Immunoglobulin Levels in Atopic Eczema

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The term "atopic dermatitis" was given by Wise and Sulzberger (1933) to those inflammatory dermatoses which are intimately associated with other allergic manifestations with a strong hereditary influence, such as hay-fever and asthma, described by Coca and Cooke (1923) as atopic.

Patients suffering from atopic conditions have a high incidence of circulating antibodies of a particular type. These antibodies, known as reagins, are responsible for positive immediate-type hypersensitivity reactions when the patient is skin-tested with a wide range of naturally occurring antigens. These antibodies cannot be demonstrated by *in vitro* techniques. However, their presence can be shown by the passive transfer technique of Prausnitz and Küstner (1921). They also differ from other circulating antibodies by having a high affinity for skin and being heat-labile. In June 1964 a committee of the World Health Organization defined proteins of animal origin with known antibody activity as immunoglobulins. The human immunoglobulins have been divided into three major groups. Two groups of molecular weight ~150,000 were called IgG (previously γ , γ_{7S} , γ_{8S} , or γ_2) and IgA (previously β_{2A} or γ_{11}). The third group consists of the macroglobulins of molecular weight ~900,000 and is called IgM (previously γ_{1M} , β_{2M} , or $19S\gamma$). Most circulating antibodies occur in the IgG fraction, though the IgM fraction contains red-cell iso-antibodies, saline anti-D (rhesus) antibodies, and immune antibodies directed against the somatic antigens of *Salmonella* spp.

Until recently many workers considered reagin-type antibodies to be immunoglobulins of the type IgA. However, more recently there has been evidence to suggest that the concept has been somewhat oversimplified, and in fact molecules of the class of IgG might be involved. As there is no doubt that reagin-type antibodies are readily demonstrated in patients with atopic eczema, the present study was undertaken to determine the levels of immunoglobulins in patients with atopic eczema as compared with those with other skin conditions, including other patterns of eczema. Until recently this has not been possible because of the lack of a simple quantitative technique for determining accurately the levels of these different forms of immunoglobulins (Fahey and McKelvey, 1965). Previous work has been of a semi-quantitative type either by the use of scanning of electrophoresis strips (Tickner, McCabe, and Mier, 1959), or, more recently, by the arbitrary assessment of the degree of precipitation in immunoelectrophoresis (Kálder, 1965).

Material and Methods

The investigation was carried out on 113 adults and 51 children.

A. The adults consisted of five groups:

1. Twenty-seven patients with atopic eczema.
2. Twenty-seven patients with eczematous conditions other than atopic eczema. These included: contact dermatitis 9 cases, eczema unclassified 4, stasis eczema with secondary spread 6, photodermatitis 3, seborrhoeic eczema 5.
3. Twenty-one patients with miscellaneous non-eczematous dermatoses. These consisted of: discoid lupus erythematosus 1, allergic vasculitis 1, mycosis fungoides 2, pityriasis rubra pilaris 1,

pityriasis versicolor 1, psoriasis 7, pemphigus erythematosus 1, pemphigoid 1, pruritus ani 1, rosacea 1, lichen planus 2, urticaria 2.

4. Twenty-one patients with asthma and no history of atopic eczema, 10 of whom were skin-test-positive to one of the standard allergens.

5. Seventeen healthy subjects without any history of skin diseases. This group included mainly medical and laboratory staff.

B. Fifty-one children ranging in age from 1 to 13 years were divided into two groups:

1. Thirty-five patients with atopic eczema.
2. Sixteen patients with other dermatoses. These were: psoriasis 7 cases, scabies 3, pityriasis lichenoides chronica 2, photodermatitis 1, discoid eczema 2, erythrodermatitis variabilis 1.

Serum was obtained under sterile conditions and stored at -20°C . It was examined within one week by the agar-gel-immuno-diffusion method. None of the patients was receiving systemic corticosteroid treatment at the time the sera were collected.

Quantitative Estimation of Serum Immunoglobulins.—Serum levels of IgG, IgA, and IgM were estimated quantitatively by a method of gel immunodiffusion. This involved the use of commercially available immunoplates (Hyland Laboratories, Los Angeles, California) in which specific antiserum to one of the human immunoglobulins is incorporated in a buffered agar gel. Samples of the test sera were allowed to diffuse from wells in the agar plates, thus forming a ring of antigen-antibody precipitate around each well. Three standard solutions of the appropriate immunoglobulin were used as reference in each plate. After filling the wells the plates were kept overnight in a humid atmosphere at 4°C . to allow development of precipitate (Fahey and McKelvey, 1965). The diameters of the precipitation rings were then measured by means of a magnifying scaleometer (Baird and Tatlock Ltd., London). Measurements were made independently by two investigators, providing duplicate readings in which the acceptable error was not greater than ± 0.1 mm. By plotting the diameters of the three standard solutions on a graph paper standard curves were obtained, and by reference to them the concentrations of the test sera were determined. In order to ascertain whether a given result could be reproduced, three samples were tested in five different plates. No differences were found in the diameters of the precipitation rings formed in the different plates.

