Mast cells in the inflammatory connective tissue diseases

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Objective: To detect mast cells in normal human skin and mast cell infiltration in skin biopsies in patients with systemic sclerosis, systemic lupus erythematosus, Raynaud’s phenomenon, and persons not suffering from any systemic disease.

Material and methods: Paraffin histological sections were stained with toluidine blue and by immunoperoxidase method for the von Willebrand factor.

Results: In normal human skin mast cells were mostly distributed in the perivascular area of the upper dermis, the perifollicular area and sweat glands. Normal skin mast cell density varied from case to case. In the initial stage, SSc skin showed a significantly increased density of mast cell infiltration as compared with normal controls. Extensive deposition of granules was observed extracellularly around mast cells. There was a wide range of variation in degranulation among the specimens. In advanced stages, SSc was accompanied by flourishing vasculitis and in the most of specimens showed a significant decrease in the density of mast cells. In the late stage of SSc, in areas where extensive deposition of collagen fibers was present and a relatively severe sclerosis was noted, few or no mast cells were present. In SLE patients, the number of mast cells and their degranulation varied depending on the stage of the disease. In patients with Raynaud’s phenomenon, mast cell infiltration usually did not differ from the patterns found in healthy controls. Expression of vWF factor was found predominantly in endothelial cells and reflected changes in the blood vessel density in different stages of the diseases under study. In edematous tissues, occasionally extravascular leakage of vWF was noted. In the late stage of SSc dominated small blood vessel occlusion, atresia and progressing of fibrosis tissues. No vWF leakage into the perivascular interstitial matrix was detectable in this stage, and the vWF staining was usually restricted to endothelial cells. No vWF leakage was found in healthy skin from control subjects.

Conclusion: Skin mast cell density considerably varies among healthy persons. In the early stage of SSc, augmentation of mast cell number and activity of degranulation were found. In the late stages of SSc, the density and activity of degranulation of mast cells decrease. In SLE and Raynaud’s phenomenon patients, generally the population and activity of mast cells varied depending on the stage of disease and were lower than in SSc.

Key words: mast cells, human skin biopsies, systemic sclerosis (SSc), systemic lupus erythematosus (SLE), Raynaud’s phenomenon (RP)

INTRODUCTION

Mast cells are potent effector cells of the immune system. Upon stimulation, they release a heterogeneous group of factors that promote inflammation and influence cell proliferation. The observations, which date back to Paul Ehrlich, suggest that these enigmatic cells are functionally relevant to many aspects of tissue physiology, inflammation and vasodilatory responses to environmental antigens (1). It is conceivable that mast cells participate in the process of inflammation, angiogenesis, matrix degradation and tissue remodeling (2). Mast cells contain a variety of potent mediators such as histamine, heparin, proteinase and multifunctional cytokines. Many of their products (for example, heparin, histamine and TNFα) are able to stimulate fibroblast proliferation and collagen...
synthesis (3–6). Tryptase and chymase are specific mast cell proteases (7). Physiologically, mast cells could be in resting or activated (degranulated) states.

Systemic sclerosis is a generalized connective tissue disorder with an unknown cause (8, 9). It is characterised by cutaneous sclerosis attributable to increased synthesis of collagen, glycosaminoglycans and other connective tissue substances by dermal fibroblasts (10). Seibold et co-authors (11) pointed out that degranulated mast cells exist as an activated form with increased mast cell numbers in the dermis of early and late phases of scleroderma. Nevertheless, other studies on mast cell numbers in skin from SSc patients have given variable results (12). It has been postulated that mast cell numbers increase in the beginning of the SSc but reduce to normal values as the disease progresses (13); when fibrosis of the skin is evident, the epidermis becomes fixed to the deeper subcutaneous tissues and vascular insufficiency occurs. However, the extent of the contribution of mast cells and their precise functional role remain to be elucidated in SSc.

Endothelial cell activation is believed to be an early event in the pathogenesis of SSc. There are anti-endothelial cell antibodies present in the serum of scleroderma patients, but their role in the pathogenesis of the disease remain obscure. Endothelial alterations probably may lead to a cascade of stimulatory changes that involve many cells, including fibroblasts, T lymphocytes, macrophages, and mast cells. In turn, the activated cells secrete a variety of substances, including cytokines and their soluble receptors and enzymes, and their inhibitors. In such circumstances, increased collagen production or disturbances in its degradation can cause excessive collagen deposition in tissues.

