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

Takashi Yoshimasu*, Nobuo Kanazawa, Naoya Mikita and Fukumi Furukawa

Department of Dermatology, Wakayama Medical University, Japan

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–medicated 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 SLC 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 immnoperoxidase 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

*Address correspondence to this author at the Institutional affiliation: Department of Dermatology, Wakayama Medical University, Location: 811-1. Kimiidera, Wakayama City, 641-0012, Japan; Tel: +81-73-441-0661; Fax: +81-73-448-1908; E-mail: 777@wakayama-med.ac.jp

1874-3722/08

antibodies (Invitrogen Ltd., Paisley, UK) were used for the H2R and H3R, and affinity purified anti mouse CD117 (eBioscience, 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 determined 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, Tokyo, 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 polymerization, each consisting of 1 min at 94˚C, 1min at 60˚C and 1.5 min at 72˚C. PCR products were electrophoresed on a 2% agarose gel and visualized by ethidium bromide staining. All assays were performed in triplicate according to the manufacturer's instructions.

Table 1. Polymerase Chain Reaction Primers

Gene		
H1R	Forward	CGACACTGTCAGCACCGCCA
H1R	Reverse	GAAGACAGTCGGAGAGGTCA
H2R	Forward	TGTGGTCGTCTGCCTGGCTG
H2R	Reverse	CAACACGGGGTACCGCAGTG
H3R	Forward	GTTGGGAGTACCTGTCCGGT
H3R	Reverse	GCCGAAGACGAGTGCGCCTC

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**). Histopathological changes including hyperkeratosis, acanthosis, lymphocyte infiltration and dense mast cell dermal infiltrations were also seen in skin lesions from MRL/l (Fig. **1c**,**d**). The average numbers of mast cells were 100±10 in one microscopic 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 microscopic 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 expression 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 expressing 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, however a few mast cells had infiltrated. Furthermore, HRs including 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.

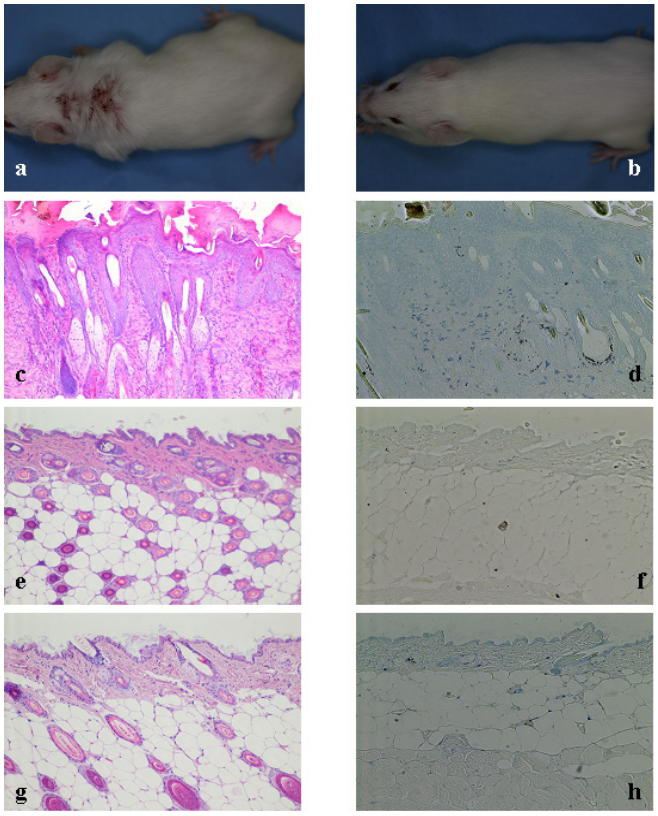

Fig. (1). Clinical findings, HE and toluidine blue staining in the skin of MRL/l mice and MRL/n mice. The skin lesion from 5-month-old MRL/l mice showed skin lesions (**a**), hyperkeratosis, acanthosis, lymphocytes infiltration (**c**) and dense mast cell dermal infiltration (**d**). 2-month-old MRL/l 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).

