Cellular hypersensitivity to components of intestinal mucosa in ulcerative colitis and Crohn's disease

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SUMMARY The migration of peripheral leucocytes *in vitro* is examined in 36 patients with ulcerative colitis, in 34 patients with Crohn's disease, in 12 patients with ulcerative colitis or Crohn's disease, and in 31 patients with other gastrointestinal disorders. In a majority of the patients with ulcerative colitis extracts of foetal, colonic, and jejunoileal mucosa inhibit migration of leucocytes. A similar reactivity is seldom seen in Crohn's disease. Extracts of liver, kidney, and adrenal gland do not inhibit the migration. The reactivity of the ulcerative colitis group was found to be significantly different from that in controls and in the Crohn group, whereas the Crohn group did not differ significantly from the controls. The examination thus reveals a biological difference between ulcerative colitis and Crohn's disease, which are otherwise separable mainly on nosological criteria.

The finding of a similar inhibition of leucocyte migration in five out of 31 patients with miscellaneous gastrointestinal disorders unrelated to ulcerative colitis and Crohn's disease was inconclusive.

An antigen-induced inhibition of leucocyte migration has been shown *in vitro* to be a correlate of cellular hypersensitivity. The immunological mechanisms behind the present system are discussed, and it is concluded that the reactivity observed probably indicates the existence in ulcerative colitis of a state of cellular hypersensitivity to components of normal foetal, colonic, and jejunoileal mucosa.

The differential diagnosis between ulcerative colitis and Crohn's disease is based upon histopathological, radiological, and clinical criteria. In some cases a classification cannot be made with certainty, especially if only the large bowel is involved. However, the conventional nosological differentiation between the two conditions corresponds well with certain immunological differences which are probably related to the pathogenesis.

In Crohn's disease some observations indicate a state of reduced immunological responsiveness (Phear, 1958; Williams, 1963; Taylor, 1965), although this supposition has not been confirmed by a more recent report (Fletcher and Hinton, 1967). Ulcerative colitis seems to be associated with an immunological hyperreactivity including signs of organ-specific hypersensitivity (Perlmann and Broberger, 1962; Watson, Styler, and Bolt, 1965; Taylor, 1965; Lagercrantz, Hammarström, Perlmann, and Gustafsson, 1966; Kraft and Kirsner, 1966; Wright and Truelove, 1966; Weeke and Bendixen, 1968). Observations in support of the latter hypo-

thesis mainly rest upon the demonstration of circulating anticolonc antibodies and are thus informative principally of the humoral (immediate) type of organ-specific hypersensitivity. To assess more completely the immunopathological mechanisms associated with ulcerative colitis, it is necessary to examine whether as well as circulating anticolonc antibodies an organ-specific hypersensitivity of the cellular (delayed) type can also be demonstrated. The existence of this type of autoreactivity in ulcerative colitis can be assumed on the basis of other observations (Perlmann and Broberger, 1962; Watson *et al*, 1965; Bendixen, 1967) but still needs confirmation on larger series.

Since 1932 (Rich and Lewis) it has been known that a specific, antigen-induced inhibition of the migration of immunocompetent cells is *in vitro* a correlate of cellular hypersensitivity. The leucocyte migration technique which has been employed in the present and a previous study (Bendixen, 1967), was developed on the basis of methods described by George and Vaughan (1962), by David,

Al-Askari, Lawrence, and Thomas (1964a), and by David, Lawrence, and Thomas (1964b). Experiments with hypersensitivity to *Brucella* have established firm evidence that this technique makes possible a demonstration *in vitro* of cellular hypersensitivity in man (Søborg and Bendixen, 1966; Søborg, 1967 and 1968), and the method further seems to permit an evaluation of cellular hypersensitivity in states which cannot be estimated by more traditional methods, for example, diseases with presumed autoimmune genesis (Bendixen, 1967 and 1968; Bendixen and Søborg, 1968a; Nerup, Andersen, and Bendixen, 1969).

