

IMMUNOFLUORESCENCE OF THE SKIN IN ALLERGIC DISEASES: AN INVESTIGATION OF PATIENTS WITH CONTACT DERMATITIS, ALLERGIC VASCULITIS AND ATOPIC DERMATITIS

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Abstract. Skin biopsies for immunofluorescent studies were taken from patients with contact dermatitis (positive patch tests), atopic dermatitis and allergic vasculitis for comparison with normal-appearing skin from the same patients, and from healthy controls. A variety of deposits of immunoglobulins, complement components and fibrinogen were demonstrated in 6 out of 20 patients with contact dermatitis, 7 out of 10 with atopic dermatitis, 8 out of 10 with allergic vasculitis, and in 4 out of 20 control individuals. No diagnostic pattern of deposits was found. Elevated serum IgE and eosinophilic counts were found in patients with atopic dermatitis, and high serum IgA and fibrinogen levels were found in the allergic vasculitis group.

Key words: Immunofluorescent studies; Skin biopsy; Contact dermatitis; Atopic dermatitis; Allergic vasculitis

Since 1963, when Burnham first published his findings on immunoglobulin deposits at the basement membrane in patients with systemic lupus erythematosus (SLE) (3), immunofluorescent studies have been applied to many other diseases involving the skin (1, 2, 12, 13). Deposits of immunoglobulins, complement factors and fibrinogen have been reported in several skin conditions and systemic diseases, and in some, such as LE and bullous skin disorders, they are considered important in confirming or invalidating the diagnosis. The present comparative investigation concerns immunoglobulin deposits in normal and lesional skin from patients with allergic skin reactions, vis-à-vis the situation in a healthy control group.

MATERIAL

This investigation comprises 20 patients with allergic contact dermatitis (13 females and 7 males, mean age 48 years, range 15-80 years) 10 patients with atopic dermatitis (3 females and 7 males, mean age 22 years, range 8-39 years), 10 patients with allergic vasculitis (2 females and 8 males, mean age 52 years, range 26-86 years), and 20 healthy individuals with no history of dermatitis (9 females and 11 males, mean age 38 years, range 18-76 years).

The patients with contact allergy had all had a recent, positive patch test, in most cases related to a current or previous eczematous disease. The diagnosis of atopic dermatitis was based on the history and the typical distribution and morphology of the skin lesions.

Three patients also suffered from allergic rhinitis, and were at the time of investigation being desensitized with weekly injections of allergen. In no cases did this therapy influence the skin changes clinically. All the patients were intermittently treated with some locally applied steroid preparation.

The diagnosis of allergic vasculitis was primarily based on the clinical appearance of the skin lesions; in 7 out of the 10 cases, the diagnosis was confirmed by conventional histological examination; in the remaining 3 cases only unspecific changes were found.

Control biopsies were obtained from individuals with apparently normal skin, and with no history of allergic skin reactions. Most of them were patients attending the VD clinic, and in whom no current infection was found, together with elderly patients undergoing orthopedic surgery.

METHODS

Skin biopsies and blood samples were obtained simultaneously from a total of 40 patients and 20 controls.

Table 1. Serological and immunochemical results (median values, ranges given in parentheses) in patients with allergic skin diseases and control individuals

