

Cell mediated immunity to corn starch in starch-induced granulomatous peritonitis

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SUMMARY Two patients with histologically diagnosed starch induced granulomatous peritonitis (SGP) have been shown to have cell mediated immunity to corn starch using the techniques of macrophage migration inhibition and lymphocyte DNA synthesis. Control groups of normal subjects, patients with uncomplicated laparotomy, and patients with Crohn's disease were negative in both tests. Lymphocytes from two patients with band adhesions, one of whom had biopsy evidence of a granulomatous reaction to starch, were sensitized to starch. Cell mediated immunity to starch may contribute to the pathogenesis of SGP, and some band adhesions may be a chronic low grade manifestation of this disorder.

Starch induced granulomatous peritonitis (SGP) after laparotomy is a continuing clinical problem that usually requires laparotomy for a confident diagnosis. The pathogenesis of the granulomatous response to the persistent starch granules is not known. Specifically, the contribution of cell mediated immunity to starch has not been determined. Here we describe studies in two patients with SGP, and conclude that cell mediated immunity to starch may be one mechanism which contributes to its pathogenesis.

Methods

PATIENTS

Starch-induced granulomatous peritonitis

A. M., a 48-year-old man, had a modified Belsey repair for a hiatus hernia. Three weeks later he developed colicky abdominal pain and fever, an epigastric mass was present, and air fluid levels were seen on an erect abdominal radiograph. At laparotomy the omentum was diffusely inflamed and thickened and subsequent biopsy revealed multiple granulomata related to particles of starch demon-

strated by 'Maltese cross' birefringence (as was starch in the other biopsies). After operation he had a spontaneous remission without steroids. Studies of cell mediated immunity to starch were carried out 10 weeks after the second laparotomy.

E. B., a 16-year-old boy, had an ileocolic resection for Crohn's disease. As he had taken steroids previously, at operation an increased dose of steroids was given, and then discontinued. Histology confirmed Crohn's disease with numerous giant cells and granulomata. Four weeks later, he developed abdominal pain and fever, and a mass was palpated in the right iliac fossa. At laparotomy, granular peritonitis was found, and the palpable mass was inflamed omentum. A diagnosis of SGP was confirmed by finding a granulomatous reaction around starch particles in an omental biopsy. Corticosteroids induced complete clinical remission, and this drug was successfully discontinued after six weeks. Lymphocytes were studied 12 weeks after the second laparotomy.

Band adhesions

D. R., a 30-year-old man, developed a small bowel obstruction four months after an ileocolic resection for Crohn's disease. A band adhesion causing the obstruction contained a granulomatous reaction to starch. Lymphocyte studies were carried out three days after operation.

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L. P., an 18 year old girl, developed a small bowel obstruction four months after an ileal resection for Crohn's disease. At laparotomy a band adhesion was removed, but not biopsied. Lymphocyte studies were carried out four months after the second operation.

Controls

Five normal subjects who had no previous laparotomies and six patients with a recent laparotomy (within six months) were studied as control groups. One patient had a band obstruction, which when biopsied showed cotton fibres but no starch granules. As three of the patients studied had Crohn's disease, five patients with this diagnosis were also studied, three of whom had had a laparotomy without complication. Some of these patients were taking sulphasalazine (Salazopyrin) but not corticosteroids when tested. No patient or control had an allergy to starch.

TECHNIQUES

Lymphocyte preparation.

Lymphocytes were prepared from heparinised peripheral blood by centrifugation through a Hypaque-ficoll gradient (Böyum, 1968). Cell viability was greater than 95%, and more than 90% of cells were lymphocytes.

Lymphocyte stimulation

Lymphocyte stimulation by antigen was measured as the increase in DNA synthesis in the presence of corn starch using a standard technique (Clancy and Bienenstock, 1974). Briefly, triplicate cultures containing 1×10^6 lymphocytes/ml in RPMI 1640 with 15% heat inactivated AB positive human serum and gentamicin (50 $\mu\text{g/ml}$) and mycostatin (100 u/ml), were incubated at 37°C for 92 h in 5% CO_2 . Corn starch was added to test cultures as Biosorb¹, at 1, 10, and 100 $\mu\text{g/ml}$. 1 μC H^3 -thymidine was added to each tube five hours before termination.

