

# Reaginic hypersensitivity in ulcerative colitis

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**SUMMARY** Reaginic hypersensitivity in ulcerative colitis has been investigated in respect of a hypersensitivity to the cow's milk proteins and the frequency of atopic asthma, hay fever, and eczema. Intradermal tests were frequently positive, especially to casein, but the results did not differ from those found in healthy individuals and in groups of patients with Crohn's disease, hypolactasia, and the irritable colon syndrome. No circulating IgE-specific antibodies to the milk proteins were found. An increased frequency of atopic diseases was found in patients suffering from ulcerative colitis (15.7%) and Crohn's disease (13.3%) compared with the findings in a control group (1.2%).

It is concluded that, if an allergy to milk proteins is a factor in the pathogenesis of ulcerative colitis, it is not mediated by reaginic antibodies. It is possible, however, that the frequent occurrence of atopy indicates a susceptibility to develop reaginic responses even though this mechanism does not apply to the milk proteins.

Clinical observation has suggested that an allergy to dietary proteins may be a factor in the aetiology of ulcerative colitis (Andresen, 1942; Mackie, 1942; Rowe, 1942; Sarles, Deck, Chalvet, and Ambrosi, 1959). The commonest food incriminated has been cow's milk and this was supported by the fact that patients in remission on a milk-free diet rapidly relapsed when challenged with milk (Truelove, 1961). Subsequently, a controlled dietary trial showed that one in five patients appeared to benefit from a milk-free diet (Wright and Truelove, 1965a). Rowe, Rowe, and Uyeyama (1955) claimed that an allergy to pollen was an important factor in some patients with the disease.

Andresen (1942) and Rowe (1942) stressed that skin tests were unhelpful in selecting the patients who would benefit from a milk-free diet. However, Rider, Moeller, Deveraux, and Wright (1960) found that patients frequently reacted within five minutes to various allergens—such as milk, wheat or egg— injected into the rectal mucosa. When the relevant allergen was removed from the diet, the patient remained in remission but no long-term study has been reported. Taylor and Truelove (1961) found that patients with ulcerative colitis had raised antibody titres to the milk proteins when compared with control subjects but Wright and Truelove (1965b) were unable to predict from the height of the antibody titres whether or not an individual patient would benefit from a milk-free diet.

The possibility that milk sensitivity in ulcerative colitis might be mediated by reaginic antibodies has been investigated in the present study by the use of intradermal testing and by measuring IgE-specific antibodies in serum, as it is now established that IgE is the carrier of reaginic antibody (Ishizaka and Ishizaka, 1967). The frequency of other reagin-mediated diseases such as asthma, hay fever, and atopic eczema occurring in ulcerative colitis has also been investigated.

## Methods

### PATIENTS STUDIED

The patients studied were those with classical ulcerative colitis diagnosed on the basis of a history of bloody diarrhoea, on the characteristic sigmoidoscopic appearances and the histological appearances of the rectal biopsy, and on the changes seen on the barium enema. The results obtained were compared with those obtained from a control group of healthy volunteers and from groups of patients with Crohn's disease, the irritable colon syndrome, hypolactasia, and coeliac disease.

### SKIN TESTS

Purified casein,  $\alpha$ -lactalbumin, and  $\beta$ -lactoglobulin were supplied by Dr R. Lyster of the National Institute for Research in Dairying. They had been prepared by the method of Gunther, Aschaffenberg, Matthews, Parish, and Coombs (1960). For intra-

dermal testing, the purified proteins were made up in a 1% solution containing 0.5% phenol by Bencards.

Of each solution, 0.01-0.02 ml was injected intradermally into the forearm and the result was read at 10 minutes. A control solution containing 0.5% phenol was also injected. The results were assessed by measuring the diameter of the wheal and were recorded as negative, 1 cm or less (positive), greater than 1 cm (strongly positive).

