INTERLEUKIN 1 AND INTERLEUKIN 1 INHIBITOR PRODUCTION BY HUMAN MACROPHAGES EXPOSED TO INFLUENZA VIRUS OR RESPIRATORY SYNCYTIAL VIRUS.

Respiratory Syncytial Virus is a Potent Inducer of Inhibitor Activity

By NORBERT J. ROBERTS, JR., ALEJANDRA H. PRILL, AND THOMAS N. MANN

From Infectious Diseases Unit, Department of Medicine, University of Rochester School of Medicine, Rochester, New York 14642

Influenza virus infection results in altered responses of human mononuclear leukocytes to mitogen and antigen stimulation. We have previously shown (1, 2) that depression of such proliferative responses to alternate (nonviral) stimuli was due to decreased monocyte-macrophage accessory cell function, with lymphocyte responsiveness preserved. It has been unclear whether altered influenza virus–infected macrophage accessory cell function was due to (*a*) inadequate presentation of mitogens, for example, together with class II HLA determinants; (*b*) inadequate production of essential cofactors, notably IL-1; (*c*) production of inhibitory factors, such as IL-1 inhibitors; (*d*) direct cell-cell inhibition; or (*e*) a combination of such processes. The studies reported herein were undertaken to determine whether the macrophages produce IL-1 or IL-1 inhibitors after exposure to the virus.

In addition, production of IL-1 and IL-1 inhibitors by respiratory syncytial virus (RSV)[1]-exposed human macrophages was examined. In contrast to influenza virus, infections with RSV commonly recur, despite serological evidence of immunity of the individual and the lack of clear evidence of antigenic shift or drift of the virus (2–4). RSV infection of human mononuclear leukocytes also results in depressed proliferative responses (2), but RSV differs significantly in ability to induce production of macrophage-derived immunoregulatory factors, such as IFN (2, 5).

Materials and Methods

Cell Collection and Separation. Mononuclear leukocytes were obtained from the peripheral blood of healthy adult donors by Ficoll-Hypaque sedimentation, and purified monocyte-derived macrophages were obtained by 24-h adherence separation of the cells in plastic culture dishes, as described previously (1, 2). Such macrophage preparations consist of 93–97% monocytes-macrophages by strict criteria for differentiation (1, 6). Unless noted otherwise, macrophages were cultured at 37°C in Eagle's MEM supplemented with 4% FCS, Hepes buffer, and penicillin (100 U/ml).

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[1] *Abbreviations used in this paper:* RSV, respiratory syncytial virus.

Exposure of Cells to Virus, and Collection of Culture Fluids. Macrophages were exposed or sham-exposed for 1 h at 37°C in serum-free medium to influenza A/AA/Marton/43 H1N1, or influenza A/Scotland/840/74 H3N2, or RSV (Long strain), which had been grown in allantoic cavities of embryonated hens' eggs or HEp-2 cell cultures, respectively (virus/macrophage ratio, 1:1) (2, 5). There were no differences in macrophage interactions with the different strains of influenza virus. Macrophages were then cultured for an additional 24 h in medium with 4% FCS, after which supernatant fluids were collected, depleted of cells by centrifugation, and stored at −70°C until assayed (7).

In a subset of experiments, macrophages from which supernatants had been collected at 24 h were fed with fresh medium, and supernatants were again collected at 72 h (representing 24–72-h mediator production). Also, in another subset of experiments, concomitant aliquots of the macrophages were exposed to allantoic fluid (without virus), LPS (20 ng/ml to 20 μg/ml), from *E. coli* (Difco Laboratories, Detroit, MI), polymyxin B (125–5,000 U/ml), or a combination of polymyxin B with virus or LPS. Polymyxin B was added to cultures to diminish any potential contribution of contaminating LPS to results observed upon exposure to the virus (8), although several series of data suggested that LPS was highly unlikely to contribute to the results.

Gel Filtration. In addition to measurements of total (or net) activity of the virus-induced crude macrophage culture supernatant fluids, assays were performed using components fractionated by Sephadex G-100 column chromatography (9) to characterize IL-1-like activity, and to determine whether multiple factors stimulating and inhibiting thymocyte proliferation were produced after exposure to virus. The void volume was determined using blue dextran, and the column was calibrated using several proteins, including BSA (66 kD) carbonic anhydrase (29 kD) and cytochrome C (12.4 kD).

