XIX. Attempts to induce subepidermal immunoglobulin deposition in MRL/Mp-+/+ mice. *Arch Dermatol Res* 285:20–26, 1993b

Furukawa F, Ohshio G, Tanaka H, Nakamura T, Ikehara S, Imamura S, Hamashima Y: Pathogenesis of lupus dermatoses in autoimmune mice. VI. correlation between positivity of lupus band test and lupus nephritis. *Arch Dermatol Res* 278:343–346, 1986

Furukawa F, Tanaka H, Sekita K, Nakamura T, Horiguchi Y, Hamashima Y: Dermatopathologic studies on skin lesions of MRL mice. *Arch Dermatol Res* 276:186–194, 1984

Galoppin L, Saurat JH: In vitro study of the binding of antiribonucleoprotein antibodies to the nucleus of isolated living keratinocytes. *J Invest Dermatol* 76:264–267, 1981

Gavrieli Y, Sherman Y, Ben-Sasson SA: Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J Cell Biol* 119:493–501, 1992

Gilliam JN, Prystowsky SD: Mixed connective tissue disease syndrome. *Arch Dermatol* 113:583–587, 1977

Horiguchi Y, Furukawa F, Hamashima Y, Imamura S: Ultrastructural lupus band test in skin of MRL/l mice. *Arch Dermatol Res* 278:474–480, 1986

Horiguchi Y, Furukawa F, Ohshio G, Horio T, Imamura S: Effects of ultraviolet light irradiation on the skin of MRL/l mice. *Arch Dermatol Res* 279:478–483, 1987

Kanauchi H, Furukawa F, Imamura S: Characterization of cutaneous infiltrates in MRL/lpr mice monitored from onset to the full development of lupus erythematosus-like skin lesions. *J Invest Dermatol* 96:478–483, 1991

Kondo S, Aso K: Establishment of a cell line of human skin squamous cell carcinoma in vitro. *Br J Dermatol* 105:125–132, 1981

Maruyama N, Furukawa F, Nakai Y, Sasaki Y, Ohta K, Ozaki S, Hirose S, Shirai T: Genetic studies of autoimmunity in New Zealand mice. IV. Contribution of NZB and NZW genes to the spontaneous occurrence of retroviral gp70 immune complexes in (NZB × NZW) F1 hybrid and the correlation to renal disease. *J Immunol* 130:740–746, 1983

Murphy ED, Roths JB: Autoimmunity and lymphoproliferation. Induction by mutant gene lpr, and acceleration by a male-associated factor in strain BXSB mice. In: Bigazzi PE, Warner NL (eds.). *Genetic Control of Autoimmune Disease.* Elsevier, New York, 1978, pp 207–221

Norris DA, Ryan SR, Fritz KA, Tan EM, Deng JS, Weston WL: The RNP, Sm, and SS-A/Ro-specific antisera from patients with lupus erythematosus in inducing antibody-dependent cellular cytotoxicity (ADCC) of targets coated with nonhistone nuclear antigens. *Clin Immunol Immunopathol* 31:311–320, 1984

Okudaira K, Yoshizawa H, Williams R: Monoclonal murine anti-DNA antibody interacts with living mononuclear cells. *Arthritis Rheum* 30:669–678, 1987

Parks DR, Bryan VM, Oi VT, Herzenberg LA: Antigen specific identification and cloning of hybridomas with a fluorescence-activated cell sorter. *Proc Natl Acad Sci USA* 76:1962–1965, 1979

Provost TT, Watson R: The MRL/lpr mouse model of cutaneous lupus. In: Wallace DJ, Hahn BH (eds.). *Dubois' Lupus Erythematosus.* 4th ed. Lea & Febiger, Philadelphia, 1993, pp 282

Sato S, Ihn H, Soma Y, Igarashi A, Tamaki T, Kikuchi K, Ishibashi Y, Takehara K: Antihistone antibodies in patients with localized scleroderma. *Arthritis Rheum* 36:1137–1141, 1993

Sharp GC, Irvin WS, Laroque RL, Velez C, Daly V, Kaiser AD, Holman HR: Association of autoantibodies to different nuclear antigens with clinical patterns of rheumatic disease and responsiveness to therapy. *J Clin Invest* 50:350–359, 1971

Shirai T, Ohta K, Kohno A, Furukawa F, Yoshida H, Maruyama N, Hirose S: Naturally occurring antibody response to DNA is associated with the response to retroviral gp70 in autoimmune New Zealand mice. *Arthritis Rheum* 29:242–250, 1986

Shu S, Provost T, Croxdale MB, Reichlin M, Beutner EH: Nuclear deposits of immunoglobulins in skin of patients with systemic lupus erythematosus. *Clin Exp Immunol* 27:238–244, 1977

Takahashi S, Nose M, Sasaki J, Yamamoto T, Kyogoku M: IgG3 production in MRL/lpr mice is responsible for development of lupus nephritis. *J Immunol* 147:515–519, 1991

Tamada Y, Takama H, Kitamura T, Yokochi K, Nitta Y, Ikeya T, Matsumoto Y: Identification of programmed cell death in normal human skin tissues by using specific labeling of fragmented DNA. *Br J Dermatol* 131:521–524, 1994

Tan EM, Kunkel HG: An immunofluorescent study of the skin lesions in systemic lupus erythematosus. *Arthritis Rheum* 9:37–46, 1966

Theofilopoulos AN: The basis of autoimmunity: Part II, Genetic predisposition. *Immunol Today* 16:150–159, 1995

Theofilopoulos AN, Dixon FJ: Etiopathogenesis of murine SLE. *Immunol Rev* 55:179–216, 1981

Tuffanelli DL: Cutaneous immunopathology: recent observations. *J Invest Dermatol* 65:143–153, 1975

Velthuis PJ, Kater L, van der Tweel I, Meyling FG, Derksen RHWM, Hene RJ, van Geutselaar JA, de la Faille HB: In vivo antinuclear antibody of the skin: diagnostic significance and association with selective antinuclear antibodies. *Ann Rheum Dis* 49:163–167, 1990

Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S: Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature (London)* 356:314–317, 1992