# STUDY OF A LYMPHOCYTOSIS-STIMULATING FACTOR OF Bordetella pertussis

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Whooping cough vaccine (6 billion bacterial cells), inactivated with merthiolate, was injected once or repeatedly intravenously into mice. This induced hyperleukocytosis affecting the lymphocytes and polymorphonuclear leukocytes. The number of monocytes, large lymphocytes, and juvenile forms of the myeloid series in the blood was increased mainly in the late stages and after repeated injections. Different changes were observed in the internal organs, reflecting a stress reaction, toxic effects, immunomorphological changes, and stimulation of hematopoiesis. Foci of myeloid hematopoiesis appeared in the liver.

Bordetella pertussis modifies the resistance of the organism to infectious, physical, and chemical agents, induces leukocytosis and lymphocytosis, and leads to changes in the internal organs in man and experimental animals [1, 4-13]. Despite much research, the genesis of the hyperleukocytosis remains largely unexplained, and even the dynamics of the changes in the blood picture during repeated successive intravenous injections of Bordetella pertussis has not yet been studied.

The object of the investigation described below was to make a cytological and histological investigation of the blood and internal organs of mice after administration of whooping cough vaccine (WCV).

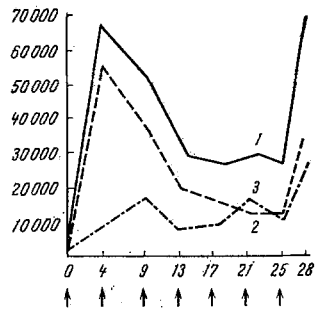


Fig. 1. Leukocytosis, lymphocytosis, and granulocytosis in BALB/c mice after repeated intravenous injections of WCV. Ordinate, number of cells in 1 $mm^3$ blood; abscissa, time of investigation in days. Arrows indicate injections of WCV: 1) leukocytosis; 2) lymphocytosis; 3) granulocytosis.

## EXPERIMENTAL METHOD

WCV, manufactured at the Moscow Mechnikov Institute of Vaccines and Sera, inactivated with 1:10 000 merthiolate and containing 90 billion bacterial cells per ml, was diluted 1:3 with physiological saline. Injections of 0.2 ml of this diluted vaccine, i.e., 6 billion bacterial cells, were given to mice.

Female and male BALB/c mice weighing 18-20 g and female $F_1$ (CBA × C57Bl/6J) mouse hybrids weighing 20-22 g were used in the experiments. The mice were divided into three groups: group 1 received a single injection of WCV; the mice of group 2 received repeated injections of WCV at intervals of 3-4 days; the mice of group 3 received repeated daily injections of WCV. The control for each group consisted of mice which were injected with 1:30 000 merthiolate in physiological saline, which corresponded to its concentration in the final dilution of vaccine, by a similar scheme.

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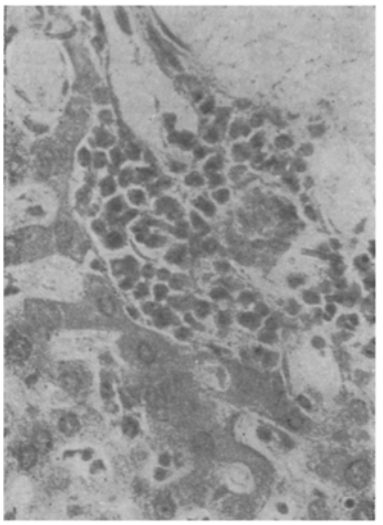

Fig. 2.

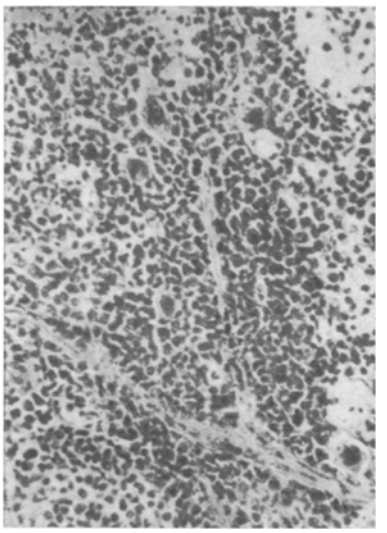

Fig. 3.

Fig. 2. Focus of myeloid hematopoiesis in the liver. Hematoxylin-eosin, 500 ×.

Fig. 3. Proliferation of reticulum and plasma cells along the course of the trabeculae and increase in number of hemocytoplasts and megakaryocytes in the red pulp of the spleen. Hematoxylin-eosin, 250 ×.

