THE LUNG IN TUBERCULOSIS AS AN IMMUNOCOMPETENT ORGAN

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Experiments on guinea pigs infected with Mycobacterium tuberculosis or vaccinated with BCG were carried out to study the dynamics of immunomorphological changes in the lung, the character of dissemination of mycobacterial antigens, and synthesis of enzymes, proteins, and nucleic acids. It is concluded from the results that the lungs participate in immunogenesis in tuberculosis.

During immunization and various pathological processes in the lungs, proliferation and immunological activity of the cells of the lymphoid and histiocytemacrophagal systems take place in the lungs [1-8].

In the investigation described below the role of the lungs in immunological reactions was studied during tuberculous infection and after BCG vaccination.

EXPERIMENTAL METHOD

Dissemination and distribution of mycobacterial antigens in the lung were studied in 180 guinea pigs infected with 0.0001 mg of an 18-day culture of $H_{37}RV$ or vaccinated with 0.1 mg of a 2-week culture of BCG (Coons' method in tissue sections and the complement fixation test in lung homogenates), proliferative synthetic and metabolic processes in the lung were investigated [staining with hematoxylin-eosin, by Van Gieson's method and for elastic tissue, for DNA by Feulgen's method and RNA by Brachet's method, for thiol groups and aromatic amino acids (tyrosine, tryptophan, histidine), and for acid and alkaline phosphatase, succinate dehydrogenase, cytochrome oxidase, NAD- and NADP-diaphorase, and lactate dehydrogenase]. Synthesis of nucleic acids also was studied by means of radioactive precursors of DNA (thymidine-H^3) and RNA (uridine-H^3), and synthesis of antibodies in the lungs was studied by the direct Coons' method.

EXPERIMENTAL RESULTS

During the first few hours after BCG vaccination and infection with $H_{37}RV$, specific antigens were found in lung homogenates (Fig. 1), and subsequently the titers of antigens increased, more especially following infection with the virulent strain. With the development of tuberculous inflammation, progressive dissemination of the antigens was observed (until 1 month). During the next 4 weeks (until 2 months), the titers of specific antigens in the lung homogenates decreased considerably, and starting from the 8th week, they again increased and continued to rise until death of the animals. The decrease in titers of antigens (4th-8th week) in the lung homogenates was probably attributed to their binding with antibodies. Territories occupied by antigen-containing cells (detected by Coons' method) progressively increased in size, and their number showed no tendency to diminish during the first 1-2 months. After vaccination, the increase in dissemination of antigens (maximal titers 2-3 weeks after vaccination and maximal territories occupied by

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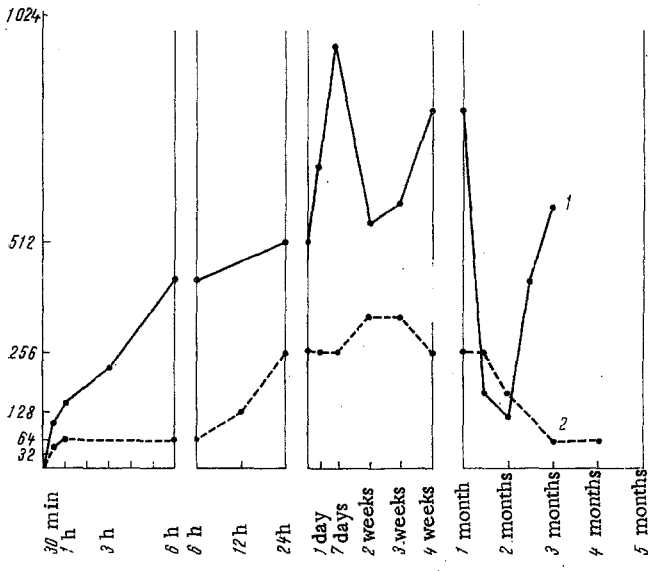

Fig. 1. Titers of antigens in lung homogenates in experimental tuberculosis and vaccination. Abscissa, time after infection or vaccination; ordinate, titer of antigens. 1) Infection with strain $H_{37}RV$; 2) BCG vaccination.

antigen-containing cells observed after 3 weeks to 3 months) was replaced by gradual "sterilization" of the body (antigens were not found in the homogenates after 4 months, or antigen-containing cells after 10-12 months).

In the lungs, just as in the body as a whole, definite patterns were observed in the succession of cells absorbing antibodies (Fig. 2a), with polymorphonuclear leukocytes playing an unimportant role, and most absorption of antigen taking place through macrophages. Antigens were also found in the small lymphocytes, especially in the late periods after vaccination and at the time of death of the animals after infection. The prolonged retention of antigen by the lymphocytes in these cases must apparently have a completely different significance.

