

IMMUNOFLUORESCENT STUDIES OF THE SKIN IN MIXED CRYOGLOBULINAEMIA AND SCHÖNLEIN-HENOCH PURPURA

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Abstract. Immunofluorescence investigations in patients with mixed cryoglobulinaemia, Schönlein-Henoch purpura and purpuric allergic reaction with disseminated intravascular coagulation have been performed. In the lesional skin from 8 patients with mixed cryoglobulinaemia, granular vascular deposits of immunoglobulins of the same classes as those of the circulating cryoglobulins were detectable in all cases; complement and fibrinogen were concomitantly present. In the unaffected skin, IgM and complement were detected in the walls of the capillaries. In the early purpuric lesions from 6 patients with Schönlein-Henoch syndrome and from a patient with disseminated vascular coagulation from acute allergic reaction to phenylbutazone, deposits of fibrinogen occurred mainly in the vessel walls.

Key words: Mixed cryoglobulinaemic purpura; Schönlein-Henoch purpura; Immunofluorescence; Vascular deposits of Ig and C

The antigen-antibody complexes circulating in the blood can intervene at different levels in the pathogenesis of the immune complex diseases, directly at the level of the platelets (thrombocytopenia crises either drug-induced or post-infectious), or at the level of the blood vessel walls, causing alteration of a phlogistic nature or favouring a disseminated intravascular coagulation. The latter two processes can also be associated variously. Among the immune complex diseases in which leukocytoclastic vasculitis represents the principal histopathological alteration, mixed cryoglobulinaemic purpura, hypergammaglobulinaemic purpura, and Schönlein-Henoch anaphylactoid purpura are of dermatological interest. The allergic vasculitis can also be considered an immune complex disease, if one takes into consideration the immunological findings at tissue level (4, 15, 26, 29), but the presence of circulating immune complexes has not yet been demonstrated clearly (1).

The mixed cryoglobulins are most frequently observed in autoimmune disease states (systemic lupus erythematosus, rheumatoid arthritis, Sjögren syndrome) and immuno-proliferative diseases (chronic lymphatic leukaemia, macroglobulinaemia, myeloma, etc.), but also in a certain number of infectious diseases (mononucleosis, syphilis, leprosy, cytomegalic inclusion disease) (14, 18, 20, 24, 25, 31, 32). The chemical and immunological properties of the cryoprecipitate are well known (generally formed by IgM-IgG complexes, more rarely by IgA-IgG complexes, and exceptionally by IgG-IgG complexes) (3, 16, 27).

The study of the tissue situation has been carried out by means of immunofluorescence techniques (IF).

Researches conducted by Miescher et al. (23) on purpuric lesions have demonstrated the presence of blood-vessel deposits of IgG, IgM in 2 out of 3 cases and of only IgG in one case. Cream (7) in 4 out of 6 patients found deposits of Ig of the same type as those of the circulating cryoglobulins, Ig of a different type and not present in the cryoprecipitate in 2 of the 4 cases, while no deposit of immunoglobulins was found in one case. The complement (β_1C and β_1A fractions) and the fibrinogen were present in all 6 cases. Nearly identical results have been obtained by other researchers (9, 10, 19). At the level of the glomerules to, in 5 cases out of 7 with renal damage, deposits of IgG and IgM, i.e. of the same immunoglobulins present in the cryoprecipitate, were demonstrated (11, 18, 13, 20).

Published observations by IF on healthy skin are few: Cream (7) found small vascular deposits of IgG in only 3 cases out of 6. The Schönlein-Henoch purpura also is generally recognized as a vasculitis of circulating immune complexes mediated by neu-

Table 1. *Mixed cryoglobulinaemic vasculitis. Immunological findings in cryoglobulin and affected and unaffected skin*

