

EXPERIMENTAL ALLERGIC ENTEROCOLITIS

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Autoimmune processes play an important role in the development of chronic nonspecific ulcerative colitis [3, 7, 11, 14]. Evidence in support of the autoallergic theory of ulcerative colitis is the discovery of autoantibodies in the blood of these patients [4, 5, 8, 13]. The results of the authors' previous investigations also showed that in the active phase of nonspecific ulcerative colitis considerable quantities of anti-intestinal antibodies may be found in the patients' serum by means of Steffen's reaction [2].

Important confirmation of the autoallergic theory would be given by the experimental reproduction of colitis by immunization of animals with homologous intestinal tissue. No such model of colitis has been described in the literature. All that has been reported is that colitis can be produced in dogs by injection of anti-intestinal rabbit cytotoxic sera into these animals [9-11]. In these experiments, however, the autoimmunization factor was missing, so that they contribute little to the elucidation of the pathogenesis of nonspecific ulcerative colitis in man.

In the present investigation an attempt was made to reproduce allergic enterocolitis in dogs by immunizing the animals with homologous tissue from the large intestine, thus employing the principle of Cavelti's well known experiment [6].

EXPERIMENTAL METHOD

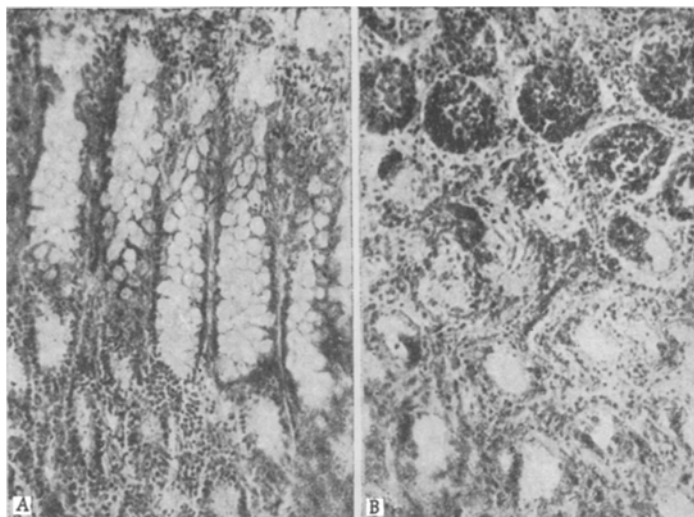
The antigen for immunization of the dogs was prepared as follows. The mucous membrane of the large intestine was dissected from a recently killed dog, washed with sterile physiological saline, and ground in a mortar with quartz sand. The tissue mince thus obtained was diluted in physiological saline in the ratio of 1:1 or 1:2 and centrifuged for 30 min at 5000 rpm. The supernatant was discarded, mertiolate was added, and the product was mixed with Freund's adjuvant in the ratio of 1:2. This mixture of intestinal antigen with Freund's adjuvant (3 ml of mixture per injection) was used to immunize the dogs subcutaneously in the region of the dorsal surfaces of all the limbs twice at intervals of 7 days. Observations were kept on the animals for between 1 and 6 months. The development of allergic enterocolitis was determined from the results of stool inspections and rectosigmoidoscopy, and from the results of the Tribulet-Vishnyakov, Coombs' and Steffen's tests. In some experimental animals the content of the intestinal enzymes enterokinase and alkaline phosphatase in the stool was investigated. At the end of the period of observation the dogs were sacrificed by air embolism and the state of the large and small intestine was investigated macroscopically and microscopically in the department of pathological anatomy of the Institute.

Experiments were carried out on 25 dogs of which 10 were controls (5 received injections of intestinal antigen without stimulator, the other 5 received Freund's adjuvant alone).

EXPERIMENTAL RESULTS

After immunization of the animals with a mixture of intestinal antigen and Freund's adjuvant, from about the 10th day after the reacting injection, all the dogs developed loss of appetite, wasting, and diarrhea with mucus and blood mixed with the stools. On microscopic investigation of the stools, leukocytes and erythrocytes were found. The Tribulet-Vishnyakov tests, demonstrating inflammatory hypersection of the intestine, became positive. The content of enterokinase and alkaline phosphatase in the stool was increased by 8-10 times. The characteristic picture of erosive-catarrhal colitis was seen on proctoscopy. In 3 dogs the enterocolitis followed a progressive course and the animals died one month after injection of the antigen with signs of increasing cachexia and diarrhea with mucus and blood in the stools. Six dogs also showed

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Preparation of the mucous membrane of the small (A) and large (B) intestine of a dog with experimental allergic colitis (experiment No. 14). A) Goblet cells are present in abundance, with hypersecretion of mucus, and proliferation of the reticulocytes of the stroma of the mucous membrane; B) degeneration and desquamation of the epithelium of the gland can be seen in the superficial layers of the mucous membrane and swelling of the partitions of stroma between the glands. Photomicrographs. Objective 8, ocular 7. Hematoxylin-eosin.

a well defined picture of enterocolitis, but later the manifestations of the disease disappeared, and the results of stool examination and rectosigmoidoscopy were restored to normal. After 2-3 months the animals were completely cured. In the remaining 6 dogs the picture of acute colitis was less marked, and clinical manifestations were seen for 1-1.5 months, after which recovery took place.

