The Expression of Histamine Receptors in Skin Lesions of MRL/MP-lpr/lpr Mice

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Abstract: Systemic lupus erythematosus (SLE) is a chronic inflammatory disease accompanied with systemic organs disorder including skin changes. The MRL/MP-lpr/lpr (MRL/l) mouse is a model of human LE. MRL/l mice skin lesions exhibit a decreased activity in histamine-N-methyltransferase (HMT) and impaired histamine metabolism. In order to clarify the role of histamine receptors (HRs) including H1R, H2R and H3R in MRL/l skin lesions, the relationship between HRs and skin lesions was assessed by immunohistochemical staining and RT-PCR methods. The expression of H2R was seen in the non-lesional skin of 2-month-old (mo) MRL/l mice; H2R expression continued for a couple of months, and then decreased in the skin lesions of 5-mo MRL/l mice. In MRL/l skin lesions, a dense mast cell infiltration expressed H2R was seen. In conclusion, an increased expression of H2R in mast cells may be associated with histamine metabolism in skin lesions from MRL/l mice.

Keywords: Histamine receptor, lupus erythematosus, model mouse.

INTRODUCTION

The etiology of human LE is not fully understood, however skin lesions have a number of infiltrating T cells and their related mediators. The MRL/l mouse is a model for the spontaneous development of skin lesions similar to those associated with human LE [1-3]. In skin lesions from MRL/l mice, a decreased activity in histamine-N-methyltransferase (HMT) is reported and impaired histamine metabolism is supposed to be a particular biochemical feature of the MRL/l skin [4]. In human SLE, irrespective of fresh lesions or perilesional sites, the HMT activity of lesions is much lower than that of controls and the decreased activity plays a particular role in the development of immune-complex–mediated skin lesions [5]. The decreased HMT of histamine metabolism was also seen in the experimental Arthus reaction [6].

Histamine is an important inflammatory mediator and can affect the function of dendritic cells, monocytes and lymphocytes. Histamine binds to 4 different receptors that transduce signals to cells through distinct pathways and can regulate antigen-specific Th1 and Th2 cells. Th1 cells mainly express the histamine H1 receptor (H1R) and produce IFN-γ, and Th2 cells mainly express H2R and induce immune-tolerance [7, 8]. In the present study, in order to clarify the role of histamine receptors (HRs) in skin lesions from MRL/l mice, we determined the relationship between HRs and the development of skin lesions.

MATERIALS AND METHODS

Female MRL/l mice and MRL/Mp- +/- (MRL/n) mice were purchased from Japan SLIC Inc. (Hamamatsu, Japan) and maintained under specific pathogen-free (SPF) conditions. Skin samples were obtained from the upper backs of all examined MRL/l mice (1-month to 5-month-old) and MRL/n mice (5-month-old), and additional samples were obtained from the lower backs to represent normal skin samples from MRL/l mice with skin lesions on their upper backs. The group of each month includes 3 mice. The experiment using MRL mice was approved according to principles of morality in animal experiment by institute of animal experiment of Wakayama Medical University.

Macroscopic and Light Microscopic Observations

Skin lesions from all examined MRL/l mice were observed every 2 weeks. Skin specimens were taken from the upper back region in all examined mice, because skin lesions appeared at this site in this particular mouse model. Specimens were fixed in 10% formalin and stained with hematoxylin-eosin (HE). Toluidine blue staining was also performed to assess the infiltration of mast cells in the skin of these examined mice.

Immunohistochemical Studies

For vertical sections, skin specimens were embedded in OCT compound (Miles Laboratory Elkhart, IN), quick frozen in an acetone bath and stored at 70°C, for subsequent immunoperoxidase staining. To determine the infiltration of T lymphocytes associated with the skin lesions of MRL/l mice, the following antibodies were used as primary antibodies: anti-CD4 (Pharmingen, San Diego, CA), anti-CD8 (Southern Biotechnology, Birmingham, AL) and the Vectastain Elite kit (anti Goat IgGs) were used as secondary antibodies. Furthermore, to assess the expression of histamine receptors in skin lesions from all examined mice, H1R, H2R, and H3R antibodies (Santa Cruz Biotechnology, Inc.) were used. Vectastain Elite kits (anti rabbit IgGs for H1R, and anti Goat IgGs for H2R and H3R) were used as secondary antibodies (Vector Laboratories, Inc.). Labeled donkey anti-goat IgG

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antibodies (Invitrogen Ltd., Paisley, UK) were used for the
H2R and H3R, and affinity purified anti mouse CD117 (eBio-
science, CA, USA) was used for staining of mast cells in
immunofluorescence study.