Histamine Receptors Expression Analyses by RT-PCR

In skin lesions from 5-month-old MRL/l mice, the expression 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

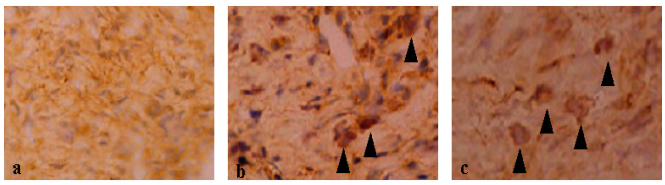

Fig. (2). Immunohistochemical staining of H1R (**a**), H2R (**b**) and H3 (**c**) in skin lesion mast cells from 5-month-old MRL/l mice (x 400). Expression of H2R was remarkably seen compared to those of H1R in skin lesion mast cells. The expression of H3R was also slightly seen in skin lesion. (Arrow heads show H2R and H3R positive mast cells).

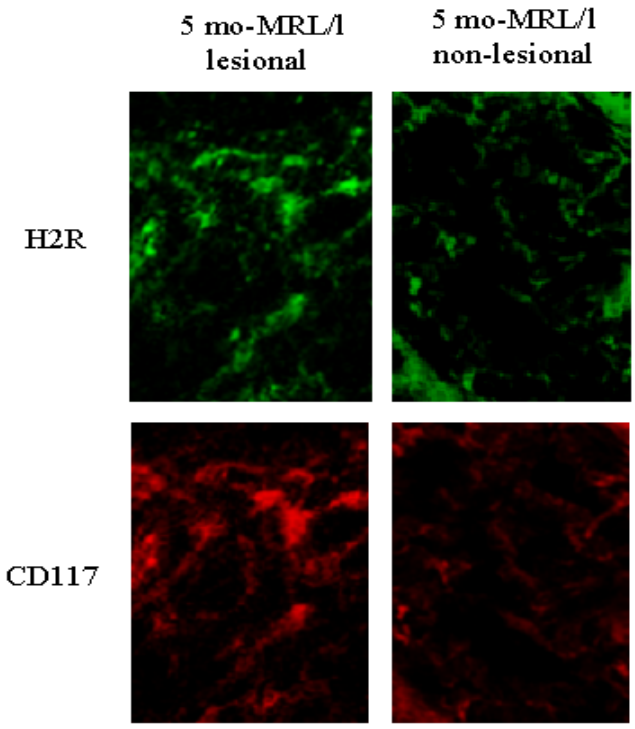

Fig. (3). Immunofluorescent studies of H2R and CD117 in lesional or non-lesional skin from 5-month-old MRL/l mice (x200). The expression of H2R was seen on CD117 positive cells infiltrated in the skin lesions from 5-month-old MRL/l mice.

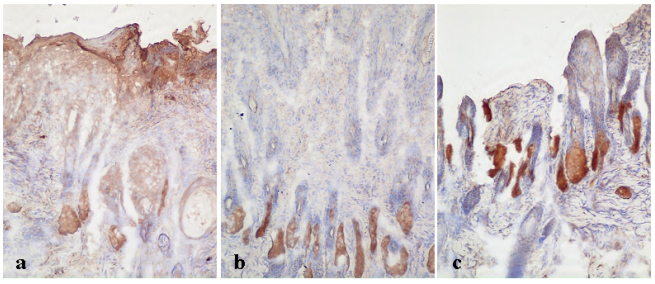

Fig. (4). Immunohistochemical staining of H1R (**a**), H2R (**b**) and H3R (**c**) in sebaceous gland of skin lesion from 5-month-old MRL/l mice (x 100). All histamine receptors are expressed in sebaceous gland.

decreased in skin lesions from 5-month-old MRL/l mice (Fig. **5**). The expression of H2R was not seen in skin from 1-month-old MRL/l and 5-month-old MRL/n mice. The expression of H3R showed an age-dependent increase in MRL/l mice. On the other hand, the expression of H3R in 5-month-old MRL/n mice was also seen and it was lower than expression from MRL/l mice at any given point in time.

