IMMUNOFLUORESCENCE AND SEROLOGIC INVESTIGATION OF EARLY REACTION TO INJECTION OF BCG AND $H_{37}Rv$ MYCOBACTERIA

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Destruction of BCG and $H_{37}Rv$ mycobacteria begins within a few minutes after their injection into guinea pigs. Virulent mycobacteria ($H_{37}Rv$) are destroyed more intensively than avirulent (BCG). Greatest accumulation of bacterial antigens is found in the regional lymph gland and also in the spleen, peripheral lymph glands, lungs, and bone marrow. Erythrocytes and lymphocytes participate in transportation of antigens in the body.

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Reactions of the body to injection of mycobacteria in the period preceding the immune response have received little study. The only reports so far published indicate that cells of Mycobacterium tuberculosis can be detected radiometrically and seeded from organs within a few hours after infection [1-3, 5] and that hyperplasia of lymphoid tissue develops soon after injection of mycobacteria [4].

In the present investigation the pathways of spread of breakdown products of BCG and $H_{37}Rv$ mycobacteria in organs, tissues, and cells were studied at times soon after infection.

EXPERIMENTAL METHOD

Experiments were carried out on 56 guinea pigs, 27 of which were injected subcutaneously with an 18-day culture of $H_{37}Rv$ (0.0001 mg per animal) and 24 with a 2-week culture of BCG (0.1 mg each); the animals were sacrificed 5 and 30 min, 1, 3, 6, and 12 h, and 1, 3, 5, 6, and 7 days after infection. In addition, 5 control animals were sacrificed. Sections were cut to a thickness of 5 μ from pieces of the internal organs embedded in paraffin wax by Sainte-Marie's method and stained by the indirect Coons' method to reveal specific tuberculosis antigens. Serum of rabbits hyperimmunized with antigen from BCG was applied to deparaffinized sections. The sections were then washed with buffered physiological saline and stained with ass serum against rabbit globulins, labeled with fluorescein isothiocyanate. After repeated washing the sections were examined under the ML-2 luminescence microscope. To detect antigens in blood cells, blood films from the infected animals were fixed in methanol and stained by the indirect Coons' method. To confirm the specificity of fluorescence in the cells and tissues, the usual controls were carried out and sections from organs and blood films from uninfected animals were stained by the indirect Coons' method. Specific fluorescence was absent from all control preparations. Antigens in organ homogenates and blood hemolysates were determined by the complement fixation reaction.

EXPERIMENTAL RESULTS

By 1 h after vaccination, immunofluorescence examination of the tissue sections revealed specific tuberculosis antigens liberated from BCG in cells of the lymphoid follicles of the regional lymph gland, by 12 h they were found in the alveolar macrophages of the lungs and in the macrophages and reticulum cells of the white pulp of the spleen and follicles of the lymph gland. The number of cells containing antigen gradually increased: after 3 days the lungs, spleen, and regional and distant lymph glands were "inundated" by them. Antigen-containing cells began to desquamate into the lumen of the alveoli and sinuses of the lymph

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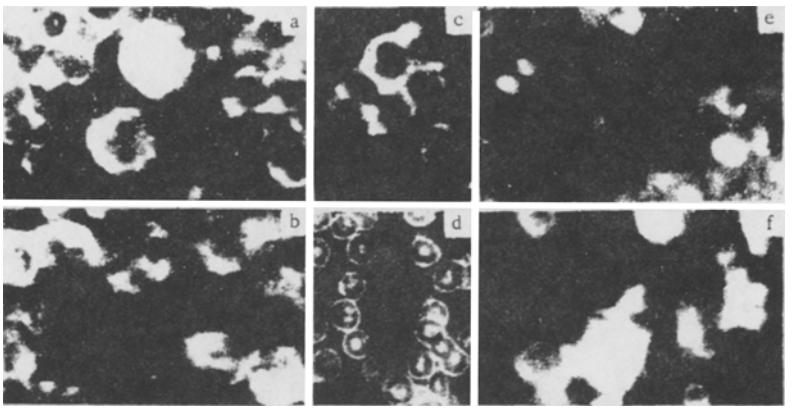

Fig. 1. Antigen-containing cells in organs and tissues of guinea pigs infected with BCG and $H_{37}Rv$. a) Antigen-containing cells in sinus of regional lymph gland 7 days after BCG vaccination; b) antigen-containing cells in red pulp of spleen (7 days after BCG vaccination); c) group of lymphocytes containing antigen in film made from suspension of lymph gland (7 days after BCG vaccination); d) antigen on erythrocytes (7 days after BCG vaccination). Objective 70, ocular 5; e) antigen-containing cells in follicle of regional lymph gland (30 days after infection with $H_{37}Rv$). Objective 40, ocular 5; f) antigen-containing cells in follicle of regional lymph gland (6 h after infection with $H_{37}Rv$). Objective 70, ocular 5.

glands; they were found in the central part of the follicles of the lymph glands and malpighian corpuscles of the spleen, and also in the medullary cords of the lymph glands and red pulp of the spleen, in the interalveolar septa and alveolar walls, and in macrophages and cells of the reticular syncytium of the bone marrow. A similar picture was observed 5-7 days after vaccination (Fig. 1, a, b). At the same time (3-7 days after vaccination) antigen began to appear in the lymphocytes of immunologically competent organs: smears prepared in Ringer–Locke solution from a suspension of cells from the regional lymph gland of a vaccinated animal, fixed in methanol and stained by the indirect Coons' method, showed intensive fluorescence of antigen in lymphocytes and other cells (Fig. 1, c). Immunofluorescence investigation of many blood films revealed fluorescence of antigen in the form of a halo on the erythrocytes in some of them (Fig. 1, g). No antigen-containing cells could be found in the liver and kidneys.

