SUPPRESSION OF THE IMMUNE RESPONSE BY LUNG CELLS
IN EXPERIMENTAL TUBERCULOSIS

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Infection of mice by virulent Mycobacterium tuberculosis cells is accompanied by generalized infection with bacteriemia, lymphadenopathy, and splenomegaly. The most marked pathological process develops in the lungs, which in tuberculosis are the site of direct interaction between pathogen and effector mechanisms of specific and nonspecific resistance [1].

Data which have now accumulated are evidence that lung tissue contains all the principal types of immunocompetent cells, including T and B lymphocytes, macrophages, dendritic cells, and natural killer cells [3, 5, 6], but their particular features in tuberculosis are unknown. This paper gives the results of a study of the immunologic properties of the interstitial cells of the lung in mice with experimental tuberculosis, evidence of activation, as a result of infection, both of T lymphocytes specific for mycobacterial antigens and of suppressor cells, adherent to plastic, which suppress proliferation of immune T cells.

EXPERIMENTAL METHOD

Mice of the inbred CBA/Sto line aged 3-4 months were obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. The mice were infected intravenously with a virulent strain of M. tuberculosis H37Rv in a dose of 25 μg/mouse. The mice were used in the experiments 3-7 weeks after infection. Sterile suspensions of the interstitial cells of the lung were obtained by enzymic dissociation. For this purpose the vascular bed was perfused with an intravenous infusion of 7 ml of Hanks' solution with antibiotics, containing 10 U/ml of heparin, after which bronchoalveolar lavage was carried out with warm physiological saline containing antibiotics, 1-1.5 ml of solution being injected through a cannula inserted into the trachea, followed by aspiration of the contents, the procedure being repeated eight times. The lungs were removed from the chest, perfused another twice in medium 199 containing antibiotics, cut into small pieces measuring about 1-2 mm³, and added to a solution containing 2 mg/ml of type I collagenase (260 IU/mg, from "Boehringer," West Germany) in medium L-15 containing 20 mM HEPES, 1% embryonic calf serum (ECS), 50 U/ml of kanamycin (all components from "Flow Laboratories," England), and 50 μg/ml of DNase (USSR). The sample was incubated at 37°C for 90 min on a planchet shaker (ABP-1).

A unicellular suspension was obtained by repeated pipetting of the suspension and passing it through a metal sieve. The cells were then washed 3 times by centrifugation at 150 g for 10 min and filtered through a cotton wool filter. The suspension thus obtained consisted of single cells with a viability of 80-90%. To remove cells adherent to plastic the suspension was incubated in complete nutrient medium (RPMI-1640, containing 10% ECS, 10 mM HEPES, 4 mM glutamine, 50 U/ml kanamycin, 1% of nonessential amino acids, 2.2 mM pyruvate, and 5·10⁻⁵ 2-mercaptoethanol; all components from "Flow Laboratories," England), on plastic Petri dishes 90 mm in diameter for 2 h at 37°C in a CO₂-incubator in a dose of (25-30)·10⁶ cells per dish. Adherent cells were removed mechanically after incubation of the monolayer with a cold solution containing 0.02% EDTA for 30 min at room temperature.
Fig. 1. Proliferative response of cells isolated from lungs (in CPM $\cdot 10^{-3}$) of mice infected with tuberculosis (I), intact mice (II), or mice vaccinated with BCG (III). a) Unfractionated lung cells, b) lung cells without those adherent to plastic, c) fraction enriched with T lymphocytes; 1) in presence of PPD (10 $\mu$g/ml), 2) in presence of con A (2.5 $\mu$g/ml), 3) without stimulation.

To obtain a cell suspension enriched with T lymphocytes nonadherent to plastic, the lung cells were applied in a concentration of 50 $\cdot$ 10^6 cells/ml to a column containing 0.7 g nylon wadding ("Femoall," USA), incubated for 60 min at 37°C, after which the nonadherent cells were eluted with warm solid medium at the rate of 1 ml/min.

Cells adherent to the nylon wadding were obtained by rinsing the column with 30 ml of medium, after which the column was filled with cold phosphate-buffered saline without Ca^{2+} and Mg^{2+}, and the wadding was pressed 3 times with the piston from the syringe used with the column. To obtain immune lymphocytes, mice were immunized in the footpads with Freund's complete adjuvant ("Sigma," USA), containing M. tuberculosis H37Rv cells or ovalbumin (100 $\mu$g/ml) or corpuscular antigen of Staphylococcus aureus (5 $\cdot$ 10^6 microbial particles in 1 ml) in Freund's incomplete adjuvant. After 9-14 days a unicellular suspension was obtained from the popliteal lymph nodes, and washed twice with medium 199 containing 2% ECS, 10 mM HEPES, and antibiotics. The proliferative response of lung and lymph node cells was assessed on the basis of uptake of $^3$H-thymidine. For this purpose 4 $\cdot$ 10^5 cells (three repetitions for each version of the test) were introduced into wells of a flat-bottomed 96-well planchet ("Nunc," Denmark) in 0.2 ml of complete nutrient medium. Proliferation was stimulated (experiment) by adding purified tuberculin (PPD, Statens Serum Institute, Denmark), concanavalin A (con A, "Pharmacia," Sweden), ovalbumin ("Sigma"), and the cytoplasmic fraction of antigens of Staphylococcus aureus, generously provided by M. M. Averbakh. Wells without addition of the antigen served as the control. After 66 h of culture, 1 $\mu$Ci of $^3$H-thymidine was added to each well, and after a further 6 h the contents of the wells were transferred to a glass fiber filter with the aid of a cell harvester, dried, and their radioactivity counted on a "Beta-2" liquid scintillation counter.

The suppressor action of the lung cells was tested by adding 1, 0.5, 0.25, or 0.125 $\cdot$ 10^5 unfractionated lung cells or of the different fractions of these cells to 4 $\cdot$ 10^5 immune lymph node cells.

**EXPERIMENTAL RESULTS**

It was possible to obtain 8-10 million viable cells by the method described above from the lungs of one intact mouse. After infection of the mice with tuberculosis the number of cells began to rise with the 10th-14th day (30-40 million cells/mouse), to reach a peak (60-70 million/mouse) in the terminal stage of the disease. The development of infection was accompanied by the appearance of giant cells, accounting for 5-10% of the total.