

Is matrix metalloproteinase-9 a culprit involved in dermatitis? Increased expression of gelatinolytic activity in allergic contact dermatitis

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Introduction

Among the 24 species of matrix metalloproteinases (MMPs) that are encoded by distinct genes in mammals, MMP-2 and MMP-9 have important functions as gelatinases (1), and MMP-9 in particular plays important roles in tissue metabolism (1–5). Concerning the role of MMP-9 in inflammation of skin tissue, the reduced formation of antigen-mediated blisters in MMP-9 null mice has been reported (1). In contrast, prolonged delayed hypersensitivity in allergic contact dermatitis in MMP-9 null mice has also been reported (2). Thus, the precise role of MMP-9 in inflammation is unclear. This study was aimed at characterizing the gelatinolytic activities in the skin lesions of contact dermatitis in mice.

Methods

The dorsal skins of age-matched hairless mice (Hos: HR-1) 12 weeks of age were sensitized topically with 0.15% 2,4-dinitrofluorobenzene on D0. On D24, after three challenge procedures on D7, D14, and D21, the mice were killed. Lesional skins and non-lesional skins (without sensitization or challenge) were harvested with

the fat tissue just above the fascia, with no visible bleeding, and were weighed. Each specimen was then homogenized in 0.15 M NaCl/Tris-HCl, pH 7.4 (200 mg/ml). After

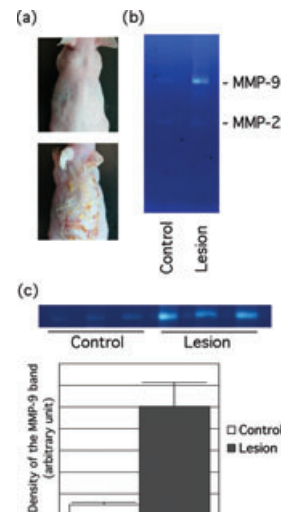


Fig. 1. (a) Clinical manifestation of mice without (upper panel) or with (lower panel) allergic contact dermatitis. (b) Gelatin-zymography patterns of skin samples from the lesion or from the site-matched and age-matched control; an aliquot of each homogenized skin sample (20 µg/lane) was analysed. (c) Gelatin-zymography patterns (upper panel) for matrix metalloproteinase (MMP)-9 in skin samples from the lesions and from the control, and the densitometric quantification of those bands in arbitrary units (lower panel); bars show averages of the quantified activities ($n = 3$) $\pm$ standard deviation.

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centrifugation at 15 000 *g* for 20 min at 4°C, an aliquot of each supernatant corresponding to 20 µg of the original weight was analysed by gelatin-zymography, and gelatinase activities were quantified in arbitrary units by densitometric analysis after scanning the gels as previously reported (6). All experiments were performed with institutional approval.

Results

Both in the non-lesional and in the lesional skin (Fig. 1a), two major bands of gelatinolytic activities, corresponding to MMP-9 and MMP-2, were detected (Fig. 1b). MMP-9 expression was especially increased in the lesional skin (Fig. 1b,c), and was approximately 10-fold higher than in the non-lesional skin (Fig. 1c).

Discussion

In contact dermatitis in humans, Giannelli et al., using immunohistochemistry, found increased expression levels of MMP-2 and MMP-9 in the involved skin of

positive patch test reactions as compared with uninvolved skin (3). Kusubata et al., using zymography, found a tendency for a temporary increase in the expression of those gelatinases in the acute phase of chronic contact dermatitis in mouse skin (4). Concerning the sensitization, in addition to the essential role of MMP-3, as shown using MMP-3 null mice (2), a role for MMP-9 in the migration of Langerhans' cells has also been proposed (1, 5). The sum of those findings indicates a role for MMP-9 in inflammatory lesions of allergic contact dermatitis, although the specific mechanism of such involvement of MMP-9 needs to be further elucidated.

This is the first study showing increased gelatinolytic activity by quantifying MMP function between lesional and non-lesional skin in allergic contact dermatitis. This approach to monitoring MMP-9 activity could be a useful tool with which to estimate the degree of inflammation in human skin tissue as well, and might prove to be a valuable model for further characterization of the pathogenesis of allergic contact dermatitis in human skin.

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