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Circulating antibodies in coeliac disease

by

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Antibodies produced against gliadin were demonstrated in the sera of patients suffering from coeliac disease as well as of immunized rabbits and rats. In the patient sera the specific antibodies proved to be of the IgG type. IgE may have a role in sensitization against gliadin only in the cell-fixed state.

In the serum of coeliac patients, precipitins and haemagglutinins produced against gliadin have been demonstrated [1]. Later these antibodies were shown to be of the types IgG, IgA and IgE [8, 10]. The present experiments were aimed at establishing whether IgE or IgG antibodies played the more important role in coeliac disease and whether the presence of IgA antibody was frequent.

Material and Method

The serum of 28 coeliac patients aged between 2 and 8 years was studied with the inverse agar diffusion technique of Mancini and passive haemagglutination [12]. The antigen was extracted from wheat-meal with water at 2—4 °C, during 34 to 36 hours, then it was centrifuged at 50,000 *g* for 60 minutes [4]. The aqueous extract was fractionated on Sephadex G-75. As a positive control, rabbits were immunized with the whole aqueous extract, giving the antigen with complete Freund's adjuvant. In this case the rabbits produced an IgG type antibody.

IgE content in the patient sera was demonstrated with the RIST technique [9]. For the demonstration of specific IgE we utilized the property of human IgE to sensitize passively the mast cells of the rat; under the action of homologous antigen, histamine is released from the cells [11]. As positive controls, rats were immunized with aqueous extract, with *Bordetella pertussis* adjuvant to enhance IgE production. With 0.5 ml serum of the animals and patients the mast cells (3.5×10^5) obtained from untreated animals were sensitized passively. The sensitized cells were incubated at 37 °C for 30 min, with 20 μg fraction A homologous antigen and the quantity of histamine released from the cells was measured by fluorimetry [5].

Finally, the serum of patients was absorbed with anti-human IgG and anti-human IgA. The antibody content of sera was determined by immune diffusion; unabsorbed serum served as the control.

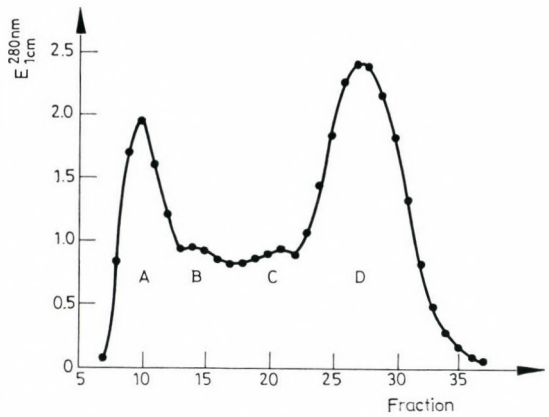

FIG. 1. Elution diagram of water soluble wheat-meal in Sephadex G-75 column. Fraction peaks marked with A, B, C, D

RESULTS

The water soluble wheat-meal was fractionated on Sephadex G-75 column. Fig. 1 illustrates the distribution of fractions marked A, B, C and D. With the technique of Mancini, fractions A showed the most distinct antigenicity against the immune serum of rabbits. After repeated fractionation this component was used as antigen, which, on the basis of its amino acid composition, could be considered to be gliadin. With the technique of Mancini the anti-gliadin content of immune serum of rabbits was between 1.75 and 2.00 mg/ml. Table I summarizes the antibody content of human serum determined with the technique of Mancini and passive haemagglutination. In the serum of the same patient we demonstrated the IgE level with the RIST technique. Finally, following passive sensitization of mast cells of rats, we studied the specific IgE content on the basis of

TABLE I

Antibody titre of the serum of coeliac patients

Determination	Number of patients	+ patients	Antibody titre
Mancini	28	28	> 1 : 16 – 1 : 64 serum dilution
Haemagglutination	28	28	> 1 : 8 serum dilution
RIST	28	9	200 – 601 IU/ml
RIST	28	2	601 – 1000 IU/ml
Histamine release	11	1	> 20% histamine release

+ patients = with increased antibody titre

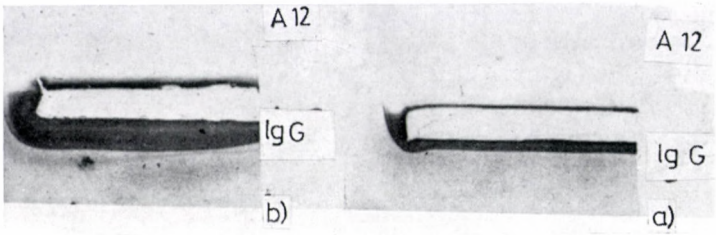

FIG. 2. Double immune diffusion of patient sera. (a) above: antigen A; middle: serum absorbed with anti-human IgG; below: anti-human IgG; (b) above: antigen A; middle: patient serum, below: anti-human IgG

histamine released from the cells on the effect of homologous antigen (fraction A). Table II illustrates the specific IgE level in patients and immunized rats.

