MORPHOLOGICAL INVESTIGATIONS IN HYPERSENSITIVITY OF DELAYED TYPE TO STREPTOCOCCAL ANTIGENS USING MESENTERIC FILM PREPARATIONS

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Skin reactions, which reach their maximal development 24-48 h after intradermal injection of specific microbial antigens, are used as one test for the detection of hypersensitivity of delayed type (HDT). The morphological picture of changes in the skin of animals at the site of injection of antigens in HDT to microbial antigens, including to streptococcal antigens, has been described by several workers [1, 2, 8-13]. Microscopic examination of sections through animals' skin has shown that cellular response, predominantly involving monocytes, develops in the loose connective tissue around small blood vessels and nerve fibers. Since the response during infection develops in the tissues of many organs, it is important to study the principles governing the development of cellular responses in the peritoneum in HDT to injection of specific antigens, in connection with the problem of development of serositis and aseptic peritonitis.

The object of this investigation was to study the morphological picture of the reaction developing in the abdomen of animals during HDT to streptococcal antigens after intraperitoneal injection of a preptation of thermostable fractions of the streptococcus. As was shown previously, marked HDT arises to antigens of the thermostable fraction (TST fraction) in animals sensitized with group A streptococcus [4, 6].

EXPERIMENTAL METHOD

Guinea pigs (21 animals) were sensitized by a single injection of $5 \cdot 10^9$ bacterial cells of group A streptococcus type 10 (strain Dochez NY-5), mixed with Freund's incomplete adjuvant (FIA), into the foot pads. The animals of the control group (17 guinea pigs) received an injection of FIA only. Intact animals (19 guinea pigs) served as the other control group. The TST-fraction was prepared by Ando's method [7] in the modification in [3] by precipitation of protein at pH 4.0-4.2 from the supernatant obtained by decanting broth cultures of Dochez NY-5 streptococcus and subsequently dissolving the residue in borate buffer at pH 8.0-8.2. To prove the development of HDT, intradermal tests were carried out on some animals by injection of 0.1 ml of a solution of TST fractions containing 6 μg protein. The peritoneal response was studied by the method of film preparations. At different times after sensitization (on the 8th, 14th, and 17th-21st days) different doses of the TST fractions (3, 30 and 300 μg protein) were injected intraperitoneally. The animals were killed with ether 1, 3, and 24 h later, and mesenteric film preparations were obtained. For this purpose, six or eight pieces of mesentery (12 × 12 mm) were excised, stretched out by means of dissecting needles on slides, dried, fixed in formalin solution, and stained with hematoxylin by Yasvoin's method [5].

EXPERIMENTAL RESULTS

The results of the intradermal tests showed that as a rule well-marked reactions of delayed type arose in the animals on the 17th day or later after sensitization with the streptococcal culture, in the form of erythema and induration of the skin (15 × 15 mm). Earlier (on the 14th and, in particular, on the 8th day) weakly positive skin reactions were found in only some animals. In guinea pigs receiving FIA and in intact animals, no reaction was obtained to intradermal injection of the same doses of the TST fraction. A study of the reaction of the peritoneum by the film preparation method 1 h after intraperitoneal injection of the TST fraction

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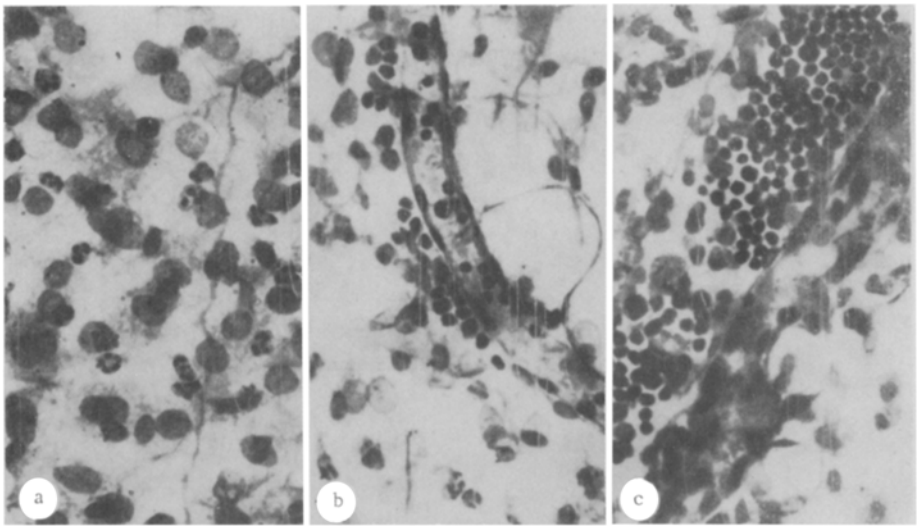