The formula of the circulating blood leukocytes was determined in the animals and the histological picture of their internal organs (thymus, spleen, lymph glands, brain, heart, liver, kidneys, adrenals, and bone marrow) was studied. Organs and blood from the caudal vein were taken from the animals of group 1 before injection of the preparation and on the 2nd, 3rd, 4th, 5th, 7th, 10th, 16th, 19th, and 23rd days thereafter, and in the animals of groups 2 and 3 after each successive injection. The mean results of blood tests on 5 mice were taken as the leukocyte count and formula. The organs were fixed in 80° ethanol or 10% formalin solution. Histological specimens were stained with hematoxylin-eosin, by Brachet's method, and with toluidine blue.

## EXPERIMENTAL RESULTS

Before the experiment the leukocyte count in the blood of the mice of group 1 and in the corresponding control animals varied from 7000 to 9000. On the 2nd day after injection of WCV the leukocyte count in the mice of the experimental group reached 28,000-39,000, and on the 4th-7th day it reached 50,000. After the 7th-10th day it began to fall, and by the 19th-23rd day the leukocyte count had returned to normal. In mice of the control group the leujocyte count fluctuated during the experiment between 5000 and 11,00. In the mice of the experimental group it was raised on account of both lymphocytes, which showed a sevenfold increase on the 4th day, and of polymorphonuclear leukocytes, with an elevenfold increase on the 5th-7th day.

The dynamics of the leukocyte count in the mice of group 2 was fluctuating in character: in mice of line BALB/c there were two maxima (Fig.1), and in the $F_1$ (CBAxC57B1/6J) hybrids there were three maxima. The highest leukocyte count ( up to 70,000) was found on the 4th, 9th,27th, and 37th days in the BALB/c mice and on the 4th, 13th, and 28th days in the $F_1$ hybrids (up to 86,000). In the former, the leukocyte count fell to 26,000-30,000 from the 13th to the 25th day, and in the latter it fell to 20,000-30,000 from the 17th to the 25th day. The leukocyte count in the experimental mice was at most 10-11 times higher than initially at its maximum, and 4-2.5 times higher at its minimum. In the control groups the leukocyte count fluctuated during the experiment from 6000 to 14,000. It rose as a result of an increase in the absolute number of both lymphocytes and polymorphonuclear leukocytes (Fig. 1). During repeated injection of WCV juvenile and primitive forms of white blood cells (myelocytes and promyelocytes, appeared in the blood stream).

In the mice of group 3 a leukocytosis (from 50,000 to 208,000) was observed from the 3rd until the 18th day, with a maximum (up to 208,000, or 60 times higher than originally) on the 11th day. In the control mice the leukocyte count fluctuated during the experiment between 9000 and 12,000. The leukocytosis in the experimental animals of this group was increased on account of both polymorphs and lymphocytes. However, the absolute number of polymorphs was 18 times higher than initially, whereas the number of lymphocytes was only 11 times higher.

It may be pointed out that the animal continued to respond to successive injection of vaccine ( in groups 2 and 3) by an increase in the leukocyte and lymphocyte counts. After repeated injections a marked absolute monocytosis and eosinophilia and also an increase in the number of large lymphocytes were observed in the mice, while juvenile and primitive forms of white blood cells appeared- in group 2 degenerative forms of lymphoctyes, with a very pale cytoplasm and "moth-eaten" nucleus also were present. In the animals of group 3 (on the 9th day and later) the bone marrow films contained mainly mature and immature neutrophils, while the erythroblastic series was severely inhibited and in some films it was completely suppressed.

Composite changes consisting evidently of stress reaction, toxic and immunomorphological changes, and stimulation of hematopoiesis were found in the internal organs. The stress reaction was manifested primarily as a decrease in size and total atrophy of the thymus gland, especially after repeated injections, and disturbance of the general structural architectonics of the lymph glands and marked hyperemia of the parenchymatous organs . The emptying of the lymph glands can be regarded both as a manifestation of stress and as the result of mobilization lymphocytes into the blood stream. Changes in the adrenals also were characteristic of the stress reaction.

Foci of necrosis, sometimes observed in the spleen, lymph glands, and liver after repeated injections, were possibly manifestations of the toxic action of changes produced in these experiments by WCV. The appearance of foci of myeloid hematopoiesis also was observed in the liver (Fig.2).

Morse [9,10] showed that the lymphocytosis observed in mice after a single injection of WCV occurs as the result of both discharge and proliferation.

The experiments with repeated injections of WCV showed that besides discharge of lymphocytes, proliferation also evidently occurred in the lymphoid tissue. Histological investigation of the organs of the experimental mice revealed hyperplasia and proliferation of the reticulum cells and also an increase in the number of polymorphs, plasma cells, hemocytoblasts, and megakaryocytes in the spleen in the late stages after a single injection of WCV (Fig.3). After repeated injections of WCV myeloid foci of hematopoiesis appeared in the liver. The increase in the number of large lymphocytes and intensified monocytic response which, according to several investigatior [2,3] are an indication of proliferation in the lymphoid tissue.

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