The purpose of the present work is to investigate by means of the leucocyte migration test the occurrence of specific cellular reactivity to components of normal intestinal mucosa in ulcerative colitis and Crohn's disease. Besides throwing further light upon mechanisms related to the pathogenesis the examination might be helpful in deciding whether the clinical entities ulcerative colitis and Crohn's disease are separable on an immunopathological basis. In addition it is intended in the light of recent knowledge to discuss the immunological mechanisms involved in the leucocyte migration system.

CLINICAL MATERIAL

CONTROLS The control group of 55 subjects consisted of 29 women (aged 15 to 65, average 38 years) and 26 men (aged 9 to 67, average 42 years). They were either healthy members of the medical staff or patients in whom disease of the gastrointestinal tract had been excluded as far as possible by ordinary medical examination during a period in hospital.

ULCERATIVE COLITIS AND CROHN'S DISEASE There were 82 patients with ulcerative colitis or Crohn's disease, 43 women (aged 10 to 75, average 36 years) and 39 men (aged 12 to 74, average 33 years). The differential diagnosis between ulcerative colitis and Crohn's disease rested upon histopathological and radiological examinations and upon generally accepted, anamnestic, and objective clinical criteria. In 32 patients a conclusive histopathological diagnosis according to the criteria described by Lockhart-Mummery and Morson (1960) was made on specimens removed by intestinal resection. In four operated cases a clear classification was not possible. In 37 patients biopsy specimens from the rectum and small bowel did not allow a definite, histopathological conclusion. Nine patients had no histopathological examination at the time of study. In all patients the radiological examination included stomach, small bowel series, and barium enemas. Most cases were examined repeatedly at intervals. Involvement of the terminal ileum or other parts of the small bowel, segmentary distribution of the lesions, and mucosal fissures with 'cobblestone' configuration were considered typical of Crohn's disease. When the colon alone was involved

with a blurred mucosal picture, ulceration and spicula formation the appearance was considered typical of ulcerative colitis.

After a careful study of each case the material was differentiated into 36 cases of ulcerative colitis (18 women and 16 men) and 34 cases of Crohn's disease (18 women and 16 men). Twelve cases could not be classified with certainty (seven women and five men).

OTHER GASTROINTESTINAL DISORDERS The third group comprised 31 patients with various gastrointestinal disorders unrelated to ulcerative colitis or Crohn's disease (14 women, aged 17 to 65, average 38 years, and 17 men, aged 15 to 70, average 43 years). The diagnoses were: idiopathic steatorrhoea (4 cases), duodenal ulcer (3), Schönlein-Henoch purpura (3), acute gastroenteritis or enteritis (3), intestinal lymphangiectasia (2), chronic diarrhoea of unknown aetiology (2), sequelae of small bowel resection (2), postgastrectomy syndrome (1), adenocarcinoma of the colon with peritoneal carcinosis (1), exsudative enteropathy (1), diffuse eosinophilic, granulomatous affection of the small bowel (1), chylascites, chylothorax, and malabsorption (1), acquired hypogammaglobulinaemia with malabsorption (1), thrombosis of the superior, mesenteric artery with the malabsorption syndrome (1), chronic pancreatitis (1), melanosis of the rectum (1), intestinal mastocytosis (1), exfoliative erythrodermia with haemorrhagic diarrhoea (1), and systemic scleroderma with gastrointestinal involvement (1).

METHODS

LEUCOCYTE MIGRATION TEST The capacity of components of various tissues to induce specific inhibition of the migration of peripheral leucocytes was estimated *in vitro* by means of the leucocyte migration test, which has been described in detail elsewhere (Bendixen and Søborg, 1969) and will only be briefly outlined. Heparinized blood from a cubital vein is left to form a sediment for one hour at 37°C. The plasma is withdrawn and the white blood cells are washed three times in Hank's balanced salt solution. The cell suspension is transferred to capillary tubes, and the leucocytes (approximately 50% granulocytes and 50% lymphocytes) are allowed to migrate for 24 hours from the open end of the capillary tube along the plain bottom of a 1 ml tissue-culture chamber containing TC 199 with 10% horse serum (Figs. 1a and b). The circular migration area is measured by paper planimetry. The migration areas of a series of cultures without antigen are put up in parallel to the migration areas of a series of antigen-containing cultures to calculate the migration index which indicates inhibition if the value is below, and stimulation if the value is above, unity.

