

# Smoking, humoral immunity, and ulcerative colitis

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## Abstract

Since ulcerative colitis predominantly affects non-smokers and ex-smokers we have examined the possibility that smoking modifies the humoral immune response to an antigenic challenge from the gut lumen. Gut lavage was used in healthy subjects and patients with ulcerative colitis, including both smokers and non-smokers. Antibodies in the intestinal fluid to *Escherichia coli* (five pooled serotypes), *Candida albicans*, gliadin, ovalbumin, and  $\beta$  lactoglobulin were measured by ELISA to determine specific antibody concentrations of IgG, IgA, and IgM classes. Total IgG, IgA, and IgM were also measured in intestinal secretions and serum. In addition, circulating antibody concentrations of IgG, IgA, and IgM to three gut commensals - *E coli* (five pooled serotypes) *C albicans*, and *Poroteus mirabilis* were measured. There was a significant reduction in the IgA concentration in intestinal fluid of smokers with ulcerative colitis compared with healthy non-smoking controls. No other significant differences were found between the groups. Overall, these data are not consistent with the idea that smoking suppresses immune responses in the gut and suggest that the effect of smoking in colitis is mediated by another mechanism.

Cigarette smoking is associated with immunological changes<sup>1,2</sup> that affect the T cell subsets,<sup>3</sup> alveolar macrophages,<sup>4,5</sup> reduce natural killer cell function,<sup>6</sup> and alter circulating immunoglobulins of the IgG and IgA classes.<sup>7-9</sup> Reduced amounts of salivary IgA have been found in heavy smokers, both in normal subjects and patients with epithelial head and neck tumours.<sup>10</sup> Since ulcerative colitis is a disease of non-smokers<sup>11</sup> and ex-smokers,<sup>12</sup> smoking may have a protective effect against colitis involving humoral immunity of the gut.<sup>13</sup> To examine this possibility, we compared healthy controls, smokers and non-smokers with colitis patients for serum immunoglobulin concentrations and specific antibodies to three gut commensals. Mucosal responses were also examined by measuring in the intestinal secretions total immunoglobulin and specific antibodies to a range of antigens.

## Methods

Two sets of measurements were made. In the first, serum concentrations of total immunoglobulins and specific antibodies to three gut commensals were measured. In the second, concentrations of immunoglobulins and specific

antibodies were measured in gut secretions obtained after intestinal lavage.

## PATIENTS

### Systemic humoral immunity

Twenty one healthy male non-smokers, aged 20 to 65 years, and receiving no medication took part in the study on systemic humoral immunity. They were age matched with 21 healthy male smokers (10 or more cigarettes daily for more than one year, mean 15 and range 10-30 per day) and 21 patients with ulcerative colitis; those with colitis were all non-smokers, in remission, and had not taken steroids in the previous six weeks but were currently taking either mesalazine or sulphasalazine. Some 10 ml of venous blood were taken from each subject and sera were stored in 1 ml aliquots at -20°C.

### Mucosal humoral immunity

Ten healthy non-smokers (two men, mean age 32.2 years) and 12 healthy smokers (five men, mean age 35.2 years) who were receiving no medication took part in the study on mucosal humoral immunity. The mean number of cigarettes consumed was 19 per day (range 3 to 30). In addition 30 patients with colitis in remission and who were not on steroids were included. Nineteen were non-smokers (13 men, mean age 47.9 years) and 11 were smokers (six men, mean age 39.2 years). The mean number of cigarettes consumed daily in this group was 16 (range 2 to 30). Twenty three of the patients with colitis underwent sigmoidoscopy before gut lavage to ensure that the disease was in remission and a rectal biopsy specimen was taken one week later to quantify inflammatory changes (Table I). Histological grades of biopsy specimens in 23 of the patients showed no acute inflammation in 16 and minimal changes in seven; four were grade 1 and three grade 2.

### LAVAGE PROTOCOL

After an overnight fast, subjects were asked to drink a solution of the bowel preparation Golytely (Seward Medical Limited) at a rate of 250 ml every 15 minutes.<sup>14</sup> This was chilled to 4°C to make it more palatable but half the subjects chose to have it by nasogastric tube. Metoclopramide 10 mg was given intramuscularly 30 minutes before the lavage, which consisted of at least 4 l. A stool collection was taken at the end of the study when clear liquid was passed rectally, free from particulate matter. About 200 ml were collected and 10 ml filtered through a cotton

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TABLE I Histological grading of acute inflammation in rectal biopsy specimens from patients with ulcerative colitis

0	No polymorphs
1	Small number of polymorphs in the lamina propria with minimal infiltration of crypts.
2	Prominent polymorphs in the lamina propria with infiltration of >50% of crypts
3	Florid polymorph infiltrate with crypt abscesses
4	Florid acute inflammation with ulceration

sheet. Specimens were centrifuged twice and treated with protease inhibitors<sup>15</sup> before storage in 1 ml aliquots at -70°C.

