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Different Effects of Influenza Virus, Respiratory Syncytial Virus, and Sendai Virus on Human Lymphocytes and Macrophages

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Influenza virus, respiratory syncytial virus, and Sendai virus depress human cell-mediated immune responses, such as mitogen-induced lymphocyte transformation, but differ in their ability to induce other immune defense mechanisms, such as interferon production. Exposure to the different viruses resulted in depressed transformation responses to the mitogen phytohemagglutinin by affecting the function of lymphocytes, or macrophages, or both cell types.

Influenza virus and respiratory syncytial virus (RSV) can cause clinically similar disease in adults (6, 15). Both influenza virus and RSV depress phytohemagglutinin (PHA)-induced transformation responses (14); however, influenza virus is a good inducer, and RSV is a poor inducer of interferon production by human mononuclear leukocytes (4). Reinfection of adults with RSV is common (15) despite genetic stability relative to influenza viruses (3, 6).

The current studies were undertaken to determine whether varied effects of influenza virus and RSV on functions of human mononuclear leukocytes result from differences in leukocyte populations affected by the viruses. Effects of Sendai virus were also examined. Sendai virus can cause disease in mice that is clinically and pathologically similar to influenza in humans (10, 11) and is commonly used to induce human leukocyte interferon production for clinical use (7). Lymphocyte transformation responses to PHA and interferon production were assayed with separated and recombined macrophages and lymphocytes, exposed concurrently to the different viruses.

Peripheral venous blood was obtained by venipuncture from eight healthy young adult donors (five male, three female) who were taking no medication. Mononuclear leukocytes were obtained from heparinized whole blood by Ficoll-Hypaque sedimentation (1). Separation of mononuclear leukocytes into adherent and nonadherent cell populations was accomplished by previously described methods (22). Briefly, mononuclear leukocytes were incubated in plastic petri dishes at 37°C, using medium 199 (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 20% autologous serum or plasma. Cells adherent to the plastic dishes (macrophages, Mϕ) were vigorously washed to remove nonadherent cells, and the nonadherent cells (lymphocytes, Ly) were further depleted of adherent cells by passage over nylon wool (Fenwal Laboratories, Inc., Morton Grove, Ill.) columns. For all experiments, viability was assayed, and cells were added to culture and tested for transformation response and interferon production according to the number of viable cells. Viability (always >80%) was similar after exposure to the different viruses.

The influenza viruses and Sendai virus were grown in allantoic cavities of 10-day-old embryonated hens' eggs, and RSV (Long strain) was grown in HEp-2 cells, as previously described (4). Virus was added to cells at a multiplicity of infection of 10. Samples of purified Ly and purified Mϕ were exposed concomitantly for 1 h at 37°C to serum-free medium alone or the medium containing one of the following viruses: influenza A/Scotland/840/74 H3N2; influenza A/AA/Marton/43 HON1; parainfluenza-1(Sendai) virus; or RSV (Long strain). After 1 h of exposure the Ly and Mϕ were washed and reincubated for an additional 24 h in medium supplemented with 20% autologous plasma. Supernatant culture fluids were then removed, and the Ly and Mϕ were collected as previously described (22). The supernatant fluids were tested for interferon activity, and the Ly and Mϕ were assayed for transformation responses to PHA alone and in combination. Previous studies showed that neither allantoic fluid nor HEp-2 culture fluids affect lymphocyte transformation responses or interferon production in the absence of virus (4, 22).

The interferon activity of supernatant fluids was assayed by the inhibition of plaque formation by vesicular stomatitis virus in human fore-

skin fibroblast (HFF) cultures, as previously described (20, 28). Minimum interferon titers of 5 U/ml were detectable in assays used in these studies. Results are presented in $\log_2$ IU/ml.

Quadruplicate cultures of purified Ly and purified Mϕ preparations alone, as well as recombinations of the two cell types (5×10^5 Ly/ml; 5×10^4 Mϕ/ml), were assayed for response to PHA in sterile microtiter plates (Microtest II; Falcon Plastics, Oxnard, Calif.), with medium supplemented with 10% autologous serum by previously described methods (22). The cells were cultured for 3 days with medium alone or medium containing PHA-M (Difco Laboratories, Detroit, Mich.) at a concentration of 160 μg/ml, shown to yield maximum lymphocyte transformation response. Results of studies using suboptimal doses of PHA did not differ from those reported and are not included in the results. Data are presented both in terms of absolute counts per minute of tritiated thymidine incorporated by the cells (in text) and in terms of percent response (in figures). The response of recombined control Ly plus control Mϕ was defined as 100% for each experiment, and responses of other single and recombined cell types were compared to that value. In all experiments, Mϕ incorporated negligible amounts of tritiated thymidine when cultured alone, and results using Mϕ alone are not presented in the figures. Comparisons of the function of individual cell types or cell mixtures were evaluated by paired *t* test.