**Histamine Receptors Expression Analyses by RT-PCR**

Levels of mRNAs for H1R, H2R and H3R were deter-
mined by reverse transcription-polymerase chain reaction
(RT-PCR) in each group of MRL/l mice and 5-month-old
MRL/n mice. Each primer was purchased from Kurabo
Medical Ltd. (Okayama, Japan). Primers are shown in Table
1. After the mice were sacrificed, each dorsal skin was
removed and immediately frozen in liquid nitrogen. Total
RNA was prepared using the RNeasy Mini Kit (Qiagen, To-
kyo, Japan). Single-stranded cDNA was reverse-transcribed
using each RNA sample and amplified by PCR (Takara PCR
Therminal Cycler, Takara, Otsu, Japan). PCR reactions were
performed by melting DNA samples incubation for 5 min at
94°C, followed by 35 cycles of melting, annealing and po-
lymerization, each consisting of 1 min at 94°C, 1 min at 60°C
and 1.5 min at 72°C. PCR products were electrophoresed on
a 2% agarose gel and visualized by ethidium bromide stain-
ing. All assays were performed in triplicate according to the
manufacturer’s instructions.

**Table 1. Polymerase Chain Reaction Primers**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>H1R</td>
<td>CGACACTGTCAGCACCGCCA</td>
<td>GAAAGACAGTCGGAGGTCA</td>
</tr>
<tr>
<td>H2R</td>
<td>TGTGGTCGTCTGCCTGGCTG</td>
<td>CAACACGGGGTACCGCAGTG</td>
</tr>
<tr>
<td>H3R</td>
<td>GTTGGGAGTACCTGTCCGGT</td>
<td>GCCGAAGACGAGTGCGCCTC</td>
</tr>
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**RESULTS**

**Macroscopic and Light Microscopic Findings**

Skin lesions in MRL/l mice had completely developed on
their upper backs at 5-months of age. The incidence of skin
lesion was 67% (2/3). Skin lesions were palpable and
showed alopecia and scab formation (Fig. 1a). Histopa-
thological changes including hyperkeratosis, acanthosis,
lymphocyte infiltration and dense mast cell dermal infiltra-
tions were also seen in skin lesions from MRL/l (Fig. 1c,d).
The average numbers of mast cells were 100±10 in one mi-
oscopic field (x100). 2-month-old MRL/l mice had not yet
developed skin lesions (0/3). The lesions also showed no
pathological changes, however there were a few mast cells
infiltrating as observed by toluidine staining (Fig. 1e,f). The
average numbers of mast cells were less than 5 in one micro-
scopic field (x100). These results were similar to those from
5-month-old MRL/n mice which did not develop skin lesions
(0/3) (Fig. 1b,g,h).

**Immunohistochemical Analysis**

CD4 and CD8 positive cells were observed in the skin
lesions from 5-month old MRL/l mice. Expression of H2R
was remarkably seen compared to those of H1R and H3R, in
skin lesion mast cells (Fig. 2a-c). Then, immunofluorescence
staining was performed to confirm the H2R expression on
mast cell in the skin lesions from MRL/l mice. The expres-
sion of H2R was seen on CD117 positive cells infiltrated in
the skin lesions from 5-month-old MRL/l mice (Fig. 3). On
the other hand, the infiltration of CD117 positive cells ex-
pressing H2R was not seen in non-lesional skin from 5-
month old MRL/l mice (Fig. 3). H2R expression was not
seen on CD4 or CD8 positive T cells in the skin lesions of
MRL/l mice. Skins from 2-month-old MRL/l mice did not
show a remarkable infiltration of lymphocytes as well as
those of non-lesional skin of 5-month-old MRL/l mice, how-
ever a few mast cells had infiltrated. Furthermore, HRs in-
cluding H1R, H2R and H3R were also seen in sebaceous
gland in skin lesions of 5-month old MRL/l mice (Fig. 4).
The development of sebaceous grand was seen as the skin
lesions of MRL/l develop.

**Histamine Receptors Expression Analyses by RT-PCR**

In skin lesions from 5-month-old MRL/l mice, the ex-
pression of H1R was very weak. H1R expression was also
weak in 2-month-old MRL/l or 5-month-old MRL/n mice as
evaluated by RT-PCR (Fig. 5). The expression of H2R was
seen in the non lesional skin of 2-month-old MRL/l mice and
its expression continued for a couple of months, and then

![Fig. (1). Clinical findings, HE and toluidine blue staining in the skin of MRL/l mice and MRL/n mice. The skin lesion from 5-
month-old MRL/l mice showed skin lesions (a), hyperkeratosis,
acanthosis, lymphocytes infiltration and dense mast cell dermal infiltra-
tions (d). 2-month-old MRL/l mice (e,f) and 5-month-old MRL/n mice (b,g,h) showed no clinical and pathological changes, however there were a few mast cells infiltration (x100).](image-url)
Histamine Receptors in MRL/MP-lpr/lpr Mice

The etiology and pathogenesis of autoimmune diseases cannot be readily analyzed without appropriate animal models. Animal models are commonly used to study the genetic, environmental and pathogenic aspects of autoimmune diseases. MRL/l mice show immune complex glomerulonephritis, arteritis, arthritis, anti-DNA antibody and spontaneous LE-like lesions with IgG deposits at the dermoepidermal junction [1, 8]. These clinical findings are largely similar to human systemic lupus erythematosus (SLE), thereby MRL/l mice are supposed to be an excellent model of SLE. In our study, we focused on the relationship of the expression of HRs and skin lesions.