#### MEASUREMENT OF ANTIBODY BY ELISA TECHNIQUES

For *Candida albicans* and *Proteus mirabilis* the method was essentially as described previously.<sup>16</sup> Aliquots (200 µl) of live bacterial suspension (absorbance=0.34–0.60 nm) in carbonate/bicarbonate coating buffer (pH 9.6) were added to half the wells of Dynatech (96 wells) micro-elisa plates with coating buffer (200 µl) added to the other half. The plates were centrifuged at 2000 rpm for 15 minutes and incubated overnight at 4°C. The bacteria were then fixed with 200 µl of 0.25% glutaraldehyde in isotonic phosphate buffered saline, pH 7.3 with 0.05% Tween 20 (PBST) for 10 minutes at room temperature washed five times with water, treated with 200 µl of 0.1 M glycine in water for 10 minutes at room temperature, and then washed five times in water. For *Escherichia coli*, a pool of antigen from five serotypes was used.<sup>17</sup>

Test sera were diluted 1/400 in PBST and 100 µl were added in triplicate to coated and uncoated wells. Lavage fluids were tested either neat or in 1/5 dilution, depending upon the antigen. After incubation for two hours at room temperature, the wells were washed three times in PBST. Some 100 µl of alkaline phosphatase conjugated anti-human IgG, IgA, or IgM (Sigma or Northeast biomedical) were used at the recommended dilution and incubated for two hours at room temperature, then washed five times in PBST. A total of 100 µl of alkaline

phosphatase substrate (Sigma 1 mg/ml in glycine buffer pH 9.8) were then added. After 30 minutes incubation at 37°C, the absorbance of 450 nm was measured by Titertek Multiskan photometer. In measuring antibodies to other antigens, plates were coated overnight at 4°C with 5 µg/ml antigen in coating buffer and blocked with buffer containing 0.05% Tween 20 and 1% adult bovine serum.

Test sera were randomised and measurements made without the knowledge of the subject group. Results were calculated by taking the mean of triplicate absorbance measurements at 405 nm of coated wells and subtracting the mean value for the corresponding uncoated wells. A pooled human serum was included as a standard on every plate and the corrected absorbance value for the test sera was expressed as a percentage of the corrected absorbance of this standard. By expressing results in this way, interplate variation is minimised.

#### TOTAL IMMUNOGLOBULIN IN INTESTINAL SECRETIONS

Total immunoglobulin concentration in intestinal secretions was measured by ELISA techniques exactly as described previously.<sup>18</sup>

#### STATISTICAL ANALYSES

The Mann-Whitney U test and Spearman's rank correlation coefficient were used for statistical analyses.

## Results

#### SYSTEMIC HUMORAL IMMUNITY

There was no significant difference in serum immunoglobulin concentrations between the three groups (Table II).

Overall there was little difference between the test groups in terms of IgG, IgA, or IgM responses to gut commensals (Table II). Healthy smokers compared with non-smokers showed reduced values of IgA antibody to *P. mirabilis* only ( $p < 0.01$ ). The colitis patients compared with controls had reduced IgG values ( $p < 0.02$ ) and increased concentrations of IgM antibody ( $p < 0.02$ ) to *C. albicans*.

#### MUCOSAL HUMORAL IMMUNITY

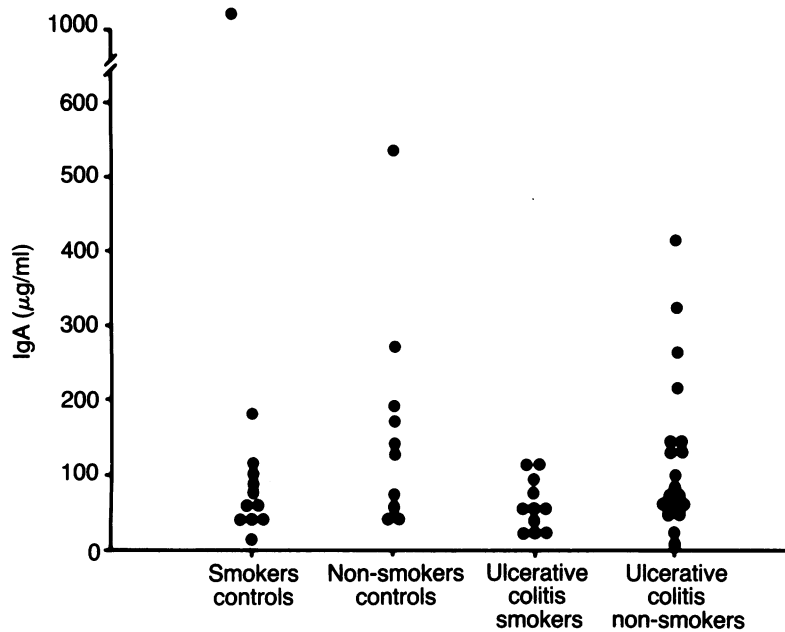
Total IgA immunoglobulin concentration was generally lower in healthy smokers compared with non-smokers and in colitis patients who smoked compared with their non-smoking counterparts, though differences were not statistically significant. The IgA immunoglobulin concentration in smokers with ulcerative colitis was significantly reduced ( $p < 0.03$ ) compared with that in healthy non-smokers (Figure, Table III). There were no significant differences between IgG and IgM immunoglobulin concentrations. Concentrations of IgA, IgM, and IgG antibodies to the various antigens studied showed no significant difference between the four groups (Table III), although for the food antigens, IgA and IgM concentra-

