

Immunological findings in primary malabsorption

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To evaluate the reactivity of patients affected by primary malabsorptive disorders, circulating and secretory IgA, antibodies to milk and gliadin, and soluble immunocomplexes were studied. Special attention has to be paid to tissue-bound immune complexes detectable by immunofluorescence, as the test may point to an advanced stage of the disease.

In the studies concerning the aetiology of coeliac disease and other malabsorptive disorders of infancy and early childhood, increasing attention has been paid to local and systemic immune responses to nutritive allergens. In this paper the importance of immunological studies in primary and secondary malabsorption has been reviewed. In our patients affected by primary and secondary malabsorption treated during the last ten years, the following tests have been carried out: measurement of immune proteins in serum and jejunal juice; antibodies to cow's milk and gliadin and their relationship to eventual IgA deficiency; measurement of circulating soluble immune complexes; and immunofluorescence of jejunal mucosa of patients affected by coeliac disease.

MATERIALS AND METHODS

In 121 children, 59 boys and 62 girls aged 8 months to 12 years, affected by malabsorption, and in 70 healthy control

children of similar age the quantity of immunoglobulins was measured by the technique of Mancini and values were related to standard values published by Uffelman et al [19] i. e. IgA, 0.72 g/l; IgM, 0.08—1.22 g/l; and secretory IgA in jejunal juice, 0.04—0.44 g/l. Antibodies to cow's milk were estimated by the passive haemagglutination test of Boyden [3] modified by Grenzmann et al [11] as described by Nagy et al [15]. Antibodies to gliadin were measured by immunodiffusion and the circulating soluble immunocomplexes by the polyethylene glycol technique of Digeon et al [9]. For immunofluorescence, the original serum of Hyland dissolved in its solvent was used. Jejunal biopsy specimens were frozen in carbon dioxide and the sections were treated with biopsy buffer; after fixation in ethanol-ether, concentrated FITC serum was applied.

RESULTS

Since in most cases of villous atrophy the serum immunoglobulin level was low [4, 5, 6, 14] and Kaufmann and Hobbe [12] had shown low serum levels of IgA in atopic populations, we performed measurements of

serum and jejunal secretion IgA values in patients affected by coeliac disease and secondary malabsorption. The group of secondary malabsorption comprised patients recovering from acute enteritis, patients affected by dyspepsia caused by giardiasis or protracted antibiotic therapy, and patients with allergic diarrhoea elicited by ingested protein antigens.

Table I shows the results obtained in these patients.

Immunodiffusion revealed low serum IgA levels in a larger proportion of patients affected by coeliac disease than among those with secondary malabsorption. Secretory IgA is frequently absent from the jejunal se-

cretion of patients with coeliac disease. The serum immunoglobulin levels have been described and evaluated in detail in a previous publication [18].

Special attention has been paid to the relationship between decrease or absence of secretory IgA and the presence of antibodies to gliadin since the secretory IgA plays an important role in mucosal defense. Those results are presented in Table II.

In patients with coeliac disease having high antibody titres, decreased values or lack of IgA in serum and, more frequently, in the secretions, were encountered especially in the acute phase of the disease. Such a

TABLE I

Secretory and circulatory immunoglobulins of patients affected by primary and secondary malabsorption

	No. of samples examined		IgA			
			in serum		in jejunal juice	
	serum	jejunal juice	low (< 0.68 g/l)	absent	low (< 0.16 g/l)	absent
Secondary malabsorption	91	82	6	—	3	6
Coeliac disease	30	26	12	—	4	22
Total	121	108	18		7	28

TABLE II

Secretory IgA in jejunal secretion and antibodies to gliadin in patients with coeliac disease, cow milk allergy, recovered from acute enterocolitis and in healthy persons

	No. of patients	Jejunal juice secretory IgA		Positive anti-gliadin antibody titre 1/8—1/16
		low (< 0.16 g/l)	absent	
Coeliac disease	32	4	28	22
Cow milk allergy	20	5	2	2
After enterocolitis	24	5	3	2
Healthy	50	6	0	0

TABLE III

Antibody levels to cow milk and gliadin in patients with various forms of malabsorption and in healthy children

	Cow milk protein		Gliadin	
	No.	Positive < 1/16	No.	Positive titre, 1/8-1/16
Healthy children	56	3.6	187	0
Allergic diarrhoea	36	22	36	14
Coeliac disease	18	39	18	83
Giardiasis	17	23	17	6
Other malabsorption	28	11	28	0

TABLE IV

Gliadin antibody titres and circulating immune complex levels in patients with coeliac disease, secondary malabsorption and in healthy children

	No. of patients	Antigliadin antibody titre		Circulating immune complex	
		≥ 1/8	< 1/8 1/16	≥ 0.12	< 0.12
Coeliac disease	20	8	12	6	14
Other malabsorption	20	16	4	17	3
Healthy children	20	18	2	17	3

high incidence of positivity was not found in the controls or in patients affected by other forms of enteritis. This seems to confirm the assumption that secretory IgA plays an outstanding role in the defense of jejunal barrier function [1, 2, 18, 20].

