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Cell-Mediated Immunity Shown by Lymphocytes from the Respiratory Tract

Abstract. *Cell-mediated immunity, as evidenced by inhibition of macrophage migration in the presence of antigen, was associated with lymphocytes obtained from the respiratory tract of guinea pigs immunized by dinitrophenylated human immunoglobulin G in nose drops, but not from those immunized parenterally. However, splenic lymphocytes from parenterally immunized animals inhibited macrophage migration while those from locally immunized animals did not.*

Lymphoid cells of the respiratory tract can produce antibody after local antigenic stimulation (*1*). This humoral response, thought to be independent of systemic antibody production, is characterized by production of antibodies belonging to the immunoglobulin A (IgA) class. However, there has been no report of a cell-mediated immune response in external secretions. The increasing awareness that cell-mediated immunity functions in a variety of defense mechanisms and disease processes suggested to us that, in addition to the induction of antibody synthesis, there might be lymphoid cells present which could specifically react with antigen and effect those reactions associated with cell-mediated immunity.

Experiments were devised to study the development of sensitized lymphocyte (*2*) production in the respiratory tract and to compare these lymphoid cells with those present in the spleens of animals after administration of antigen, either locally (nose drops), or by injection of antigen in complete Freund's adjuvant into the footpad. The results suggest the existence of cell-mediated immunity in cells obtained from the respiratory tract after local antigenic stimulation. After systemic immunization, sensitized lymphocytes could be demonstrated in the spleen but not in cells from the respiratory tract.

Twelve adult Hartley guinea pigs (approximately 400 g) were immunized with 50 μg of dinitrophenylated human immunoglobulin G (DNP-HGG) in complete Freund's adjuvant injected into the rear footpads. Another group of 12 animals was lightly anesthetized (with sodium pentobarbitalum injected intraperitoneally) and given approximately 200 μg of the same antigen in 0.15 ml of phosphate-buffered saline nose drops. Three animals from each group were exsanguinated 11, 15, 19, and 26 days after administration of antigen, and the spleens, tracheae, bronchi, and lungs were removed. The lower respiratory tracts were externally washed, and 5 ml of sterile Eagle's medium was instilled into the main-stem bronchi in 1-ml portions. The cell suspension was aspirated and then centrifuged at 1500 rev/min for 5 minutes; the resulting pellet was resuspended and washed twice with Eagle's medium containing 10 percent inactivated calf serum. The final suspension contained between 3.1×10^6 and 5.0×10^6 lymphocytes and 4.6×10^6 to 7.5×10^6 macrophages per animal.

Table 1. The effect of local and systemic immunization on the development of sensitized lymphocytes. Lymphocytes were obtained after immunization with nose drops (200 μg DNP-HGG) or with 50 μg of DNP-HGG in Freund's adjuvant injected into the footpads.

Time after stimulation (days)	Mean macrophage migration inhibition by lymphocytes after immunization by:	
	Nose drops	Footpads
	Lymphocytes from spleen	
11	5.1 ± 4.5*	57 ± 6.9
15	5.0 ± 4.2	58 ± 3.8
19	10.0 ± 4.7	62 ± 9.6
26	5.0 ± 3.2	11.5 ± 4.0
	Lymphocytes from bronchial washing	
11	60 ± 7.7	0
15	59 ± 9.0	31 ± 3.5
19	26 ± 6.5	0
26	8.2 ± 5.5	0.5 ± 0.2

* Mean of six determinations and standard error of the mean.

The spleens were homogenized by hand in a glass grinder containing modified Eagle's medium containing fourfold amounts of amino acids and vitamins. The cell suspension was centrifuged at 1500 rev/min for 5 minutes, and the sediment was resuspended and allowed to stand for 1 hour at 4°C. The cells remaining in the supernatant were sedimented by centrifugation at 1500 rev/min for 5 minutes and resuspended in 0.83 percent sterile NH_4Cl to induce erythrocyte lysis. Thirty minutes later the remaining lymphoid cells were again sedimented and washed twice in Eagle's medium containing 10 percent inactivated calf serum. The number of viable lymphocytes distinguished by the Trypan blue exclusion test were determined with a hemocytometer.

Lymphocyte preparations from the spleens and bronchial washings of the same animals were assessed for the presence of specifically sensitized lymphocytes by estimation of their capacity to inhibit macrophage migration in the presence of DNP-HGG (*3*). Cells from normal guinea pig peritoneal exudate were harvested aseptically 48 hours after the administration of 20 ml of paraffin oil intraperitoneally (*4*). The average number of macrophages thus obtained was 1.3×10^8 per animal. Splenic lymphocytes were mixed with peritoneal macrophages in Eagle's medium containing 10 percent inactivated calf serum or in the same medium containing 300 μg of DNP-HGG per milliliter, so that the final suspension contained 5×10^6 lymphocytes and 25×10^6 macrophages per milliliter. Capillary tubes were filled from each cell suspension and sealed with paraffin wax. The filled tubes were centrifuged, cut cleanly at the cell interface, and mounted into wax-paraffin chambers filled with Eagle's medium containing 10 percent inactivated calf serum, with or without the addition of 300 μg of DNP-HGG per milliliter. The chambers were then stored in a CO_2 incubator at 37°C for 48 hours. The extent of macrophage migration was assessed at 24 and 48 hours by projecting the migration areas onto a glass screen and then tracing and weighing them. The effect of antigen on migration was assessed by calculating the migration in the presence of antigen as a percentage of that obtained in its absence.