Macrophage inhibition factor

Macrophage inhibition factor (MIF) was assayed by the mixed direct technique (Marsman *et al.*, 1972). In brief, guinea-pig peritoneal macrophages were used as indicator cells, mixed with the patient's lymphocytes, which are present at a concentration of 20%. Each test is done in triplicate, and the cell mixture migrates from a capillary tube into medium to which the test antigen (corn starch) has been added at concentrations of 1, 10, and 100 $\mu\text{g/ml}$. The areas of migration are calculated by projection after 18 h at 37°C and a migration index is calculated as:

$$\frac{\text{mean area of migration in presence of antigen}}{\text{mean area of migration in absence of antigen}}$$

Anti-corn starch antibody

Serial dilutions of patient's serum were layered over an equal volume of a 1 mg/ml suspension of Biosorb, in a vertically placed capillary tube, and allowed to mix.

Results

CONTROLS

Results obtained from the three groups of controls (normal subjects, patients having a laparotomy within six months, and patients with Crohn's disease) did not demonstrate lymphocyte sensitization as evidenced by inhibition of macrophage migration or lymphocyte stimulation in the presence of corn starch. Thus the migration indices in each group were between 0.8 and 1.17 for antigen concentrations of 1, 10, and 100 $\mu\text{g/ml}$ and no mitogenic effect was detected when lymphocytes were incubated with the same antigen range. Lymphocyte stimulation with an antigen concentration of 1 $\mu\text{g/ml}$ is seen in Fig. 2, and migration indices at 10 $\mu\text{g/ml}$ are seen in Fig. 1. As a control for the two patients with band adhesions, the six subjects with an uncomplicated laparotomy had a mean migration index of 0.99 with a range of 0.83 to 1.17 at an antigen concentration of 100 $\mu\text{g/ml}$. Antibody to corn starch was not detected in any subject.

STARCH-INDUCED GRANULOMATOUS PERITONITIS

One patient (A. M.) demonstrated clear evidence of cell mediated immunity to corn starch. The initial study two weeks after the clinical episode showed migration indices (MI) of 0.2, 0.35, at 100 and 10 $\mu\text{g/ml}$ concentrations of antigen. Two months later the MIs were 0.6 at concentrations of 100 and 10 $\mu\text{g/ml}$, and 0.65 at 1 $\mu\text{g/ml}$ (Fig. 1). At that time lymphocyte DNA synthesis was stimulated from 450 CPM to 1300 CPM at a concentration of 1 $\mu\text{g/ml}$ corn starch (Fig. 2). No anti-corn starch antibody was detected at that time.

The second patient (E. B.) was studied three months after an episode of SGP, and both the MI at 10 $\mu\text{g/ml}$ corn starch (Fig. 1) and the level of lymphocyte stimulation at 1 $\mu\text{g/ml}$ of antigen (Fig. 2) was less impressive than the values quoted for patient A. M., but in each case were greater than those found for the control groups. No anti-corn starch antibody was detected.

The spontaneous level of DNA synthesis was higher than controls in each case (Fig. 2).

¹Biosorb starch glove powder (Ethicon Ltd) is prepared from maize starch treated with epichlorhydrin, forming 1-3 diether glycerin groups.

determine the significance of cell mediated immunity to starch in the pathogenesis of obstructing band adhesions.

The studies reported here, however, suggest that there may be a spectrum of clinically relevant sensitization to corn starch. Thus some patients may have a chronic low grade cell mediated immune response with the gradual formation of mature fibrous bands, while in others a more acute and exuberant response causes an acute granulomatous peritonitis. Factors modifying this host response are not clear but probably include individual susceptibility, the dose of antigen, and the way corn starch is handled by the peritoneum. While corn starch is rapidly absorbed from the peritoneum (Marsman *et al.*, 1972), local factors—for example, fibrin formation—may trap starch particles (Turk, 1971) and account for the localization of a subsequent immunologically-mediated inflammatory response.

It is emphasized that reduction of peritoneal contamination with corn starch by thorough washing of surgeons' gloves may reduce the complications of corn starch sensitization. The potential of the macrophage migration inhibition assay rapidly to detect cell mediated immunity to corn starch may provide a useful diagnostic technique, and thus help avoid unnecessary laparotomies in patients suspected of having SGP.

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