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AN IMMUNOCHEMICAL STUDY ON MASTOCYTOSIS

(Urticaria Pigmentosa)

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In two cases of Urticaria Pigmentosa (skin mastocytosis) Möslein (6) demonstrated a marked relative increase of albumin, and a slight decrease of alfa and gamma globulins in the electrophoretic pattern of the serum proteins. However, as each electrophoretic fraction covers several independent antigens (*vide infra*) with different and specific biological functions, the changes found by Möslein cannot give direct indication of a probable pathogenetic significance of the above-mentioned changes. As part of current studies of urticaria pigmentosa, the present paper deals with the individual changes in serum antigens of seven patients suffering from urticaria pigmentosa, of which one was of the bullous diffuse type. The changes are compared with normal serum findings and with those of scleroderma.

Material and Methods

Serum specimens were obtained from 28 normal individuals of both sexes, six patients with scleroderma, and eight patients with urticaria pigmentosa. The normal individuals had all been without infections three weeks prior to estimation of the content of serum immunoglobulin. Serum as well as bullous fluid were obtained from one patient with the bullous diffuse variant of urticaria pigmentosa. The venous blood was allowed to clot for a few hours at room temperature. The clotted blood was centrifuged at 2500 g for 15 minutes, after which it was stored in a deep-freezer at $-20°C$ for subsequent analysis.

All chemicals used were of the highest purity obtainable from British Drug Houses, with the exception of Noble Agar.[1]

Immunochemical Methods

Micro-immuno-electrophoresis (8) was used for qualitative screening of the serum and blister fluids for proteins. The immuno-electrophoresis was performed in agar-buffer (1 % w/v in sodium-diethyl-barbiturate buffer, 0.05M, pH 8.6), 1 mm thick, on microscopic glass slides (7.6×2.6 cm). The pattern consisting of two holes (diameter 1 mm) 0.5 cm apart from the common antibody trough was made by a template (Shandon Ltd. London). The experimental conditions used were carefully standardized as described by Clausen in 1969 (1). 1.5 μl undiluted serum or blister fluid was studied using 80 μl undiluted antiserum. Each pattern was compared on the same slide with that of the pooled normal human serum.

The antisera used consisted of a polyvalent horse antiserum against pooled hu-

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[1] Obtained from Difco Lab., USA.

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man serum, and specific rabbit antisera against albumin, alpha-2-M, alpha-2-haptoglobin, transferrin, β-1-AC, IgG, IgA and IgM. The antisera used were all obtained from the Red Cross Central Laboratory of Amsterdam.

The electrophoresis (7 volt/cm, 1 1/2 h at 25°C) was followed by immunodiffusion for 16 h at 25°C in the electrophoretic chamber saturated with water. The lines were interpreted as described by Clausen (1) on slides washed for two days in 0.9 % (w/v) NaCl and 1 hour in distilled water, then dried and stained with Amido Black.

Quantitative radial immunodiffusion (5) was used for quantitative determination of the immunoglobulins IgG, IgA, and IgM. IgG was determined in plastic Petri-dishes (diameter 5 cm, height 0.5 cm) filled with two ml of an equal mixture of 45°C warm agar buffer (1 % w/v agar in 0.050 M sodium-diethyl-barbiturate buffer pH 8.6) and specific rabbit antiserum against IgG diluted 20 times with sodium-diethyl-barbiturate buffer (*vide supra*). This antiserum was made by immunization of rabbits five times with 250 µl IgG globulin (5 % w/v) dissolved in 0.9 % (w/v) NaCl (1).

The immunologically pure IgG globulin was isolated from normal human serum by column chromatography on DEAE cellulose as described by Fahey and Horbett (2). The IgG content was estimated on 1.5 µl samples applied into 1 mm holes of the agar with an equilibrated Hamilton syringe. The holes were placed 1 mm apart 1.5 cm from the center of the dish. After immunodiffusion for two days the leading edges of the immunoprecipitates did not move. The content of IgG was estimated on the basis of the diameters measured using suitably diluted immunologically pure IgG as standard. IgA and IgM were estimated on "Partigen plates", using a standard serum.[2] The experimental conditions were as described above (1) except that the 2.0 µl antigen sample was applied onto the Mancini plates.