The migration of peritoneal macrophages was specifically and markedly inhibited in the presence of antigen and splenic lymphocytes from animals immunized with DNP-HGG injected into the footpads (Table 1). This inhibition was similar (57 to 62 percent) between days 11 and 19, but by day 26 showed a marked decline (11 percent inhibition). On the other hand, lymphocytes from the spleens of animals immunized with DNP-HGG in nose drops failed to give significant inhibition of macrophage migration in the presence of antigen. Splenic lymphocytes from unimmunized animals were likewise not inhibitory.

Similar studies were made with lymphocytes obtained from the bronchial washings of animals of the two groups. Individual washings were adjusted to contain 5×10^6 lymphocytes and 25×10^6 macrophages per milliliter by the addition of cells from normal guinea pig peritoneal exudate. The effect of DNP-HGG on the migration of these suspensions of macrophages and lymphocytes was assessed in a manner identical to that used for the spleens. Lymphocytes from the respiratory tracts of animals immunized with DNP-HGG injected into the rear footpads, unlike their splenic lymphocytes, which were markedly inhibitory throughout the first 19 days, were only weakly and transiently inhibitory in the presence of antigen (Table 1). Respiratory tract lymphocytes from animals given DNP-HGG in nose drops, however, were strongly inhibitory in the presence of antigen. This inhibitory effect was most noticeable in preparations of bronchial lymphocytes harvested 11 and 15 days after administration of the antigen and by day 26 had virtually disappeared. Populations of lymphocytes from the bronchial washings of unimmunized animals were never inhibitory.

It thus appears that sensitized lymphocytes may be caused to appear either in the spleen or in the bronchial tract according to the mode of antigen presentation.

The initial studies with lymphocytes from bronchial washings were performed on the whole cell populations containing about 60 percent macrophages. It was considered necessary, to ensure that the phenomenon observed was not related to the presence of macrophages from the immunized animal, to show that purified preparations of lymphocytes also gave the same effect. Consequently, the bronchial washings of six animals, collected 14 days after the administration of 200 μg of DNP-HGG in nose drops, were pooled, and the cells present were collected as previously. The initial preparation showed 60 percent of the cells phagocytosed colloidal carbon; these were assumed to be macrophages (*5*). Lymphocytes were isolated from this mixture by the method of Mosier (*6*). After three sequential transfers (at hourly intervals) of this cell preparation from sterile glass Petri dishes, the resulting nonadherent cell population contained less than 1 percent of cells which phagocytosed carbon. The capacity of this cell population to inhibit the migration of peritoneal macrophages in the presence of antigen was assessed as described above. They were extremely inhibitory, the macrophages migrating 27 percent of the distance that they did in the absence of antigen. A similar lymphocyte population from the bronchial washings of unimmunized animals did not inhibit the migration of the same macrophage population in the presence of antigen.

Table 2. Production of migration inhibitory factor by respiratory tract cells incubated with antigen. The percentage of migration is the mean migration compared to the migration of normal cells in media without addition of supernatant fluids (± standard error of the mean). Cells were obtained from (A) eight guinea pigs immunized with nose drops and from (B) three guinea pigs immunized in the footpads.

Supernatant of incubation mixture of:		Migration (%)
Bronchial washing cells	Antigen	
A	DNP-HGG	58 (±10)
A	0	96 (±6.2)
B	DNP-HGG	106 (±18)
B	0	98 (±14)

To investigate whether antibody or some other noncellular substance present in the respiratory tract functioned in the observed inhibition of macrophage migration, we obtained pooled bronchial washings from locally immunized animals. Cells and supernatant fluid were separated by centrifugation, and the cells shown to be active in inhibition. The supernatant fluid was added to the cells of bronchial washings from normal unimmunized animals. No inhibition of migration was observed, an indication that the inhibition observed with cells from the respiratory tract of immunized animals was associated with the cellular, and not the humoral, component of the washings.

The specific inhibition of macrophage migration has been associated with a nondialyzable, heat-stable substance called migration inhibitory factor (MIF) (*7*). To investigate whether a similar substance was produced by respiratory tract lymphocytes, we incubated cells from bronchial washing of animals immunized either subcutaneously or with nose drops for 24 hours in medium with or without DNP-HGG. After centrifugation and millipore filtration, the cell-free supernatants were added to cells of normal peritoneal exudate and the extent of macrophage migration was observed. The results indicate that in the presence of antigen a substance similar to MIF was elaborated by cells from the respiratory tract of animals immunized locally, but not from animals immunized parenterally (Table 2).

Lymphoid cells obtained from the bronchial washings of animals immunized with antigen in nose drops thus exhibit a characteristic of cell-mediated immunity in that they specifically prevent macrophage migration in the presence of antigen. Splenic lymphocytes from these same animals do not cause such inhibition. The converse is true of animals immunized parenterally.

These findings suggest the local induction of cell-mediated immunity in the respiratory tract. Thus, this immunity might be considered a part of the secretory immunologic system, previously described only in terms of humoral immunity with secretory IgA antibody as its predominant component.

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References and Notes

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