

**Induction in rats of serum agglutinins to *Eubacterium*,
Peptostreptococcus and *Coprococcus* species by the faecal flora
from patients with Crohn's disease and healthy subjects**

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SUMMARY

Sera from patients with Crohn's disease more often than those from other subjects contain agglutinins against anaerobic coccoid rods, identified as *Peptostreptococcus productus*, *Eubacterium contortum* (two strains) and *Coprococcus comes*. The presence of antigens of the four coccoid rods in faeces of patients with Crohn's disease and healthy subjects was investigated by inoculation of rats with faecal suspensions. Antigens of the coccoid rods were detected in faeces from both patients with Crohn's disease and healthy subjects.

INTRODUCTION

The anaerobic faecal flora of patients with Crohn's disease (CD) has been found to differ from the flora of healthy subjects by higher numbers of gram-positive coccoid rods and gram-negative rods (Wensinek *et al.*, 1981; Ruseler-Van Embden & Both-Patoir, 1983). Agglutinating antibodies to four strains, identified as *Peptostreptococcus productus* (strain C18), *Eubacterium contortum* (strains Me44 and Me47) and *Coprococcus comes* (strain Me46) have been found in patients with CD in 55, 62, 37 and 49%, and in healthy subjects in 5, 15, 4 and 1%, respectively. The agglutination reactions have shown to be useful as a diagnostic test for CD (Van de Merwe, Schmitz & Wensinek, 1981; Wensinek & Van de Merwe, 1981; Wensinek, Van de Merwe & Mayberry, 1983).

In the present study, the occurrence of the four coccoid rods in faeces of patients with CD and healthy subjects was investigated by determination of agglutinins in sera of rats after intraperitoneal injection with sterilized faecal suspensions.

MATERIALS AND METHODS

Human subjects

Faeces and sera were obtained from seven apparently healthy laboratory workers and seven patients with CD. Patients with positive agglutination reactions were selected from the group described previously (Van de Merwe, 1980). Agglutination reactions with the four strains of the subjects are given in Table 1.

Table 1. *Agglutination reactions with coccoid anaerobes of sera from patients with Crohn's disease and healthy subjects*

	Agglutination reaction			
	Me44	C18	Me46	Me47
Crohn's disease				
1	3	1	2	1
2	2	3	3	1
3	3	3	3	2
4	3	3	3	0
5	3	2	3	0
6	1	1	2	1
7	1	1	2	0
Healthy subjects				
1	0	1	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0

Rats

Conventional rats (Wag/Cpb), age 3 months, were bred in the laboratory Animal Center TNO Zeist, The Netherlands.

Agglutination reactions

Sera were tested for the presence of agglutinating antibodies within 3 days after collection. The suspensions of strains Me44, C18, Me46 and Me47 (5×10^8 /ml) were prepared as described previously (Wensink & Van de Merwe, 1981). Results were scored as negative (0) or positive (1, 2 or 3 according to strength).

Induction of agglutinins

The induction of agglutinins to the coccoid rods in groups of five rats was tested by induction of faecal suspensions as well as by suspensions of each of the coccoid rods.

Faecal samples were diluted six times, sterilized (at 120 °C, 5 min) and injected intraperitoneally (0.25 ml) twice a week as described previously (Hazenberg *et al.* 1982).

Rats were inoculated similarly with suspensions of strain Me44, C18, Me46 and Me47.

The presence of serum agglutinins was tested before the first injection and thereafter at weekly intervals for 3 weeks.

Statistical methods

Results were compared with the Mann-Whitney U test or with Fisher's exact test. *P* values were derived from two-tailed tests.

Table 2. Induction of serum agglutinins in rats inoculated with diluted faecal suspensions from patients with Crohn's disease and healthy subjects or with suspensions of coccoid rods

