

DEPOSITION OF FIBRINOGEN (FR-ANTIGEN) IN SKIN DISEASES

1. *Psoriasis Vulgaris and Psoriasis Arthropathica (with Special Reference to Heparin-precipitable Fraction)*

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Abstract. Twenty-five in-patients with psoriasis arthropathica (ps.a.) and 18 in-patients with psoriasis vulgaris (ps.v.) were examined for cryofibrinogen as heparin-precipitable fraction (HPF), for total fibrinogen in plasma and by immunofluorescence (IF) technique for FR-antigen in tissue sections of affected and unaffected skin. A control group of 14 in-patients with various non-psoriatic dermatoses were examined with IF for FR-antigen in unaffected skin. Pathological quantities of HPF were found in 6% in ps.v. and in 48% in ps.a. Total fibrinogen in plasma was normal in ps.v. (365 mg%), and moderately elevated in ps.a. (471 mg%). In psoriatics, FR-antigen was found in unaffected skin in ps.v. in 50%, in ps.a. in 72%. In affected skin, the figures were 67% and 84% respectively. No direct relationship could be found between the incidence and the degree of HPF, and depositions of FR-antigen in the tissue sections in the skin at the time of the examination. In the control group, no FR-antigen could be found in unaffected skin, except in one case of acne cystica, with traces of FR-antigen in a few areas of the tissue section.

Key words: Psoriasis; Arthropathica; Plasmafibrinogen; Heparin-precipitable fraction; Skin-FR-antigen

The extravascular deposition of fibrin as a response to tissue injury is a prerequisite for normal tissue repair, together with subsequent fibrinolysis (1). Increased fibrin formation and deposition is, furthermore, a component of the inflammatory process (2). Inflammation and vascular reaction is a part of the histological picture in psoriasis (10, 11).

In the demonstration of fibrin or fibrinogen, the traditional staining methods are relatively insensitive, compared with immuno-histochemical methods (5). As fibrin and fibrinogen have a common antigenicity (16), fluorescein-labelled antibodies against fibrinogen have been used in the detection of fibrinogen, fibrin, or its degradation products (FR-antigen) in the skin in a number of dermatological diseases such as lichen ruber planus (3, 19), derma-

titis herpetiformis (9, 14, 18) and lupus erythematosus (19).

The detection of FR-antigen by means of fluorochrome-labelled antifibrinogen in the psoriasis group, has been contradictory (8, 20). Of the two available studies, one concludes with the detection of depositions of fibrinogen/fibrin in the skin lesions in 62% of the cases with "unstable psoriasis", generalized psoriasis pustulosa, or atypical eczema-like psoriasis, compared with 11% in psoriasis vulgaris (20). The other study (8) found "only scattered fluorescence after application of anti-fibrinogen in involved and uninvolved skin in 18 cases with psoriasis vulgaris, 10 cases with psoriasis arthropathy, 2 cases with psoriasis erythrodermica and a control group of 30 patients with various dermatosis".

As the psoriasis arthropathica group has a higher incidence of cryofibrinogen, measured as heparin precipitable fraction in plasma (7), the present investigation has been performed to study the deposition of FR-antigen in the affected and unaffected skin in patients with psoriasis vulgaris and psoriasis arthropathica, to see if there are any apparent connections between the deposition of FR-antigen in the skin, heparin-precipitable fraction, and total fibrinogen in plasma.

MATERIALS AND METHODS

Patients

Twenty-five in-patients with psoriasis arthropathica (ps.a.) and eighteen in-patients with psoriasis vulgaris (ps.v.) from the Department of Dermatology, Rikshospitalet, Oslo, were examined for FR-antigen in affected and unaffected skin, for cryofibrinogen as heparin-precipitable fraction (HPF) in heparinized plasma and for total fibrinogen in citrated

plasma. The ps.a. group consisted of 9 females from 35 to 70 years of age, mean 53 years, and 16 men from 26 to 77 years of age, mean 46 years. The ps.v. group had 4 females from 22 to 63 years of age, mean 34 years, and 14 men from 17 to 61 years of age, mean 45 years. Only patients with clinically typical psoriasis were selected. Patients with arthralgia, with or without additional edema and degenerative joint changes were classified as ps.a. All patients with ps.a. had negative Waaler-Rose reaction and RA-latex test. Ps.v. patients had symptom-free joints during the 6-month period prior to examination. Practically all patients had been subjected to topical treatment before entering the ward, most of them regularly with fluorinated corticosteroids. None had received systematic corticosteroids prior to the investigation. Patients with additional diseases liable to cause an unspecific rise in HPF were excluded from the material.