Assays for IL-1 and IL-1 Inhibitors. IL-1 activity was measured using the standard mouse thymocyte assay (10). Briefly, 6–8-wk-old C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, ME) were killed by cervical dislocation. Using aseptic techniques, the thymus glands were removed, teased into single-cell suspensions, and passed through a wire gauge to remove particulates, washed twice in PBS, and resuspended in Eagle's MEM supplemented with 4% FCS, Hepes buffer, penicillin, and 2-ME (2.5×10^{-5} M). Thymocytes were cultured for 72 h at 10^6 cells/well in 96-well flat-bottomed plates (Costar 3596) in the presence of 5 μg/ml PHA and serial dilutions of the test samples. Cultures were terminally pulsed with tritiated thymidine, the cells were collected with a semiautomatic cell harvester (Brandell Inc., Gaithersburg, MD), and cpm incorporated were determined using a liquid scintillation counter.

The assays for IL-1 incorporated several relevant controls, such as virus-containing culture medium (without cell-derived factors). The viruses, when carried over into mouse thymocyte cultures, did not themselves induce proliferation. All assays incorporated (as a standard) a purified IL-1 preparation (Ultrapure Interleukin-1; Genzyme, Boston, MA). The purified IL-1 was also used, in combination with test samples in culture wells, in assays for IL-1 inhibitors.

Assays for IL-2 and IL-2 Inhibitors. Supernatants were tested for IL-2 activity by their ability to stimulate the incorporation of tritiated thymidine by IL-2-dependent murine T lymphocytes (HT-2), using standard methods (10,11). The incorporated cpm induced by dilutions of the various supernatants were compared to cpm induced by standard IL-2 preparations, including recombinant DNA–produced IL-2 (Genzyme). The standard IL-2 preparations were also used in combination with test supernatants to detect inhibitors of IL-2 activity.

Results

Supernatant fluids derived from human macrophages that had been exposed to influenza virus 24 h previously contained substantial amounts of IL-1 activity, as measured by the standard mouse thymocyte proliferation assay (Fig. 1). The amount of IL-1 produced in response to influenza virus was in excess of that induced by as much as 20 μg/ml of LPS, and was produced despite concomitant

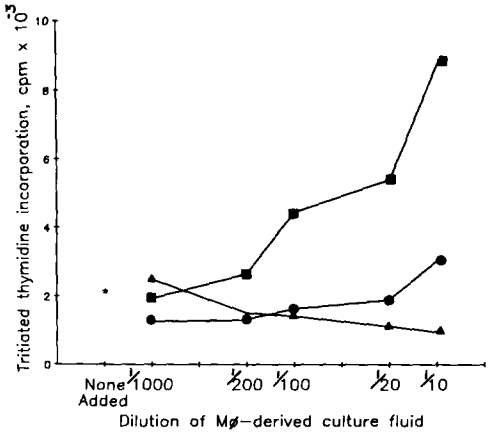

FIGURE 1. IL-1 activity produced in 24 h by control macrophages (Mϕ) (●) and macrophages exposed to influenza virus (■) or RSV (▲). ★, thymocyte proliferation in the absence of macrophage fluids.

addition of polymyxin B (up to 5,000 U/ml) to the cultures (data not shown). Macrophages exposed to virus-free allantoic fluid did not produce IL-1. Uninfected (control) macrophages also did not produce IL-1, and macrophages exposed to RSV appeared not to produce IL-1 in the thymocyte comitogen assay using crude supernatant fluids (Fig. 1).

Since exposure to either of the viruses can result in depressed lymphocyte proliferative responses (2), we next examined whether IL-1 inhibitors might be produced concomitantly (Fig. 2). Control macrophages did not produce IL-1 inhibitor activity. Overall, there was no net inhibition of IL-1 activity by supernatants derived from influenza virus–infected macrophages at the dilutions tested. In individual experiments, however, there was often, at higher concentrations of fluid, inhibition which could be removed by serial dilution, leaving substantial residual IL-1 activity. In contrast, there was marked production of IL-1 inhibitor activity by macrophages exposed to RSV (Fig. 2). Thus, experiments measuring activity of the crude supernatant fluids did not determine whether RSV also induced production of IL-1.