Total protein was determined as described by Lowry *et al.* (3) using tyrosine as standard.

Results

The immunoelectrophoretic studies of serum from the six cases of Urticaria Pigmentosa and eight of Scleroderma revealed the presence of all serum proteins. No abnormalities in the shape of immunoprecipitates could be traced apart from a transformation of the β-1-C globulin into β-1-A (inactive third part of complement). However, this cannot be evaluated, since freezing and storage can cause similar changes (4).

Immunoelectrophoretic studies of the blister fluid revealed the presence of all serum proteins, including the high molecular species α-2-macroglobulin, α-2-lipoprotein, and IgM. No immunoprecipitates revealed abnormalities in shape, but the precipitates for IgA and IgG were distinct indicating both these immunoglobulins to be present (*vide infra*).

The quantitative data are indicated in Table 1. The data are correlated to those obtained from the normal material (28 determinations). The normal adult limits were similar to those obtained by Schwick and Störiko (8) apart from the fact that the IgA and IgM content was found to possess a narrower range than indicated by these authors. In siblings and children aged below 2 years there was evidence of lower IgG and IgA values than in the adults. In all urticaria pigmentosa patients but one, the quantitative radial immunodiffusion revealed a normal serum IgA content (one patient showed a value below the lower limit). All patients but one had increased IgG. The IgM values were all increased. The Urticaria Pigmentosa blister showed significant amounts of all three immunoglobulins. The ratio between the content of IgG and IgA was 20 : 1 (w/w) contrasting to a value of 10 : 1 in normal serum.

Five Scleroderma patients studied showed an increase of all immunoglobulins, one patient had a normal IgA level.

[2] Behringwerke, Germany.

Table 1

Disease		Code No	IgG G/100 ml	IgA G/100 ml	IgM G/100 ml
Urticaria Pigmentosa	Serum	02 11 50	2.96	0.120	0.204
		08 07 64	1.08	0.016	0.160
		26 11 50	2.52	0.184	0.308
		23 10 60	1.88	0.096	0.176
		30 07 44	2.42	0.156	0.216
		29 04 47	1.74	0.172	0.286
		03 10 66	1.50	—	0.296
		24 02 28	2.06	0.200	0.280
	Bulla		0.738	0.035	0.068
Scleroderma Serum		04 03 48	2.42	0.216	0.240
		16 02 22	2.02	0.344	0.240
		11 08 18	2.70	0.280	0.308
		25 03 15	2.30	0.584	0.204
		31 92 09	2.69	0.328	0.216
		05 03 24	2.69	0.248	0.240
Normal range, adults 18–37 years of age			0.93 to 1.40	0.067 to 0.253	0.048 to 0.123
Mean			1.17	0.162	0.084
Normal range, siblings and children <2 years			0.74 to 1.17	0.021 to 0.064	0.126 to 0.144
Mean			0.88	0.032	0.136

Discussion

The present data correlate the qualitative and quantitative abnormalities in the protein pattern of serum of patients suffering from Urticaria Pigmentosa with those of Scleroderma that has a possible autoimmune genesis, and further correlate these two disorders with the protein pattern of normal serum.

All Urticaria Pigmentosa patients showed normal or decreased IgA levels. Seven out of eight patients showed increased IgG, and all had increased IgM levels. These findings are different from those found in Scleroderma, where only 16 % showed an IgA level within the normal range. The "dissociated" immune response with increase in the serum levels of IgG and IgM and a normal IgA level in urticaria pigmentosa are unexplained since no known immunological processes are associated with this disorder.

SUMMARY

The concentration of the three main immunoglobulins of human serum (IgG, IgA, and IgM) was determined by quantitative radial immunodiffusion in eight specimens of serum from patients with Urticaria Pigmentosa, and six specimens from patients with Scleroderma. The results obtained were correlated with normal serum findings. In Urticaria Pigmentosa, a "dissociated" immune response was found with an increase in IgG and IgM associated with normal (7 cases) or decreased (one case) IgA levels. This was in contrast to the findings in Scleroderma, where, in all but one case, the three immunoglobulins were found to be increased. No abnormalities

were found in the immunoelectrophoretic mobilities and the shapes of the precipitation lines.

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