Ly, Mϕ, and recombinations of the two cell types were tested for PHA responses. There was considerable variation in the absolute counts per minute of tritiated thymidine incorporated by the PHA-stimulated cells of different individuals, as previously established (18). However, within each individual experiment, the relative responses of cell types and recombinations of cells were consistent. For example, in every experiment, control Ly plus Mϕ exposed to influenza virus showed markedly less thymidine incorporation than did control Ly plus control Mϕ.

There were no significant differences between responses of control Ly and Ly exposed to influenza virus, whether tested alone (7,370 cpm ± 2,563 and 10,919 cpm ± 2,605, respectively), in combination with control Mϕ (18,624 cpm ± 2,605 and 19,273 cpm ± 3,641, respectively), or in combination with Mϕ exposed to influenza virus (9,378 cpm ± 2,562 and 11,627 cpm ± 2,478, respectively). These results are presented in terms of percent response (relative to the response of control Ly plus control Mϕ, for each individual) in Fig. 1 and 2. In contrast, Mϕ exposed to influenza virus were less effective than control Mϕ ($P < 0.001$) in enhancing the responses of either control Ly (9,378 cpm ± 2,562 and 18,624 cpm ± 3,606, respectively) or Ly exposed to influenza virus (11,627 cpm ± 2,478 and 19,273 cpm ± 3,641, respectively). These data confirmed earlier observations (22), but were determined concurrently in these studies for comparison with the effects of exposure to RSV (Fig. 1) or Sendai virus (Fig. 2).

RSV, unlike influenza virus, did not adversely affect the ability of Mϕ to enhance Ly transformation response to PHA (Fig. 1). There were no significant differences between the responses of control Ly combined with control Mϕ and the responses of control Ly combined with Mϕ exposed to RSV (19,056 cpm ± 5,100 and 20,361 ± 5,528, respectively). However, Ly exposed to RSV showed markedly depressed responses to PHA relative to control Ly ($P < 0.001$), whether assayed alone (1,242 cpm ± 428 and 7,140 cpm ± 4,417, respectively), in combination with control Mϕ (1,995 cpm ± 514 and 19,056 cpm ± 5,100, respectively), or in combination with Mϕ exposed to RSV (2,222 cpm ± 738 and 20,361 cpm ± 5,528, respectively).

Sendai virus adversely affected the responses of both Ly and Mϕ (Fig. 2). Ly exposed to Sendai virus showed depressed PHA responses relative to control Ly, whether tested alone (3,228 cpm ± 1,376 and 7,599 cpm ± 3,333, respectively) or in combination with control Mϕ (7,954 cpm ± 4,333 and 18,193 cpm ± 5,875, respectively) ($P < 0.005$). In addition, Mϕ exposed to Sendai virus were less effective than control Mϕ ($P < 0.005$) in enhancing the responses of control Ly (9,561 cpm ± 4,585 and 18,193 cpm ± 5,875, respectively). Ly exposed to Sendai virus showed equivalent responses, whether combined with control Mϕ or Mϕ exposed to Sendai virus (7,954 cpm ± 4,333 and 7,589 cpm ± 4,306, respectively).

In no experiment was interferon produced by control Ly, control Mϕ, or Ly exposed to any of the viruses. Mϕ exposed to RSV produced low titers of interferon compared with Mϕ exposed concurrently to influenza virus (3.32 $\log_2$ IU/ml ± 0.41 and 10.96 $\log_2$ IU/ml ± 1.38, respectively; $P < 0.02$). In contrast, Mϕ exposed concurrently to influenza viruses or to Sendai virus produced equivalent titers of interferon (6.15 $\log_2$ IU/ml ± 0.49 and 5.90 $\log_2$ IU/ml ± 0.67, respectively).

Viral infections have long been associated with depressed cell-mediated immune responses (12, 17, 30). Direct addition to human mononuclear leukocyte cultures of measles, rubella, polio, mumps, influenza A, RSV, Sendai, and other viruses has been shown to depress lymphocyte transformation responses to mitogens (14, 17). Both monocyte-macrophages and lymphocytes are necessary to develop such respons-