TABLE II

Specific IgE content of antisera on the basis of histamine released from the mast cells

		Histamine release (%)
Rat: sensitized	(8)	48.85±2.06
control	(10)	23.60±1.81
Human: patient	(11)	17.61±1.72
control	(10)	16.50±1.43

In brackets, number of individuals

From both the qualitative and quantitative points of view, the results obtained with passive haemagglutination supported the quantity of antibody in the serum of coeliac patients demonstrated with the inverse technique of Mancini. IgE was present in a smaller quantity in the serum of the 11 patients. In these cases, specific IgE was not demonstrable. On the other hand, the immunized rats showed a specific IgE level.

The sera of patients absorbed with anti-human IgG did not present a precipitation curve against antigen A; the concentration decreased in accordance with the absorption (Fig. 2a). On the other hand, the unabsorbed sera of patients showed a precipitation curve with anti-IgG and accordingly reacted with antigen A (Fig. 2b). Absorption with anti-IgA did not influence the reaction with the antigen.

DISCUSSION

The technique used for the demonstration of specific IgE is equivalent to the RAST technique [11]. The results excluded the presence of circulating specific IgE in the patient sera. Considering the high cytophilic ability of IgE, in coeliac patients it seems to occur bound to cells [3]. Our observations indicate that the circulating antibody produced against gliadin is of the IgG type.

The disturbed absorption in the background of coeliac disease may be associated with a lack of tissue proteases [7]. To this contributes the

absence of IgA demonstrable locally. These circumstances may cause that gliadin is converted into an antigen. As the antigen persists in the patients, an opportunity may present itself in a certain phase of the disease for the formation of an immune complex. Following jejunal biopsy it was possible to demonstrate the presence of IgG, IgM and C_3 in the epithelial basal membrane [6] and this may be regarded as an evidence of immune complex deposition.

Thus, a mechanism more complex than the atopic one might explain the immunologic manifestation in coeliac disease [2].

References

1. Beckwith, A. C., Heiner, D. C.: An immunological study of wheat gluten proteins and derivatives. Arch. Biochem. **117,** 239 (1966).
2. Brant, L., Stenstam, M.: Subnormal lymphocyte counts in adult coeliac disease. Lancet **1,** 978 (1975).
3. Brown, W. R., Borthistle, B. K., Chen, S. T.: Immunoglobin E (IgE) and IgE-containing cells in human gastrointestinal fluids and tissues. Clin. exp. Immunol. **20,** 227 (1975).
4. Csorba, S., Szabolcs, M., Kávai, M., Jezerniczky, J., Szabó, B.: Über die Antigenität des Gliadins und die Antikörper gegen Gliadin. Acta paediat. Acad. Sci. hung. **16,** 249 (1975).
5. Evans, D. P., Thomson, D. S.: Histamine release from rat mast cells passively sensitised with homocytotropic (IgE) antibody. Int. Arch. Allergy **43,** 217 (1972).
6. Green, H. Y., Carty, J. E.: Coeliac disease and autoimmunity. Lancet **1,** 964 (1976).
7. Grüttner, R.: Die Coeliakie. Mschr. Kinderheilk. **114,** 485 (1966).
8. Heiner, D. C., Rose, B. J.: Elevated levels of γE/IgE in conditions other than classical allergy. Allergy **45,** 30 (1970).
9. Johansson, S. G. O., Bennich, H. H., Berg, T.: The clinical significance of IgE. In: Schwartz, R. S. (ed.): Progress in Clinical Immunology. Grune and Stratton, New York 1972.
10. Mietens, C.: Immunologische Aspekte der Coeliakie. Pädiat. u. Pädol. **8,** 68 (1973).
11. Perelmutter, L., Liakopoulu, A.: Comparison between morphological changes and histamine release induced in rat mast cells by human allergic sera and specific allergen. Acta allerg. (Kbh.) **29,** 444 (1974).
12. Suzuki, T., Tanaka, S., Kawanishi, Y.: An improved method for preparation of stable antigen coupled erythrocytes for passive hemolysis. Immunochemistry **11,** 391 (1974).

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