

Tissue inhibitors of matrix metalloproteinase-1 levels are increased in serum of patients with allergic contact dermatitis

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Allergic contact dermatitis (ACD) is an antigen-specific, T-cell-mediated skin inflammatory disease. Matrix metalloproteinases (MMPs) and their tissue inhibitors of matrix metalloproteinases (TIMPs) play a role in degradation of extracellular matrix and subsequent tissue remodelling during inflammatory process. The objective of this study was to examine a possible role of TIMP-1 and MMP-9 in the pathogenesis of ACD. The serum levels of MMP-9 and TIMP-1 have been measured using enzyme-linked immunosorbent assay in patients with disseminated ACD during exacerbation of skin lesions and the remission stage ($n = 20$) and were compared with healthy subjects ($n = 20$). The mean serum levels of TIMP-1 were significantly higher in patients with ACD than in control group (42.8 ± 4.9 ng/ml). This difference was more prominent in patients with ACD during remission (69.01 ± 6.99 ng/ml, $P < 0.0001$) than in patients with exacerbation of disease (46.8 ± 3.6 ng/ml, $P = 0.0054$). Mean values of serum MMP-9 did not differ significantly between patients with ACD, both in the acute and in the remission stage, compared with healthy persons ($P = 0.76$ and $P = 0.29$, respectively). TIMP-1 might be involved in pathogenesis of ACD. It seems that moderately increased levels of TIMP-1 could reflect degree of activity of skin inflammation, whereas markedly increased levels could contribute to the maintenance of the remission of disease.

Key words: allergic contact dermatitis; ELISA; MMP-9; TIMP-1. © Blackwell Munksgaard, 2007.

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Allergic contact dermatitis (ACD) is an antigen-specific, T-cell-mediated skin inflammatory disease. Despite better knowledge about pathophysiology of contact hypersensitivity (CHS) reaction, ACD is remaining common skin disease contributing to serious problems in occupational and daily living conditions. ACD is induced by contact with haptens in sensitized individuals. In the elicitation phase of disease, these are Langerhans cells, which play a key role by binding, processing, and presentation of allergen to T lymphocyte in the draining lymph nodes. The effector skin reaction is mediated by cytotoxic CD8+ T1 cells, which are recruited into the skin at the site of hapten challenge and induce keratinocyte apoptosis (1, 2). In some cases, ACD can develop to disseminated or even generalized form, but little is known about mechanisms responsible for such course.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases linked to degradation of extracellular matrix and tissue remodelling. They are associated with different

pathological processes that might result in infection, cancer, or degenerative and inflammatory diseases. Tissue inhibitors of matrix metalloproteinases (TIMPs), which affect normal and pathologic matrix remodelling, regulate activity of MMPs.

Among the MMP family, some of them have been implicated in pathophysiology of ACD. Immune cells, including T cells, Langerhans cells, and macrophages, produce MMPs such as stromelysin-1 (MMP-3) and gelatinase B (MMP-9). MMP-9 is highly efficient in the cleavage of denatured collagens of all genetic types and also degrades collagen IV, fibronectin, and elastin (3, 4). It was shown that MMP-3 is essential for the initiation of CHS by induction of T lymphocyte proliferation in response to specific antigen, and it was also shown that MMP-9 is necessary for the resolution of skin inflammation (5). It has been shown in mice (6, 7) and humans (8) that MMP-9 is essential for migration of Langerhans and dendritic cells from the skin in response to

epicutaneous application of sensitizer. The microarray studies in rats with chemically induced ACD also presented the evidence that of more than 8000 studied genes specifically upregulated is a group of MMPs, including MMP-9 along with TIMP-1 (9).

In another study, it was also shown that MMP-9 and gelatinase A (MMP-2) were increased in the involved skin of patients with ACD; however, serum MMP-2 and MMP-9 levels did not differ compared with healthy subjects (10).