In control experiments no pathological signs were found in the intestines throughout the period of observation.

The results of immunological investigations showed that during the first week after the end of immunization no incomplete antibodies were found in the blood and the Coombs' and Steffen's tests were negative. In the second week these tests became weakly positive; after 3 weeks and more, when the full clinical picture of enterocolitis had developed, both reactions were positive.

The following case will serve as an illustration.

Experiment No. 14, the dog Sheika. On October 29, 1963, 0.75 ml of a mixture of intestinal antigen with Freund's adjuvant was injected into each limb, and a further injection of the mixture was given on November 5. On November 10, ulcers appeared at the site of injection of the antigen in all the limbs. From this time the animal's condition worsened; the dog took its food reluctantly, grew visibly thinner, and developed diarrhea with stools mixed with mucus and blood. Stool examination revealed numerous leukocytes and the Tribulet-Vishnyakov test became strongly positive. The content of enzymes in the stools increased to 7500 units/g (phosphatase) and 2000 units/g (enterokinase). At this period Steffen's test was weakly positive. The titer of antiglobulin serum in the experimental tube fell only to half the level in the control tube. The animal's condition worsened, the diarrhea became continuous in character, the excretion of mucus and blood increased, and petechial hemorrhages, erosions and ulcers appeared on the mucous membrane of the large intestine. The enzyme-secreting function of the small intestine was profoundly disturbed. The excretion of alkaline phosphatase with the stools increased to 45,000 units/g, and that of enterokinase to 16,000 units/g. Anti-intestinal (Steffen's test) and anti-erythrocyte (Coombs' test) antibodies were found in the blood serum taken 1.5 months after the beginning of the experiment.

In the dogs dying from enterocolitis or sacrificed at the height of the disease, a marked hyperemia of the mucous membrane of the large intestine was observed, and multiple erosions and ulcers were seen

in the distal portions. Isolated areas of hyperemia and edema and single hemorrhages were observed in the mucous membrane of the small intestine. Histological investigation revealed a well-developed pattern of inflammation.

As an example a short extract is given from the records of the daily observations on the animal's condition and the autopsy findings.

Experiment No. 4, the dog Verevochka. On March 5, 1962 a mixture of antigen with Freund's adjuvant was injected subcutaneously as in the previous experiment.

On the 15th day from the beginning of the experiment liquid stools with mucous were observed for the first time. On the following days the diarrhea continued and large quantities of mucous and flecks of blood were excreted. Examination of the stools on March 17 and 25 revealed numerous leukocytes and erythrocytes in all fields of vision, and the Tribulet-Vishnyakov became strongly positive.

Rectosigmoidoscopy on March 17 showed: the anus was gaping; the sigmoidoscope was introduced for a distance of 25 cm; the mucous membrane was hyperemic and edematous, with petechial erosions; mucous present in the lumen of the intestine, the mucous membrane in the region of the sphincter was hyperemic and eroded. The animal's condition progressively worsened, vomiting developed, and food began to be refused. On April 2, 1962 the animal died. At autopsy infiltration of the tissues was present at the site of injection of the antigen in the subcutaneous cellular tissue of the limbs. The abdominal cavity contained 0.5 liter of transparent fluid. The stomach and intestine were empty. The mucous membrane of the large intestine was acutely hyperemic and edematous, with numerous hemorrhages and erosions, and three ulcers were found in the distal portion of the intestine. The histological findings confirmed the presence of well-developed inflammation of the large intestine (see the figure A and B).

In the animals sacrificed after observations for 4, 5, and 6 months, as in all the control animals, no signs of inflammation of the large or small intestine were found at autopsy.

The results of these experimental investigations thus showed that a model of allergic enterocolitis can be reproduced by isoimmunization with the tissues of the mucous membrane of the large intestine. This provides indirect evidence in support of the autoallergic theory of the pathogenesis of nonspecific ulcerative colitis in man. The model of enterocolitis developed in dogs would appear to be very promising for the study of the role of the allergic component in the mechanism of developing of the enterocolitis and methods of their experimental treatment.

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