ROLE OF THE LUNGS IN CAPTURE OF A SUBCUTANEOUSLY INJECTED ANTIGEN

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Experiments by the fluorescent antibody method on guinea pigs showed that after 1 or 2 injections of complete typhoid antigen into the hind foot pad, the antigen can be found in the macrophages of the lungs within days of the injection (or within hours of reimmunization), and it persists there for up to 30 days. There is a tendency for antigen-containing macrophages to concentrate in the perivascular and peribronchial tissue. Meanwhile, the dynamics of spread of typhoid antigen among the lymphoid organs can be described.

KEY WORDS: lungs; subcutaneous immunization; typhoid antigen; spread of antigen.

The spread of antigens after their subcutaneous injection has frequently been studied, but in most cases attention has been concentrated on antigen capture by cells of the various lymphoid organs and on the mechanism of transmission of antigenic information to the immunocompetent cells. The question of concentration of antigens in the various nonlymphoid organs has received much less attention and most investigations devoted to this subject have been concerned with the liver and bone marrow. Nevertheless, there is evidence that the lungs actively capture an antigen even if administered by subcutaneous injection [2-4] rather than via the respiratory passages [7-9]. The active role of the lungs in immunization is shown by the development of an immunomorphological response in them to subcutaneous injection of various antigens [1, 6].

It was accordingly decided to attempt to discover subcutaneously injected antigen in lung tissue and, at the same time, to study its distribution among lymphoid organs.

EXPERIMENTAL METHOD

Experiments were carried out on 38 guinea pigs weighing 250-300 g receiving one or two injections of 0.1-0.15 mg complete typhoid antigen in 0.1 ml physiological saline into the hind foot pad. The popliteal and paratracheal lymph glands, spleen, and lungs, embedded in paraffin wax by Sainte-Marie's method [10], were investigated by the indirect Coons' method in Lebedev's modification [5] at various times ranging from 1 h to 30 days after a single immunization and up to 14 days after reimmunization. The following control tests were used: 1) treatment with exhausted specific serum, 2) the use of heterogeneic immune serum, 3) treatment with labeled antiserum against rabbit γ-globulin without application of the specific antiserum, 4) application of specific and antirabbit sera to sections of organs of intact animals. The specimens were examined in the ML-2B microscope and photographed on RF-3 film.

EXPERIMENTAL RESULTS

Bright fluorescence of antigen, located chiefly in macrophages present in the sinuses, and also concentrated around the postcapillary venules, was observed in the regional lymph

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Fig. 1 Fig. 2

Fig. 1. Group of macrophages with antigen in spleen 24 h after reimmunization (90×).

Fig. 2. Antigen-containing macrophages located around blood vessels and in the interalveolar septum 24 h after reimmunization (90×).

gland (RLL) 1 h after a single injection of the antigen. Fluorescence of the cytoplasm of the sinus endothelium also was observed.

The macrophagal response in RLL was intensified after 5 h, when cells exhibiting definite specific fluorescence and morphologically indistinguishable from macrophages were also found in the lumen of blood vessels.

Later, on the first to third days, the macrophagal reaction as before was very strong in RLL and the intensity of fluorescence of individual cells was considerable. This reaction continued until the ninth day, when the number of antigen-containing macrophages began to fall, so that by the 30th day only a few cells could be detected in RLL. The intensity of their fluorescence also fell gradually, although to different degrees.

In the spleen, specifically fluorescent macrophages began to appear about 24 h after immunization. Their number increased over 2-3 days. The same picture was observed in a distant lymph gland, but starting from the second day.

From the ninth and, in particular, from the 14th day, the number of fluorescent macrophages in the distant lymphoid structures fell progressively, although they were still fairly numerous in the spleen. It must be emphasized in general that the number of antigen-containing cells in the spleen at all times of the investigation was greater than the number found in the distant lymph gland. The intensity of fluorescence of cells in the spleen and in the paratracheal lymph gland varied from weak to considerable; in the spleen, however, it was higher than in the distant lymph gland.

