

FIBRINOGEN/FIBRIN DEGRADATION PRODUCTS IN EXUDATES FROM BULLOUS DERMATOSES

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Abstract. Inflammatory exudates from 10 patients with bullous skin diseases were analysed by immunochemical techniques including crossed immunoelectrophoresis. The results were compared with those obtained in fluid from suction bullae obtained in normal skin in 13 control subjects and synovial fluid from 20 patients with rheumatoid arthritis. Abnormal fibrinogen degradation products identical with those found in synovial fluid from patients with rheumatoid arthritis were detected in exudates from each of the patients with bullous dermatoses, whereas significantly smaller amounts of fibrinogen antigenic material were detected in fluid obtained by suction. The fibrinogen antigenic material demonstrated in exudates from pathological bullae, immunochemically similar to that found in rheumatoid synovial fluid, indicates that the presence of these products reflects the more general features of an inflammatory exudate.

Key words: Fibrinogen/fibrin; Bullous dermatoses; Inflammatory exudates; Synovial fluid; Suction bullae

Abnormal fibrinolytic activity has previously been described in various cutaneous disorders, including vasculitis (4), psoriasis (6, 8) and bullous diseases—notably dermatitis herpetiformis (11, 18). Common features in the deposition of fibrinogen/fibrin antigenic material in skin and synovial tissue from patients with psoriatic arthritis may reflect common mechanisms of formation in the tissues. In synovial fluid in patients with rheumatoid arthritis, fibrinogen degradation products of possible pathogenetic significance have been detected recently (3).

These degradation products seem unrelated to the fibrinolytic system (3). Their presence in inflamed rheumatoid joints may not necessarily be specific for this disease. They may equally well be non-specific products of an inflammatory reaction, subsequently detectable in other types of inflammatory exudates including easily accessible skin exudates.

We have therefore studied inflammatory exudates in a group of patients with various bullous skin diseases and compared these results with those obtained in a control group in whom suction bullae were raised on normal non-inflamed skin. The results have been compared with those found in rheumatoid synovial fluid.

MATERIAL AND METHODS

Inflammatory exudates from patients with bullous skin diseases, fluid from suction bullae obtained in normal skin and synovial fluid from patients with rheumatoid arthritis were analysed by immunochemical techniques as described by Clemmensen et al. (2) including crossed immunoelectrophoresis.

Ten patients (3 men and 7 women, mean age 68 years) with bullous diseases were examined. The diagnoses were bullous pemphigus (1), stasis bullae (1), bullous erysipelas (5), bullous impetigo (2) and zoster (1). Cell counts for erythrocytes and leukocytes were performed in exudates from 7 patients with bullous dermatoses and plasma fibrinogen levels were also determined in this group. Fluid from bullae raised by suction (12) on normal non-inflamed abdominal skin in 13 volunteer patients (9 men and 4 women, mean age 61 years) was examined by the same methods. As a further control, suction bullae were raised in 2 of the patients with bullous dermatoses. Synovial fluid from 20 patients (6 men and 14 women, mean age 62 years) with rheumatoid arthritis was also analysed.

Bullous fluid was collected by puncture of the bullae in 0.11 M trisodium citrate (volume fraction = 0.10) and stored at -20°C.

Synovial fluid was collected and treated with hyaluronidase as described previously (2).

The concentration of individual proteins was determined by electroimmuno-assay in agarose gel essentially as described by Laurell (13) using Protein-Standard-Plasma, Protein-Standard-Serum (Behringwerke, Marburg-Lahn, West Germany) and a crude primary plasmin inhibitor preparation (14) as reference. Crossed immunoelectrophoresis was performed essentially as described by Ganrot (9) with barbiturate buffer at pH 8.6.

Estimation of the concentration of plasmin-inhibitor complex was performed as described by Weeke (19).