In the present study, we examined the role of TIMP-1 and MMP-9 in the pathogenesis of ACD by evaluating their serum levels in patients with ACD in the acute stage of disease and in the remission. Results were related to those obtained in healthy individuals.

Patients and Methods

20 patients hospitalized because of disseminated form of ACD were selected for this study. The diagnosis of ACD was established in each patient on detailed history made before and after patch testing and clinical picture of skin lesions. The duration time of disease was expressed in mean 4.8 years (ranging from 2 weeks to 25 years) and time of the last exacerbation was in mean 2.8 weeks (range 1–6 weeks). Dissemination of the lesions was preceded by localized eczema area at the site of allergen challenge. The reason for dissemination of skin lesions depends on many established factors such as workplace condition, massive contact with allergens, and other difficult to precise factors; however, no history of systemically introduced allergens was evident. At the time of examination, all patients had multiple typical eczema areas of inflammatory skin lesions with indefinite borders and with presence of papules, vesicles, and erosions. Extent and severity of skin lesions were calculated using the eczema area and severity index (EASI) scoring system for assessing the activity of atopic dermatitis (AD), which ranges from 0 to maximum 72 scores (11). It seemed to us most appropriate to use. The mean value of the eruption score in a group of examined patients was 25.01 ± 10.5 (range 10.9–53.0).

Peripheral blood samples were collected twice: during exacerbation of disease (presence of papules, vesicles, erosions, and acute inflammation of the skin) and next in the stage of remission of skin lesions.

20 healthy individuals (5 women and 15 men) with the mean age of 47.9 ± 6.4 years (range 35–60 years) participated in this study as a control group. Individuals with familial or personal history of atopy were excluded. A venous antecubital

blood sample was collected from all patients before administration of any other medication and serum was stored at -70° Celsius in preparation for testing as a batch in the MMP-9 activity and TIMP-1 assays.

The commercially available enzyme-linked immunosorbent assay (ELISA) kit [Biotrak MMP-9 activity assay system (lower detection threshold: 0.5 ng/ml) and TIMP-1 Human; Biotrak Assay (lower detection threshold: 3.13 ng/ml), both from Amersham Pharmacia Biotechnology, Uppsala, Sweden] shows total MMP-9 activity (inactivated proenzyme and activated enzyme form) through activation of a modified proenzyme and subsequent cleavage of its chromogenic peptide substrate. TIMP-1 assay recognizes free TIMP-1 and that complexed with the active form of different MMPs. Duplicate samples were tested and averaged. The methods used were those described in the protocols accompanying the ELISA kits and are described in details for different options of MMP activity assays elsewhere (12, 13).

Data are presented as mean (ng/ml) of the serum TIMP-1 and MMP-9 values (in ng/ml) and standard deviation of the whole group. After checking of the normal distribution of the data, the serum values of TIMP-1 and MMP-9 in patients with disseminated contact dermatitis with each stage of ACD (acute or remission) were compared with each other and with those of control subjects, using two-tailed, non-paired *t*-test. Pearson's correlation test was used to assess the correlation between serum levels of MMP-9 and TIMP-1 and extent and intensity of the skin lesions as shown by EASI score.

A *P*-value of less than 0.05 was regarded as statistically significant.

Results

Characteristics of the examined and control groups are presented in Table 1.

The serum levels of TIMP-1 in patients with disseminated ACD in acute stage of disease ranged from 36.9 to 50.9 ng/ml, whereas in the remission, it ranged from 53.99 to 81.18 ng/ml. In the control group, the values ranged from

Table 1. Demographic characteristics of examined patients with ACD and control subjects

	Patients with ACD (<i>n</i> = 20)	Control group (<i>n</i> = 20)
Sex (% of women)	35.0	20.0
Age, mean (range), in years	53.8 ± 15.2 (26–78)	47.95 ± 6.4 (35–60)

ACD, allergic contact dermatitis.

