

OCCURRENCE OF ANTINUCLEAR FACTORS AND IMMUNOGLOBULINS BOUND TO THE DERMO-EPIDERMAL JUNCTION IN PATIENTS WITH CHRONIC BIOLOGICAL FALSE POSITIVE (CBFP) REACTIONS FOR SYPHILIS

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Abstract. Sixty-eight patients with chronic biological false positive (CBFP) reactions for syphilis were screened for immunoglobulins bound to the dermo-epidermal junction, and for serum antinuclear factors. The series included 9 patients with definite and 11 patients with probable systemic lupus erythematosus (SLE). Twenty-three patients were found to have bound immunoglobulins, and 35, antinuclear factors in their serums. Both aberrations were more common in the cases of SLE, but the presence of bound immunoglobulins in the uninvolved skin seemed to be more specific for SLE. The IgM class of immunoglobulins was most frequently found both in the dermo-epidermal junction and in tests for antinuclear factors. Circulating auto-antibodies occurred more frequently in the patient group with bound immunoglobulins than in the group lacking this phenomenon. None of the patients with a false positive reaction only in a complement-fixation test with lipoidal antigen had immunoglobulins bound to the basal membrane area.

The *in vivo* binding of immunoglobulins and complement to the dermo-epidermal junction is a characteristic feature of lupus erythematosus (5, 6, 14, 25). This binding occurs in the skin lesions and uninvolved skin of systemic lupus erythematosus (SLE) (6, 14, 26). IgG is also bound to the dermo-epidermal junction in bullous pemphigoid (3). On the other hand, it is known that chronic biological false positive serological tests (CBFP) can precede the appearance of systemic lupus erythematosus by many years, especially in young women (10, 12, 19). Therefore, it seemed to be of interest to combine these methods of investigation and to study a series of patients with CBFP reactions by immunofluorescence examination of the skin.

PATIENTS AND METHODS

The series consisted of 68 CBFP reactors examined at the Department of Dermatology, University Central Hospital,

Helsinki, in 1969-1971. Of the 68 patients, 54 were women and 14 men. Their ages ranged from 12 to 80 years (mean 43 years).

The lipoidal tests applied in this study were the Kahn, VDRL slide, and Kolmer complement-fixation tests (22). Only patients with a negative result in both the *Treponema Pallidum* Immobilization (TPI) and Fluorescent Treponemal Antibody-Absorption (FTA-ABS) tests were included in the study (15).

The diagnosis of definite or probable SLE was, with one modification, based on the criteria proposed by Mustakallio et al. (16). The diagnosis of probable SLE was not established unless the antinuclear factor test gave a positive result. Patients with clinical and serological signs generally known to be connected with connective tissue diseases but not sufficient for any specific diagnosis were classified as having indefinite connective tissue disease.

In addition to the laboratory examinations included in the 25 criteria for SLE in 1966 (16), the following serological tests were performed:

Antinuclear factor test

Antinuclear factors were tested by an indirect FA technique (9). Titres of $\geq 1:10$ were regarded as positive. In positive cases, the immunoglobulin class of ANF was studied by specific antisera against IgG and IgM, using a method described by Wager et al. (29).

Rheumatoid factor tests

The technique of the Waaler-Rose test has been described earlier (23). For the latex test, the one-tube technique of Singer and Plotz (24) was used.

Thyroid antibodies

Antibodies to thyroglobulin were studied by the thyroglobulin tanned red cell (TRC) haemagglutination method; in addition, antibodies to thyroid microsomal antigen were sought by a complement-fixation (CF) method (20). In the TRC-test titres of $\geq 1:25$ and in the CF-test titres of $> 1:4$ were regarded as positive.

Screening for cryoglobulins and cryoprecipitation

For cryoglobulins, the technique was that described by Mustakallio et al. (18). Screening for cryoprecipitation was

Table I. *The clinical diagnoses and main clinical symptoms of the 68 CBFP reactors in correlation with bound immunoglobulins in the dermo-epidermal junction*

	Bound immunoglobulins		
	Present	Not present	Total
Definite SLE	7	2	9
Probable SLE	6	5	11
Definite or probable RA	1	3	4
Indefinite connective tissue disease	0	5	5
Autoimmune thyroiditis	2	1	3
Cryoglobulinaemic purpura	0	2	2
Cold agglutinin syndrome (chronic)	2	0	2
Polyarteritis nodosa	0	1	1
Sarcoidosis	0	1	1
Epilepsy	0	1	1
Eczema solare	1	1	2
Urticaria	0	2	2
Erythrodermia	0	1	1
Psoriasis vulgaris	0	1	1
Vitiligo	0	1	1
Hepatomegaly	1	4	5
Hepatosplenomegaly	1	1	2
Serological changes only	2	11	13
Clinically healthy	0	2	2

carried out by the technique described by Wager et al. (28) with a 4-day observation time.

Coombs test

A direct Coombs antiglobulin test was performed according to the microtechnique developed by Wager et al. (27).