During the first day after introduction of mycobacteria of both strains, intensification of migratory, proliferative, and synthetic reactions of various cells was observed in the lungs, as has also been described by Puzik and other workers [2, 7-11]. Mobilization of the blood cells occurred, with their departure from the capillaries and lymphatics, and these cells proliferated after leaving the vessels. Intensive proliferation also developed among active connective-tissue cells. Macrophages of both hematogenous and local origin infiltrated into the alveolar septa and escaped into the lumen of the alveoli. During hyperplasia and transformation of the cellular structures, reactions for ribonucleoproteins and protein components of the cells (aromatic amino acids and thiol groups) became stronger. The intensity of the early proliferative reactions was greater in the case of infection with the virulent strain.

Vaccination with BCG in its purest form reproduced immunomorphological changes in the organs in which both specific and nonspecific components could be distinguished. Both specific and nonspecific reactions developed in a particularly clear form in the lungs, lymph glands, and spleen. Intensive infiltration of the alveolar septa with macrophages and lymphocytes developed in the lungs, and the number of lymphoid nodules increased considerably. Proliferative and infiltrative reactions sometimes spread over wide areas, especially in the subpleural regions ("white pneumonia"), while at the same time specific vaccinal structures developed, consisting of epithelioid and lymphoid cells and also giant cells of Langhans. The proliferative reactions reached their maximum on or after the 3rd-4th week, and regression began to take place 4 months after vaccination. At these same times (1-4 months), metabolic and synthetic processes reached their level of intensity.

The study of the synthesis and content of nucleic acids in the lungs showed that both DNA and RNA were synthesized in this organ much more intensively after immunization than in control animals (the H^3 index for DNA synthesis between 1 week and 6 months after infection was between 2.16 and 2.66, compared

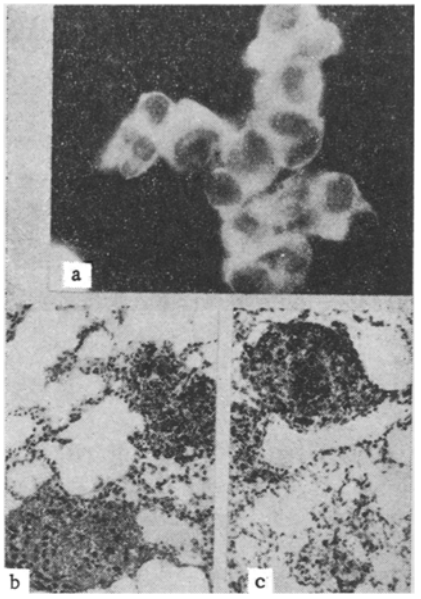

Fig. 2. Lung tissue of guinea pigs after infection with tuberculosis or BCG vaccination: a) antigen-containing cells in lung tissue 2 months after infection with $H_{37}RV$; indirect Coons' method (350 ×); b) DNA synthesis in lung tissue 1.5 months after vaccination (200 ×); c) RNA synthesis in lung tissue 3 months after vaccination (200 ×).

with 0.98 in the control; for RNA synthesis from 1 week to 8 months after infection the H^3 index was 1.68-2.2, compared with 0.54 in the control). Between 1 week and 6-8 months after infection a much more intensive incorporation of precursors of nucleic acids into the cells of the interstitial tissues of the lungs and of the specific vaccinal structures (activated reticular cells, blast cells, epithelioid cells, immature plasma cells) was observed (Fig. 2b,c).

The course of experimental tuberculosis, like that of vaccination, was characterized by a regular succession of different cells performing various immunological functions. Until 1.5 months, active proliferation of lymphoid and plasma cells took place in the lungs, and synthesis of nucleic acids and proteins was intensified; corresponding changes also took place in metabolism. However, in the early stages, the virulent properties of the agent apparently left an impression on the development of the immune reaction, for they were apparently in competition with nonspecific changes and, later, specific tuberculous changes. Nevertheless, in the lungs of the infected animals proliferative and synthetic reactions continued to occur until the last days of life among cells of the histiocyte, macrophagal, and lymphoid systems (gradually weakening after 1.5 months).

Of the specific defensive reactions in the lungs, antibody formation was studied, using the direct Coons' method (with antigen from BCG, PPD, and antiserum against guinea pig globulins, labeled with fluorescein isocyanate). After BCG vaccination the dynamics of antibody formation in the lungs, as in the other immunocompetent organs, was found to coincide in time with the dynamics of development of immunity against tuberculosis: the number of cells synthesizing antibodies increased to a maximum and decreased parallel with changes in the intensity of immunity. In experimental tuberculosis the number of antibody-synthesizing cells in the lungs increased until the development of "frank tuberculosis" (1 month), remained at a high level in the period of maximum development of the specific disease (to 1.5 months), and then decreased considerably as the tuberculosis became generalized and hyperergic in character (2-3 months). In the lungs, just as in the other organs, gradual maturation of antibody-synthesizing cells was observed. Whereas during the first 2-3 weeks after infection with mycobacteria, antibodies were synthesized principally by blast cells and immature plasma cells, later, both in experimental tuberculosis and after vaccination, most antibody-synthesizing cells were mature plasma cells.

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