

EFFECT OF HYPOXIA ON THE DEVELOPMENT OF IMMUNOMORPHOLOGICAL REACTIONS IN LYMPHOID ORGANS

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A previous investigation [1] showed that if mice are kept for two weeks in a pressure chamber at an atmospheric pressure corresponding to an altitude of 2000 m, it has no effect on the state of cellular and humoral immunity, whereas repeated "ascents" of the mice to an "altitude" of 4000-6500 m leads to some depression of antibody formation in immunized animals.

This paper describes the results of a histochemical investigation of the lymphoid organs and subcutaneous cellular tissue at the site of injection of antigen into immunized mice, and also gives the histological picture of the lymphoid organs of unimmunized mice kept at a low atmospheric pressure.

EXPERIMENTAL METHOD

The investigation was conducted on 150 male mice of the Balb/c line, weighing about 20 g. Experimental group 1 included 40 animals kept for two weeks in a pressure chamber at an atmospheric pressure of 596 mm Hg (corresponding to an altitude of 2000 m). Experimental group 2 also consisted of 40 mice which were kept for 6 h daily for 10 days in a pressure chamber in which the pressure was lowered every second day by 25 mm Hg (a value corresponding to an ascent of 500 m), the initial pressure in the chamber being 462 mm Hg and the final pressure 330 mm Hg (corresponding to altitudes of 4000 and 6500 m). The mice of group 3 (control), also 40 in number, were kept throughout the experiment at a normal atmospheric pressure. Two hours after the end of the experiment all the mice received a subcutaneous injection into the right thigh of an alcohol-killed typhoid vaccine in a dose of 400 million cells. The mice were sacrificed 3 hours, and 3, 4, 5, 7, 10, and 14 days after immunization. The subcutaneous cellular tissue at the site of injection of the antigen, the regional (inguinal) lymph gland, and the spleen were fixed in cold acetone and embedded in paraffin wax. Serial sections were stained with haematoxylin-eosin, methyl green-pyronine (control with ribonuclease), and by the PAS method (control with amylase). Similar methods were used to study the lymph glands and spleen of 30 unimmunized mice kept together with the animals of groups 1 and 2.

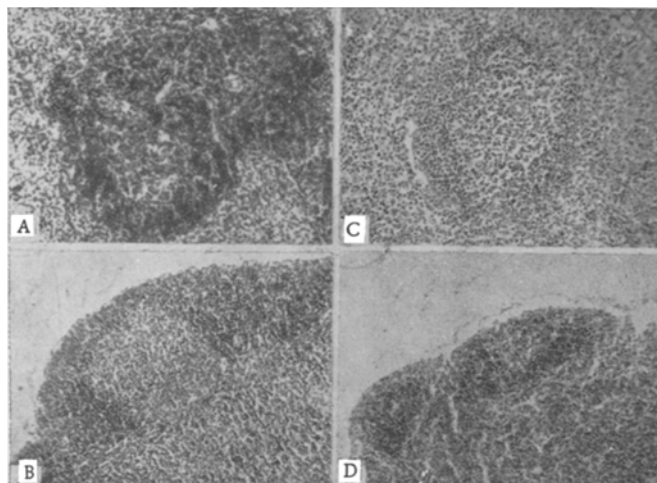
The intensity of the plasma-cell hyperplasia in the regional lymph gland of the immunized mice was determined by the method of V. P. Mikhailov and co-workers [2]. The numerical results were subjected to statistical analysis. The difference between the mean indices in the mice of different groups was regarded as significant if $P < 0.05$.

EXPERIMENTAL RESULTS

Lymphoid organs of unimmunized mice. Histological changes were found in the lymph glands and spleen only of the mice of experimental group 2: the lymphoid follicles and their pale centers were reduced in size (see the figure a and b), the number of cells in the red pulp of the spleen was reduced, mainly on account of lymphocytes; in the pale centers of the lymphoid follicles of the spleen and lymph glands, mitoses were more rarely seen.

Similar changes in the lymphoid organs of rats under the influence of hypoxic hypoxia have been observed by other authors [3, 4], who in addition described a marked decrease in the weight and size of the lymph glands in the experimental animals. Under the influence of hypoxia some inhibition of the process of lymphocyte reproduction evidently takes place and this accounts for the hypoplasia of the lymphoid tissue.