Specifically fluorescent macrophages appeared in the lungs after the third day. They were mainly perivascular in distribution, but some were found also in the interalveolar septa, in the lumen of the alveoli, and in the submucosa of the bronchi. These cells usually were few in number and the intensity of their fluorescence varied from weak to moderate. Later, the number of antigen-containing cells remained at the same level, falling toward the 30th day, although there was a definite tendency for these cells to be concentrated in the perivascular and peribronchial connective tissue.

After reimmunization many antigen-containing macrophages were observed in RLL between 1 h and 9 days later, but by the 14th day their number had fallen appreciably. Most of them, just as after a single immunization, were situated in sinuses and the medullary cords, but others could be seen in the parafollicular zone also. The intensity of fluorescence of most macrophages was high, but towards the 14th day it diminished.

In the spleen, unlike after a single immunization, antigen-containing macrophages were found as early as 1 h after reimmunization. However, they were few in number and the intensity of their fluorescence was weak or moderate. After 5 h their number and the intensity of their fluorescence began to increase and continued to do so until the ninth day, when they started to fall again (Fig. 1). The dynamics of accumulation of antigen-containing cells in the distant lymph gland was similar in general except that fluorescent cells started to appear a little later and in smaller numbers. The intensity of their fluorescence also was lower.

Individual fluorescent cells appeared in the lungs 1 h after reimmunization, with the same distribution as after primary immunization. The number of antigen-containing cells was increased after 5 h and later, and a tendency was found for them to accumulate around the bronchi and vessels (Fig. 2). In addition, large cells with a fairly large nucleus, the cytoplasm of which gave a definite specific fluorescence, could be seen in the lumen of the septal capillaries and in some branches of the pulmonary artery. These cells could not be precisely identified.

After subcutaneous immunization of guinea pigs with typhoid antigen the latter thus spread rapidly throughout the body. Disseminated antigen-containing macrophages were held up in distant lymphoid organs, and more of them were found in the spleen than in the lymph glands. The explanation of this fact may be that the antigen reached the distant lymphoid organs not only when phagocytosed by macrophages, but also in the native state, and in the spleen there were many more macrophages than in the lymph glands.

In confirmation of immunological data in the literature, antigen-containing macrophages were found in the lungs. Their tendency to localize around the bronchi and also their discovery in the lumen of the alveoli suggest that some part of the antigen is eliminated from the body in this way. Antigen-containing macrophages reach the lungs evidently by the hematogenous route. This is confirmed indirectly by the discovery of cells with specific fluorescence in the lumen of the postcapillary venules and RLL, and also in the lumen of blood vessels and in the perivascular tissue of the lungs. However, the possibility of capture of native antigen, disseminated by the blood stream, directly by the alveolar macrophages cannot be ruled out.

As these results show, after reimmunization antigen-containing cells are found in the lungs and spleen as early as 1 h later, i.e., at the earliest time of investigation. The evident explanation of this fact is that a certain number of macrophages containing antigen injected at the first immunization still remained in these organs at the time of reimmunization. This is confirmed by the discovery of such cells 30 days after primary injection of the antigen.

LITERATURE CITED

1. M. M. Averbakh and V. I. Litvinov, The Immunobiological Basis of Vaccination against Tuberculosis [in Russian], Moscow (1970).
2. S. I. Ginzburg-Kalinina, Transactions of the Moscow Research Institute of Vaccines and Sera [in Russian], Vol. 8, Moscow (1956), pp. 160-170.
3. S. I. Ginzburg-Kalinina, Zh. Mikrobiol., No. 9, 52 (1971).
4. S. I. Ginzburg-Kalinina and E. F. Vakarina, in: Problems in Bacteriology, Virology, and Immunology [in Russian], Moscow (1959), pp. 85-93.
5. K. A. Lebedev, in: Principles and Methods of Histochemical Analysis in Pathology [in Russian], Leningrad (1971), p. 254.
6. B. B. Shul'man-Satin, Zh. Mikrobiol., No. 11, 87 (1971).
7. G. Blandford and R. B. Heath, Immunology, 22, 637 (1972).
8. L. J. Goldberg and W. R. Lief, Science, 112, 299 (1950).
9. J. E. Logan, Canad. J. Microbiol., 2, 565 (1956).
10. G. Sainte-Marie, J. Histochem. Cytochem., 10, 250 (1962).