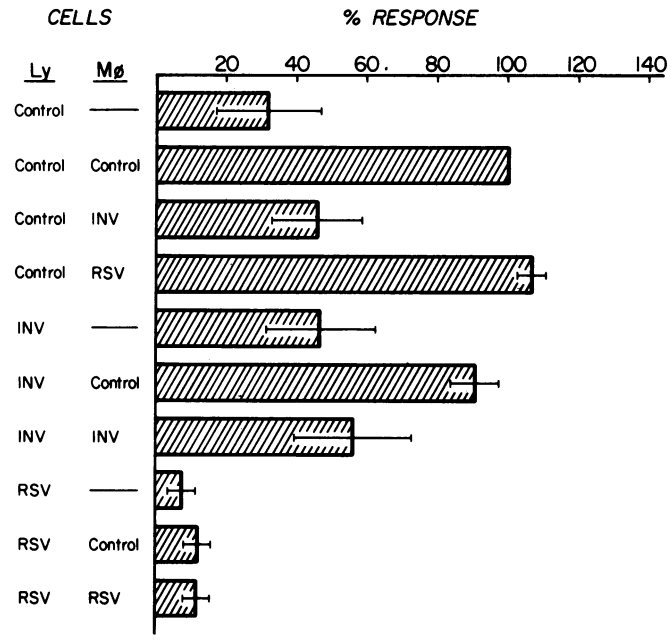

FIG. 1. PHA responses of Ly and Mϕ exposed to influenza A/AA/Marton/43 HON1 (INV) or RSV. The data represent mean percent responses ± standard error from four experiments with concurrent determinations. The response of recombined control Ly plus control Mϕ was defined as 100% for each experiment.

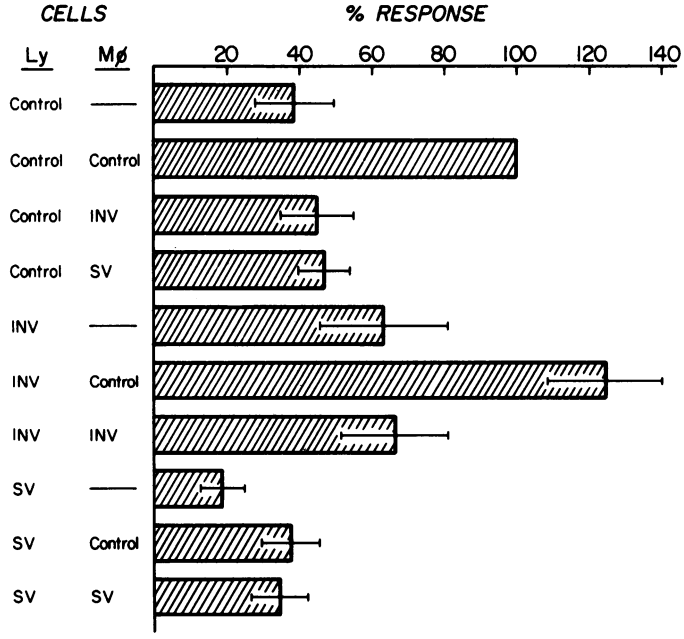

FIG. 2. PHA responses of Ly and Mϕ exposed to influenza virus (INV) or Sendai virus (SV). The data represent mean percent responses ± standard error from four experiments, using concurrent determinations. In three experiments INV A/AA/Marton/43 HON1 was used, and in one experiment INV A/Scotland/840/74 H3N2 was used. There were no differences between results, using the different influenza virus strains. The response of recombined control Ly plus control Mϕ was defined as 100% for each experiment.

es (13, 22, 24, 25). The current studies showed that three viruses, associated with similar respiratory disease (6, 11, 15), affected different populations of human mononuclear leukocytes, resulting in depressed lymphocyte transformation responses to mitogen stimulation. Exposure to influenza A virus resulted in normal lymphocyte function and depressed macrophage function, whereas exposure to RSV resulted in depressed lymphocyte function and normal macrophage function. Exposure to Sendai virus resulted in depression of both lymphocyte and macrophage function. The purified macrophages produced large amounts of interferon after exposure to either the influenza viruses or Sendai virus, but produced minimal amounts of interferon after exposure to RSV.

It is unclear why the viruses differ substantially in their effects on human mononuclear leukocyte subpopulations. The differences cannot be explained by available data regarding infectivity of the different viruses for the cells. The ability of RSV to replicate in human leukocytes has not been reported. Both influenza and Sendai viruses are rapidly inactivated by mononuclear leukocytes, and the infection of both lymphocytes and macrophages has been shown to be abortive (2, 5, 8, 9, 16, 23, 26, 27, 29, 31; N. J. Roberts, Jr., R. G. Douglas, Jr., unpublished data). Our earlier studies showed that inactivated influenza virus does not cause a depression of macrophage accessory cell function for lymphocyte transformation responses (19), but does induce interferon production in high titers by the macrophages (19, 21). Thus, differences in interferon production (between RSV and the other viruses) may not be due to differences in replication of the viruses in the cells.

The differences in effects due to the viruses cannot be explained by available data regarding the impact of prior exposure and immunity to the virus strains. All of our leukocyte donors are seropositive for RSV. Seropositivity to the influenza virus subtype or specific strain does not appear to modify the infectious virus-induced depression of human macrophage accessory cell function for lymphocyte transformation responses (19), or the titers or type of interferon produced by human mononuclear leukocytes (4) or purified macrophages (19).

The current studies suggest that effects on human immune function determined for one of these respiratory viruses cannot be attributed automatically to the others.

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