#### IgE ANTIBODIES

Antibodies to the milk proteins of the IgE immunoglobulin class were measured using the red-cell-linked antigen-antiglobulin reaction. Details of the method have been published elsewhere (Jewell and Truelove, 1972). Specific IgE antiserum was supplied by Dr D. S. Rowe (WHO). The antiserum had been prepared by immunizing sheep with the Fc-fragment of IgE isolated from a pool of African sera. It had been absorbed with normal serum of low IgE content and shown to be specific for IgE by immunoelectrophoresis and by gel diffusion. It was used at a dilution of 1 in 500.

#### Results

##### SKIN TESTS

The results of skin testing with the purified milk proteins are shown in Table I. It can be seen that the percentages of positive reactions obtained in each diagnostic category were similar. When the positive reactions were divided into those that were positive (1 cm or less in diameter) and those that were strongly positive (greater than 1 cm), no differences emerged between the various groups of patients and the healthy control group.

Diagnosis	No. Tested	Percentage Positive Tests		
		Casein	$\alpha$ -Lactalbumin	$\beta$ -Lactoglobulin
Ulcerative colitis	39	59	10	13
Crohn's disease	19	68	21	12
Hypolactasia	11	45	10	18
Normal	20	75	20	20
Irritable colon syndrome	19	68	16	16

Table I Frequency of positive skin tests to milk proteins

When the results from all the 111 subjects were combined, 64% of them showed a positive reaction to casein, 14% to  $\alpha$ -lactalbumin, and 16% to  $\beta$ -lactoglobulin.

All the subjects who were skin tested to the milk proteins had been tested for circulating antibodies to the milk proteins using the red-cell-linked antigen-antiglobulin reaction (Jewell and Truelove, 1972).

No correlation existed between the presence of IgA or IgG antibodies in the serum and a positive skin test. Subjects who had direct agglutinating antibodies to casein in their sera were more likely to have positive skin tests than were subjects without such antibodies although the differences are only at the borderline of statistical significance (Table II).

Result of Skin Test	Direct Agglutinating Antibodies		Total
	Negative	Positive	
Negative	39	1	40
Weakly positive	47	8	55
Strongly positive	13	3	16
Total	99	12	111

Table II Results of skin tests to casein correlated with the presence of direct agglutinating antibodies to casein in serum<sup>1</sup>

<sup>1</sup>By Fisher's exact test,  $P = 0.062$ . When the positive skin tests are grouped together,  $P = 0.053$ .

#### IgE ANTIBODIES

Sera from 167 subjects were tested. They were obtained from patients with ulcerative colitis (51), Crohn's disease (30), hypolactasia (13), the irritable colon syndrome (24), and coeliac disease (11). Sera from 38 healthy volunteers, who had no history of milk intolerance, were also tested.

No IgE antibodies to the milk proteins were detected in any of the sera tested. Three patients (two with ulcerative colitis and one with the irritable colon syndrome), who had strongly positive skin tests to all three proteins, were studied in more detail. None of the three sera contained direct agglutinating antibodies and they were therefore tested using anti-IgE antiserum diluted in a range from 1 in 50 to 1 in 5 000. No IgE antibodies could be demonstrated.

#### FREQUENCY OF ATOPY

Eight patients with ulcerative colitis (15.7%) and four patients with Crohn's disease (13.3%) had asthma, hay fever, or atopic eczema, either singly or in combination (Table III). In contrast, only one patient (1.2%) out of the other 86 patients studied had an atopic disease. The greater frequency of these

Diagnosis	Total No.	Asthma	Hay Fever	Eczema
Ulcerative colitis	51	4	6	3
Crohn's disease	30	2	1	2
Hypolactasia	13	0	0	0
Coeliac disease	11	0	0	0
Normal controls	38	0	0	0
Irritable colon syndrome	24	0	0	1

Table III The frequency of atopic conditions

diseases observed in the patients with ulcerative colitis or Crohn's disease, compared with the control subjects, was statistically significant ( $\chi^2 = 10.97$ ,  $n = 2$ ,  $P < 0.01$ ).

A higher proportion of the patients with these atopic diseases gave positive skin tests to the purified cow's milk proteins compared with the results obtained for the whole series of patients (Table IV). Patients with asthma, hay fever, or eczema did not have greater titres of circulating antibodies to the milk proteins than did those subjects who did not have these diseases.