	Contact dermatitis (n=20)	Atopic dermatitis (n=10)	Allergic vasculitis (n=10)	Control group (n=20)
IgA (g/l)	1.9 (1.0–3.6)	1.1 (0.6–2.2)	2.3 ^a (1.2–7.5)	1.4 (0.4–4.2)
IgM (g/l)	0.6 (0.2–1.1)	0.4 (0.3–1.0)	0.4 (0.2–0.6)	0.5 (0.2–1.5)
IgG (g/l)	11.7 (6.6–15.3)	12.5 (6.8–15.3)	11.7 (9.5–20.0)	11.6 (7.6–16.3)
IgD (mg/l)	18 (0–125)	9 (0–70)	12 (0–52)	14 (0–115)
IgE (U/l)	33 (7–186)	661 ^a (26–3 380)	52 (16–2 145)	27 (7–860)
C1q (U/l)	0.81 (0.73–1.03)	0.78 (0.68–0.93)	0.80 (0.51–1.20)	0.79 (0.58–1.20)
C4 (U/l)	1.01 (0.59–1.74)	0.81 (0.35–1.20)	1.00 (0.18–1.69)	0.82 (0.60–2.43)
C3 (U/l)	0.96 (0.69–1.62)	1.03 (0.71–1.16)	1.06 (0.60–1.49)	1.04 (0.56–1.30)
C5 (U/l)	1.20 (0.45–1.65)	0.97 (0.73–1.35)	1.10 (0.78–1.50)	1.15 (0.80–2.00)
C9 (U/l)	1.23 (0.66–1.90)	1.14 (0.60–2.40)	1.15 (0.81–2.00)	1.35 (0.71–2.40)
Fibrinogen (g/l)	7.5 (2.9–16.2)	9.3 (3.3–14.2)	12.4 ^a (6.8–23.2)	9.9 (3.4–21.2)
White cell count × 10 ⁹ /l	6 350 (3 900–13 000)	6 500 (1 600–9 200)	6 600 (2 200–12 300)	6 500 (2 800–9 700)
Eosinophilic count (%)	0.03 (0.00–0.08)	0.07 ^a (0.01–0.20)	0.03 (0.00–0.09)	0.03 (0.00–0.10)

^a $p < 0.05$.

Each patient had skin biopsies taken from lesional and normal skin at symmetrical, non-exposed sites on the back. This was not possible in some atopics and patients with allergic vasculitis, in whom biopsies were taken from the flexural aspect of the arm.

In 18 out of 20 cases of contact dermatitis the biopsy from lesional skin was taken from the positive patch test. Following the regular application for 48 hours, the positive patch was reapplied for another 48 hours, and the biopsy was then taken on the fifth day. In 2 patients the biopsies were taken from areas with acute exacerbation of a chronic, patch-test verified, contact dermatitis.

Ethyl chloride spray was used as a local anaesthetic. Three mm punch biopsies were taken in duplicate: one for immunofluorescence, and one for conventional histological examination. The tissue for immunofluorescence was frozen immediately and stored at -70°C , until 4–6 μm thick sections were cut in a cryostat. The sections were air-dried for 15 min, washed in phosphate-buffered saline (PBS) pH 7.2 for 30 min, and incubated with one drop of diluted conjugate in a moist chamber for 30 min. Sections not incubated with conjugate were controlled for auto-fluorescence, and sections blocked with unconjugated antisera were included as controls.

Conjugates. Fluorescein isothiocyanate (FITC) labelled rabbit IgG specific for human γ , α and μ chains, complement C3 and fibrinogen (Dakopatts A/S, Copenhagen) were used. FITC-labelled anti-human C4 conjugate, and rabbit antisera specific for human δ and ϵ chains and complement C1q, and C9 were from Behringwerke, G.F.R. The isolated IgG fractions from these antisera were labelled with FITC (14). The anti-human immunoglobulins were tested for specificity on IgG, IgM, IgA, IgD and IgE monoclonal bone marrow specimens from patients with multiple myeloma and macroglobulinaemia (14). Before FITC-labelling, the conjugates all showed monospecific reactions in crossed immunoelectrophoresis. The fluorescein/protein ratios as estimated by OD 496/280 nm were 0.5–0.8. The sections were

examined in a Leitz Orthoplan fluorescence microscope. No non-specific staining was seen at the dilution used.

Blood samples were taken for the determination of total and differential white cell count. Serum values of IgA, IgG, IgM and IgD, together with complement components C3, C4, C5 and C9, were determined by quantitative immunoelectrophoresis. Serum IgE was determined by radioimmunosorbent test. Sera were investigated for the presence and titre of IgG (≥ 16) and complement C3 fixing (undiluted) granulocyte-specific and organ-nonspecific antinuclear factors (GS- and ON-ANF) (14). Rat liver cryostat sections and smears of isolated and washed human leukocytes served as nuclear substrates (14). Rheumatoid factors were demonstrated by the FII latex fixation slide test (Behringwerke). Titres ≥ 32 were considered abnormal. Anti-basement membrane antibodies were investigated using normal human skin and guinea pig lip as antigens. The five immunoglobulins were used as conjugates, and titres of sera ≥ 40 were considered abnormal.