Fig. 1. Peritoneum of animal sensitized with culture of streptoccus 24 h after intraperitoneal injection of thermostable fraction of streptococcus. a) Diffuse infiltration of peritoneum by cells, mainly polymorphs; b) dilatation of a vessel, leukocytes distributed along its walls; perivascular concentration of a few monocytes; c) dilatation of prelymphatic accompanying vein, with small concentration of lymphocytes. Mesenteric film preparations, Yasvoin's iron-hematoxylin.

(300 μg) showed that in animals both of the experimental group (sensitized with streptococcus) and in the control group (only FIA was injected into the footpads), the peritoneum and blood vessels showed no visible changes. In the lumen of the vessels the number of polymorphs was slightly increased. A uniform and diffuse infiltration of the peritoneum with polymorphs and an increase in the number of polynuclear and mononuclear cells in the lumen of the vessels were observed 3 h after injection of the same dose of TST fractions into the animals of these groups. In animals sensitized with streptococcus, small concentrations of lymphocytes could be observed near some vessels, evidently in connection with the initial stage of lymphostasis in the lymphatic circulation. Diffuse infiltration of the peritoneum, of considerable intensity, with mononuclear and polynuclear cells was observed in the peritoneum of guinea pigs sensitized with the streptococcal culture, 24 h after the reacting injection of TST fraction (3-30-300 μg). It was found under these circumstances that if relatively small doses of TST fraction (3-30 μg) were injected, mononuclear cells predominated, whereas if the dose was increased (150-300 μg) the number of polymorphs in the peritoneum rose sharply (Fig. 1a). In the region adjacent to the blood vessels, infiltration of the peritoneum was more marked. Under these circumstances the mesenteric blood vessels as a rule were dilated and filled with blood. The number of leukocytes in their lumen increased (Fig. 1b). In the lymphatics accompanying the neurovascular bundles of the mesentery, multiple concentrations of lymphocytes of different sizes were observed. In seven of 11 animals, multiple foci of concentration of large numbers of lymphocytes were found not only on the 19th day, but also at earlier times after sensitization with streptococcus (on the 8th and 14th days), in response to intraperitoneal injection of both 300 μg and 3 μg of the TST fraction, in the region of the small blood vessels. A study of the reaction of the peritoneum in animals of the control group, receiving FIA only, 24 h after injection of 3-30 μg of the TST fraction showed diffuse infiltration of the peritoneum of the mesentery with polymorphs, with some intermingling of monocytes, whereas in response to injection of 300 μg the infiltration was predominantly polynuclear in character. It should be noted that in the animals of this group, the infiltration was on average less intensive than in the animals of the experimental group. A study of the reaction of the vessels in individual guinea pigs revealed (in one of six preparations) slight dilatation of the lumen (in two of ten animals), with solitary foci of lymphostatis (in three animals) or focal infiltration of low intensity of the tissues around the vessels with cells (in two animals). This last picture was observed only in animals receiving 300 μg of the TST fraction. On examination of preparations of the mesentery of unsensitized guinea pigs, obtained 24 h after intraperitoneal injection of the TST fraction (3-30-300 μg), diffuse infiltration of the peritoneum, of low intensity, with monocytes was observed in five of the seven animals, whereas after injection of 300 μg of the TST fraction infiltration with some admixture of polymorphs was observed. No visible changes were found in the cells and blood vessels of the peritoneum. When borate buffer or 0.85% NaCl solution was injected intraperitoneally into ani-

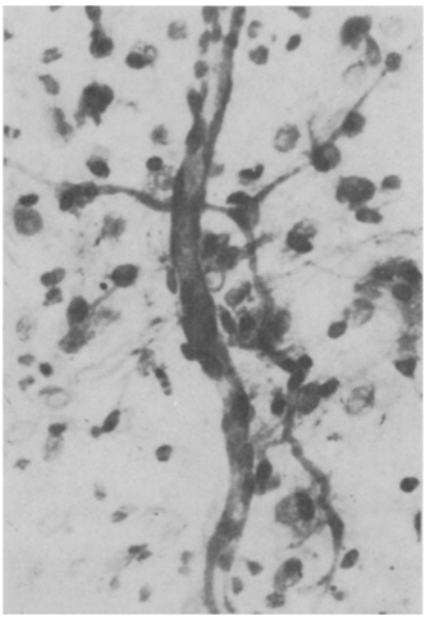

Fig. 2. Peritoneum of unsensitized animal (control). Mesenteric film preparations. Stained by Yasvoin's method, 280×.