TABLE II Specific antibody concentrations of IgG, IgM, and IgA to *Proteus mirabilis*, *Candida albicans*, and *Escherichia coli* (5 pooled serotypes) measured by ELISA with total IgG, IgM, and IgA concentrations measured in 20 male smokers, 21 non-smokers and 21 smokers with ulcerative colitis (UC)

Organism	Antibody	Median (range) antibody concentration absorbance (450 nm) × 10 <sup>3</sup>		
		Smokers (n=20)	Non-smokers (n=21)	UC (non-smokers) (n=21)
<i>P. mirabilis</i>	IgG	90 (50–180)	98 (60–197)	95 (50–184)
	IgM	100 (45–268)	131 (56–258)	113 (47–256)
	IgA	62 (20–151)*	127 (28–211)	88 (31–215)
<i>C. albicans</i>	IgG	107 (1–157)	100 (21–156)	80 (1–176)†
	IgM	76 (1–725)	172 (41–267)	147 (40–672)†
	IgA	106 (17–205)	123 (31–206)	84 (25–198)
Immunoglobulin concentration				
<i>E. coli</i> (5 pooled serotypes)	IgG	99 (21–160)	95 (46–196)	107 (53–203)
	IgM	59 (25–151)	73 (5–329)	84 (29–232)
	IgA	127 (1–944)	120 (1–600)	194 (1–76)
Total immunoglobulin concentration (µg/ml)	IgG	18 (10–27)	19 (10–39)	16 (10–27)
	IgM	1 (1–2)	1 (1–5)	1 (1–7)
	IgA	4 (2–7)	4 (2–7)	5 (2–9)

\**P. mirabilis* IgA antibody reduced in smokers compared with non-smokers,  $p < 0.01$ ;

†*C. albicans* IgG antibody reduced in colitis compared with non-smokers,  $p < 0.02$ , and IgM antibody increased in colitis compared with smokers,  $p < 0.02$ .



The IgA immunoglobulin concentration measured in intestinal lavage fluid after 4 l of Golytely solution in 12 healthy smokers, 10 healthy non-smokers, and patients with ulcerative colitis in remission, 11 smokers and 19 non-smokers. Colitis smokers compared with control non-smokers  $p < 0.03$ .

tions were all lower in healthy smokers than in non-smokers, especially IgA, although there is a wide scatter. This was not seen with *E coli* and *C albicans*, and was not found when smokers and non-smokers with colitis were compared.

There was no correlation between the number of cigarettes smoked and IgA immunoglobulin concentration for each group.

### Discussion

The circulating antibodies measured for each immunoglobulin class – IgG, IgA, and IgM – for three common gut commensals in the three groups of subjects gave 27 sets of data for comparison, but only three significant differences were identified; IgA antibody to *P mirabilis* was reduced in smokers compared with non-smokers, while IgG and IgM antibodies to

*C albicans* showed reduced and raised concentrations respectively in patients with colitis. The only significant finding in our work with intestinal secretions after gut lavage was a reduced concentration of total IgA immunoglobulin in smokers with colitis compared with healthy non-smokers.

The data are probably valid in these groups since the ELISA technique is a sensitive way of measuring total immunoglobulin and antibodies for the different isotypes. Gut lavage as a way of obtaining intestinal secretion is a simple, safe, and non-invasive technique; the solution has the additional advantage of being poorly absorbed with little effect on fluid and electrolyte balance.<sup>19, 20</sup> Protease inhibitors were added to prevent significant proteolysis of immunoglobulin<sup>17, 18</sup> before measurement by ELISA. Five pooled serotypes of *E coli* were used since intestinal *E coli* flora are not consistent and often vary between individuals.<sup>21</sup> *C albicans* was used since it is a common gut commensal with limited serotypes and most immunocompetent individuals have responded by producing antibodies.<sup>22</sup> Three common dietary antigens, gliadin,  $\beta$  lactoglobulin, and ovalbumin were also included.

