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آموخته مهارت‌های کاربردی در ندوین و چاپ مقاله
Dermal Wound Fibroblasts and Matrix Metaloproteinases (MMPs): Their Possible Role in Allergic Contact Dermatitis

Mohammad Reza Khorramizadeh, Reza Falak, Mohammad Pezeshki, Farnaz Safavifar, Parvin Mansouri, Aziz Gahary, Farshid Saadat, and Kamran Varshokar

1Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2Hematology, Oncology and BMT Research Center, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
3Department of Dermatology, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran
4Faculty of Medicine, University of Alberta, Edmonton, AB, Canada

ABSTRACT

This study was conducted to examine if allergic contact dermatitis (ACD) alters the expression of MMPs in human dermal fibroblasts. Fibroblasts are the primary source for MMP and matrix production in skin. MMPs are known to involve in a number of physiological and pathological processes. Some published data indicated a gelatinase-like activity in acute and chronic phases of allergic contact dermatitis. However, no exact source of gelatinase activity was demonstrated. Moreover, little is known about the role of MMPs in immune responses.

To study and predict the pathophysiological effects of (MMP-2) in allergic contact dermatitic (ACD) patients, we established an in vitro tissue culture survey based on fibroblast explanted from ACD wounds and normal tissues respectively. We also employed a precise proliferation assay [i.e. MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to analyze and compare three ACD vs. three normal cell strains. Parallel to MTT assay, we assessed the activity as well as the kinetics of gelatinase (MMP-2) in conditioned media using a zymogeraphy analysis.

RESULTS: There was a significant difference in proliferation capacity between mean ACD fibroblast strains vs. mean normal cells, particularly in days 6 to 8 post explantation, 492.5±6.6 vs. 361.75±8.25 respectively. Zymoanalyses indicated significant differences between ACD cells and normal fibroblasts both in time-course and MMP-2 activity per cell fashions, 163.7±16.21 for mean ACD fibroblasts vs. 130±9.09 for normal cells respectively.

These data suggest that fibroblasts overproliferated in the process of ACD. Moreover, simultaneous overexpression of MMPs observed in ACD fibroblasts vs. normal strains, is indicative of altered fibroblast functionality in the process of allergic contact
INTRODUCTION

Allergic contact dermatitis (ACD) is a T cell-mediated immune response. This process is initiated by a challenge with an immunogen, followed by processing of antigen through skin dendritic cells (i.e., Langerhans cells), migration of antigen-bearing Langerhans cells to regional lymph nodes and stimulation of naive T cells.1,6 A second exposure of the same antigen to skin, then results in local influx of antigen-specific T cells which release cytokines and inflammatory mediators. These components attract other inflammatory cells to the site of exposure, dilate cutaneous blood vessels, and cause dermal edema.7

Matrix metalloproteinases (MMPs) are a family of highly homologous, zinc-dependent endopeptidases involved in extracellular matrix turnover, connective tissue damage, inflammation and cell proliferation. In spite of identifying several pathways of the degradation of ECM, most investigators admitted that matrix metalloproteinases (MMPs) are critical enzymes in ACD process.8,9 Among members of this family of human MMPs, Gelatinase A and B (MMP2 and 9) degrade basement membrane collagens type IV, gelatin and other proteoglycan component of the ECM.10,12 It has been shown that collagen in skin with chronic contact dermatitis comprised 60% of type I collagen and 40% of type III collagen, the latter being higher than the content of type III collagen in control normal skin. An increased collagen-degrading activity was also observed in fibroblasts when incubated in contact with collagen of chronically inflamed skin.13 It has been suggested that MMP-2 and MMP-9 could play a role in the mechanisms including alterations of the epidermal architecture, and in the pathogenesis of ACD lesions. In contrast, no MMP level difference was observed between serum of ACD patients and healthy subjects.14 In the present study, we sought to determine whether the expression of MMPs in human allergic contact dermatitic fibroblasts was altered or not.

MATERIALS AND METHODS

Collection of Biopsies: Upon individual written consents, six allergic contact dermatitic (ACD) patients (aged between 20 to 40 years) and two normal individuals (aged 25 and 35 years, respectively) were included in this study. Punch skin biopsies from upper abdomen were collected under sterile conditions. Initial and confirmatory medical examinations as well as collection of the biopsies were performed by an authorized specialist at Dermatology Ward, Imam Khomein Hospital, Tehran University of Medical Sciences. Biopsies were received in DMEM+Antibiotics, on ice, to the Tissue Culturer Lab. Then, Dermal side was chosen for explantation and dissociated by two scalpels into small pieces placed in 25 cm² tissue culture flasks and incubated at 37oC, 5% CO2 and saturated humidity. After monitoring for fibroblast migration and 50-75% confluency, pieces were moved to another set of flasks, and the above procedure repeated. Synchronous cultures at passages 3 to 7 were used for MTT and Zymography analyses.