Raynaud’s phenomenon, a vascular disorder triggered by cold or emotional stress, results from an exaggerated vasoconstriction and vasospasm of the digital arteries and arterioles. The fingers and, less often, the toes are affected; ear lobes, lips, nose, and nipples may also be clinically involved. There are differences between primary and secondary Raynaud’s phenomenon (14). Unlike SSc, no extent fibrosis accompanies Raynaud’s phenomenon.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by immunological hyperactivity, vascularitis and multi-system organ damage. In contrast to SSc, fibrosis and vascular atresia are not characteristic of SLE.

The aim of the present study was to analyze mast cell infiltration in pathogenetically different systemic connective tissue diseases in which different kinds of vascular dysfunction, vasculitides and fibrotic changes could be diagnosed.

MATERIALS AND METHODS

Patients and biopsies

Twelve SSc patients aged 29–60 years (mean, 46.75), thirteen SLE patients aged 25–73 years (mean, 43.62), seven Raynaud’s phenomenon patients aged 24–54 years (mean, 37.57), and thirteen subjects not suffering from any systemic diseases were examined physically before a biopsy was taken. All patients were hospitalized, gave their informed consent and met the diagnostic criteria established for a particular disease under study. All biopsies were from the forearm skin. The specimens were fixed in 10% neutral formalin, embedded in paraffin and processed for staining with hematoxylin-eosin, toluidine blue at pH 2.0 for mast cell detection and for immunohistochemical staining to detect the von Willebrand factor (vWF).

Immunohistochemistry

The primary antibodies used were serum protein absorbed rabbit anti-human vWF IgG (1:500, Dakopats A/S, Glostrup, Denmark). Paraffin sections (5 µm) were mounted on DAKO Capillary slides (TechMate™ DAKO, Glostrup, Denmark), deparaffinized in xylene and rehydrated in a graded ethanol series and 10 mM phosphate-buffered, 0.9 M saline, pH 7.4 (PBS). For antigen retrieval, the slides were pretreated with 0.4% pepsin in 1N HCl at +37°C for 30 minutes. Then the slides were washed and stained automatically in a staining robot by the following protocol: 1) the primary antibody, diluted with DAKO ChemMate™ antibody diluent, for 30 minutes; 2) secondary antibody containing biotinylated goat anti-rabbit IgG for 30 minutes; 3) peroxidase block for 30 minutes; 4) peroxidase-conjugated streptavidin 3 times for 3 minutes; 5) HRP Substrate Buffer, and finally 6) substrate working solution containing 3,3’-diaminobenzidine tetrahydrochloride (ChemMate™ Detection Kit) for 5 minutes. Between each step, the sections were washed with DAKO ChemMate™ washing buffers three times and dried in absorbent pads. After staining the sections were removed from the robot, counterstained with hematoxylin or left without counterstaining, washed, dehydrated in ethanol series, cleared in xylene and mounted in synthetic mounting medium (Diatax, Beckers Industrifärg AB, Märsta, Sweden). Replacement of the primary antibody with normal rabbit IgG in corresponding dilution was used as a negative staining control. All incubations were performed at +22°C.

Microscopic examination

Mast cells were counted under high magnification (×400) of a light microscope. The number of metachromatic mast cells was counted in ten high power fields of the specimen, from the papillary to subcutaneous layer. The total number of cells was counted. Statistical analyses were performed by Student’s test.

Diffusion of vWF from the blood vessels into extracellular/perivascular space, and perivascular mast cell degranulation were scored as none (0), mild (+), moderate (+++) or strong (++++)+. The score evaluation was done by two microscopists independently.

RESULTS

Mast cells were found predominantly in the subepithelial tissue near blood vessels and nerves and usually were sprinkled diffusely without forming clusters, and their
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cytoplasm contained metachromatic granules. Toluidine blue stained mast cells red-purple (metachromatic staining) and the background blue (orthochromatic staining). In tissue sections stained with hematoxylin and eosin, mast cells usually displayed a round-to-oval nucleus with clumped chromatin and indistinct nucleoli. Mast cells had moderately abundant cytoplasm and were oval, spindle, or polygonal in shape. The cytoplasm was amphophilic, and small slightly eosinophilic granules were visible.

In normal skin, mast cells were preferentially distributed in the perivascular area of the upper dermis, the perifollicular area and the sweat glands. Normal skin mast cell density was slightly variable in the perifollicular and sweat gland areas of each specimen. Grade 1 SSc skin (Fig. 1A, table) showed a significantly increased density of mast cell infiltration as compared with normal controls. Extensive deposition of granules was observed in extraacellular locations around mast cells. However, there was a wide variation among the specimens. Grade 2 of SSc accompanied by flourishing vasculitis showed a significant decrease in the density of mast cells, or even their absence (Fig. 1 B). In these specimens, oedema between collagen bundles was outstanding. In the late stage of SSc, in areas where extensive deposition of collagen fibers was present and relatively severe sclerosis was noted, few or no mast cells were present (Fig. 1 C).