Skin Tests.—Skin tests to various common allergens were performed on 23 out of the 27 atopic adults and on 28 out of the 35 atopic children for detection of immediate-type hypersensitivity. The standard solutions of allergens obtained from Bencard Allergy Division, Beecham Research Laboratories, Brentford, were used on both adults and children. They consisted of pollens and other common inhalants, foods, fabrics, tobacco, yeasts, and moulds. The tests were performed on the flexor surface of the forearms by scarifications and intradermal injections, and the sites were observed throughout the first 30 minutes.

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Results

Table I shows the serum levels of immunoglobulins in adults and children. The results are expressed as mean values $\pm$ the standard deviation. It is apparent that the level of IgG in patients with atopic eczema is higher than that in the other groups, in adults as well as in children. Adults with atopic eczema had a mean value of 21.2 mg./ml. In patients with other eczematous conditions, other dermatoses, or asthma the mean concentration of IgG was 15.2 mg./ml. In normal subjects it was 13.6 mg./ml. In the atopic children the mean value of IgG was 20.6 mg./ml., whereas in the children with other dermatoses it was 14.0 mg./ml. Statistical analysis shows that the probability of difference between the atopic eczema group and the other groups in both adults and children is less than 0.001.

TABLE I.—Levels of Immunoglobulins (mg./ml.) in Patients with Atopic Eczema as Compared with Patients with Other Skin Diseases. (Results Expressed as Mean $\pm$ Standard Deviation)

	Total	IgG	IgA	IgM
Adults:				
Atopic eczema ..	27	21.2 $\pm$ 3.8	3.52 $\pm$ 1.08	0.87 $\pm$ 0.40
Other eczemas ..	27	15.2 $\pm$ 5.6	3.82 $\pm$ 1.17	0.86 $\pm$ 0.35
Other dermatoses ..	21	15.2 $\pm$ 4.8	2.80 $\pm$ 1.50	0.79 $\pm$ 0.25
Asthmatics ..	21	15.2 $\pm$ 2.3	2.87 $\pm$ 0.99	0.89 $\pm$ 0.12
Normal subjects ..	17	13.6 $\pm$ 3.1	2.92 $\pm$ 0.72	0.83 $\pm$ 0.20
Children:				
Atopic eczema ..	35	20.6 $\pm$ 4.2	1.97 $\pm$ 0.89	0.84 $\pm$ 0.26
Other dermatoses ..	16	14.1 $\pm$ 1.5	1.98 $\pm$ 0.79	0.69 $\pm$ 0.28

* Probability of difference between two groups < 0.001.

There was no difference between the IgA levels in adult patients with atopic eczema and those with other eczemas. However, the IgA level in patients with eczema, whatever the cause, was greater than that found in normal subjects or those with other skin diseases. This was demonstrated when the IgA levels of patients with atopic eczema and eczema due to other causes were grouped together and compared with the levels found in patients with other dermatoses or asthma and in normal subjects. A significant increase in the IgA levels was found in the serum of patients with eczema irrespective of cause ($P < 0.001$). No difference was found in the IgA level of children with atopic eczema when compared with that found in children with other dermatoses. Also, no differences were found in the IgM levels in all groups examined.

In Table II the serum IgG concentrations have been separated into three groups (<17 mg./ml., 17–19 mg./ml. and >19 mg./ml.), and the numbers of patients are expressed as percentages in the various disease groups. It is apparent that 71% of the adults with atopic eczema and 66% of the children with atopic eczema had serum levels of IgG over 19 mg./ml., whereas only 8% of patients with other eczemas and none of those with other dermatoses had IgG levels over 19 mg./ml. None of the normal adults and none of the children with other dermatoses had IgG levels of that magnitude. On the other hand, 74% of the adults with non-atopic eczema, 95% of the adults with non-eczematous dermatoses, all the normal adults, and all the children with non-atopic dermatoses had IgG levels less than 17 mg./ml.

None of the patients with atopic eczema who had IgG levels greater than 19 mg./ml. had IgA levels greater than 5 mg./ml.

TABLE II.—Percentage of Patients in Each Group with IgG Levels of >19, 17–9, and <17 mg./ml.