Processing of skin biopsies

Immunofluorescent examination of the skin was performed with biopsies taken from the uninvolved skin of the forearm. The biopsies were immediately frozen with carbon dioxide and sectioned in a cryostat at 6 μ . The slides were washed for 30 min in phosphate-buffered saline and incubated for 30 min in a moist chamber with FITC conjugated antisera. After incubation, the slides were

Table II. *Occurrence of different immunoglobulins in the dermo-epidermal junction, and immunoglobulin class of the antinuclear factors*

	IgM	IgG	IgM- IgG	IgM- IgG- IgA	IgG- IgA	Total
Bound immunoglobulins	9	1	2	2	1	15
Antinuclear factors	15	2	13	0	0	30

again washed for 30 min with PBS. The following antisera were used: for screening purposes, all the biopsies were stained with horse anti-human globulin conjugated with FITC (Progressive Laboratories, Inc., Baltimore, Md., USA; distributed by Roboz). Positive biopsies in the screening examination were studied by FITC-conjugated antisera against IgG, IgM, and IgA, manufactured by Nordic Pharmaceuticals, Holland.

Microscopy was performed with Leitz Orthoplan fluorescence microscope using incident light, Xenon light source, AL 485 exciter, and OG 515 barrier filters.

RESULTS

The occurrence of immunoglobulins in the dermo-epidermal junction

In 23 out of the 68 CBFP reactors, bound immunoglobulins could be found in the dermo-epidermal junction. Of these 23 patients, 17 were females and 6 males. Ten of the women were less than 40 years old.

The clinical diagnoses or main clinical symptoms of the 68 CBFP reactors are presented in Table I in correlation with the occurrence of bound immunoglobulins. As seen from the Table, in 2 of the 9 patients with definite SLE, bound immunoglobulins could not be found in the dermo-epidermal junction. One of them, a young woman having corticosteroid treatment, had suffered from skin lesions typical of SLE, though at the time of the study her skin was normal. The other one, a male patient, had never had skin lesions. He had been treated with corticosteroids 5 years previously, but at the time of the study he was in clinical remission.

Six of the 11 patients, diagnosed as having probable SLE, had bound immunoglobulins in the skin. None of the 5 patients in whom bound immunoglobulins could not be demonstrated in the dermo-epidermal junction, was having corticosteroid therapy, but 1 patient was receiving anti-malarial drugs. Of the 4 patients diagnosed as having definite or probable rheumatoid arthritis (RA), only 1 had bound immunoglobulins in the dermo-epidermal junction. This patient was suffering from severe mutilating RA with systemic manifestations having, in addition, bullous pemphigoid. The patient's serum contained antibodies reacting with the basal membrane.

As seen from Table I, cryoglobulinaemic purpura was the main diagnosis in 2 cases. One of them displayed the purpura-arthritis-rheumatoid factor syndrome with cryoglobulinaemia of the

mixed IgG-IgM type. In none of them could immunoglobulins be demonstrated in the dermo-epidermal junction, but cutaneous vessels showed fluorescence with the IF staining. Only one of the patients with miscellaneous dermatoses had immunoglobulins in the dermo-epidermal junction. She was a 28-year-old-woman, admitted to our hospital because of light eruption. However, at the time of the study, her skin was normal. Except for arthralgia and migraine, she was clinically in apparent health. In the dermo-epidermal junction, immunoglobulins IgG, IgM, and IgA were demonstrated. In addition, she had antinuclear factors, thyroid antibodies, and cold agglutinins in her serum.

Classification of the bound immunoglobulins

By the use of specific antisera, sections of biopsies of 15 patients were studied to ascertain the occurrence of IgG, IgM, and IgA in the dermo-epidermal junction. The occurrence and class of bound immunoglobulins are presented in Table II in correlation with the occurrence and class of ANF in the serum of the CBFP reactors.

IgM was the most frequent immunoglobulin seen in the dermo-epidermal junction, found in 13 out of the 15 tested cases. IgM was the sole class of immunoglobulin in 9 cases, IgG in 1 case. This was the patient with bullous pemphigoid and confirmed RA. Another patient with probable SLE had IgG and IgA bound to the dermo-epidermal junction. He had small multiple leg ulcers, purpura, and recurrent deep thromboses despite strong circulating anticoagulant activity of his

Table III. *The occurrence of bound immunoglobulins in correlation with the occurrence of circulating auto-antibodies in the serum*

Percentages within parentheses

	Bound immunoglobulins		
	Present	Not present	Total
No. of patients ...	23	45	68
Antinuclear factors	18 (78%)	17 (38%)	35
Rheumatoid factors	10 (43%)	10 (22%)	20
Thyroid antibodies	10 (43%)	13 (29%)	23
Cryoglobulins	12 (52%)	18 (40%)	30
Direct Coombs test, positive	7 (30%)	3 (7%)	10

Table IV. *The occurrence of antinuclear factors and immunoglobulins in the dermo-epidermal junction compared with the results of standard serological tests for syphilis (STS)*

	ANF +		ANF -	
	Ig+	Ig-	Ig+	Ig-
Bound immunoglobulins (Ig)				
Positive flocculation test	4	4	0	13
Positive complement-fixation test	0	4	0	1
Both tests positive	14	9	5	14

serum. In addition, in 1 female patient IgM was found in the discoid lupus erythematosus lesions. In the uninvolved skin, no immunoglobulins could be demonstrated; she had therefore been included in the patients lacking bound immunoglobulins in the dermo-epidermal junction.