Statistical methods

The rank sum test and Fischer's four-fold table test were used (6), and a significance level of 5% was chosen.

RESULTS

The serological and immunochemical results, set out in Table 1, show that the demonstration of high serum IgE and eosinophilic count were most useful in diagnosing atopic dermatitis. Blood fibrinogen levels were high in the patients with allergic vasculitis, as also were serum IgA values.

Abnormal latex titres were found in 10 patients: 8 with contact dermatitis, one with cutaneous vasculitis and in 2 patients with atopic dermatitis.

Five patients with contact dermatitis had IgG

Table II. Immunofluorescent analysis of biopsied skin

DE=dermo-epidermal zone, VW=vessel walls

	Contact dermatitis (n=20)				Atopic dermatitis (n=10)				Allergic vasculitis (n=10)				Controls (n=20)	
	Normal skin		Lesional skin		Normal skin		Lesional skin		Normal skin		Lesional skin		Normal skin	
	DE	VW	DE	VW	DE	VW	DE	VW	DE	VW	DE	VW	DE	VW
IgG	-	-	-	-	-	-	1	-	1	-	3	-	-	2
IgA	-	-	1	1	-	-	-	-	-	-	1	-	-	-
IgM	2	-	2	-	3	-	2	-	-	-	1	1	2	-
C1q	-	-	-	-	-	-	-	-	-	-	1	-	-	-
C4	-	-	-	1	-	-	-	-	-	-	1	1	-	-
C3	-	-	2	-	2	-	3	-	2	-	6	6	-	-
C9	-	-	-	-	-	-	1	-	-	-	1	1	-	-
Fib.	1	-	-	1	4	-	6	-	2	-	6	1	1	-

ON-ANF, and 2 fixed complement C3, all in low titres.

In no case were basement membrane antibodies found in any group.

Immunofluorescent Studies

Contact dermatitis

Deposits were found in 6 patients (Table II). Two had IgM in both eczematous and normal skin; one of them had complement C3 in lesional skin as well. One had IgA, C3 and fibrinogen in lesional skin, and IgA was found in the blood vessel walls. One patient had fibrinogen in the blood vessels of lesional skin; one had complement C4 similarly located. In one patient, fibrinogen was found at the dermo-epidermal junction of healthy skin. This was one of the patients from whom the biopsy was not taken from a patch test; the other one had no deposits.

Atopic dermatitis

Of the 10 patients, 7 had a variety of deposits. Of these, 6 had fibrinogen in lesional skin, 4 patients had it in normal skin as well. C3 was demonstrated in lesional skin of 3 patients; 2 of these also had it in normal skin. Two patients had IgM in normal and lesional skin; one had it in normal skin only. In addition to IgM and complement C3 in normal and lesional skin, one patient had IgG and C9 in lesional skin.

Allergic vasculitis

Eight out of 10 patients had one or more deposits in the skin. Six patients had C3 in lesional skin

(including blood vessels); 2 had it in normal skin as well. IgG was found in lesional skin of 3 patients, one of whom also had it in apparently normal skin. This patient also had IgA in lesional skin. One patient had IgM, C1q, C4 and C9 in lesional skin (including blood vessels, where fibrinogen was also found); C1q and fibrinogen was demonstrated in normal skin. In all, fibrinogen was found in lesional skin of 6 patients; in 2 patients it was also demonstrated in normal skin.

Control group

Only 4 out of 20 patients had deposits. 2 had IgM at the dermo-epidermal zone, one of them also had fibrinogen, 2 had IgG in the vessel walls.

DISCUSSION

Deposits of immunoglobulins, complement components and fibrinogen are found more often in patients with the skin diseases investigated (by us), than in healthy controls. It also appears that when deposits are demonstrated, they are likely to be present in both lesional and clinically healthy skin, although minor variations are seen (10). In our patients the deposits vary so much within the different groups that no clear—and still less diagnostically certain—pattern appears. It is remarkable that in no case were deposits of IgD or IgE demonstrated. Deposits of IgD in patients with cutaneous vasculitis have been reported by others (13), but only in connection with polymorph nuclear leukocytic infiltration. In our material, the infiltrate was of round cell type in 8 out of 10 cases. Further-

more, none of the patients had lung involvement, nor were immunological abnormalities detected in the complement components (2).