32.1 to 52.8 ng/ml. Mean values of serum TIMP-1 were significantly higher in patients with ACD, both in acute (46.8 ± 3.6 ng/ml) and in the remission stage (69.01 ± 6.99 ng/ml), than in healthy individuals (42.8 ± 4.9 ng/ml) ($P = 0.0054$ and $P < 0.0001$, respectively). Elevation of mean TIMP-1 serum levels was more prominent in patients in the remission stage than in those with exacerbation of disease ($P < 0.0001$) (Fig. 1).

The serum values of MMP-9 in patients with acute ACD ranged from 2.4 to 25.2 ng/ml and from 3.19 to 15.73 ng/ml in the remission stage. The mean values of serum MMP-9 were not significantly different between patients with acute ACD (11.86 ± 8.01 ng/ml) and those in the remission stage (9.32 ± 2.89 ng/ml) compared with control subjects (11.15 ± 7.16 ng/ml) ($P = 0.76$ and $P = 0.29$, respectively) (Fig. 2). Mean values of MMP-9 did not differ significantly between two examined stages of ACD ($P = 0.19$).

The ratio TIMP-1/MMP-9 did not differ significantly between patients with acute ACD (7.19 ± 5.86) and healthy individuals (7.46 ± 7.21) ($P = 0.895$) and also in patients in the remission stage (8.01 ± 4.35) compared with the control group ($P = 0.77$). No differences was observed in TIMP-1/MMP-9 ratios between patients with ACD with acute and remission stages ($P = 0.61$) (Table 2). There was significant correlation neither between serum level of MMP-9 and EASI ($P = 0.967$) nor between TIMP-1 serum level and EASI ($P = 0.29$) (Fig. 3).

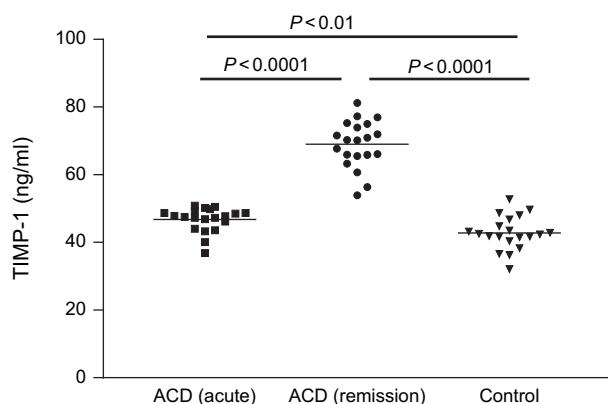


Fig. 1. Serum levels of tissue inhibitors of matrix metalloproteinases (TIMP-1) in patients with disseminated allergic contact dermatitis (ACD) and control subjects. Mean values of serum TIMP-1 were significantly higher in patients in the acute (46.8 ± 3.6 ng/ml) and in the remission stage (69.01 ± 6.99 ng/ml) than in healthy individuals (42.8 ± 4.9 ng/ml) ($P = 0.0054$ and $P < 0.0001$, respectively). Elevation of mean TIMP-1 serum levels was more prominent in patients in the remission stage than in those in exacerbation of disease ($P < 0.0001$).

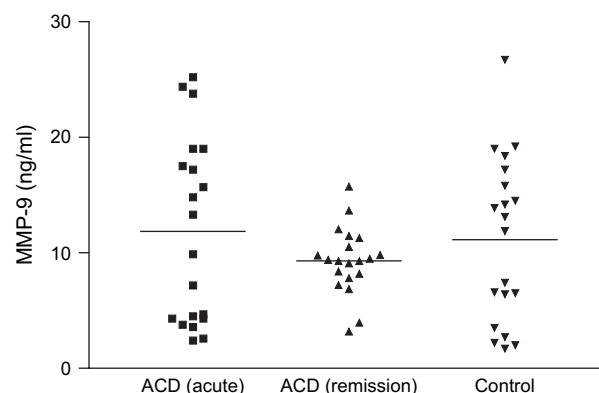


Fig. 2. Serum values of matrix metalloproteinase (MMP)-9 in patients with disseminated allergic contact dermatitis (ACD) and control subjects. MMP-9 concentrations did not differ significantly between control subject and patients with ACD both in acute stage of disease ($P = 0.76$) and in the remission stage ($P = 0.29$). MMP-9 values did not differ significantly between patients with ACD in acute and remission stage ($P = 0.19$) of disease.