Source of faeces	Agglutination reaction*			
	Me44	C18	Me46	Me47
Crohn's disease				
1	0 (0, 0, 0)	7 (0, 0, 1)	0 (0, 0, 0)	13 (1, 1, 0)
2	67 (4, 3, 3)	0 (0, 0, 0)	40 (4, 1, 1)	0 (0, 0, 0)
3	7 (0, 0, 1)	0 (0, 0, 0)	0 (0, 0, 0)	7 (0, 0, 1)
4	7 (0, 0, 1)	0 (0, 0, 0)	13 (0, 1, 1)	0 (0, 0, 0)
5	20 (0, 2, 1)	0 (0, 0, 0)	13 (2, 0, 0)	7 (0, 1, 0)
6	73 (2, 5, 4)	20 (1, 1, 1)	7 (0, 1, 0)	13 (0, 0, 2)
7	13 (0, 2, 0)	80 (3, 5, 4)	40 (4, 2, 0)	0 (0, 0, 0)
Healthy subjects				
1	0 (0, 0, 0)	40 (1, 1, 4)	13 (0, 0, 2)	13 (0, 0, 2)
2	7 (0, 0, 1)	100 (5, 5, 5)	20 (1, 1, 1)	73 (4, 3, 4)
3	0 (0, 0, 0)	93 (4, 5, 5)	13 (0, 1, 1)	27 (0, 2, 2)
4	53 (4, 3, 1)	7 (0, 0, 1)	20 (0, 2, 1)	0 (0, 0, 0)
5	40 (4, 1, 1)	80 (4, 5, 3)	7 (1, 0, 0)	0 (0, 0, 0)
6	53 (5, 1, 2)	0 (0, 0, 0)	13 (2, 0, 0)	0 (0, 0, 0)
7	0 (0, 0, 0)	0 (0, 0, 0)	7 (1, 0, 0)	0 (0, 0, 0)
Bacterial suspension				
Me44	100 (5, 5, 5)	0 (0, 0, 0)	0 (0, 0, 0)	0 (0, 0, 0)
C18	0 (0, 0, 0)	87 (4, 5, 4)	0 (0, 0, 0)	33 (1, 2, 2)
Me46	0 (0, 0, 0)	0 (0, 0, 0)	40 (2, 1, 3)	0 (0, 0, 0)
Me47	0 (0, 0, 0)	0 (0, 0, 0)	0 (0, 0, 0)	53 (3, 3, 2)

* Positive/total (%) in a 3-week period. The number of positive sera in five rats at weekly intervals during a 3-week period is given in parentheses.

RESULTS

Serum agglutinins against coccoid antigens were induced by inoculation of rats with faeces from patients with CD as well as from healthy subjects. In Table 2 the presence of agglutinins for a period of 3 weeks after the first injection are given. The results are expressed as the percentage of rats with serum agglutinins. Large differences were seen in individual faecal suspensions from CD patients and healthy subjects to induce serum agglutinins. Individual rats also had different capacities to produce agglutinins. Comparison of results with faecal suspensions from healthy subjects and CD patients shows that, except for C18, they equally induced agglutinins against separate strains. Agglutinins to C18 were more often induced by faecal suspensions from healthy subjects than by those from CD patients ($P = 0.0125$, $P = 0.019$ and $P = 0.005$ at week 1, 2 and 3 respectively).

Suspensions of all strains induced agglutinins but frequencies differed (Me44, C18, Me47 and Me46, in decreasing order).

Agglutinins to the four strains were not induced in rats injected with suspensions of their own faeces or with saline.

DISCUSSION

Serum agglutinins against four strains of anaerobic coccoid rods were found in rats injected with corresponding bacterial suspensions or with faeces from both healthy subjects and patients with CD. This finding extends previous results (Hazenberg *et al.* 1982) and indicates that antigens of *Peptostreptococcus productus*, *Eubacterium contortum* (two strains) and *Coprococcus comes* used in the agglutination test for CD, are also present in faeces from healthy subjects. Although large individual differences were seen, faeces of patients with CD did not induce more frequent agglutinins to Me44, Me46 and Me47 than faeces of healthy subjects. Agglutinins to C18 were more frequently present in rats inoculated with faecal suspensions of healthy subjects.

Wensinck *et al.* (1981) found more coccoid rods in the faecal flora of CD patients than in those from healthy subjects. Agglutinins to the coccoid rods were detected much more frequently in sera from CD patients than in those from healthy subjects (Wensinck & Van de Merwe, 1981). The present study indicates that the coccoid antigens also occur in faeces of healthy subjects and can induce serum agglutinins. The way in which antigens are presented to the host, therefore, obviously determines the induction of serum agglutinins.

These results are in line with the association between serum agglutinins and the presence of fistulae and levels of serum IgG and IgM (Van de Merwe *et al.* 1982).

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