	Adults				Children	
	Atopic Eczema	Other Eczemas	Other Dermatoses	Normal Subjects	Atopic Eczema	Other Dermatoses
No. of patients examined ..	27	27	21	17	35	10
IgG level > 19 mg./ml. ..	71	8*	0	0	66	0
IgG level 17–19 mg./ml. ..	11	18†	5	0	17	0
IgG level < 17 mg./ml. ..	18	74	95	100	17	100

* All had IgA levels > 5 mg./ml.

† 2 out of 5 patients in this group had IgA levels > 5 mg./ml.

However, the two patients with eczema from other causes with IgG levels greater than 19 mg./ml. and two of the five patients in this group with IgG levels between 17 and 19 mg./ml. had IgA levels greater than 5 mg./ml. Table III gives an indication of the IgG levels in children with and without atopic eczema. Most of the atopic children with IgG levels below 17 mg./ml. were in the younger age group, although 11 children were found between the ages of 1 and 4 with levels greater than 19 mg./ml. It will be noticed that atopic eczema covered a different age distribution in children when compared with other dermatoses. It was not possible to compare children with atopic eczema with children suffering from other dermatoses completely according to age. Twelve (34%) children and 15 (55%) adults with atopic eczema had asthma as well. No significant differences were found in the immunoglobulin levels in the serum of those with and without asthma.

TABLE III.—Levels of IgG in Children of Different Ages

Age Group	Total	< 17 mg./ml.	17–19 mg./ml.	> 19 mg./ml.
Atopic Eczema				
1–4	19	5	3	11
5–8	12	1	2	9
9–13	4	0	0	4
Other Dermatoses				
1–4	5	5	0	0
5–8	5	5	0	0
9–13	6	6	0	0

Immediate hypersensitivity of the weal-and-flare type was detected to pollen and other inhalants in 60% of adults and 67% of children with atopic eczema who were skin-tested with standard allergens. Again, no significant differences in the levels of immunoglobulins were found between positive and negative skin reactors.

Discussion

IgG levels in patients with atopic eczema are shown to be significantly higher than those in patients with other eczemas, other dermatoses, or asthma. IgA levels in adults were also somewhat higher in patients with atopic eczema than the levels found in normal subjects or those with other dermatoses. These increased levels of IgA were not specific to the atopic state, as they were also found in patients with other patterns of eczema. IgM levels were not raised in any of the skin diseases investigated. It was particularly significant that IgG levels were raised not only in adults with atopic eczema but also in children, even at the age of 1 year.

No significant differences were found in the immunoglobulin levels in the serum of patients with atopic eczema with and without asthma. Similarly there were no differences in the levels in those patients showing hypersensitivity of weal-and-flare type, when compared with those giving negative skin responses.

The significance of the raised levels of IgG in patients with atopic eczema must be discussed in relation to the recent suggestion that reagins which can be readily demonstrated in atopic diseases belong to the class of immunoglobulins known as IgA. Fireman, Vannier, and Goodman (1963) demonstrated that the removal of IgA globulins from the sera of ragweed-sensitive people by immune absorption was associated with a loss of reagins, as tested by the Prausnitz-Küstner reaction. Ishizaka, Ishizaka, and Hornbrook (1963) also showed that a normal human IgA globulin preparation was capable of blocking passive sensitization of non-allergic human skin reagins.

Although Fireman *et al.* (1963) were unable to remove reagent activity by absorption of their sera with specific antisera against IgG, and Ishizaka *et al.* (1963) were unable to block the Prausnitz-Küstner reaction with IgG, evidence has been accumulating to suggest that reagent-type antibodies can under certain conditions occur in the IgG fraction. Loveless (1964)

showed that a patient without any detectable IgA could form reagin-type antibodies. Fireman, Boesman, and Gitlin (1965) have also investigated a patient with ataxia telangiectasia who had no detectable IgA and demonstrated weal-and-flare skin reactions to cows' milk protein. Skin sensitizing antibody to this protein was demonstrated in the patient's serum by the Prausnitz-Küstner test. The IgG globulin fraction isolated from the patient's serum by chromatography was found to contain the reagin-like activity.

Stanworth (1965) has discussed the structure of reagins more fully. He studied reagins by ultracentrifugation and found that they have a sedimentation constant higher than that found for immunoglobulins of molecular weight $\sim 150,000$. Moreover, on diethylaminoethyl cellulose chromatography reagins from normal subjects were detected in a fraction midway between the IgG and IgA fractions. He has therefore suggested the possibility that reagins could consist of end-to-end dimers, one monomer unit being IgA and the other unit IgG.