Fourteen non-psoriatic in-patients with various skin diseases served as a control group for the biopsies of the unaffected skin (acne cystica, acne vulgaris, systemic scleroderma, hypostatic and contact eczema, chronic urticaria, ulcer cruris, keratosis palmo-plantaris).

Tissues

Skin biopsies were taken 1–3 days after entering the department. They were taken from psoriatic lesions and from uninvolved skin, both mostly in the same body region and in unexposed areas, always at a distance from each other of at least 10–20 cm. In the control group, the biopsies of the uninvolved skin were mostly taken from the anterior part of the upper right arm. Local anaesthesia was performed with regional infiltration of 1% lidocain chloride (Xylocain®) without addition of vasoactive substances. The biopsy was taken with a no. 11 scalpel, in affected skin mostly from the older psoriasis lesions.

The biopsy material was then immediately transported in physiological saline solution to the Institute of Immunology and Rheumatology, Rikshospitalet, Oslo. The samples were diagnostically unmarked and the IF examiner did not know the diagnosis of the patients. The tissues were immediately embedded in Ames OCT compound (Ames Comp., USA) and quick frozen.

Immunohistochemical techniques

Anti-fibrinogen was raised in rabbits by injections of purified, human fibrinogen in Freund's complete adjuvant (13). IgG was separated from the rabbit serum by passing through an anion exchange resin (DEAE-cellulose, Whatman DE 52). Labelling with fluorescein isothiocyanate (FITC) was performed according to Nairn (15). Unbound dye was removed by dialysis against PBS, followed by gel filtration (Sephadex G-25). To avoid unspecific staining, the conjugate was absorbed with acetone-dried calf liver powder. The FITC-labelled anti-fibrinogen antiserum was used at a final protein concentration of 0.5 mg/ml. For control, a FITC-labelled normal rabbit serum was used.

Immunofluorescence (IF) staining was carried out on 4 µm frozen tissue sections which had been fixed in cold acetone. Stained sections were inspected in a Leitz Orthoplan Microscope. Ultraviolet light source was 200 W Mercury lamp. Microphotographs were taken by incident light through a Leitz vertical illuminator with an Orthomat camera. Filter

combinations for FITC were 0.5-mm BG 12, AL 490 nm and secondary filter K 530.

By the injection of fibrinogen in animals, antibodies are produced against fibrinogen, fibrin and its degradation products, therefore the term FR-antigen is used.

Sera and plasma

The next morning after entering the ward, a venepuncture was made in the cubital vein of the patients, under venestasis. The first 10 ml of blood were used for other purposes, after which blood for the examination of HPF was obtained and examined as described elsewhere (7). In this study, the HPF-pattern characteristic of healthy individuals was excluded from the material (7). Total fibrinogen in citrated plasma was measured according to routine methods at the Central Laboratory, Rikshospitalet, Oslo. Waaler-Rose reaction and RA-Latex tests, were performed according to routine methods in use at the Institute of Immunology and Rheumatology, Rikshospitalet, Oslo.

RESULTS

HPF

HPF was found in plasma of 6% of the patients with ps.v. compared with 48% of the patients with ps.a. (Fig. 1). The mean values of HPF in the ps.a. group was 0.34 mg/ml whole blood. No direct correlation could be found between the degree and the incidence of HPF and the deposition of FR-antigen in the skin.

Immunohistochemical detection of FR-antigen

FR-antigen detected by IF technique was found in affected and in unaffected skin in ps.v. and ps.a. The depositions were located in the dermis just

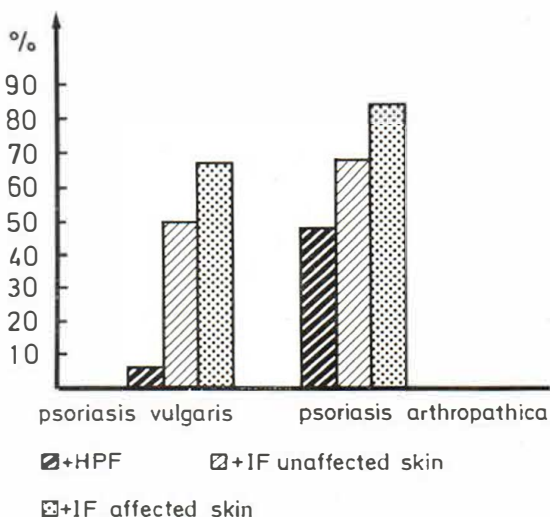


Fig. 1. Incidence of HPF and IF for FR-antigen in affected and unaffected skin in patients with psoriasis vulgaris and psoriasis arthropathica.



