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Immunoglobulin-Bearing Cells in Lungs of Mice Infected with Influenza Virus

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In sequential studies of cellular infiltrates from influenza-infected mouse lungs, increased populations of lymphocytes with surface immunoglobulins were observed, and immunoglobulin A-bearing cells exhibited the greatest relative increase.

Numerous investigations have demonstrated the importance of a secretory immunological system in defense against diseases of the respiratory tract (6, 15, 17). Most reports agree that resistance to respiratory infection correlates better with antibody in respiratory secretions than with humoral antibody. Artenstein et al. (2) found both immunoglobulin A (IgA) and IgG antibodies for several types of viruses in nasal secretions. According to Rossen et al. (11-13), most of the IgA in nasal secretions is the 11S secretory type with lesser amounts in the 7S category. Immunofluorescent studies by Tomasi and Bienenstock (16), Brandtzaeg et al. (4), and R. P. Rossen, K. C. Hsu, and H. M. Rose (Clin. Res. 15:298, 1967) provided indirect evidence that secretory Ig's are synthesized locally in submucosal plasma-like cells in glandular areas and secretory ducts. They found good correlation between the predominance of a particular Ig class in a secretion and the distribution of plasma cells stained with specific antisera in the secretory tissue.

In the present study, quantitative changes in the distribution of Ig-bearing lymphoid cells in the lungs of mice were investigated during response to a sublethal infection with influenza virus. Groups of mice (7 weeks old, male and female, Crl:COBS^{R}CD^{R}-1 [ICR]BR) were infected by exposure to 30 median egg infectious doses of mouse-adapted influenza virus, A/Aichi/2/68 (H3N2), in small aerosol particles (mass median diameter, 2 μm). Before infection and at weekly intervals for 4 weeks postinfection, lungs from 14 mice were pooled for examination. Lungs were removed by resecting the main bronchi at the hilus; an attempt was made to strip all mediastinal nodes from the lungs. The lungs were minced, teased, and washed through a sterile, 60-mesh, stainless-steel screen with approximately 3 ml of RPMI 1640 medium (GIBCO, Grand Island, N.Y.) per lung. The cell suspension was layered onto a Ficoll-Hypaque solution (LSM solution, Litton Bionetics, Inc., Kensington, Md.); mononuclear cells were recovered after centrifugation at 400 $\times$ g for 30 min at 20 C as described by Böyum (3). The cells were washed and dispersed in RPMI 1640. Total cell counts were performed in a hemocytometer. Trypan blue dye exclusion was used to determine cell viability. Differential counts for morphological classification were made as described by Shortman et al. (14).

Lymphoid cell-enriched suspensions were held for 3 h at 25 C to permit regeneration of cell surface Ig's removed during processing. The proportion of nucleated cells that could be stained with fluorescein isothiocyanate-labeled polyvalent antibodies for mouse IgA, IgG, and IgM or with labeled monospecific antibodies for IgA or IgG were determined as described by Rabellino et al. (10). Two hundred cells in each preparation were examined; each microscope field was examined first with ultraviolet and then with tungsten light to count both the number of stained cells and the total number of lymphocytes.

Cell counts recorded in Table 1 represent arithmetic means for two or three replicate experiments. Ten to 15% of the cells recovered from uninfected lungs were lymphocytes; the remainder were primarily of erythrocytes, monocytes, foamy cells, and epithelial cells. More than 90% of the lymphoid cells were viable, and 40% stained with labeled polyvalent antiserum. In noninfected lung suspensions, the ratio of lymphocytes with surface IgG to those with surface IgA was approximately 4:1. This abundance of IgG cells in suspensions prepared from whole mouse lungs contrasts with the reported preponderance of IgA cells observed in sections of normal human nasal and bronchial tissues (16); however, it is consistent with observed concentrations of the respective Ig classes in bronchoalveolar lavage fluids from volunteers (17) and from mice (9). Typical infec-

TABLE 1. *Distribution of lymphoid cells in mouse lungs during infection with influenza virus*

Cell population	Mean cell count[a] × 10^4 by days postinfection			
	0	8	14	28
Total recovered/lung	81 ± 31	285 ± 47	457 ± 30	200 ± 40
Lymphoid/lung	11 ± 4.0	211 ± 51	223 ± 32	32 ± 8.0
Stained with:				
Polyvalent anti-IgA, IgM, and IgG	4.5 ± 0.5	46 ± 15	114 ± 18	6.0 ± 2.0
Monospecific anti-IgA	1.2 ± 0.7	17 ± 13	45 ± 18	4.0 ± 2.0
Monospecific anti-IgG	4.0 ± 0.0	26 ± 8.0	78 ± 24	2.0 ± 1.0

[a] Mean of two or three replicate experiments ± standard error.

tion-induced lymphoid cell infiltration was observed in infected lungs (7). The number of lymphoid cells increased 19-fold within 8 days and remained elevated through 14 days but returned to near base-line values within 28 days. These infiltrates contained increased numbers of lymphoid cells with Ig on their surface. Maximum recovery of Ig-bearing cells was observed 14 days postinfection, and 51% of the lymphoid population reacted with anti-Ig serum. Cells bearing IgG predominated during the early and acute phases of disease, but IgA-bearing cells exhibited a greater relative increase, and by 28 days postinfection their absolute number exceeded that of cells with IgG. Cells bearing IgM were not counted. However, within each sample the sum for cells identified with anti-IgA and with anti-IgG closely approximated the number of cells stainable with polyvalent antiserum, indicating that IgM-bearing cells were minimal during all stages of infection. The increase in IgA- and IgG-bearing cells in the lungs is consistent with observed increases of IgA and IgG antibodies in bronchoalveolar fluids of mice during and after sublethal influenza infection (G. H. Scott and R. Sydiskis, submitted for publication). Other investigators (T. B. Tomasi, Ph.D. thesis, Rockefeller Univ., New York, N.Y., 1965) have found that the Ig class in a secretion is correlated with the plasma cells which stain for that specific Ig in sections of secretory tissue. Our findings were similar in that the Ig classes in bronchoalveolar secretions have a distribution similar to the number of lymphoid cells bearing similar surface Ig's isolated from homogenates of lung tissue.

It has been suggested that the essential event for initiation of an immune response is the direct interaction of antigen with specific, antibody-like receptors on the surface of lymphocytes (1, 5, 8). Thus, in infected lungs the increased number of Ig-bearing cells that are in close proximity to the replicating antigen may facilitate this first essential event. Subsequently, differentiation of these "primed" cells and development of antibody-producing plasma cells in lung tissue could account for the IgA and IgG antibodies that are found in respiratory secretions of infected mice.

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