AGGLUTININS TO ANAEROBIC BACTERIA IN CROHN'S DISEASE AND IN INDIAN PATIENTS WITH DIARRHOEA

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SUMMARY. Agglutinins to certain species of *Eubacterium* and *Peptostreptococcus* have been reported in sera from a high proportion of patients with Crohn's disease. Because this might be a non-specific finding common to patients with diarrhoea associated with damaged intestinal mucosa, we have compared the incidence of such agglutinins in patients with Crohn's disease with that seen in patients in North-East India with acute or chronic diarrhoea. The incidence of agglutinins in Crohn's disease was 44%, compared with 11% in acute and 17% in chronic diarrhoea. These figures suggest that mucosal damage alone does not explain the high incidence of agglutinins in Crohn's disease.

INTRODUCTION

Although the cause of Crohn's disease remains unknown, several studies have shown that certain bacteria may play a role (Burnham *et al.*, 1978; Swarbrick *et al.*, 1979; Matthews *et al.*, 1980; Wensinck *et al.*, 1981). In Rotterdam, a much higher incidence of agglutinins to anaerobic bacteria of *Eubacterium* and *Peptostreptococcus* species was found in the sera of patients with Crohn's disease than in those from healthy controls (Wensinck, 1975 and 1976; Van de Merwe, Schmitz and Wensinck, 1981; Wensinck and Van de Merwe, 1981). These workers also identified increased numbers of these bacteria in the stools of patients with the disease (Wensinck *et al.*, 1981). In Cardiff, we have confirmed their serological findings and shown that the incidence of agglutinins to eubacteria and peptostreptococci was between 54 and 65% in Crohn's disease, compared with 11-28% in ulcerative colitis and only an occasional positive result in healthy controls (Matthews *et al.*, 1980; Mayberry *et al.*, 1981).

The increased incidence of agglutinins in Crohn's disease may reflect either a role for the bacteria in the disease process or simply a secondary phenomenon due to increased exposure of the immune system to gut bacteria as a result of a damaged intestinal mucosa. In an attempt to distinguish between these possibilities, we have looked for agglutinins to anaerobic bacteria in sera from patients with gastro-intestinal disease other than Crohn's disease.

MATERIALS AND METHODS

Collection of sera. Samples from India were obtained from patients at the Duncan Hospital,

Received 29 Jul. 1983; revised version accepted 24 Oct. 1983.

Raxaul, a border town in North-East India which serves the surrounding Nepalese and Indian populations. Blood was taken from 19 subjects with acute diarrhoea (defined as less than 10 days of diarrhoea with at least three watery stools/day), from 12 other subjects who had diarrhoea for more than 10 days, and from 44 control patients who had been admitted for non-intestinal illnesses. The mean ages of the three groups were similar (28–32 years). The blood samples were centrifuged and the sera refrigerated, with sodium azide added to prevent deterioration.

In Cardiff, samples were taken from 61 out-patients who were known to have established Crohn's disease, from 42 patients with long-standing ulcerative colitis, and from 47 controls who were mostly hospital personnel. The mean ages of the three groups were similar; that of the Crohn's disease group was somewhat below those of the other two (38.5 years compared with 47 years for the colitis group and 42 years for the controls).

Agglutination tests. Work in Rotterdam suggested that patients with Crohn's disease often had serum antibodies which agglutinated certain bacteria, in particular two strains of *Eubacterium contortum* (ME₄₄ and ME₄₇), one of *E. rectale* (ME₄₆) and one of *Peptostreptococcus productus* (C₁₈) (Wensinck and Van der Merwe, 1981). Suspensions of these organisms were kindly provided by Professor Wensinck. Forty μ l of serum were added to 20 μ l of each bacterial suspension in the wells of flat bottomed microtest trays and thoroughly mixed for 5 min on a Dynatech microshaker. Agglutination patterns were examined with an inverted microscope at $\times 125$ magnification. Agglutination was scored as either positive or negative for each bacterial strain and the overall result for individual patients was recorded as positive if agglutination occurred with strain ME₄₆ or any two of the other strains. All of the samples were examined without knowledge of the group of patients to which they belonged, and results were checked independently by two observers; there were no significant disagreements about the readings.

RESULTS AND DISCUSSION

The results are summarised in the table. All three groups of Indian patients had higher incidences of agglutinins than the control group from Cardiff. However, neither the acute nor the chronic diarrhoea group had an incidence approaching that seen in the patients with Crohn's disease. If it is assumed that the Indian and European subjects have comparable numbers of peptostreptococci and eubacteria in their intestinal flora, these findings suggest that mucosal damage alone may not be sufficient to cause an increased incidence of agglutinins to *Peptostreptococcus* and *Eubacterium* species. The increased antibody response to these organisms in Crohn's disease may, therefore, reflect some additional factor such as an allergic response or a large antigenic load.

TABLE

Incidence of agglutinins to eubacteria and peptostreptococci in sera from patients and controls

<i>Subject groups</i>	<i>Number (percentage) of subjects with serum agglutinins</i>
North-East India:	
Patients with acute diarrhoea	2/19 (11)
Patients with chronic diarrhoea	2/12 (17)
Controls	5/44 (11)
Cardiff:	
Patients with Crohn's disease	27/61 (44)
Patients with ulcerative colitis	5/42 (12)
Controls	2/47 (4)

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