Fig. 2. Immunofluorescence micrograph. Deposition of FR-antigen in dermis just below the dermal-epidermal junction as a continuous network of thin fluorescent threads probably related to capillaries ($\times 460$).

beneath the dermo-epidermal junction as a continuous network of thin fluorescent threads probably related to capillaries (Figs. 2, 3). Sometimes, however, the depositions consisted of thicker elements with a tendency to confluence (Fig. 4). A semi-quantitation of the deposits of FR-antigen was made by grading from $+$ to $+++$ according to the strength of fluorescence and the size of the deposits.

No fluorescence was obtained with FITC-labelled normal rabbit IgG. Pretreatment of the sections with unconjugated anti-FR-antigen completely pre-

vented the fluorescence, while pretreatment with a normal rabbit serum did not influence the fluorescence obtained with the FITC-labelled anti-FR-



Fig. 3. Immunofluorescence micrograph. Detail of Fig. 2 at higher magnification ($\times 1150$).



Fig. 4. Immunofluorescence micrograph. Deposition of FR-antigen in dermis consisting of partly confluent thicker elements ($\times 530$).

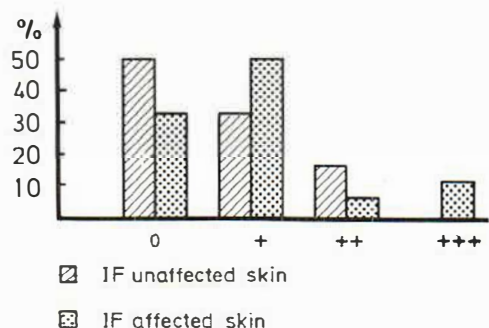


Fig. 5. Degree of IF for FR-antigen in affected and unaffected skin in patients with psoriasis vulgaris.

antigen antiserum. No fluorescence was obtained when the antiserum was absorbed with fibrinogen before application on the sections.

In affected skin, FR-antigen was found in 67% of cases with ps.v. and 84% of cases with ps.a. (Fig. 1). When quantitated according to the degree of IF, 50% of the ps.v. group showed + and 17% ++/+++, while in the ps.a. group, 24% showed + and 60% showed ++/+++ (Figs. 5, 6).

In unaffected skin, FR-antigen was found in 50% of the cases with ps.v., compared with 72% of the cases with ps.a. (Fig. 1). The degree of IF was sometimes equal to, but mostly higher in the affected skin, than in the unaffected skin areas. A higher degree of IF in unaffected than in the corresponding affected skin areas, was never observed.

Ten cases of the 43 patients examined showed negative IF in affected and unaffected skin. No cases could be detected with positive IF in the unaffected skin, and negative IF in the affected skin specimens, whereas in 6 cases with positive IF in the affected skin, the IF in the corresponding unaffected skin was negative.

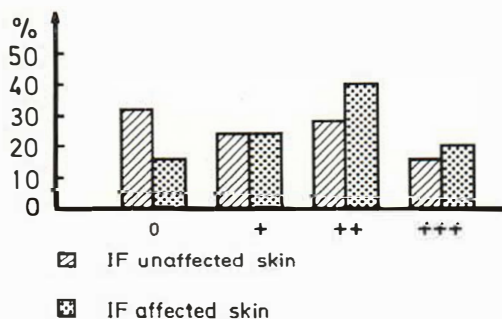


Fig. 6. Degree of IF for FR-antigen in affected and unaffected skin in patients with psoriasis arthropathica.

In the control group, one patient with acne cystica had traces of FR-antigen (+) below the dermo-epidermal junction in a few areas of the skin biopsy. The rest of the group had no detectable FR-antigen in the unaffected skin.

Total plasma fibrinogen

In the ps.v. group the mean value of fibrinogen in the blood measured in citrated plasma was 365 mg% (from 328 to 556 mg%), compared with 471 mg% (from 302 to 774 mg%) in the ps.a. group (normal range 200–400 mg%).