In previous work, concentrations of circulating immunoglobulins in patients with ulcerative colitis have been either normal or raised.<sup>23</sup> There is a fourfold greater synthesis of IgA by colonic biopsy specimens cultured from patients with ulcerative colitis compared with controls.<sup>24</sup> Although there has been little work on the effect of smoking on the humoral immune response in colitis, this relation has been examined in the respiratory tract. Smokers have a reduced systemic response to antigens encountered by nasal and respiratory mucosae.<sup>25, 26</sup> At mucosal level, however, conflicting results have been reported for total IgA and bronchoalveolar lavage fluid with either a decrease<sup>27</sup> or a three to fourfold increase reported.<sup>28</sup> In nasal mucosa, a decrease in local antibody concentrations in smokers is reported.<sup>29</sup> Studies on extrinsic allergic alveolitis, a disease more common in non-smokers,<sup>30, 31</sup> showed that antibody to the antigen responsible for the disease was significantly depressed in smokers and this effect seemed related to the severity of the illness.

From our data we are reluctant to attribute much importance to the differences in systemic humoral response since previous work has shown a poor relation between mucosal events and the systemic humoral response. Changes in the intestinal secretions are more likely to reflect the mucosal response to antigenic challenge.<sup>32</sup> Our findings show no significant difference in total immunoglobulin or mucosal immunity to commensal bacteria or food antigens in healthy smokers and non-smokers. Similarly, there was no significant difference in these parameters between non-smoking and smoking patients with colitis.

The simplest interpretation of these data is that smoking does not affect mucosal antibody responses in the gut and the apparently beneficial effects of smoking in colitis are probably mediated by other mechanisms. Possibilities are that smoking affects mucus production,<sup>33</sup> rectal blood flow,<sup>34</sup> intestinal permeability,<sup>35</sup> and the

TABLE III Specific antibody concentrations of IgA, IgM, and IgG classes to ovalbumin,  $\beta$  lactoglobulin, gliadin, *Escherichia coli* (5 pooled serotypes) and *Candida albicans* together with total IgA, IgM, and IgG immunoglobulin concentrations in healthy subjects and patients with ulcerative colitis

		Median (range) antibody concentration absorbance (450 nm) $\times 10^3$			
		Controls		Ulcerative colitis	
Antigen	Antibody	Smokers (n=12)	Non-smokers (n=10)	Smokers (n=11)	Non-smokers (n=19)
Ovalbumin	IgA	128 (20–496)	339 (7–7600)	253 (12–403)	188 (52–357)
	IgM	58 (0–297)	172 (0–458)	207 (0–505)	183 (0–418)
	IgG	17 (0–104)	20 (0–81)	73 (0–124)	83 (0–322)
$\beta$ lactoglobulin	IgA	108 (0–409)	210 (5–329)	156 (0–595)	112 (0–1027)
	IgM	45 (0–162)	87 (0–252)	91 (0–497)	85 (0–214)
	IgG	6 (0–53)	9 (0–47)	46 (0–58)	44 (0–82)
Gliadin	IgA	66 (0–885)	226 (0–604)	358 (0–934)	129 (12–815)
	IgM	51 (0–386)	173 (0–1113)	197 (0–623)	111 (0–415)
	IgG	7 (0–56)	32 (0–48)	48 (0–67)	47 (0–106)
<i>E coli</i> (5 pooled serotypes)	IgA	135 (60–212)	92 (1–158)	116 (51–172)	880 (1–176)
<i>C. Albicans</i>	IgA	20 (1–149)	7 (1–165)	45 (13–170)	37 (1–481)
Total immunoglobulin concentration ( $\mu$ g/ml)	IgA	81 (11–105)	137 (43–274)*	56 (30–117)*	87 (7–418)
	IgM	6 (2–21)	8 (2–36)	7 (2–20)	5 (1–26)
	IgG	0 (0–2)	1 (0–3)	0 (0–10)	0 (0–20)

\*Total IgA concentration ulcerative colitis smokers compared with healthy non-smokers  $p < 0.03$ .



production of oxygen free radicals.<sup>36</sup> This is a preliminary approach to the study of smoking, humoral immunity, and ulcerative colitis. Previous work on saliva<sup>10</sup> has shown that only heavy smokers had differences from control subjects and most smokers in the present study were light smokers. Clearly, if the protective phenomenon of smoking in colitis is via immunity, the effect is more subtle than we have been able to record in these experiments with steady state immunoglobulin concentrations and antibodies to regularly encountered foods and commensals.

Nevertheless, although not significant, the results are consistent with previous observations on smoking and mucosal IgA deficiency which showed a significant decrease in total salivary IgA in heavy smokers relative to non-smokers. More significant change may have been seen in the smoking group with a higher cigarette consumption but most patients with colitis who smoke, are only light smokers.

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