Patient	Cryoglobulin component			Skin	D.I.F. (vessel wall or perivascular)				
	IgG	IgM	IgA		IgG	IgM	IgA	C	F
1	+	+	—	Purpura	+	+	—	+	+
				Unaffected	±	+	—	+	—
2	+	+	—	Purpura	+	+	—	+	+
				Unaffected	—	+	—	±	—
3	+	+	—	Purpura	+	+	—	+	+
				Unaffected	—	+	—	+	—
4	+	+	—	Purpura	+	+	—	+	+
				Unaffected	—	+	—	±	—
5	+	+	—	Purpura	+	+	—	+	+
				Unaffected	—	+	—	+	—
6	+	+	—	Purpura	+	+	—	+	+
				Unaffected	—	+	—	±	—
7	+	+	—	Purpura	+	+	—	+	+
				Unaffected	—	+	—	+	—
8	+	—	+	Purpura	+	—	+	+	+
				Unaffected	—	—	+	±	—

trophils, even if in certain cases the demonstration of some essential data is lacking for the definition of an immune complex disease: hypocomplementaemia in only 10 out of 104 cases (12, 17, 22). IF data demonstrate in the majority of the cases the presence of Ig (above all, IgG, IgM and IgD) and C at the level of the blood vessel walls (26). Nevertheless in the most recent lesions the fibrinogen deposits are more conspicuous and more negligible than those of Ig and C (30). In the pathogenetic mechanisms of S.H. purpura, phenomena of disseminated intravascular coagulation intervene beside the vasculitis.

Disseminated intravascular coagulation is a third possible histopathological event in immune complex damage. This supports some well-defined clinical situations such as the haemolytic-uraemic syndrome, the thrombotic thrombocytopenic purpura, and some cases of acute allergic reaction to drugs (21). This is a pathological condition of obvious dermatological interest for which we believe IF research has not been conducted.

We report here the results of IF investigation carried out in 8 patients with mixed cryoglobulin, 6 patients with anaphylactoid purpura and one case of fatal drug allergy purpura in which the pathological dominant observation was disseminated intravascular coagulation.

MATERIAL

(a) *Mixed cryoglobulinaemia*. The material comprises 8 typical cases of mixed cryoglobulinaemia. Cutaneous biopsies were taken from purpuric lesions of the lower limbs (8 cases), from healthy skin around the lesions (8 cases) while in 2 cases healthy skin was biopsied from the forearm where lesions were not present.

(b) *Schönlein-Henoch purpura*. Cutaneous biopsies were obtained from incipient purpuric lesions on the lower limbs of 6 patients with anaphylactoid purpura.

(c) *Purpura with disseminated intravascular coagulation*. The periphery of a hemorrhagic-necrotic patch of a patient with disseminated vascular coagulation from acute drug allergy reaction (phenylbutazone) was biopsied.

The patients were not undergoing therapy at the time of the biopsy.

METHODS

In vivo IF studies. The preparation of the skin sections was done according to the method described in a previous paper (5).

The sections of skin were studied by the direct immunofluorescence method to demonstrate the three major types of immunoglobulins (IgG, IgM, IgA), the complement (β_1C/β_1A fractions) and the fibrinogen.

Sera. In the cases of mixed cryoglobulinaemia the cryoprecipitate was studied by ultracentrifugal and immunoelectrophoretic analysis at the laboratory of the Department of Haematology at the University General Hospital of Pavia.

Microscopy. The filters and mirrors used for incident

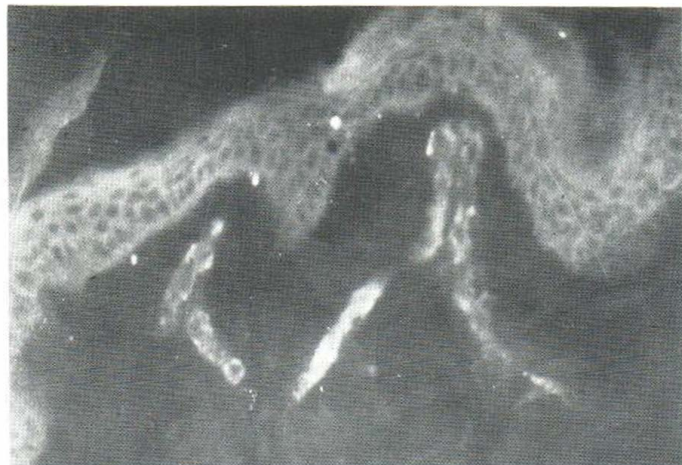


Fig. 1. Mixed cryoglobulinaemia, lesional skin, D.I.F. method. Granular deposits of IgM in dermal capillaries ($\times 460$). Similar patterns were demonstrated for IgG and β_2C/β_2A .

