MORPHOLOGICAL STUDY OF THE PATHOGENIC ACTION OF VACCINE STRAIN EV OF *Yersinia pestis* IN INBRED MICE

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EV living plague vaccine possesses residual virulence, to which are attributed the morphological changes taking place in the internal organs of experimental animals [1, 2]. The aim of the investigation described below was to study how the character and severity of the changes in structure of the internal organs depend on the animals' genotype in the early stages of development of immunity to plague.

EXPERIMENTAL METHODS

Inbred mice aged 1.5-2 months belonging to the following four haplotypes were used: H-2^k (CBA/LacJ), H-2^a (A/SnJ), H-2^d (BALB/cJ), H-2^b (C57Bl/6J, CC57W/J, B10CW/J). All the mice were females except those of line B10CW, which were males. The animals were kept under identical conditions on a standard diet. Mice of all lines received a subcutaneous injection of standard vaccine strain EV of *Yersinia pestis* in a dose of $5 \cdot 10^3$ or 10^5 bacterial cells (b.c.). These doses were used because the smaller of them is the minimal immunizing dose, whereas the larger causes the formation of strong immunity to plague. Pathological changes in the heart, lungs, liver, spleen, kidneys, adrenals, and thymus were studied 1, 3, 5, 7, and 13 days after vaccination. Paraffin sections were stained with hematoxylin and eosin, by Van Gieson's or Mallory's method, by Brachet's method, and with Sudan Black. Blood films were stained by the Romanovsky–Giemsa method and by Pigarevskii's method [5]. Activity of acid and alkaline phosphatases was studied by Gomori's method in frozen sections of unfixed splenic tissue.

EXPERIMENTAL RESULTS

Macroscopic examination of mice immunized with $5 \cdot 10^3$ b.c. of strain EV from the 5th to the 13th days revealed congestion of the internal organs and enlargement of the regional lymph nodes, liver, and spleen, and in the mice of line C57Bl/6, gray miliary nodules also were found, single in the liver and multiple in the spleen. On the 13th day, the spleen of certain individual C57Bl/6 and B10CW mice was grossly enlarged, and very firm and grayish in color. Strain EV in a dose of 10^5 b.c. caused similar but more marked macroscopic changes in the internal organs, which were observed earlier. Miliary nodules were recorded in all strains except CBA, in the spleen. Signs of cloudy swelling were observed in the liver, kidneys, and myocardium, and small focal hemorrhages were present in the lungs of B10CW mice.

Microscopically, after injection of $5 \cdot 10^3$ b.c. of strain EV, nonsuppurative (5th day) or suppurative (7th day) splenitis, with single or multiple foci of necrosis, followed by scar formation, were found in the spleen of CC57W and, in particular, of B10CW and C57Bl/6 mice. On the 13th day nearly all the splenic tissue in some mice of lines B10CW and C57Bl/6 was replaced by areas of postinflammatory fibrosis. Multiple perivascular foci of lymphohistiocytic infiltration appeared on the 3rd day in the liver of the mice of the sensitive lines mentioned above (Fig. 1a), followed by small (5th day) or large (7th day) epithelioid granulomas in place of the dying groups of liver cells (Fig. 1b), with some admixtures of lymphocytes, single polymorphonuclear leukocytes (polymorphs) and clearly defined scar forma-

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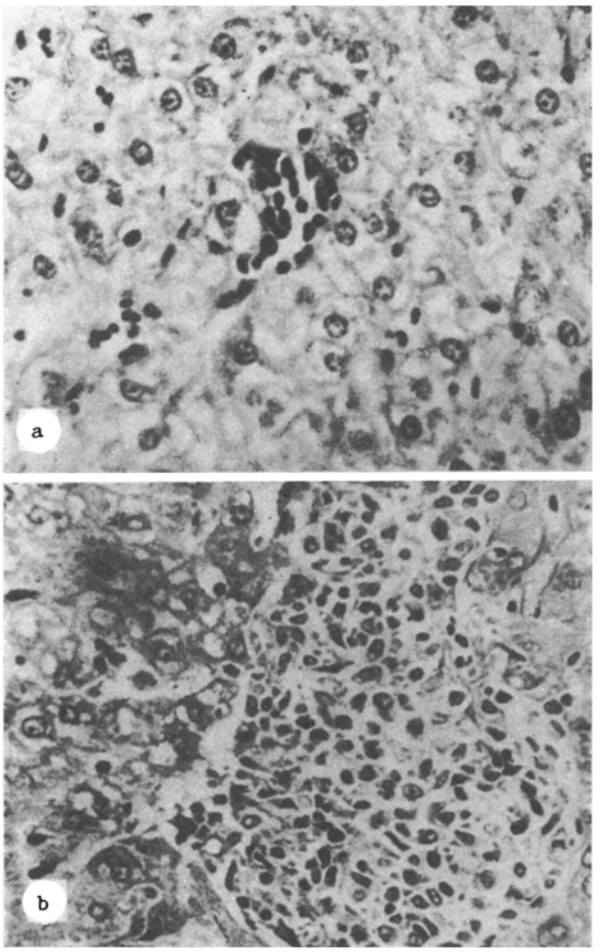