There is therefore strong evidence that immunoglobulins of the type IgG can be involved in the structure of reagin-type antibodies. This may explain the raised level of IgG in atopic eczema found in the present study. However, it is possible that, as in patients with asthma alone, the concentration of reagins in the serum of patients with atopic eczema is not high enough to contribute significantly to the level of circulating immunoglobulins. If this is so the marked increase in IgG level in atopic eczema could be due to some other, probably unrelated, phenomenon.

The clinical diagnosis of atopic eczema does not usually present any problem, but in some cases there may be difficulty in differentiating atopic from other patterns of eczema. In the present series approximately 70% of the patients with atopic eczema examined were found to have IgG levels >19 mg./ml. and these were associated with IgA levels <5 mg./ml. In con-

trast to this, only two of the 27 patients with other eczematous conditions had comparable IgG levels, and in both these cases the IgA levels were higher than 5 mg./ml. We would therefore suggest that assay of immunoglobulins could be a useful tool in the differential diagnosis of eczematous conditions. A level of IgG >19 mg./ml. associated with a level of IgA <5 mg./ml. would be highly suggestive of the diagnosis of atopic eczema.

Summary

A highly significant rise in the level of circulating immunoglobulins of the class IgG was detected in both adults and children with atopic eczema when compared with patients showing other patterns of eczema or asthma. These results are discussed in the light of recent evidence relating reagenic antibodies to both IgG and IgA immunoglobulins. It is suggested that assay of the immunoglobulin levels in the serum could be useful in the differential diagnosis of atopic eczema.

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REFERENCES

- Coca, R. F., and Cooke, R. A. (1923). *J. Immunol.*, **8**, 163.
 Fahey, J. L., and McKelvey, E. M. (1965). *Ibid.*, **94**, 84.
 Fireman, P., Boesman, M., and Gitlin, D. (1965). *J. Allergy*, **36**, 194.
 — Vannier, W. E., and Goodman, H. C. (1963). *J. exp. Med.*, **117**, 603.
 Ishizaka, K., Ishizaka, T., and Hornbrook, M. M. (1963). *J. Allergy*, **34**, 395.
 Káldor, I. (1965). *Trans. St John's Hosp. Derm. Soc. (Lond.)*, **51**, 73.
 Loveless, M. H. (1964). *Fed. Proc.*, **23**, 403.
 Prausnitz, C., and Küstner, H. (1921). *Zbl. Bakt., I Abt. Orig.*, **86**, 160.
 Stanworth, D. R. (1965). *Int. Arch. Allergy*, **28**, 71.
 Tickner, A., McCabe, M. G. P., and Mier, P. D. (1959). *Brit. J. Derm.*, **71**, 351.
 Wise, F., and Sulzberger, M. B. (1933). *Year Book of Dermatology and Syphilology*. Chicago.

New Test for Finger-tip Sensation

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This paper describes a simple objective test for finger-tip sensation which is believed to be a more accurate index of tactile sensation and is less time-consuming than the conventional tests.

The two-point discrimination threshold is the average of several measurements of two compass points approximated until they can be felt as one, in the longitudinal and transverse directions. When carefully carried out it is probably the most useful objective and quantitative test of sensation. It is subject to error when the patient is fatigued, and the period of rest required when several areas are tested makes it a time-consuming examination (Mannerfelt, 1962). Not infrequently, when testing poorly innervated skin, the results are invalidated by one point being felt as two.

Moberg's (1958) picking-up test is an indication of the total sensory impression on gripping with the index finger and thumb. Small objects are handled and identified with the eyes open and then blindfolded. Moberg describes the failure to use the index and thumb as a loss of "tactile gnosis," a term

first used by Broman (1945) as the complex sensation which makes the fingers "seeing." Though patently simple and functional, this test is limited to the index finger and thumb. It is also subject to false positives, as only one normally innervated shank of the grip is required (Sturman and Duran, 1963).

Mannerfelt (1962), assessing reinnervation of skin grafts, found Frey's hairs and Seddon's coin test to be of limited value, showing little correlation with the two-point discrimination threshold.

As an objective test of sensation the sweat test is limited. The volume of sweat is difficult to measure, and it bears no relation to sensory recovery in nerve lesions proximal to the grey rami or in skin grafts. It is a useful corollary test, as are the "signs of trauma" (Moberg, 1962) and the volume test, colour test, and finger-print test (Davis *et al.*, 1964).

The test described below arose from a need for a simple test to measure the reinnervation of skin grafts or flaps to the finger-tips which would be objective, functional, and specific for the tactile areas of all the fingers.

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