The described effects on thymocyte proliferation did not appear to be due to IL-2 or IL-2 inhibitor activities. Neither the crude supernatants from control macrophages nor those from macrophages exposed to influenza virus or RSV contained IL-2 activity, nor did the fluids inhibit IL-2 activity in the standard murine IL-2-dependent lymphocyte proliferation assay (data not shown).

Supernatant fluids with macrophage-derived factors produced 24–72 h after exposure to influenza virus or RSV showed net IL-1 and IL-1 inhibitor activities similar to those present in the 24-h fluids (Fig. 3), although greater net IL-1 activity appeared to be present in the influenza virus–induced fluids.

The crude virus-induced macrophage-produced fluids were examined further to determine the component activities contributing to the net effects on thymocyte proliferation described above. Fractions of influenza virus–induced macrophage fluids, separated by Sephadex gel filtration, were tested for IL-1 activity (Fig. 4). The major peak of IL-1 activity eluted at ~14.5 kD, as expected. Such

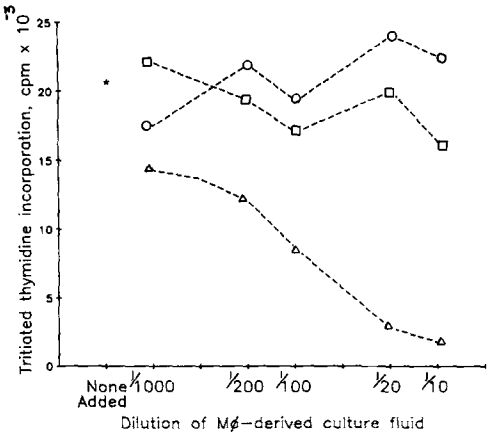

FIGURE 2. IL-1 inhibitor activity produced in 24 h by control macrophages (○) and macrophages exposed to influenza virus (□) or RSV (△). ★, thymocyte proliferation in the presence of purified IL-1 in the absence of macrophage fluids.

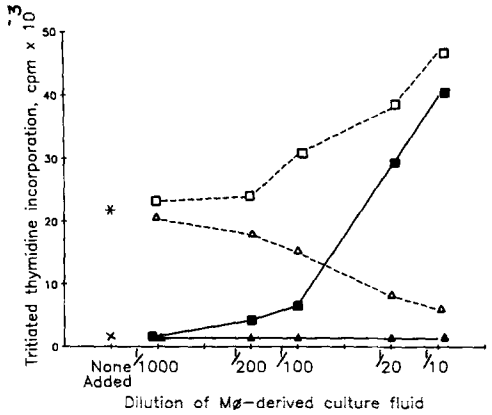

FIGURE 3. IL-1 (solid line) and IL-1 inhibitor (broken line) activities produced from 24 to 72 h by macrophages exposed to influenza virus (■, □, respectively) or RSV (▲, △, respectively). ×, thymocyte proliferation in the absence of macrophage fluids; *, thymocyte proliferation in the presence of purified IL-1 in the absence of macrophage fluids.

fractions, with substantial IL-1 activity, had no detectable IL-2 activity. An additional smaller degree of IL-1-like activity eluted at ~81 kD. The major IL-1 activity eluted in fractions without detectable total protein concentrations.

The different fractions of influenza virus–induced macrophage fluids were also tested for IL-1 inhibitor activity (Fig. 5). Suppression of thymocyte proliferation in response to IL-1 was noted primarily with influenza virus–induced fluid fractions of ~99 kD, with a lesser amount of inhibitor activity at ~3–5 kD.