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Spleen and regional lymph gland of mice kept at a low atmospheric pressure. a) Lymphoid follicle of the spleen of a normal mouse; b) hypoplasia of a follicle with a sharp decrease in the number of lymphocytes in the spleen of a mouse repeatedly "raised" to an "altitude" of 4000-6500 m; c) lymphoid follicle with a well marked center of irritation in the regional lymph gland of a mouse immunized with typhoid vaccine; d) hypoplasia and absence of a center of irritation in a follicle of the regional lymph gland of an immunized mouse repeatedly "raised" to an "altitude" of 4000-6500 m. Hematoxylin-eosin, 60 \times .

Changes in the subcutaneous cellular tissue at the site of injection of antigen in immunized mice.

In the animals of the control group 3 h after injection of the antigen, marked exudative and infiltrative changes were observed in the subcutaneous cellular tissue. After 24 h, an extensive zone of infiltration was found at the site of injection of the antigen, consisting almost entirely of neutrophils, and the cytoplasm of most of these cells contained glycogen granules. On the 3rd day many of the neutrophils had disintegrated, and this process was preceded by disappearance of or a decrease in the content of the glycogen in the cytoplasm. Evidently in the process of phagocytosis the neutrophils utilized glycogen, and only after the energy resources of the cell were exhausted, did disintegration take place. Massive destruction of the neutrophils was accompanied by a sharp decrease in their number and by a simultaneous and progressive increase in the number of macrophages, which reached its maximum on the 5th-7th day after injection of the antigen and then gradually fell. The cytoplasm of the macrophages contained granules of RNA and substances stained by the PAS method evidently glycoproteins, because the staining was not abolished by preliminary treatment of the sections with amylase). On the 10th-14th day after injection of the antigen, newly formed connective tissue was observed in the subcutaneous cellular tissue.

Small collections, mainly of immature plasma cells, were observed on the 7th-10th day after injection of the antigen around the blood vessels situated a short distance from the main focus of inflammation.

The changes in the subcutaneous cellular tissue at the site of injection of the antigen in the mice of experimental groups 1 and 2 were identical with those in the control animals. The only difference observed in the mice of group 2 was the decrease in the glycogen content in the neutrophils in the early periods after immunization.

Changes in the regional (inguinal) lymph glands of the immunized mice. The first changes in the regional lymph gland of the control mice, in the form of a desquamative catarrh of the sinuses, were observed 3 days after injection of the antigen. Meanwhile, in the medullary cords of the lymph glands a marked proliferation of the reticular cells was observed, differentiating in the direction of the formation of elements of the plasma-cell series (plasmablasts, immature plasma cells). The cytoplasm of the plasma cells contained large amounts of RNA. Plasma-cell hyperplasia in the medullary cords of the lymph glands reached its maximum on the 4th-5th day after immunization, and then gradually diminished. Mature plasma

cells began to predominate in the medullary cords on the 5th day after injection of the antigen. The development of plasma-cell hyperplasia in the regional lymph glands took place slightly (approximately 24 h) before the appearance of antibodies in the blood.

Simultaneously with the development of plasma-cell hyperplasia in the medullary cords of the lymph gland, a marked increase in the size of the pale centers of the lymphoid follicles, which consisted of proliferating reticular cells and blasts, was observed. The RNA content in the cytoplasm of the proliferating cells of the pale centers of the follicles was high.

The results of this study of plasma-cell hyperplasia in the medullary cords of the lymph glands showed that its intensity was the same in the animals of all the groups (the difference between the mean values for the mice of the experimental and control groups was not statistically significant). So far as the pale centers of the lymphoid follicles are concerned, in the mice of experimental group 2 the reactive changes in these centers were less intensive and the number of follicles in the lymph gland was smaller than in the mice of the control group (see the figure c and d).

Since several authors [5-7] have shown that besides elements of the plasma-cell series, antibodies are also produced (although in smaller quantities, by the cells of the pale centers of the lymphoid follicles, it may be supposed that the slight decrease in the titers of antibodies in the mice of group 2 was due to the presence of fewer cells producing antibodies in the pale centers.

No changes associated with immunization could be discovered in the spleen of the experimental and control mice.

It may thus be concluded that keeping mice for 2 weeks at an atmospheric pressure of 596 mm Hg does not cause changes in their immunomorphological reaction developing in the subcutaneous cellular tissue at the site of injection of the antigen, and in the regional lymph glands of immunized mice. Repeated "ascents" of the mice to an "altitude" of 4000-6500 m lead to moderate hypoplasia of the lymphoid tissue, a decrease in the glycogen content in the neutrophils infiltrating the tissue at the site of injection of the antigen, and a decrease in the size of the reactive centers in the follicles of the regional lymph glands of the immunized mice. The results of immunomorphological investigation correlate closely with the results of serological investigations reported previously [1].

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