Table III shows the level of antibodies against cow's milk and gliadin. It can be seen that in some control persons a cow's milk antibody titre exceeding 1:32 could be demonstrated. Such a titre was found in a high proportion of the patients affected by either form of malabsorption.

In nearly all patients acutely affected by coeliac disease an antibody

titre against gliadin exceeding 1:16 was demonstrated. This finding is similar to that encountered by Csorba et al [7] and Rossipal [17], although these authors used [different techniques. An elevated level may occasionally be found in enteritis caused by giardiasis and also in other forms. In the secondary forms, however, the phenomenon is only transitory while in gliadin sensitive coeliac disease it is a constant finding and the level of antibody decreases or disappears only after protracted dietary therapy.

Accumulation of deposits containing immunoglobulins in the jejunal mucosa of patients affected by coeliac

disease is a well-documented fact [5, 10, 18]; this has prompted us to extend our studies to immune complexes in the circulation. As can be seen from the data shown in Table IV, there is a numerical correlation between the immune complex content and the anti-gliadin antibody titre of the sera: similar findings have been described by Kávai et al [13]. In our experience a titre higher than 1:8 is of diagnostic value; in all cases

having a titre as high there was an elevated level of circulating immune complex. In some cases, however, increased immune complex levels were associated with low antibody values.

In most cases the soluble immune complex level was high. Further direct analysis of its composition was not possible, we looked therefore for indirect methods of identification: immune complex deposits in the jejunal mucosa of 30 patients with coeliac

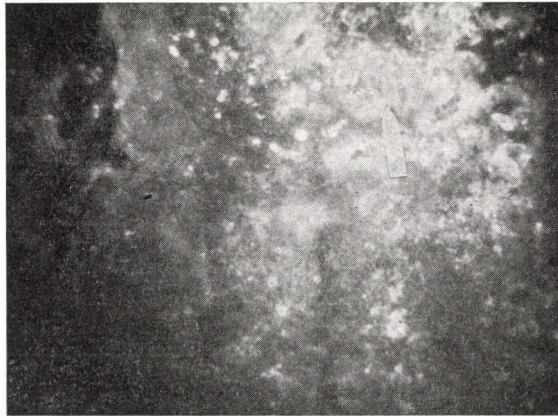


FIG. 1. Immunofluorescent picture of specimen from a 8-year-old boy



FIG. 2. Immunofluorescent picture of specimen from a 2-year-old girl. IgA particles within the duodenal and jejunal mucosa. Magnification, $\times 720$

disease were examined by immunofluorescence. Figures 1 and 2 represent the immunofluorescent picture of specimens obtained from two of our patients. The specimens were tested for the presence of IgA, IgG, IgM and complement. The highest incidence, 30%, was observed with IgA. The next most frequent positive finding was the presence of complement (10%). Nearly all the patients exhibiting immune particles were above two years of age; no immune particles were detectable in infants with gluten sensitive enteropathy. It may thus be concluded that immune particles appear only when the disease has existed for a long time.

DISCUSSION

Several methods are now available for examining the organism's reactivity. In patients affected by primary or secondary malabsorption, determination of serum and secretory IgA and antibodies to cow's milk and gliadin is most rewarding. These methods help much in differential diagnosis and judgement concerning the severity of these disorders.

Absence of secretory IgA in jejunal juice points to a weakening of the local defense of the intestinal mucosa and this may be a pathogenetic factor important in coeliac disease and cow's milk allergy [20]. The simultaneous presence of antibodies against cow's milk and gliadin suggests that gliadin sensitivity may lead to secondary hypersensitivity to cow's

milk, in other words, demonstration of antibodies against cow's milk does not exclude the presence of coeliac disease. On the contrary, their coexistence with antibodies to gliadin corroborates the diagnosis.

Our findings confirm the observations of Mietens [14]. In patients with cow's milk allergy, elimination of clinical symptoms and abnormal laboratory findings can be achieved by early institution of an adequate diet for 6—22 months [16].

A titre of 1:16 or higher can be found in nearly all patients affected by acute coeliac disease; in addition, it may be encountered in occasional cases of chronic enteritis and with certain intestinal parasites. While in secondary malabsorption the finding is transitory, it is a constant and stable abnormality in patients with coeliac disease, where the antibody level decreases only after prolonged dietary treatment; similar findings have been published by Rossipal [17].

Demonstration of IgA, less frequently IgG or complement in the immune deposits found in jejunal mucosal specimens obtained by suction biopsy may support the pathogenetic role of circulating immune complexes captured by the jejunal mucosa. The level of circulating soluble immune complex is in some degree related to the severity of the disorder or to the remission achieved by treatment. The site of deposition of the immune complex may be specified by immunofluorescence of the jejunal mucosa. In our experience the complex is usually bound to the

glandular substance adjacent to the basal membrane.

Immunological studies are extremely helpful in determining the actual reactivity of patients afflicted by primary malabsorption. In spite of growing insight since the description of the role of gliadin in the pathomechanism of coeliac disease [8], the diagnosis is still difficult. Our findings are believed to have contributed to the better understanding of the immunological aspects of this disorder.

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