Protein	No. of Patients	Skin Tests	
		- ve	+ ve
<i>Asthma</i>			
Casein	5	2	3
$\alpha$ -Lactalbumin	5	3	2
$\beta$ -Lactoglobulin	5	2	3
<i>Hay fever</i>			
Casein	5	1	4
$\alpha$ -Lactalbumin	5	2	3
$\beta$ -Lactoglobulin	5	2	3
<i>Eczema</i>			
Casein	5	1	4
$\alpha$ -Lactalbumin	5	1	4
$\beta$ -Lactoglobulin	5	1	4

Table IV Results of skin tests in patients with ulcerative colitis or Crohn's disease with atopic conditions

## Discussion

The results of this study do not support the hypothesis that a reaginic hypersensitivity to cow's milk proteins is a factor in the pathogenesis of ulcerative colitis. Although wheal and flare reactions to the purified milk proteins injected intradermally were commonly found, the frequency of positive reactions did not differ from that seen in the other groups of patients studied or in the group of healthy individuals. Furthermore, no circulating IgE specific antibodies to the milk proteins were demonstrated.

When the results of all the 111 subjects who were skin tested are combined, 64% reacted to casein, 14% to  $\alpha$ -lactalbumin and 16% to  $\beta$ -lactoglobulin. This indicates that sensitization to the milk proteins is common and the higher proportion of positive tests to casein compared with the other two proteins probably reflects the protein composition of milk. The casein in cow's milk comprises 80% of the total protein content (approximately 2.3 g/100 ml whole milk) whereas the whey proteins, which include  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin, are contained in the remaining 20%.

There is now good evidence that immediate type I skin tests are mediated by IgE antibody bound to

cells, probably mast cells. The P-K titre correlates well with the titre of IgE specific antibody (Ishizaka, Ishizaka, and Hornbrook, 1966) and the P-K reaction can be blocked by IgE (Stanworth, Humphrey, Bennich, and Johansson, 1967). Furthermore, a good correlation exists between the presence of IgE-specific antibody and positive skin provocation tests in situations of clinical hypersensitivity (Wide, Bennich, and Johansson, 1967; Stenius and Wide, 1969).

The failure to find IgE antibodies to milk proteins in the serum of patients who gave positive skin reactions may have a number of explanations. Milk proteins are absorbed from the gastrointestinal tract in minute amounts and may therefore provide a weak antigenic challenge. Since IgE is strongly homocytotropic, small amounts of specific antibody formed in response to such a challenge may be largely bound to cells, and the amount circulating in the blood may therefore be too small to measure. None of the patients who had positive reactions had an obvious clinical allergy to milk and it is possible that the presence of blocking antibody not only prevented clinical allergy, but also prevented the demonstration of IgE-specific antibody. Finally, the red-cell-linked, antigen-antiglobulin test may not have been sensitive enough to detect IgE antibodies. However, it has provided a sensitive and reliable method for the detection of antibodies of other immunoglobulin classes (Coombs, Jonas, Lachmann, and Feinstein, 1965; Jewell and Truelove, 1972), and it has been used to detect IgE antibodies to castor bean in patients with castor bean allergy (Coombs, Hunter, Jonas, Bennich, Johansson, and Panzani, 1968). Another possibility is that the presence of a direct agglutinating antibody masked low titres of IgE antibody.

The increased frequency of asthma, hay fever, and eczema observed in patients with ulcerative colitis and Crohn's disease was an interesting finding and confirms the observations of Hammer, Ashurst, and Naish (1968) who found a similar increased frequency compared with controls. Since these atopic diseases are mediated by reaginic antibody and the affected subjects usually have raised levels of IgE in their serum (Berg and Johansson, 1969; Juhlin, Johansson, Bennich, Högman, and Thyresson, 1969), it is possible that patients with ulcerative colitis or Crohn's disease may be prone to develop reagin-mediated allergies. If such a susceptibility plays a part in the aetiology of the two diseases, then the allergens involved have yet to be defined. Certainly the present study indicates that a reaginic response to cow's milk proteins is not involved in either ulcerative colitis or Crohn's disease, but does not exclude such a response to other exogenous allergens.

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