Duration of the skin lesions, and the time of biopsy in relation to that, are probably critical factors and may explain some of the differences in various investigations.

The deposits at the dermo-epidermal junction mainly exhibited a granular immunofluorescence staining pattern and no circulating basement membrane antibodies suggesting immune complexes have been found. Subclinical immune complexes in the glomeruli have been found in otherwise normal kidneys from both mice and human beings (7, 11). The basement membrane represents a focal point where these complexes are clearing by fixation, and may be a common route for the catabolism of antigen-antibody complexes during the natural history of a majority of antibody responses in vivo. The antigen component involved in these complexes may be an infectious agent, a drug (or one of its metabolites such as hapten), cell components, or tumour antigens (1, 2, 7, 8, 11).

Regarding the high serum IgE level and eosinophilic count in patients with atopic dermatitis, these are in agreement with other reports (4, 9, 15). The high values of fibrinogen in allergic vasculitis may be due to the decreased fibrinolysis found in this condition (5).

From the results reported above, it is our experience that immunofluorescence studies in the skin diseases investigated by us are at present of limited diagnostic value.

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REFERENCES

1. Baart de la Faille-Kuyper, E. H.: Lupus erythematosus. An immunohistochemical and clinical study of 485 patients. Ph.D. Thesis, University of Utrecht, 1969.
2. Beutner, E. H., Chorzelski, T. P., Bean, S. & Jordon, R. E.: Immunopathology of the Skin. Hutchinson & Ross, Dowden, 1973.
3. Burnham, T. K., Neblett, R. R. & Fine, G.: The application of the fluorescent antibody technic to the investigation of lupus erythematosus and various dermatoses. *J Invest Dermatol* 41: 451, 1963.
4. Carapeto, F. J., Winkelmann, R. K. & Jordon, R. E.: T and B lymphocytes in contact and atopic dermatitis. *Arch Dermatol* 112: 1095, 1976.
5. Conn, D. L., McDuffie, F. C., Holley, K. E. & Schroeter, A. L.: Immunologic mechanisms in systemic vasculitis. *Mayo Clin Proc* 51: 511, 1976.
6. Diem, K. & Lentner, C. (ed.): *Documenta Geigy Scientific Tables*, 7th ed. J. R. Geigy, S. A. Basel, 1970.
7. Markham, R. V., Jr, Sutherland, J. C. & Mardiney, M. R., Jr: The ubiquitous occurrence of immune complex localization in the renal glomeruli of normal mice. *Lab Invest* 29: 111, 1973.
8. Permin, H. & Sestoft, L.: Deposits of plasma proteins in the skin during treatment with carbamazepine and diphenylhydantoin. *Acta Med Scand* 202: 113, 1977.
9. Secher, L., Svejgaard, E. & S nderstrup, G.: T and B lymphocytes in patients with atopic and contact dermatitis. *Br J Dermatol* 97: 537, 1977.
10. Solley, G. O., Gleich, G. J., Jordon, R. E. & Schroeter, A. L.: The late phase of the immediate wheal and flare skin reaction. *J Clin Invest* 58: 408, 1976.
11. Sutherland, J. C., Markham, R. V., Jr & Mardiney, M. R., Jr: Subclinical immune complexes in the glomeruli of kidneys postmortem. *Am J Med* 57: 536, 1974.
12. Ullmann, S., Halberg, P., Nielsen, B., J rgensen, F. & Bals v, J. T.: Deposits of immunoglobulins and complement in the dermo-epidermal junction of patients with anaphylactoid purpura. *Acta Dermatovener (Stockholm)* 55: 359, 1975.
13. Weidner, F.: Immunofluorescent investigations in cutaneous vasculitis. *Arch Derm Res* 254: 215, 1975.
14. Wiik, A. & Munthe, E.: Restrictions among heavy and light chain determinants of granulocyte-specific antinuclear factors. *Immunol* 23: 153, 1972.
15.  hman, S. & Johansson, S. G. O.: Immunoglobulins in atopic dermatitis. *Acta Dermatovener (Stockholm)* 54: 193, 1974.

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