RSV-induced macrophage fluids were also separated by gel filtration and assayed for IL-1 activity (Fig. 6). The data showed that RSV also induced production of IL-1, undetectable in the crude supernatant fluids because of the substantial amount of inhibitor activity present. In fact, since equivalent concentrations of influenza virus– and RSV-induced macrophage fluids were applied to the column, it appeared that RSV in fact induced IL-1 production to a degree

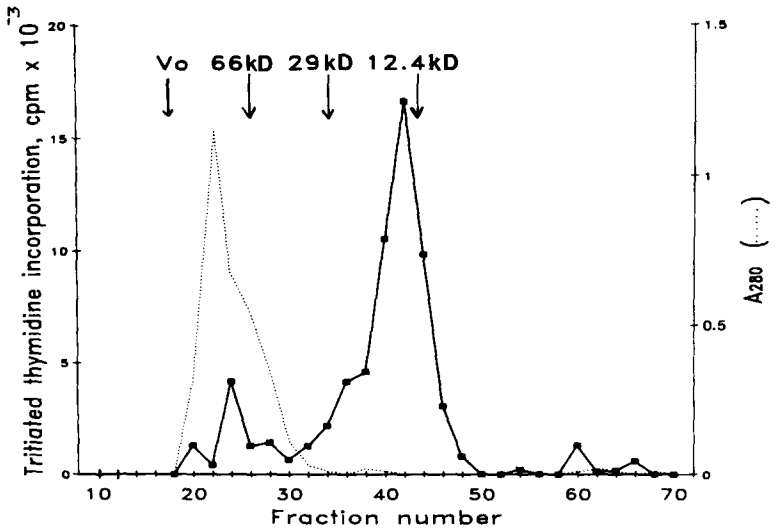

FIGURE 4. IL-1 activity in Sephadex gel filtration–separated fractions of fluids derived from macrophages exposed to influenza virus.

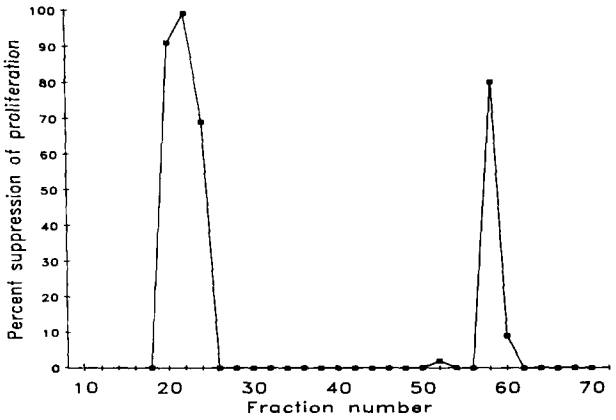

FIGURE 5. IL-1 inhibitor activity in Sephadex gel filtration–separated fractions of fluids derived from macrophages exposed to influenza virus. Activity is expressed as percent suppression relative to responses of thymocytes cultured in the presence of purified IL-1 alone.

equivalent to that induced by influenza virus. The IL-1 activity of the influenza virus–induced fluids is also shown in Fig. 6 for comparison. The IL-1 inhibitor activity in the fractions of RSV-induced fluids eluted with patterns similar to those of influenza virus–induce fluids. Furthermore, the higher molecular mass inhibitor (99 kD) did not inhibit IL-2 activity.

Discussion

These studies suggest that both influenza virus and RSV induce production of IL-1 by human peripheral blood–derived macrophages. Furthermore, both viruses concomitantly induce production of IL-1 inhibitors. The net macrophage-derived mediator activity produced after exposure to influenza virus resulted in

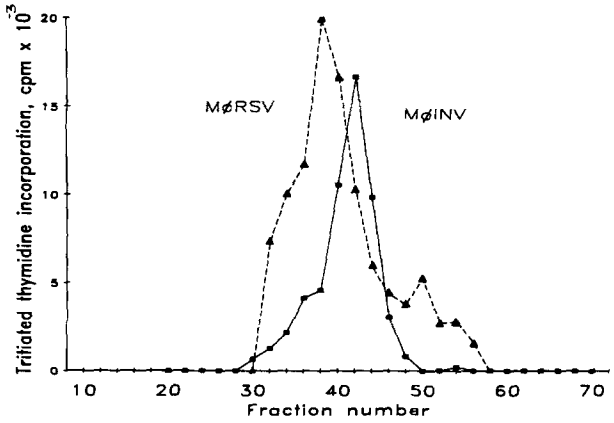

FIGURE 6. IL-1 activity in Sephadex gel filtration–separated fractions of fluids derived from macrophages exposed to influenza virus (■) or RSV (▲).

enhanced mouse thymocyte proliferation in response to the mitogen PHA. In contrast, the net mediator activity produced after exposure to RSV resulted in marked inhibition of thymocyte proliferation, either in response to any IL-1 activity that was concurrently produced, or in response to exogenous IL-1 added to the cultures.