Fig. 1

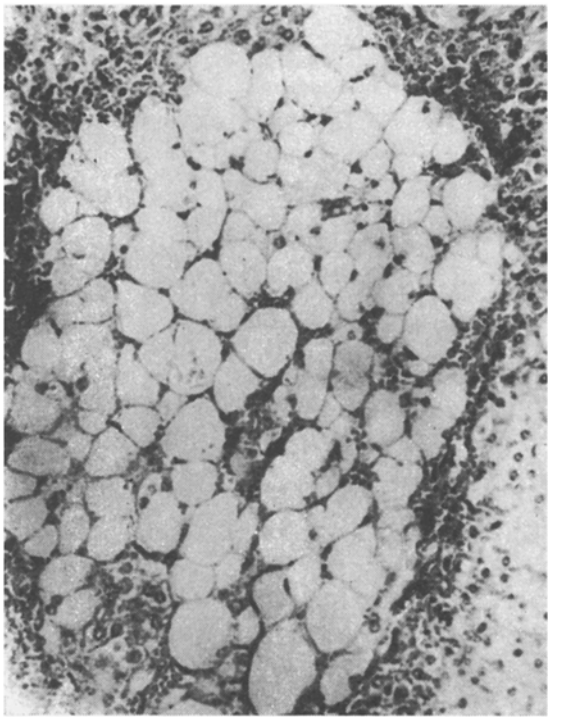

Fig. 2

Fig. 1. Liver of a CC57W mouse vaccinated with $5 \cdot 10^3$ b.c. of vaccine strain EV. a) Activation of Kupfer cells and small focus of lymphohistiocytic infiltration on 3rd day; b) large granuloma on 7th day. Here and in Fig. 2: strained with hematoxylin and eosin, 160×.

Fig. 2. Adrenal of a C57Bl/6 mouse 7 days after injection of 10^5 b.c. of vaccine strain EV: accumulation of cells resembling fat cells.

tion in the later stages. Considerable vacuolar or granular degeneration of the hepatocytes was observed, the degree of which diminished toward the 13th day. The cortex of the thymus appeared narrowed and with a reduced number of small lymphocytes; the medullary layer was widened, and contained an increased number of lymphoid cells. Hassall's corpuscles were enlarged, and had cavities containing cell debris and eosinophilic inclusions. Moderately, severe or sometimes severe cloudy swelling degeneration was found in the kidneys and myocardium. In mice of the more resistant lines (CBA, A/Sn) signs of activation of cells of the mononuclear phagocytic system predominated in the liver and spleen, and immunomorphological reconstruction took place in the secondary lymphoid organs. After injection of EV in a dose of 10^5 b.c. the most marked morphological changes in the internal organs were found in C57Bl/6 mice, the least in CBA. In the former, until the 7th day there were signs of suppurative splenitis and of suppurative interstitial hepatitis. Signs of severe disturbance of protein and lipid metabolism were found in the parenchymatous cells of the liver, kidneys, and myocardium. Moderately severe focal productive interstitial myocarditis developed. Cells in the medullary layer of the adrenals, after intensive degranulation, resembled fat cells (Fig. 2). Toward the end of the period of observation, exudative changes were replaced by fibroblastic changes. In mice of the other lines, the productive component of inflammation predominated from the very beginning. However, the changes can be regarded as excessive in mice of all lines except CBA. In the latter the predominant features were hyperplastic processes, more especially in the spleen.