mals of any of the groups, the peritoneum, including the blood vessels, usually showed no visible changes (Fig. 2). In some cases, after injection of the TST fraction, adhesion of cells of the peritoneal exudate to the surface of the mesentery, with a similar cellular composition to that found in foci of infiltration of the tissues, was observed in the animals of all three groups.

The results of these investigations showed that 24 h after intraperitoneal injection of small doses of TST fraction (not exceeding 30 μg) into animals sensitized with streptococcus, diffuse infiltration of the peritoneum by lymphocytes, monocytes, and polymorphs, predominantly by mononuclear cells, appeared. With an increase in the dose of the TST fraction (300 μg) the number of polymorphs increased sharply, and in some cases they completely masked the reaction. In a previous investigation [1], devoted to the study of cellular reactions at the site of intradermal injection of PST fraction into guinea pigs with HDT to streptococcal antigens and with unsensitized animals, similar results were obtained as a result of investigation of tissue sections. Infiltration of the peritoneum with polymorphs is evidently a nonspecific response to injection of large doses of substances foreign for the recipient, and is unconnected with HDT. This explanation is supported by the discovery of cellular responses of polynuclear type after intraperitoneal injection of TST fraction, in the group of animals receiving FIA alone. Similar results also were obtained by other workers [8] after intradermal injection of relatively large doses of tuberculin. In the present investigation the discovery of concentrations of lymphocytes in the region of the mesenteric blood vessels in animals with HDT to streptococcal antigens after intraperitoneal injection of the TST fraction is particularly interesting. Characteristic concentrations of many lymphocytes in lymphatic sinuses, accompanying blood vessels and nerve trunks, are evidently identical with the "muffs" described by Waksman [12], during HDT, in skin sections around the neurovascular bundle at the site of injection of the specific antigen.

Film preparations of the mesentery were thus used in this investigation as the object for morphological study of cellular reactions connected with HDT. Because of the comparative simplicity of the method it is possible to study the reaction not only over a wide area of the peritoneum, but also along a considerable length of its vessels. The discovery of perivascular concentrations of lymphocytes in the peritoneum, specific for HDT, in response to injection of the corresponding dose of antigen into the peritoneal cavity of a sensitized animal enables the method of film preparations to be recommended for the detection and study of HDT to microbial antigens under experimental conditions.

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STUDY OF THE ANTIGENIC PROPERTIES OF AN ALLERGOID FROM RAGWEED POLLEN

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The most effect method of treatment of allergic diseases at the present time is by repeated and prolonged administration of increasing doses of allergens to the patients. Disadvantages of this method include cases of the development of general and local allergic manifestations. These complications can largely be avoided by the use of allergoids and, in particular, of formalinized derivatives of allergens from plant pollen or other objects [2, 3].

However, whereas the mechanism of formation of toxoids, substances to some degree analogous with allergoids, under the influence of formaldehyde has been comparatively extensively studied, the conditions which determine loss of allergenic properties by allergens although they retain their immunogenicity have been inadequately discussed in the current literature. Because of the inadequacy of this research, methods of obtaining such preparations on a commercial scale are not yet available.

The object of the present investigation was to study the allergenic properties of an allergoid from ragweed pollen, prepared by the method of Haddad et al. [3], with the aim of establishing a basis for the technology of production of a Soviet allergoid from ragweed pollen on a commercial scale.

EXPERIMENTAL METHOD

Preparation of the allergoid was based on formalin treatment of a dialyzed extract from plant pollen for 32 days at a temperature of 32°C. Two preparations not treated with formalin served as the control: the initial allergen, kept at 4°C, and also the allergen heated to 32°C for 32 days. Three batches of allergoid and three batches of each of the control preparations were obtained.

The properties of the preparations were studied in Ouchterlony's precipitation test with antisera prepared by hyperimmunization of rabbits with the test preparations by Averkina's scheme [1] and also by scarification skin tests on 30 patients sensitized to ragweed pollen and on 30 healthy subjects.

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