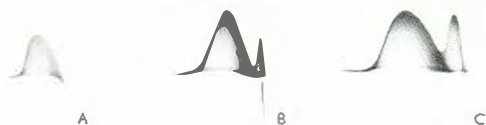


Fig 1. Pattern of immunoprecipitates obtained by crossed immunoelectrophoresis at pH 8.6 of fibrinogen antigenic material. The figure shows normal fibrinogen from plasma (A), material from rheumatoid synovial fluid (B), and material in exudate from a patient with bullous erysipelas. The first dimension electrophoresis was run in agarose gel (anode to left). The second dimension electrophoresis (anode at top) was run in agarose gel containing immunoglobulin directed against fibrinogen (volume fraction in gel 0.02). The sample applied in the slit contained (A) 10 μ l plasma (B) 10 μ l rheumatoid synovial fluid and (C) 10 μ l pathological bulla fluid.

Estimation of clottable fibrinogen was performed as previously described (3).

Platelet-poor plasma. Plasma was separated by centrifugation from freshly drawn blood collected in 0.11 M trisodium citrate (volume fraction = 0.10) and stored at -20°C .

Barbiturate buffer. Sodium barbiturate, 64 mM/l; barbiturate acid, 11 mM/l; calcium lactate, 2 mM/l, pH 8.6.

Agarose. Indubiose® A 45 (L'industrie Biologique, Gennevilliers, France).

Specific rabbit immunoglobulin against the primary plasmin inhibitor was prepared as previously described (14).

Specific rabbit immunoglobulins against human α_2 -macroglobulin, fibrinogen and plasminogen, dissolved in NaCl (0.1 M/l), NaN_3 (0.015 M/l), were obtained from Dakopatts, Copenhagen, Denmark.

RESULTS

Abnormal fibrinogen degradation products identical with those found in synovial fluid from patients with rheumatoid arthritis were detected in exudates from each of the patients with bullous diseases (Fig. 1). By contrast, no such abnormal products could be detected in suction bullae. The quantitative estimation of fibrinogen antigenic materials in the three types of exudate, viz. of synovial fluid, suction, and pathological bullae, is given in Table I. By crossed immunoelectrophoresis of fluid from suction bullae into immunoglobulin against fibrinogen, the precipitate displayed gammaglobulin mobility as does fibrinogen (Fig. 1 A). Upon addition of thrombin to the suction bulla fluid no precipitate was formed by

crossed immunoelectrophoresis indicating that the material was fibrinogen. Crossed immunoelectrophoresis of fluid from bullous dermatoses revealed immunoprecipitates in the α_2 , β_1 and γ -globulin regions of serum proteins, identical with the precipitates found in synovial fluid (Fig. 1 B, C). When thrombin was added to the fluid, no difference in the immunoprecipitates occurred, indicating that this material was not fibrinogen.

The concentration of fibrinogen antigenic material in the pathological bullae varied between 0.11 and 16.83, α_2 -macroglobulin between 0.12 and 3.28 and plasminogen between 0.20 and 3.84 $\mu\text{M/l}$ (Table I). The corresponding values for rheumatoid synovial fluid are listed in the table. By contrast, only trace amounts of fibrinogen antigenic material were detected in fluid obtained by suction and the values were significantly lower ($p < 0.05$) when compared with pathological bullae and rheumatoid synovial fluid. Only α_2 -inhibitor was found (in almost the same concentrations) in suction bullae and in pathological bullae, 0.31 and 0.42 $\mu\text{M/l}$ respectively (Table I). In rheumatoid synovial fluid the corresponding value was 0.55 $\mu\text{M/l}$. A complex between plasmin and α_2 -inhibitor was also found in pathological bulla fluid identical with that found in rheumatoid synovial fluid.

Fluid from suction bullae was examined in 2 patients with bullous dermatoses (bullous pemphigoid and stasis bullae). After 3 weeks on systemic steroid treatment resulting in clinical improvement, fluid from a suction bulla in the patient with bullous pemphigoid contained only trace amounts of fibrinogen and no abnormal fibrinogen degradation products. In the patient with bullous stasis eczema, no abnormalities were found in simultaneously induced suction bullae raised on the abdomen on clinically normal skin.