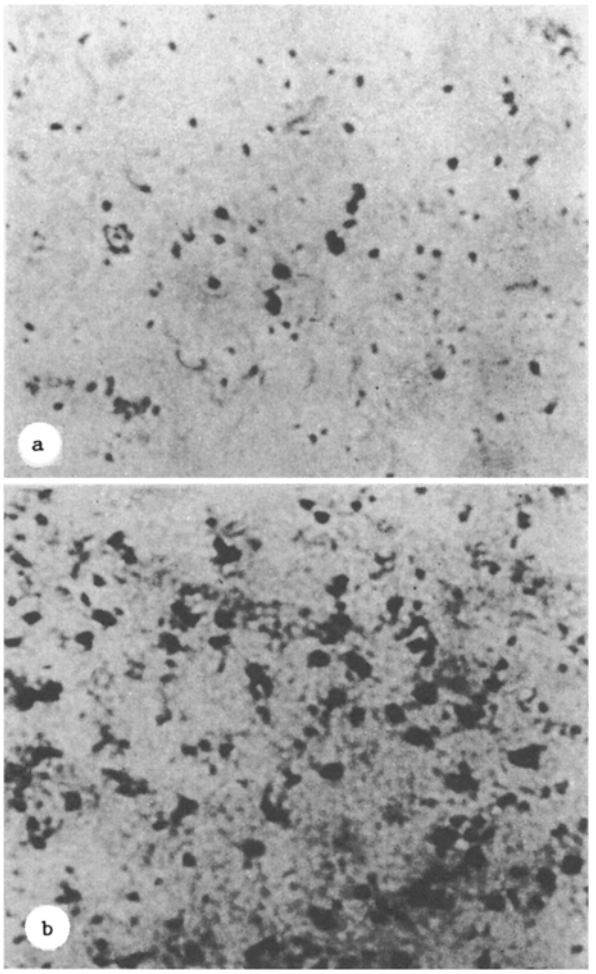

Fig. 3. Macrophages in spleen with high acid phosphatase activity 24 h after injection of 10^5 b.c. of vaccine strain EV. a) In a CBA mouse; b) in a C57Bl/6 mouse. Gomori's method, 160×.

In mice of the more resistant lines (A/Sn and CBA) there was a marked increase in the number of macrophages with high acid phosphatase activity, involved in antigen processing and in the formation of antigenic information [7], as early as after the 1st day in the red pulp of the spleen (Fig. 3a). These cells were fewer in number in mice of the other lines, especially B10CW and C57B1/6 (Fig. 3b). On the 5th day the number of macrophages with high acid phosphatase activity was high in all animals except CBA. One week after vaccination the number of cells of the mononuclear phagocytic system and their enzyme activity fell in the spleen of all lines of mice, but fibroblasts with high alkaline phosphatase activity appeared.

The number of polymorphs and monocytes in the peripheral blood was increased in animals of all lines after 24 h. The level of cationic proteins in the polymorphs was lowered, but on the 3rd day living microorganisms stained a lilac color with azure were found in the cytoplasm. Some polymorphs in C57B1/6 mice were destroyed. After the 7th day an increase was observed in the number of lymphocytes, among which there were many large and blast forms, whereas levels of polymorphs and monocytes were lowered. Toward the end of the period of observation the leukocyte formula came back close to normal.

The investigations thus showed that the sensitivity of animals to the pathogenic action of vaccine strain EV of Yersinia pestis is genetically determined, for it differs in magnitude in animals of different haplotypes. The least marked changes in structure of the internal organs and the smallest decrease in enzyme activity in the spleen were observed in mice with the H-2^k and H-2^a haplotype (CBA/Lac and A/Sn), and the greatest changes were found in animals with the H-2 and H-2^b haplotype (BALB/c, CC57W, C57B1/6, and B10CW). The

most resistant line was CBA and the most sensitive was C57Bl/6. The practical use of animals of these two lines, opposite as regards their sensitivity to the pathogenic action of living EV microbes, and differing from one another in their levels of corticosteroids, activity of nonspecific defense factors, and mobility of phagocytes [3, 4, 6], can be recommended for the study of the mechanisms of natural and acquired resistance to plague infection, and also for the study of residual virulence of strains of Y. pestis proposed for use as vaccine strains.

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ANALYSIS OF THE COMPOSITION OF SKELETAL MUSCLE FIBERS IN SKATERS' MUSCLES

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The present view is that the relative percentage of slow type I muscle fibers (MF) in human muscles is genetically determined and remains unchanged during training [1-4]. Hence it follows that the best opportunities for the achievement of good results during endurance training, other conditions being the same, will be a feature of persons whose skeletal muscles contain many MF of this type, and that successful performance in speed and strength training will correlate with a large number of intermediate type IIa MF and of fast type IIb MF. Racing skaters compete over distances ranging from 500 to 10,000 m. The skaters prefer to compete over distances at which they have exhibited their best results. That is why this type of sport is a good model with which to study the relationship between athletic results and the composition of MF in the skeletal muscles. No research devoted to the study of the composition of MF in skaters' muscles and its correlation with the chosen distance and athletic achievement could be found in the accessible literature.

EXPERIMENTAL METHODS

Fragments of the vastus lateralis muscle at the boundary between its middle and lower thirds were obtained by punch biopsy from 103 volunteer skaters aged 18-25 years, of whom 27 specialized in short and 76 in long distances. The muscle fragments were frozen in cold (−78°C) petroleum benzin and serial sections 12 μ thick were cut in a cryostat, and used to determine myosin ATPase activity after preincubation at pH 4.6 and 10.4 [5]. The relative percentages of MF of different types were determined by comparing the results of the reaction for ATPase after the two different preincubations. Sections containing fewer than a total of 100 muscle fibers were not investigated. The results were subjected to statistical analysis by the usual methods.

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