The occurrence of circulating auto-antibodies in the serum in correlation with bound immunoglobulins in the dermo-epidermal junction

The sera of the 68 CBFP reactors were further studied for the occurrence of circulating auto-antibodies (Table III). As seen in the Table, the occurrence of antinuclear factors, rheumatoid factors, thyroid antibodies, and antiglobulin antibodies (direct Coombs test) was higher in the 23 patients with bound immunoglobulins than in the 45 patients lacking bound immunoglobulins in the dermo-epidermal junction. On the other hand, as seen from the Table, in the occurrence of cryoglobulins there was only a slight difference between the two patient groups.

The standard serological tests for syphilis (STS)

The 68 CBFP reactors were evaluated with two flocculation tests and one complement-fixation test for syphilis. In Table IV, the results of these tests are presented in correlation with the occurrence of bound immunoglobulins and antinuclear factors. The Table shows that 5 of the patients had a positive result only in the complement-fixation test; in none of them could immunoglobulins be demonstrated in the dermo-epidermal junction. Nineteen of the patients with bound immunoglobulins showed a positive result in both flocculation and complement-fixation tests.

DISCUSSION

The finding of bound immunoglobulins in the dermo-epidermal junction of the uninvolved skin has generally been accepted as an important diagnostic criterion for SLE (5, 6, 14, 25, 26). However, this phenomenon has also been observed in the basal membrane zone of the skin of patients with bullous pemphigoid (3), and in some cases of rosacea (2, 11, 21).

In the present series of CBFP reactors, immunoglobulins occurred mostly in the uninvolved skin of patients diagnosed as having definite or probable SLE. Moreover, in the majority of the remaining cases with demonstrable immunoglobulins, there was clinical and/or serological evidence of an autoimmune disorder, though insufficient for a diagnosis of SLE.

Cormane et al. (7) have reported that in their series, IgG was apparently always present in the skin lesions of discoid lupus erythematosus (DLE) and also in the uninvolved skin of SLE. IgM and IgA were only coincidentally present. In rosacea, the classification of immunoglobulin has yielded results similar to those obtained in SLE. (2, 11).

In the present series, IgM was demonstrated in the dermo-epidermal junction in 13 out of the 15 cases tested with specific antisera. IgG as the sole immunoglobulin was seen in only one case, a patient with bullous pemphigoid and rheumatoid arthritis. Furthermore, IgM also seemed to be the most common immunoglobulin class with ANF-activity in our series. From the point of view of this finding, attention must be drawn to the occurrence of rheumatoid factor activity (8). However, only 7 out of the 28 patients with ANF of the IgM or IgG-IgM type had rheumatoid factor activity in their serum.

The presence of bound immunoglobulins may be considered as a more specific finding for SLE than the serum ANF-activity. Only a few of the patients lacking sufficient evidence for a diagnosis of either definite or probable SLE in the present series showed bound immunoglobulins as compared with ANF serum activity. In accordance with the findings of Burnham et al. (4), circulating auto-antibodies occurred more frequently in the patient group with bound immunoglobulins.

It was of special interest that in CBFP reactors, the IgM class of immunoglobulins was most frequently found both in the dermo-epidermal junction and in tests for ANF. On the other

hand, Aho (1) has shown that the BFP reactivity is mainly detected in 19-S globulins, while syphilitic lipoidal antibodies in latent syphilis are usually of the 7-S type. It is also known that SLE connected with a CBFP reaction (12) or rheumatoid factor activity (17) seems to have a milder clinical course than in cases without these serological aberrations. A predominance of IgM type antibodies in cases with SLE may therefore suggest a better prognosis (cf. 12).

The results of the standard serological tests for syphilis showed that none of the patients with a false positive reaction only in the complement-fixation test, had immunoglobulins bound to the basal membrane zone. This is in accordance with the findings of Johansson et al. (13) who showed that patients with a false positive reaction only in a complement-fixation test, are usually elderly and lack signs of SLE. By contrast, such signs were often found in patients with false positive reactions in both flocculation and complement-fixation tests or only in a flocculation test.

The most consistent finding in the present study was the predominance of IgM both in the dermo-epidermal junction and in ANF-activity. It also became clear that CBFP reactors with immunoglobulins bound to the basal membrane zone show a greater tendency to systemic autoimmune disease, especially of the SLE type, than patients lacking demonstrable immunoglobulins in the skin. The immunofluorescence method may therefore be a useful means of selecting CBFP reactors requiring a follow-up study, as it is well known that the CBFP phenomenon may precede the development of an autoimmune disorder of SLE type by many years.

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