We confirmed that MRL/l mice completely develop spontaneous LE-like skin lesions by 5-months of age. In the aforementioned skin lesions, lymphocytes infiltrate and numerous mast cells infiltrations were induced and the expression of H2R was seen on mast cells by immunostaining. The expression of H2R was present in significant levels in the skin of 2-month-old MRL/l mice and H2R expression was continued and then decreased within skin lesions at 5 months of age as determined by RT-PCR. Previously, we detected significant expression of IL-2, IL-10, IL-12 and TNF-α mRNAs in skin lesions from 5-month-old MRL/l mice, compared with non-lesional skin from 2-month-old MRL/l mice [9]. It is possible that the accumulation of mast cells (CD117 (+)) expressing H2R occurs to suppress the productions of Th1 and Th2 cytokine expressed in the skin lesions of MRL/l mice. The expression of H2R induces an immune-tolerance and decreases the Th1 and Th2 response [7, 10].

As shown in Fig. (4), the expression of HRs, especially H2R and H3R were observed in sebaceous gland of lesional skin of MRL/l mice. Furthermore, the expressions of HRs including H1R, H2R and H3R were also observed in sebaceous gland of non lesional MRL/l skin (data not shown).
H3R has a pro-inflammatory activity and an increased antigen presenting cell capacity as H1R has [7]. The expression of H3R showed an age-dependent increase in MRL/l mice by RT-PCR. The development of sebaceous gland was seen as the skin lesions of MRL/l mice develop. The distribution of HRs except for mast cells might depend on sebaceous gland of MRL/l skin lesions. It is substantiated the identification of histamine receptors on sebaceous gland by reducing squalene level by anti-histamine [11]. The relationship of H2R and H3R expressed mast cells and sebaceous gland is still obscure in the skin lesions of MRL/l mice

On the other hand, we recently found that the expression of H2R was observed on both mast cells and CD4 positive cells in skin lesions of drug-induced discoid lupus erythematosus (DLE) model using T cell receptor α chain knock out mice (manuscript in preparation). The expression of H2R on lymphocytes was not observed in the skin lesions of MRL/l. The expressions of IL-12 and TNF-α were common in the skin lesions of drug-induced DLE model and MRL/l mice, while the expression patterns of IFN-γ and IL-10 differs in these mice [9]. The difference of cytokine productions in these lupus models might depend on the location of HRs expression.

Previously, we reported that the activity of HMT in the skin of 2-month-old MRL/l mice was higher than that of skin lesions from 5-month-old MRL/l mice [4]. The decreased activity of HMT and the impaired metabolism of histamine are supposed to be associated with the development of skin lesions in MRL/l mice. It is interesting that the activity of HMT runs in parallel with the expression of H2R in the time course of skin changes of MRL/l mice, however their relationship between these two remains obscure.

In humans, HR expression is seen on mast cells and dermal dendritic cells (Lippert JID 2004) [12] (Ohtani JID 2003) [13]. We also investigated the relationship between human lupus skin lesions and the expressions of HRs. Mast cells infiltration was assessed by toluidine blue staining and the expressions of H1R and H2R in the skin lesions were assessed by immunohistochemical staining. Infiltration of a number of mast cells in involved skin lesions of lupus patients were observed compared with those of non-involved skin lesions. The expressions of H2R in skin lesions of human lupus were seen, but not H1R. At present, it is not identified which cells express H2R (data not shown). Our study of lupus model suggested the involvement of HRs in skin lesions of human LE.

The expression of H2R and H3R was seen on mast cells and sebaceous gland in skin lesions from MRL/l mice in our study. Functional differences of cytokine productions and CD14 expression between human and murine mast cells are reported [14]. Recent observations indicate that mast cells play a key role in coordinating the early phase of autoimmune disease [15] and mast cells are associated with the development of adaptive immune responses [16]. Furthermore, it was recently reported that mast cells could produce an array of both pro- and anti-inflammatory mediators, and intermediate regulatory T cell tolerance [17]. A better understanding of the development of skin lesions in MRL/l mice will ensue once the multi-function of mast cells expressing H2R and the relationship between H2R and H3R is clarified.

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REFERENCES


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