SSc gradation was as follows: grade 1, edema in both papillary and reticulardermis with partial homogenization of collagen bundles in the reticular dermis; grade 2, homogenization of collagen bundles in the reticular but not in the papillary dermis; and grade 3, homogenization of collagen bundles in both papillary and reticulardermis.

Blood vessel walls underwent necrobiosis and dystrophy (Fig. 1 B), their lumina gradually narrowed. The endothelial cells were shrunken.

Increased numbers of mast cells were noted in the papillary dermis of grade 1 skin of scleroderma. Reticular dermis of grade 2 and 3 skin did not show any difference in mast cell distribution from the normal skin.

We have shown an increased mast cell number and intensity of their degranulation in SSc patients with early edematous and indurative phase of the disease. It was especially remarkable in the papillary dermis. In patients with the late fibrotic phase of SSc, the mast cell number and degranulation decreased compared with the initial phases of the disease and the controls. At this stage the papillary layer was filled with homogeneous collagen bundles. A change in mast cell number and the intensity of degranulation reflected the change in interstitial collagen bundles in the papillary layer.

In SLE patients, the number of mast cells and their degranulation varied depending on the stage of the disease (Fig. 1 D, E). In the patients with Raynaud’s phenomenon mast cell infiltration usually did not differ from the patterns found in healthy controls (Fig. 1 F).

Expression of the von Willebrand factor was found predominantly in the endothelial cells and well reflected changes in blood vessel density in different stages of the diseases under study and the state of these vessels (Fig. 2).

| Table. Mast cell number per HPF (high power field) |
|-----------------|-----------------|-----------------|
|                 | Patients, n | Value          |
| SSc             | 12           | 5.92 ± 6.68    |
| SLE             | 13           | 9.31 ± 4.57    |

p = 0.037 (independent sample t test).

Fig. 1. Mast cells in the skin of patients with connective tissue diseases. A, B, C – in systemic sclerosis; D, E – in systemic lupus erythematosus; F – in Raynaud’s syndrome. Staining with toluidine blue. Original magnification ×400. Abbreviations: bv – blood vessel, ep – epidermis. Arrows indicate mast cells.

Fig. 2. Immunohistochemical staining of the endothelium. Expression of vWF in inflamed skin vessels of SLE patient. Original magnification ×400. Arrows indicate sites of vWF expression.
In the edematous stages, occasionally extravascular leakage of vWF was noted. In the late stages of SSc dominated small blood vessel occlusion, atresia and progressing tissue fibrosis. In the advanced fibrotic stage of SSc when the blood vessel network was dramatically reduced, no vWF leakage into the perivascular interstitial matrix was detectable, and the vWF staining was usually restricted to endothelial cells. No vWF leakage was found in the healthy skin from control subjects.

DISCUSSION

The distribution of mast cells in normal skin found in our observations corresponds to the data of other authors (13, 15). We have shown an increased mast cell population in the clinically involved skin of patients with early edematous and indurative phases of progressive systemic sclerosis, however, mast cell numbers were about normal in patients with later stages of the disease. Augmented mast cell infiltration was specific of clinically involved skin at this stage of the disease. Mast cell numbers varied in normal uninvolved skin of the same patients. The discrepancy of data from different authors concerning mast cell infiltration is likely due to different techniques of examination of skin tissues. We used a simple and reliable mast cell staining with toluidine blue at low pH and vWF immunostaining to detect small blood vessels. We found that the number and activity of mast cells depended on the histologic stage of a disease and on the primary status of mast cells in a particular patient. Skin mast cell density is highly variable among healthy persons (16) and known to decrease with age. No significant difference in dermal mast cell concentration between men and women was seen. The reversion of mast cell density to levels comparable with normal ones in longstanding skin diseases presumably reflects the pleiotropic functions of these cells. Local mast cell hyperplasia has been noted in association with inflammation and extensive deposition of connective tissue. Mast cells infiltrated and degranulated in a close spatial relation between mast cells and fibroblasts are involved in the fibrotic response in the area. Some mast cells secreted their granules into the dermis.