Table 2. The values (mean $\pm$ SD) of TIMP-1/MMP-9 ratios in patients with ACD and healthy individuals

	ACD exacerbation (n = 20)	ACD remission (n = 20)	Control (n = 20)
Mean	7.185	8.010	7.461
SD	5.855	4.353	7.211

ACD, allergic contact dermatitis; MMP, matrix metalloproteinases; TIMP-1, tissue inhibitor of metalloproteinase-1.

Discussion

In this study, we present the first evidence of increased serum levels of TIMP-1 in patients with disseminated form of ACD both in the acute and the remission stage of disease. The evidence was obtained with a commercially available MMP-9 and TIMP-1 ELISAs.

Elevated serum TIMP-1 levels in patients in acute stage of disseminated form of ACD compared with healthy individuals is a novel finding; however, the elevated serum TIMP-1 values have already been seen in atopic dermatitis during exacerbation of skin lesions, which correlated positively to clinical and biochemical predictors of AD activity as well as to chronicity score (14). In a very recent animal study, Hartmann et al. performed transcriptional profiling of the skin of sensitized rats with chemically (2,4-dinitrofluorobenzene, DNFB) elicited CHS. They used broad rat genome-specific microarrays, containing 8799 probe sets comprising approximately 7000 full-length sequences and approximately 1000 expressed sequence tag clusters. After thorough analysis, it was found that there exist four specific expression patterns of which several genes like MMP-3,

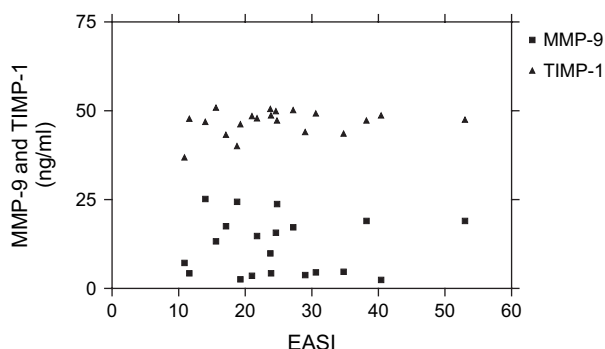


Fig. 3. Correlation of serum levels of matrix metalloproteinase (MMP)-9, tissue inhibitors of matrix metalloproteinases (TIMP-1), and eczema area and severity index (EASI). There was significant correlation neither (Pearson's test) between MMP-9 serum levels and EASI ($P = 0.96$) nor between TIMP-1 serum levels and EASI ($P = 0.29$).

MMP-9, MMP-12, MMP-13 and TIMP-1 were found to be significantly upregulated 1 day after DNFB challenge. It was presumed that those genes might potentially contribute to the mobility of invading and extravasating cells during ACD (9).

TIMP-1 is produced by keratinocytes, endothelial cells, and fibroblasts and together with MMPs by dendritic cells; however, the source of TIMP-1 both in the patients with AD and ACD is not yet clear. The pathophysiology of inflammatory reaction in skin lesions during ACD is similar to that developed in later stages of inflammatory lesions from atopic dermatitis, which is characterized by predominant role of Th1 lymphocyte (1). The characteristic feature of skin specimen from patients with ACD as well as from those with AD is a high intensity of skin inflammation owing to the activation of keratinocytes that has been shown to produce TIMP-1 (15). It could explain the increase of TIMP-1 secretion by keratinocytes. It is therefore feasible to assume that the elevated levels of TIMP-1 in the skin and serum might reflect the degree of intensity (activity) of the inflammatory process in the skin. Moreover, the peak of ACD reaction is followed by the beginning of the resolution of the acute inflammation and subsequent remodelling of the skin – possibly resulting from further increase in TIMP-1 levels. The mechanism by which MMPs and TIMPs are differentially regulated in different pathophysiological states is not fully understood. There have been quite a few situations reported where locally secreted MMPs also exert systemic effects. It can either take place in a neoplastic process or in chronic inflammatory states of different diseases. For example, in one of the more recent studies, it was shown that the serum levels of TIMP-1 are significantly higher in patients with chronic