DISCUSSION

Cryoprecipitation in plasma is a mixture of proteins occurring in different pathological conditions. It consists of fibrinogen in an unchanged or possibly slightly changed state, and of another protein. These two proteins are probably complexing, forming a cryolabile structure (12) which is rendered more sensitive to cold in heparinized plasmas such as HPF. This is probably due to the highly negative charged polysaccharides of heparin, known to form complexes with various proteins, such as fibrinogen (6).

HPF is not uncommonly found in low concentrations in plasmas of healthy persons, 37% in one study (7). Some authors therefore deny the clinical significance of this HPF-formation, also because of its incidence in a variety of clinical conditions. The pattern of cryoprecipitation is characteristic, however, and different from the pattern in diseases (7). These cases can therefore easily be excluded from the material to be examined, as has been done in the present investigation.

Cases of arthritis often show HPF in plasma—in a recent study, in 80% of the patients with ps.a., compared with 4% in ps.v. (7). In the present study, the figures of the incidence in patients with ps.a. are lower (48%), whereas the ps.v. group corresponds well, with 6%.

The ps.v. group had a normal mean value of fibrinogen in the blood (365 mg%), but in ps.a. this value was elevated (471 mg%). The number of patients was too small to ascertain if there was any connection between HPF and plasma fibrinogen. There was no direct correlation between the amount of plasma-fibrinogen and FR-antigen in the skin, as detected by the IF-method at the time of the examination.

One of the above-mentioned studies (8) concluded that there was no typical deposition of FR-antigen in either involved or in uninvolved skin in ps.v. and ps.a., in comparison with a control group. In contrast to this is the other study (20), stating that in affected skin, deposition of FR-antigen was found in ps.v. in 11 %, and in "complicated psoriasis", in 62 %. Uninvolved skin was not examined. These figures are different from those of the present study where IF was found against FR-antigen in unaffected skin in 50 % and in affected skin in 67 % in ps.v., and in ps.a. in 74 % and 84 % respectively.

Slight changes in the neighbouring unaffected skin around psoriatic lesions have been demonstrated (4). These changes are inversely proportional to the distance from the active lesions, representing a broadening of the intermediary zone in the epidermis. It is unlikely that the high incidence of FR-antigen in the clinically unaffected skin areas can be explained from these changes, because of their discrete nature and the relatively large distances between the biopsy site in affected and unaffected skin. It is possible that at an early stage of the pathological psoriatic process, FR-antigen may constitute an important factor in the subsequent inflammatory picture of the disease. In active psoriatic lesions, inflammation is a dominant part of the histological picture, with migration of leucocytes and exudation from the capillaries of the papillary bodies in the dermis. As fibrinogen, fibrin and its degradation products are leucocytotactic, they may cause migration of neutrophilic leucocytes. Granulocytes also contain inhibitors of the fibrinolytic system.

The incidence and the quantity of the FR-antigen deposition is more pronounced in ps.a. than in ps.v., although the active lesions in the skin clinically and histologically are similar in both conditions. It is an interesting fact that although FR-antigen can be found in affected skin and be missing from the unaffected skin in the same patient, FR-antigen was never found in the unaffected skin of patients with negative IF in active lesions.

In the healthy organism, the formation of fibrin deposits is probably a regular phenomenon, together with subsequent fibrinolysis in a dynamic equilibrium. Each of the two processes is regulated by a multitude of activating and inhibitory systems (1). With the use of the fibrin plate technique and frozen tissue sections, it has been demonstrated that plasminogen-activators are localized to the vascular

endothelium. From here they are released, and account for the circulating activators (21, 22). This activation of fibrinolysis may be influenced by an inhibitor produced in the epidermis (17). The main activity of fibrinolysis is localized to the middle and deeper layer of the dermis, whereas the sub-papillary vessels are less active (23). This may be of importance in psoriasis, as the deposition of FR-antigen measured by the IF technique is most pronounced in the upper layer of the dermis.

In the psoriatic condition, inflammatory changes may be induced by the deposition of fibrinogen, fibrin, or its degradation products in the unaffected skin. Reduced fibrinolysis in the vessel walls and inhibition of fibrinolysis by the granulocytes in inflammation might result in a further concentration of FR-antigen in certain stages of inflammation. In psoriasis, this might explain the more pronounced deposition of FR-antigen in affected skin areas. Continued investigation of the state of fibrinolysis in cases with deposition of FR-antigen in the skin and HPF in plasma may produce important results.

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