Viability Analysis of Cultured Cells by MTT Assay: 2x104 cells in a volume of 200ul DMEM [supplemented with 5% fetal bovine serum (FBS), antibiotics (100U/ml penicillin and 100ug/ml streptomycin)] were plated to each well of a 96-well microplate, in a pentaplicate fashion. After overnight incubation at 37°C in 5% CO2, medium was replaced with 200ul per well of fresh medium containing 10mM HEPES (pH 7.4), immediately followed by addition of 50ul MTT in PBS at a concentration of 5mg/ml. The plates were wrapped in foil and incubated for 4hrs at 37°C in 5% CO2. The medium was then replaced with 200ul DMSO(dimethyl sulfoxide) per well, followed by 25ul Sorensen’s glycin buffer(0.1M glycin plus 0.1M NaCl equilibrated to pH
10.5 with 0.1M NaOH). After 15 min incubation in dark O.D. of the plates were read at 570nm on a ELISA plate reader. Cell density was calculated according to a previously determined standard curve.

Collection of Samples for Assessment of Gelatinase: Equal number of synchronized normal skin and ACD fibroblasts were separately placed in 96-well microplates, in a pentaplicate fashion. Every day a set of wells (4-8 well) was chosen from each plate for gelatinase-A (MMP-2) activity assay and the collected medium was centrifuged at 8000g, for clarification.

Zymoanalysis: This technique has been used for the detection of gelatinase (collagenase type IV or matrix metalloproteinase type 2, MMP-2) and MMP-9, in conditioned-media according to Heussen and Dowdle method with some modifications. Briefly, aliquots of conditioned media were subjected to electrophoresis in (2mg/mL) gelatin containing polyacrylamide gels, in the presence of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing conditions. The gels underwent electrophoresis for 3 hours at a constant voltage of 80 volts. After electrophoresis, the gels were washed and gently shaken in three consecutive washings in 2.5% Triton X100 solution to remove SDS. The gel slabs were then incubated at 37°C overnight in 0.1 M Tris HCl gelatinase activation buffer (pH 7.4) containing 10mM CaCl₂ and subsequently stained with 0.5% Coomassie Blue. After intensive destaining, proteolysis areas appeared as clear bands against a blue background. Using a UVI Pro gel documentation system (GDS-8000 System), quantitative evaluation of both surface and intensity of lysis bands, on the basis of grey levels, were compared relative to non-treated control wells and expressed as “Relative Expression” of gelatinolytic activity.

Statistical Analyses: The differences in cell prolif-
eration and gelatinase activity were compared using the Student’s t test. P values <0.05 were considered significant.

RESULTS

We established six different specimens of fibroblasts from dermatitis wounds and two normal dermal fibroblasts as controls. As depicted in Figure 1, the difference in proliferation capacity between mean ACD fibroblast strains vs. mean normal cells, particularly in days 6 to 8 post explantation was 492.5±6.6 vs. 361.75±8.25 respectively. Likewise, the results of proliferation assay revealed a significantly (p<0.05) higher MTT for ACD fibroblasts than those derived from normal skin.

As shown in Figure 2 cultured allergic contact dermatitic fibroblasts (P.1 to P.6), subjected to gelatin-A zymography, exhibits significantly higher MMP-2 activities, as compared to normal cells (N.1 and N.2, respectively).

The results of zymoanalyses indicated significant differences (p<0.05) between ACD cells and normal fibroblasts both in time-course and MMP-2 activity per cell fashions, which was 163.7±16.21 for ACD fibroblasts vs. 130±9.09 for normal cells respectively (Figure 3).

DISCUSSION

Matrix metalloproteases (MMPs) are proteolytic enzymes involved in tissue remodelling and ECM turnover. They are secreted in a latent form and activated at the cellular surface by a membrane type-1 MMP (MT1-MMP) and a tissue inhibitor of MMP-2 (TIMP-2) which is also responsible for striking a balance between the proteolytic enzymes and TIMP-2.17,19 Matrix metalloproteases (MMPs) may be involved in one or more steps of ACD sensitization. It is probable that MMPs are required for detaching the resident Langerhans cells in the suprabasal portion of the epidermis, from adjacent keratinocytes through the basement membrane at the dermo-epidermal junction.20,21

In the present study, we showed that ACD fibroblasts exhibit overproliferative capacity with simultaneous overexpression of MMP-2 activity. Concerning the results of relative activity of MMP per cell analysis, MMP-2 expression in ACD fibroblasts is independent of cell number. These observed alterations in ACD fibroblasts, vs. normal cells, are indicative of altered fibroblast functionality in the process of allergic contact dermatitis. A correlation of IL-10 expression with MMP-2 has been shown in a mouse model suggesting a role for Th1/Th2 switching in the pathogenesis of ACD.22 Altogether, we can conclude that MMP-2 could play a major role in the mechanisms inducing alterations of the epidermal architecture, however, more studies are required to elucidate intra-or inter-cellular control signaling alteration involved in exhibition of ACD fibroblasts hyper-responsiveness to mitogenic or fibrogenic stimulants.

REFERENCES

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