An increment of the mast cell number was noted in both papillary and reticular dermis. Mast cell increment and then decrement in papillary dermis was shown in another study (17). Histamin is a major component of mast cell granules and is responsible for vascular permeability leading to edema (18). Granules in mast cells can stimulate collagenase and beta-hexosaminidase production by fibroblasts, and these enzymes can cause destruction of the connective tissue. Earlier, it had been found that inflammatory cell infiltration coincided with the increased numbers of mast cells in sclerotic skin (19, 20). Mast cells in scleroderma have been discussed for the past decades (17, 21) without any definite conclusion. It is almost obvious that depending on the tissue environment and condition mast cells express different patterns of surface receptor profile. The mere presence of mast cells alone is not sufficient for the development of fibrosis. It has been shown that the absence of mast cells has no effect on the proliferative aspects of wound healing, including reepithelialization, collagen synthesis, and angiogenesis (22). The frequency of vascular changes in progressive systemic sclerosis is inversely proportional to the size of blood vessel. Hand and finger arteries are often involved, arterioles and capillaries still more frequently. Whether scleroderma is a collagen or a microvascular disease remains an open question. Systemic sclerosis is a widespread connective tissue disease characterized by inflammatory, fibrotic, and degenerative changes in the skin, digital circulation, synovium, skeletal muscle, and certain internal organs (23, 24). The spectrum of SSc ranges from rapidly progressive generalized skin thickening (diffuse cutaneous involvement) to a more indolent form in which skin thickening is most frequently restricted to the fingers and face (limited cutaneous involvement). There are evidences that mast cells are involved in the development of interstitial edema in very early stages of SSc pathogenesis (3, 7). In the present study, in patients with an initial stage of SSc we found atypical capillary proliferation–bushy capillaries (25). This is in agreement with experimental observations (26) that SSc initially can evoke angiogenesis. We found that mast cells are numerous at this stage. In the late stages of SSc we found only blood vessel deterioration and atresia, and few or no mast cells in the area. In surrounding vessel-free tissues, firm fibrosis dominated. Other, kinds of damage typical of SSc blood vessel (27–29) were also found in our study.

Significant positive correlations between interstitial mast cell count and relative interstitial volume support the role of these cells in the development of interstitial fibrosis, however, this relationship needs further investigations (30). Our analysis has confirmed earlier observations that there is no correlation between the intensity of lymphoid infiltration and the number of mast cells (31).

Over the past number of years numerous data have been published regarding increased atherosclerosis in patients with systemic lupus erythematosus (SLE), and it has been shown that premature or accelerated atherosclerosis is an important cause of morbidity and mortality in these patients. Besides the traditional risk factors for cardiovascular disease, the association between SLE and atherosclerosis can be attributed to additional risk factors closely related to inflammation and autoimmunity. In particular, several autoantibodies and their respective autoantigens have been identified as possible factors in the development and progression of the atherosclerotic process in SLE. The understanding of SLE-related risk factors for enhanced atherosclerosis could shed more light on disease mechanisms, leading to new therapeutic strategies for the treatment of cardiovascular diseases in SLE patients (32). In our study of SLE skin samples, the number and activity of mast cells varied depending on the stage and activity of the disease.
Although an association between mast cell expansion and skin fibrosis has been reported and is confirmed in our study, the precise mechanisms underlying mast cell accumulation remain unclear (33). There was a considerable overlap between the numbers of mast cells in mastocytosis and normal skin (34). In agreement with earlier observations (35), it is reasonable to conclude that mast cells participate in a broad spectrum of interactions in the pathogenesis of autoimmune diseases and regulate many connective tissue functions.

CONCLUSION

Skin mast cell density is rather variable among healthy persons. In early stages of SSc, augmentation of mast cell number and degranulation activity was found. In the late stages of SSc, the density and activity of degranulation of mast cells decrease. In SLE and Raynaud’s phenomenon patients, generally the population and activity of mast cells varied depending on the stage of disease and were lower than in SSc.

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References


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PUTLIOSIOS ĖMENĖS UŽDEGIS
JUNGiamojo audino patologijoje

Santrauka
Darbo tikslas: Nustatyti putlių ląstelių infiltraciją audinio odos biopatuose sergentiems mastcellėms, sistemine raudojana viškige ir Raynaud’o sindromu.

Medžiaga ir metodai: Parafinų pjūviių dažynas tolius mėlij mėliu ir imunoperoksidazinis metodas von Wilebrando veiksnui nustatyti.


Išvada: Putlių ląstelių tankis sveikių žmonių odoje svyravo. Ankstyvoje SSc vystymosi stadijoje buvo pastebėtas putlių ląstelių kiekio ir degranuliacijos padidėjimas. Vėlyvos SSc stadijoje putlių ląstelių kiekis ir degranuliacija sumažėdavo. SLE ir Raynaud'o fenomeno atvejais dažniausiai putlių ląstelių skaičius ir degranuliacija priklausė nuo ligos stadijos ir buvo mažesni, lyginant su SSc.

Raktažodžiai: putliosios ląstelės, žmogaus oda, sistemė sklerozė (SSc), sistemė raudojana viškigė (SLE), Raynaud'o (Reino) sindromas (RP).