The proliferative factors produced in response to the viruses appeared to have several of the characteristics of IL-1 (9, 12), and have been termed IL-1, but precise identification awaits eventual sequencing (as in other such studies). Similarly, the inhibitors produced in response to the viruses have been termed IL-1 inhibitors, but the exact mechanism(s) of their action are as yet undetermined. It does appear from the current data that the inhibitors of thymocyte proliferation do not act by inhibition of IL-2 activity, nor do they have direct cytostatic or cytotoxic effects on the thymocytes (data not shown). It is possible that the higher molecular mass inhibitor is related to that which others have found to be present in the urine of febrile individuals (13), but further physiochemical characterization would be required to establish such a relationship.

Viruses have long been considered to be effective inducers of IL-1 (12, 14). The data regarding virus-induced IL-1 production have been almost entirely derived from early animal studies. IL-1 production was suggested by virus-induced fever, mainly in rabbits, wtih assays for circulating endogenous pyrogen (15–22), or by measurements of endogenous pyrogen release after exposure of rabbit blood cells to virus (23). Influenza virus was used in many of the animal studies. Before our current studies, however, there were no reports of the ability of viruses to stimulate human IL-1 production in vitro (24).

We have previously shown (7) that human macrophages exposed to influenza virus produce a soluble fibroblast proliferation–stimulating factor or factors. It is not yet established whether the influenza virus–induced macrophage factor, or factors that stimulate fibroblast proliferation are the same as the factor(s) described in the current studies (putative IL-1 activity), which stimulate mouse thymocyte proliferation. Certainly the crude influenza virus–induced macrophage supernatant fluids contain other immunoregulatory proteins as well, such

as IFN (2, 25, 26). It will be important to determine whether the stimulatory and inhibitory factors described in the current studies have similar effects on human lymphocyte proliferative responses.

Our data suggest that influenza virus–induced depression of human macrophage accessory cell support for lymphocyte proliferative responses to mitogens and antigens (1, 2, 26) is not due to an inability of the infected macrophage to produce IL-1. Thus, the role of the IL-1 inhibitors, concurrently produced, in particular warrant further investigation, including determinations of the effects of the mediators on human lymphocyte proliferative responses. Effects on mitogen-stimulated thymocyte proliferation, or potentially on human lymphocyte proliferation, may reflect the total or net activity of numerous composite factors, the balance of which, or the precise molecular identity of which, varies according to the challenge, (e.g., influenza virus vs. RSV). Thus, these data also raise the possibility that one mechanism whereby RSV infection is able to recur frequently in supposedly immune individuals is by induction of macrophage-derived factors that suppress anamnestic responses by host mononuclear leukocytes. Such a possibility also warrants further investigation.

Summary

Respiratory viral infections are commonly associated with altered immune responses, such as proliferative responses to mitogens and antigens. To examine potential mechanisms, we examined production of IL-1 and IL-1 inhibitors by purified human peripheral blood–derived macrophages exposed to influenza virus or respiratory syncytial virus (RSV). IL-1 and IL-1 inhibitor activities in supernatant fluids from macrophages exposed to the viruses 24 h previously were measured using the standard mouse thymocyte comitogen assay. Crude fluids from macrophages exposed to influenza virus contained substantial IL-1 activity, whereas crude fluids from macrophages exposed to RSV contained marked IL-1 inhibitor activity. Assays with gel filtration–separated fractions revealed that both influenza virus and RSV induced production of both IL-1 and IL-1 inhibitors. Neither IL-2 nor IL-2 inhibitor activities were detected. Thus, effects of human macrophage-derived factors on thymocyte proliferation, or potentially on human lymphocyte proliferation, may reflect the total or net activity of multiple composite factors, the balance of which varies according to the challenge. The data raise the possibility that marked production of IL-1 inhibitor activity in response to RSV plays a role in the clinical recurrence of RSV infection despite the absence of clear evidence for antigenic shift or drift of the virus.

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