PREPARATION OF ANTIGENS Foetal tissue was recovered and prepared aseptically in order to avoid contamination with bacterial antigen. The mucosa of the foetal colon and small bowel was easily stripped from the muscular wall. Microscopic examination showed that the separation took place in the submucosae. The mucosae of eight to

FIG. 1a.

FIG. 1b.

FIG. 1. Twenty-four hour leucocyte migration cultures from a patient with ulcerative colitis: (a) nothing added, (b) inhibition of migration induced by extract of colonic mucosa.

10 colons or small intestines were cut in small bits, pooled separately, washed twice in Hank's balanced salt solution, homogenized in a Warren blender at 15,000 rpm for four minutes, left at 4°C for 24 hours, and centrifuged at 1,000 *g* for 20 minutes. The supernatant was separated, lyophilized, and stored at 4°C. Before use the lyophilized mucosal extracts were reliquified with the proper volume of sterile water, and the solution was standardized by protein determination (Lowry's method). The concentration of intestinal mucosa protein in the cultures was normally 50 µg per ml culture medium, which appeared to be the highest concentration not causing non-specific inhibition in normal controls. Extracts of liver, kidney, and adrenal glands were prepared in the same way. The highest, non-toxic concentration as measured by protein contents proved to be 100 µg per ml with kidney extract, 150 µg per ml with liver extract, and 200 µg per ml with adrenal extract. With liver non-specific stimulation was sometimes seen. In the first part of the study more crude tissue homogenates were employed as

centrifugation was omitted. Comparison of the crude and the purified homogenates (extracts) showed that the preparations were tolerated equally well by the leucocytes as measured by the migration capacity. The potency for inducing specific inhibition of the migration appeared to be confined to the supernatant, as the two preparations had the same activity per microgram soluble protein and as no activity was left in the washed sediment.

RESULTS

CONTROLS The normal range (mean $\pm$ 2 SD) of the leucocyte migration test with colonic and jejunoileal extracts was calculated on the basis of 55 controls as presented in Figure 2. The leucocyte migration was barely influenced by the presence of colonic (migration index = 0.79 to 1.11) or jejunoileal (migration index = 0.78 to 1.10) extracts in the concentration employed. With kidney extract a normal range (mean $\pm$ 2 SD) of 0.81 to 1.11 was calculated on the basis of 19 controls; with liver extract similarly a normal range of 0.86 to 1.22 was calculated on the basis of 10 controls; and with adrenal extract a normal range of 0.83 to 1.07 was calculated on the basis of 18 controls.

FIG. 2. Leucocyte migration test in normal material, with extracts of foetal, colonic, and jejunoileal mucosa in 55 controls.

ULCERATIVE COLITIS AND CROHN'S DISEASE The correlation between the leucocyte migration test with colonic and jejunoileal extracts and the diagnosis is presented in Figure 3. It appears that in a majority of the cases diagnosed as ulcerative colitis leucocyte migration *in vitro* is inhibited by components of normal, foetal intestinal mucosa, colonic as well as jejunoileal, and that a similar reactivity is infrequent in cases diagnosed as Crohn's disease. The distribution of the migration index

FIG. 3. Leucocyte migration test in 36 patients with ulcerative colitis, 34 patients with Crohn's disease, and 12 unclassified cases. Antigen: pooled extract of foetal, colonic (CM), or jejunoileal mucosa (JM).

values is significantly different from the normal ($P < 0.001$) in ulcerative colitis as well as in the Crohn ($P < 0.001$) material. In Crohn's disease only a few indices are below normal, and distribution does not differ significantly from the normal material. The ulcerative colitis group included seven cases of haemorrhagic proctitis without other signs of gastrointestinal involvement and in good general condition. Five of these had migration index values within the normal range. The Crohn material included 14 cases with the terminal ileum affected only. This group did not differ significantly from the remaining 20 Crohn patients with colonic involvement.