Similar results were obtained in the immunofluorescent investigation of sections of organs from animals infected with $H_{37}Rv$, but fluorescence of antigen was observed sooner in the tissues of this group of animals: by 30 min after infection in individual cells and follicles of the regional lymph gland (Fig. 1, e), and 6 h after infection (staining by Coons' method) in macrophages and reticulum cells of the regional (Fig. 1, f) and peripheral lymph glands, in cells of the malpighian corpuscles of the spleen, bone marrow cells, and cells of the interalveolar septa of the lungs. From 1 to 7 days after infection the composition of antigen-containing cells and the areas occupied by them were similar to those observed after BCG vaccination.

A parallel serologic investigation (using the complement fixation reaction; CFR) showed that after BCG vaccination specific tuberculosis antigens were present in lung homogenates 30 min after vaccination (in a titer of 1:48), in the spleen and kidneys after 1 h (1:64), and in the liver after 1 day (1:64). The mean titers of antigen in homogenates of liver and kidneys remained at almost the same level for 7 days (1:107 in the kidneys, 1:64 in the liver), but in the lungs and spleen they increased considerably: 1:171 in the spleen and 1:343 in the lungs after 7 days (Fig. 2). Antigens detected by the CFR were present in tissue cells and in leukocytes and erythrocytes of blood remaining in the capillaries of the organs. Antigens were also detected serologically by the CFR in blood hemolysates but were not found in the serum, indicating that most antigens are carried by the blood cells. Most of the antigens discovered in liver and kidney homogenates were probably contained in the blood cells in the capillaries, as indicated by absence of fluorescence of antigen in the cells of these organs when stained by Coons' method.

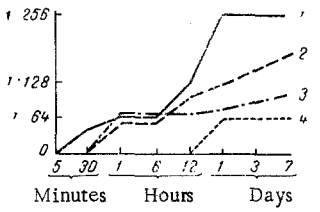

Fig. 2

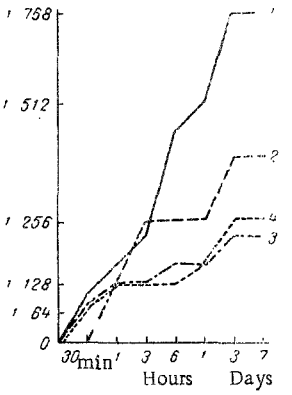

Fig. 3

Fig. 2. Titers of specific antigens in organ homogenates from guinea pigs vaccinated with BCG. 1) Lung; 2) spleen; 3) kidney; 4) liver.

Fig. 3. Curves showing titers of specific antigens in organ homogenates of guinea pigs infected with $H_{37}Rv$. Legend as in Fig. 2.

The CFR with organ homogenates of animals infected with $H_{37}Rv$ revealed antigens after 30 min in fairly high titers: 1:91 in the liver, 1:85 in the spleen, 1:107 in the lung, and 1:128 (after 1 h) in the kidney. The increase in titers was more marked in the lungs (1:766 after 7 days) and spleen (1:341) and less marked in the liver and kidneys: 1:256 and 1:213 respectively (Fig. 3). The earlier appearance and the more rapid increase in titers of the antigen after $H_{37}Rv$ infection suggest the more intensive destruction of mycobacteria of this strain in the organs.

It can be concluded from these results that within a few minutes after infection of experimental animals with BCG and $H_{37}Rv$ mycobacteria, interaction between the bacteria and cells and tissues of the body begins. The results of this interaction are, first, changes in the properties and destruction of the mycobacteria, and second, changes in the tissue and cells of the organs under the influence of liberated antigens, subsequently leading to the development of the immune response. One week after infection (the period of this investigation) $H_{37}Rv$ mycobacteria no longer exhibit their virulent properties, although interaction between the host and virulent and avirulent bacteria shows certain differences. Destruction of the virulent bacteria begins sooner than that of the avirulent, and proceeds more rapidly and intensively. The greatest accumulation of antigen is observed in the lymph glands (primarily the regional glands), spleen, lungs, and bone marrow, i.e., in those organs which subsequently play an active role in the immune response. Antigen liberated from the mycobacteria are ingested by phagocytes and distributed over the body. Erythrocytes on which the antigens are fixed play an important role in their distribution. An active role in antigen transportation is also played by lymphocytes, and this role becomes increasingly active as changes take place in the immunologically competent organs during preparation for the immune response. The lymphocytes as it were distribute antigenic information, preparing the immunologically competent tissues for antibody synthesis. All the immunologic and immunomorphological changes described above are almost equally characteristic in the early period of infection by both BCG and $H_{37}Rv$.

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