In bullous dermatoses and in synovial fluid the mass concentration of the estimated protein was found to be about 50 % of the plasma concentration. In suction bullae the mass concentration of the proteins was only about 1/10 of the plasma concentration, except for the primary plasmin inhibitor (α_2 inhibitor, Table I). The mean plasma fibrinogen concentration in the patients with bullous dermatoses was 10.4 $\mu\text{M/l}$, which was within the normal range for this protein (6–10 $\mu\text{M/l}$).

The ratio between erythrocytes and leukocytes was similar (4 : 1) in exudates from suction bullae and bullous dermatoses, but the total number of

Table I. Concentration of proteins in exudates from bullous dermatoses, suction bullae and synovial fluid. The figures represent mean $\pm$ S.E.M.

	Fibrinogen antigenic material (μ M)	Alpha ₂ macroglobulin (μ M)	Plasminogen (μ M)	Alpha ₂ inhibitor (μ M)
Bullous dermatoses ($n=10$)	$4.63^a \pm 1.64$	$1.14^a \pm 0.35$	$1.12^a \pm 0.41$	0.42 ± 0.07
Suction bullae ($n=13$)	0.33 ± 0.10	0.26 ± 0.03	0.35 ± 0.04	0.31 ± 0.03
Synovial fluid ($n=20$)	3.82 ± 1.42	0.90 ± 0.42	1.11 ± 0.40	0.55 ± 0.10

^a Values significantly different from suction bullae values at the 5% level of probability.

cells was about 100 times higher in bullous dermatoses. In bullous dermatoses the mean value of the number of leukocytes was 0.2×10^6 and in suction bullae the corresponding value was 0.2×10^8 . The number of cells was not determined in synovial fluid.

DISCUSSION

The present results have clearly shown that there is an "abnormal" fibrinolytic activity in exudates from patients with bullous dermatoses when compared with suction bullae obtained in normal uninflamed skin. Furthermore, the fibrinogen degradation products in the exudates from the pathological bullae were immunochemically similar to those present in rheumatoid synovial fluid. In suction bullae the material detected probably represents fibrinogen escaped from the circulation, since it was clottable. In fluid from bullous dermatoses and in rheumatoid synovial fluid no clottable fibrinogen was present. The plasma fibrinogen in patients with bullous dermatoses was within the normal range and particularly not elevated.

Inflammation with formation of fibrin deposits and subsequent fibrinolysis is a normal response to tissue injury, leading to tissue repair (1). The formation of fibrinogen degradation products in excess amounts in rheumatoid synovial fluid and pathological bullae might to some extent account for the exudate formation, since this material is highly hydrophilic (3).

By using immunofluorescence techniques, fibrinogen antigenic material has previously been detected close to the dermo-epidermal junction in psoriasis (6, 8), vasculitis (4) rheumatoid arthritis (5) and in certain bullous diseases, notably dermatitis herpetiformis (11, 18).

However, with immunofluorescence techniques it is only possible to determine that the material is fibrinogen antigenic. By contrast, immunochemical techniques such as crossed immunoelectrophoresis permit evaluation of the molecular structure and conclusions to be drawn concerning the nature of the material. Thus by means of crossed immunoelectrophoresis it is possible to dissociate fibrinogen from fibrin degradation products. Attempts to use immunochemical methods in those conditions where fibrinogen antigenic material has been demonstrated by immunofluorescence techniques might yield valuable new information about the chemical nature and possible pathogenetic significance of these substances.

As might be expected, leukocytes were present in greater amounts in pathological bullae than in suction bullae. Leukocytes are known to migrate into inflammatory sites when a fibrin network has been formed (17). Furthermore, neutrophil leukocytes contain activators of fibrinolysis (10) and leukocytic neutral proteases have been held responsible for the formation of various fibrinogen degradation products (15), but whether this is the case or not in rheumatoid fluid and pathological bullae remains to be determined.

The demonstrated fibrinogen antigenic material in exudates from pathological bullae, immunochemically similar to that found in rheumatoid synovial fluid, indicates that the presence of these products reflects more general features of an inflammatory exudate.

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