blue, narrow band excitations as applied for the examination of the sections in this study were the same as those described by Cormane et al. (5).

RESULTS

Mixed cryoglobulinaemia purpura

In 7 cases of mixed cryoglobulinaemia the centrifugal and immunoelectrophoretic analysis revealed that the cryoprecipitate was formed by an IgM-IgG complex, whereas in one case it was composed by an IgA-IgG complex. In the biopsies of the purpuric lesions the IF showed in 7 cases granular vascular deposits of IgM, IgG, C and fibrinogen; in the remaining case IgA, IgG, C and fibrinogen were detected. In the healthy skin around and even

away from the lesions (2 cases) granular deposits of IgM and C are evident (Table I, Figs. 1 and 2).

Anaphylactoid purpura

IF on incipient purpuric lesions reveals the presence of a considerable quantity of fibrinogen at the level of the blood vessel walls and scanty amounts of IgG are present in 3 cases, always at the level of the blood vessels (Table II) (Fig. 3).

Drug allergic purpura with disseminated intravascular coagulation

The IF picture is quite modest, showing only deposits of fibrinogen in and around the blood vessels and contrasts with the largeness of the thrombosis and vasculitis (Table III).

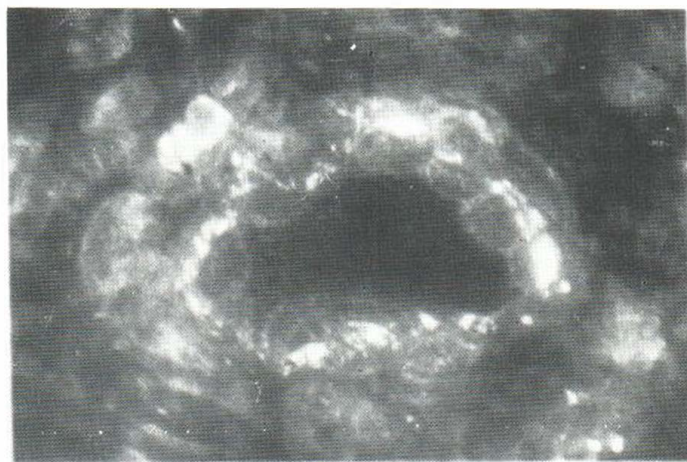


Fig. 2. Mixed cryoglobulinaemia, lesional skin, D.I.F. method. Granular deposits of IgG in a vessel wall from the middle dermis ($\times 460$). Similar patterns were demonstrated for IgM and β_2C/β_2A .