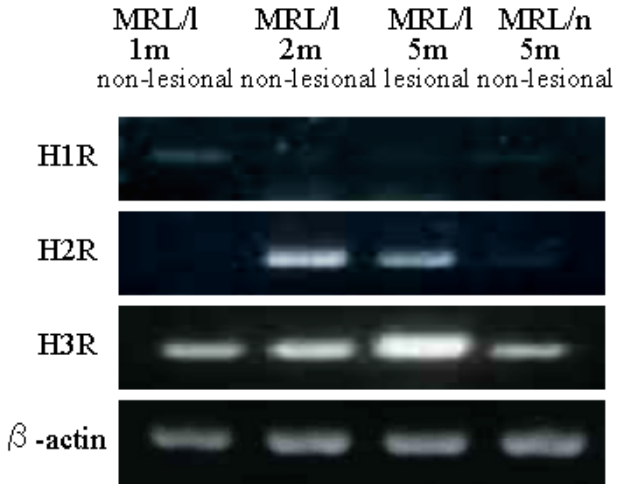

Fig. (5). HRs analyses by RT-PCR. H1R expression was very weak in the skin of MRL/l mice at any given point in time and 5-month-old MRL/n mice. The expression of H2R was seen in the non lesional skin 2-month-old MRL/l mice and its expression decreased in skin lesions from 5-month-old MRL/l mice.

DISCUSSION

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 seen 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-hisitamine [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 CD 14 expression between human and murine mast cells are reported [14]. Resent 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.

ACKNOWLEDGEMENTS

We wish to thank Taisuke Itou, Hamamatsu Medical University Japan, for his excellent advice to our immunofluorescent studies. This work was supported in part by Grants-in-Aid for Scientific Research of Japan.

REFERENCES

[1] Furukawa F, Tanaka H, Sekita K, Nakamura T, Horiguchi Y, Hamashima Y. Dermatopathological studies on skin lesions of MRL mice. Arch Dermatol Res 1984; 276: 186-194.

[2] Horiguchi Y, Furukawa F, Hamashima Y, Imamura S. Ultrastructual lupus band test in skin of MRL/lpr mice. Arch Dermatol Res 1986; 278: 474-480.

[3] 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 1991; 96: 478-483.

[4] Tachibana T, Furukawa F, Taniguchi S, Hamashima Y, Imamura S. Histamine metabolism in skin of MRL/lpr mice. Arch Dermatol Res 1985 278: 57-60.

[5] Furukawa F, Tachibana T, Taniguchi S, Imamura S. Significance of histamine metabolism in skin lesions of human and murine lupus erythematosus. Dermatologica 1989; 179: 128.

[6] Tachibana T, Taniguchi S, Furukawa F, Imamura S. Histamine metabolism in the Arthus reaction. Exp Mol Pathol 1986; 44: 76-82.

[7] Akdis CA, Blaser K. Histamine in the immune regulation of allergic inflammation. J Allergy Clin Immunol 2003; 112: 15- 22.

[8] Theofilopoulos AN, Dixon FJ. Etiopathogenesis of murine sle. Immunological Rev 1981, 55: 179-215.

[9] Nishide T, Yoshimasu T, Ikeda T, Seo N, Ohtani T, Furukawa F. Cytokine profiles of skin lesions in murine lupus models. J Dermatol Sci 2005; 1: s37-s43.

[10] Jutel M, Watanabe T, Klunker S, *et al.* Histamine regulates T-cells and antibody responses by differential expression of H1 andH2 receptors. Nature 2001; 413: 420-425.

[11] Pelle E, McCarthy J, Seltmann H, *et al.* Identification of histamine receptors and reduction of squalene levels by an antihistamine in sebocytes. J Invest Dermatol 2007; 128: 1280-1285.

[12] Lippert U, Artuc M, Grutzkau A, *et al.* Human skin mast cells express H2 and H4, but not H3 receptor. J Invest Dermatol 2004; 123: 116-123.

[13] Ohtani T, Aiba S, Mizutani M, Mollah ZU, Nakagawa S, Tagami H. H1 and H2 histamine receptors are absent on langerhans cells and present on dermal dendritic cells. J Invest Dermatol 2003; 121: 1073-1079.

[14] Bischoff SC. Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data. Nature Rev 2007; 7: 93-103.

[15] Benoist C, Mathis D. Mast cells in autoimmune disease. Nature 2002; 420: 875-878.

[16] Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. Nature Immunol 2005; 6: 135-142.

[17] Lu LF, Lind EF, Gondek DC, *et al.* Mast cells are essential intermediaries in regulatory T-cell tolerance. Nature 2006; 442: 997-1002.

Received: June 10, 2008 Revised: June 18, 2008 Accepted: June 25, 2008