obstructive pulmonary disease (COPD) than in controls; however, no differences in serum MMP-9 concentrations was observed. It was concluded that excess amounts of tissue inhibitors of metalloproteinase-1 may be associated with remodelling of lungs that occurs in COPD (16). It seems feasible that inhibition of the proteolytic action of MMP-9 by TIMP-1 in the skin is sufficient to prevent MMP-9 from being secreted by cellular compounds of the skin and consequently can limit its detection in the serum.

It is interesting that increased TIMP-1 serum levels were more prominent in patients with remission of ACD. An increased TIMP-2 level has been found in uninvolved skin specimens in opposition to site of positive patch test reaction in patients with ACD (8). In the early stage of effector phase of ACD, specific CD8+ effector cells are rapidly recruited to the site of hapten challenge before the onset of clinical and histological signs of inflammation. In the further course, CD4+ T cells are recruited, which coincides with both peak of CHS reaction and the beginning of the resolution of skin lesions. This CD4+ subset of T cells has inhibitory effect on CD8+ cells, which is mediated by interleukin (IL)-10 (17). It has been shown that IL-10 promotes production of TIMP-1 by macrophages (18).

MMP-9 serum levels were not found to be increased in patients with disseminated contact dermatitis although it could be expected in the acute stage of disease, when skin destruction is predominant. This, however, may be explained by specific timing of MMP-9 secretion. It is secreted by Langerhans cells, which play a key role in the sensitization phase of ACD (7, 9, 19). Because all patients in our study had the already developed stage of disease, it could at least partly explain why their sera did not show an increased activity of MMP-9.

To our knowledge, no alternations in serum MMPs and TIMPs levels was found in patients with ACD, but all the studies described cases that involved patients with only localized lesions or skin site of the positive patch tests reactions. All of our patients presented disseminated or generalized form of ACD.

We realize that it is difficult to draw lasting and firm conclusions on the pathogenic role of MMP-9 and TIMP-1 in ACD because no other types of inflammatory skin lesions has been investigated. However, an important input as to MMP-9 also comes from genetically modified models. In one of the earlier studies on MMP-9-deficient mice with DNFB-induced CHS and those in which an acute non-immune-mediated process of irritation was provoked by cutaneously applied phenol (20). It was shown that DNFB-treated animals

exhibited a CHS response, which persisted significantly longer compared with wild-type control mice, whereas normal rate of resolution of inflammation was observed after phenol treatment. The results allowed the authors to conclude that MMP-9 plays a critical role in the resolution of immunologically mediated CHS but most likely not in non-immunological type of lesion (20).

Results obtained by others and our own indicate that MMP-9 and particularly TIMP-1 are likely to be involved in the pathogenesis of ACD. The moderately increased TIMP-1 levels in acute stage of ACD may reflect degree of activity of skin inflammatory process. High serum values of TIMP-1 in the remission stage of ACD may reflect their role in the maintenance of dominance by repression of activity of metalloproteinases and retention of the dendritic cells in the skin as well as inhibition of T-cell migration to skin (20, 21). We believe that detection of TIMP-1 levels in serum during contact dermatitis, along with normally used patch tests, might be potentially considered as a combined approach for detection and evaluation of the likelihood of progression or remission of contact dermatitis.

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