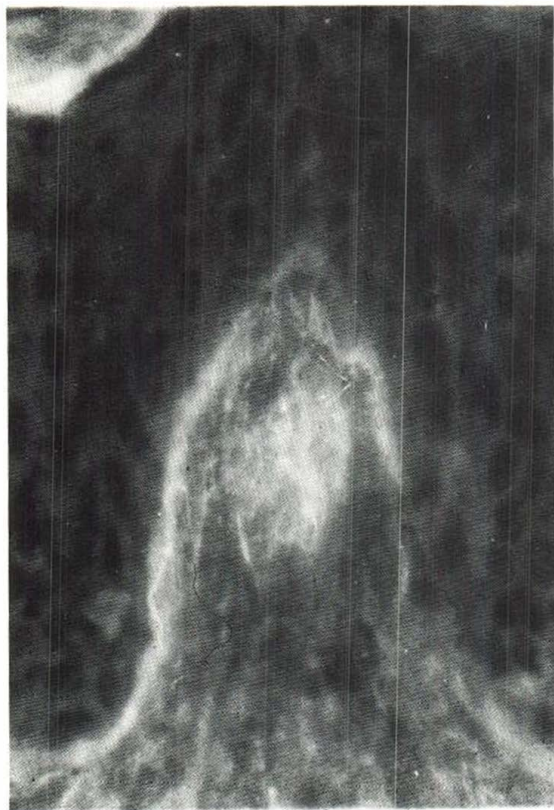


Fig. 3. Schönlein-Henoch purpura, lesional skin, D.I.F. method. Conspicuous deposits of fibrinogen in and around the dermal capillary and junctional zone ($\times 600$).

DISCUSSION

Mixed cryoglobulinaemia purpura

The data obtained on the diseased skin agree with those of Miescher and Cream, indirectly confirming the nature of the immune complexes of the pathological condition.

The presence of IgM and C deposits at the level of the blood vessel walls in healthy skin, even in

Table II. *Schönlein-Henoch purpura. D.I.F. (early lesions)*

Patient	IgG	IgA	IgM	C	F
1	—	—	—	—	+
2	—	—	—	—	+
3	±	—	—	+	+
4	±	—	—	—	+
5	±	—	—	+	+
6	—	—	—	+	+

Table III. *Histological and immunological findings in the purpuric lesions of a patient with disseminated intravascular coagulation*

Histology	Fibrinous and hyaline thrombi Leukocytoclastic angitis				
	IgG	IgM	IgA	C	F
D.I.F.:	—	—	—	—	+

areas far from the lesions (in 2 cases the lesions were confined to the lower limbs and the biopsy was performed on the forearm), and also the presence of the same deposits in a patient during complete clinical recovery, merits some remarks. These findings are not entirely surprising if one remembers that the circulating immune complex in the blood deposits itself everywhere in the cutaneous districts. Why the vasculitis and thus the clinical lesions are manifested only in certain areas, we do not know. The central role of the mechanisms of coagulation and fibrinolysis (8) has been emphasised in this connection but a rational and complete justification has still not been supplied. On the other hand, not in all the pathological conditions in which mixed cryoglobulins are found, do these complexes precipitate and cause vasculitis. It is also known that the incidence of the vasculitis is not related to the amount of the circulating cryoglobulins (20) and that the cryoglobulinaemia can be persistent, but the vasculitis intermittent.

The histological examination of the healthy skin around and away from the lesions showed evidence of complete normality, as if the deposits of Ig and C were not able to prime the reaction which induces the vasculitis. This is perhaps due to the intervention of local cellular factors of protection (reticular-endothelial system able to phagocytize the circulating complexes deposited in the tissue). This makes sense if one does not consider these findings on the healthy skin to be aspecific, as suggested by recent research (2). However, we do not believe we can share this point of view on the basis of our laboratory experience.

Anaphylactoid purpura of Schönlein-Henoch

The findings obtained from the purpuric lesions confirm the data of Urizar & Herdman (30) on renal biopsies: the prevailing presence of fibrinogen deposits in the absence of or with weak concentrations of Ig and C would lessen the pathogenetic importance of these factors only secondarily pres-

ent and perhaps only due to alterations in blood vessel permeability. On the other hand, in localized leukocytoclastic allergic vasculitis (Gougerot-Ruiter type), Cormane (6) demonstrated the presence of C_1Q at the level of the vessel walls without the presence of antigen-antibody complexes, as if the activation of the complement could proceed without the presence of the immune complex.

Drug allergy purpura with CID

The immunofluorescence data (deposits of fibrinogen only) find a significant confirmation in the experimental finding of Selye & Tuckweber (28) who induced purpura and nephritis in the rat by an intravenous administration of agar and subcutaneous administration of adrenalin, with a predominant pathological picture of disseminated intravascular coagulation and of phlogosis like the Schwartzman phenomenon, and in which deposits of fibrin are found, but not those of Ig and C.

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