With kidney extract the migration index was within the normal range in 79 of the 82 patients with ulcerative colitis or Crohn's disease and below normal in three cases (0.79, 0.80, and 0.62). Two of these had normal migration index values with colonic as well as jejunoileal antigen (one case of ulcerative colitis and one case of Crohn's disease). In the third patient the inhibition of kidney extract could not be reproduced at repeated examinations, whereas the migration index with intestinal mucosa antigens remained below normal (one case of ulcerative colitis). In none of these three patients could nephropathy be demonstrated. The patients examined with liver (14) and adrenal (12) extracts had migration index values within the respective normal ranges.

OTHER GASTROINTESTINAL DISEASES Of the 31 patients with various gastrointestinal diseases unrelated to ulcerative colitis and Crohn's disease,

five showed inhibition in the leucocyte migration test with colonic or jejunoileal homogenates. The diagnoses were (1) adenocarcinoma of the colon with peritoneal metastases; (2) exfoliative erythrodermia with haemorrhagic diarrhoea; (3) Schönlein-Henoch purpura; (4) intestinal mastocytosis; and (5) unspecified, acute enteritis associated with severe haemorrhagic diarrhoea. The migration index of the patient with Schönlein-Henoch purpura was significantly inhibited by kidney extract as well. Clinically he had, besides initial diarrhoea, severe renal involvement with microscopically verified proliferative glomerular changes and impaired renal function. The remaining 26 patients in this group, including two additional cases of Schönlein-Henoch purpura, all had normal migration index values with colonic as well as jejunoileal extracts.

DISCUSSION

Examination of 82 patients with ulcerative colitis or Crohn's disease showed that the migration of peripheral leucocytes *in vitro* was inhibited by foetal, colonic, or jejunoileal mucosa components in 31 cases. The group showing inhibition appeared to coincide more or less with the cases diagnosed as ulcerative colitis, whereas inhibition was seldom found in patients with Crohn's disease. The study thus clearly reveals a biological difference between the two conditions, which are otherwise separable mainly on nosographic criteria. As the leucocyte migration was not inhibited by extracts of other organs (kidney, liver, adrenal gland), the reactivity seems to be associated with certain component(s) of intestinal mucosa.

Lymphocytes of the peripheral blood can be assumed to possess immunological competence, and the technique thus makes it possible to register the effect of an antigen upon a mixture of immunocompetent cells derived from several sites. Animal experiments with immunocompetent cells from peritoneal exudate, lymph nodes, spleen, bone marrow, and peripheral blood indicate that the migration inhibition response is a parameter of cellular hypersensitivity (Rich and Lewis, 1932; Moen and Swift, 1936; Raffel, 1948; Heilman, Howard, and Carpenter, 1958; Darlington and Scherago, 1960; Johnson and Scherago, 1960; Svejcar and Johanovský, 1961; George and Vaughan, 1962; Heilman, 1963; Carpenter and Brandriss, 1964; David, 1964a and 1964b) and a cell population from human peripheral blood has been shown to possess the same kind of reactivity (Søborg and Bendixen, 1967; Søborg 1967 and 1968).

On account of the antigenic heterogeneity of the

present model one should consider whether the immunobiological mechanisms behind the reactivity observed are of a similar nature. Whether extracellular immunoglobulins could have any influence upon the reactivity observed should also be considered. It is assumed that all detectable antibody is removed from the cell surfaces through four cell washings (Favour, 1964), whereas possible cytophilic antibody (Sorkin, 1963) remains fixed on the cells. Thus all capacity for specific immunological reactivity entering the system is primarily derived from cell-associated material. Furthermore it can be demonstrated that the addition of serum from patients with a positive leucocyte migration test does not convey the specific reactivity to normal control cultures (Bendixen and Søborg, 1968b).

Antigen-induced synthesis *in vitro* and excretion of specific immunoglobulin would mean a correlation with humoral hypersensitivity *in vitro*, but cannot be expected to occur within the brief culture period of 24 hours and accordingly cannot be regarded as a cause of the inhibition. This possibility is further excluded by the fact that the inhibition can sometimes be observed as early as four hours after the start of the migration. Inhibition of the migration released by blood group isoantibody reacting with isoantigen of the surface of the migrating cells can be excluded likewise, as the culture medium is prepared with horse serum. With the heterogenous antigen employed in the present study cell material containing foetal blood group isoantigen is introduced into the system, but again interaction with isoantibody cannot influence the reaction, as homologous serum is not present. Blood residue in the foetal tissue extracts might contain maternal isoantibody, which could possibly influence the migration of cells with a corresponding isoantigenic pattern. As, however, inhibition was not observed in normal controls and occurred quite unrelated to the blood types of the cell donors this possibility can be ruled out.

In the light of these considerations extracellular immunoglobulins can probably be excluded as being responsible for the migration inhibition, and it seems relevant to look for a mediator of specific reactivity belonging to or derived from the cells proper, primarily the lymphocytes, as these cells in similar systems have been found to be potent carriers of specificity (David *et al.*, 1964b; David, 1966; Bloom and Bennett, 1966; Thor, 1967). The existence of lymphocyte-derived factors with the ability to mediate the cellular immune response has been confirmed in migration culture experiments. Bloom and Bennett (1966), Bennett and Bloom (1967), and David (1966) have demonstrated that peritoneal exudate lymphocytes from tuberculin hypersen-

sitive guinea pigs upon interaction with the specific antigen produce a soluble, non-dialyzable material, which inhibits the migration *in vitro* of normal peritoneal exudate cells, and Svejcar *et al.* (1967a and b) have found a similar mediator of specific reactivity in spleen cell cultures. In man Thor (1967) has reported that an RNA fraction of hypersensitive human lymph node cells can convey to precultured, normal lymph node cells the capacity for specific, antigen-induced inhibition in migration cultures. These factors are probably different from the transfer factor of Lawrence (1960), but at present no further conclusion can be drawn as to the identity or interrelationship of soluble, lymphocyte-derived, extracellular mediators of cellular hypersensitivity. Consequently it is not possible at the moment to give a full explanation of the immunobiological mechanisms behind the specific, antigen-induced inhibition of white cell migration. The above considerations, however, all tend to indicate that the antigen-induced inhibition of leucocyte migration is unrelated to extracellular immunoglobulins and is mediated by specifically reactive lymphocytes. This implies that the reactivity is a correlate *in vitro* of cellular hypersensitivity.

With this background the present findings suggest an association between ulcerative colitis and a state of organ-specific, cellular hypersensitivity directed towards antigen components of normal, foetal, colonic, or jejunoileal mucosa. Cell-mediated, organ-specific hypersensitivity reactions may accordingly be involved in the pathogenesis of ulcerative colitis. A similar reactivity is not associated with the clinical syndrome known as Crohn's disease. This finding, however, does not necessarily indicate that the two syndromes are aetiologically different. The differences in clinical picture, organic involvement, and course of disease may just as well primarily depend upon individual variations in immunological reactivity to the same aetiological agent. The occurrence in both syndromes of normal and subnormal migration indices may support this point of view. The low frequency of abnormal leucocyte migration tests in the Crohn group might advocate the use of the leucocyte migration test as a supplementary differential diagnostic procedure in the two conditions, especially if a more specific antigenic component could be isolated.

The leucocyte migration was inhibited by intestinal mucosa components in five out of 31 patients with various gastrointestinal diseases unrelated to ulcerative colitis and Crohn's disease. The reactivity thus does not seem to be a common feature of gastrointestinal disorders on the whole. On the contrary, it was an expected finding in, for example, Schönlein-Henoch purpura, in exfoliative dermatitis

with intestinal involvement, and in adenocarcinoma of the colon (Gold, Gold, and Freedman, 1968). These findings indicate that further applications of the technique in the field of gastroenterology will be of interest, preferably with more specific antigenic material.

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