Production of Interferon by Alveolar Macrophages

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ABSTRACT

ACTON, JEAN D. (Bowman Gray School of Medicine, Winston-Salem, N.C.), AND QUENTIN N. MYRVIK. Production of interferon by alveolar macrophages. J. Bacteriol. 91:2300–2304. 1966.—Rabbit alveolar macrophages inoculated with para-influenza-3 virus in vitro produce a viral inhibitor which possesses the properties of interferon. The interferon is nondialyzable, is stable at pH 4, is not sedimented at 100,000 × g, exhibits species specificity, and can passively protect other alveolar macrophages from infection with virulent rabbitpox virus. The possible significance of alveolar macrophage-produced interferon is discussed.

In spite of increased interest in the interaction of viruses with the cells of the reticuloendothelial system (RES), there exists a paucity of investigations on the function of alveolar macrophages in viral infections with respect either to their role in immunity to viral respiratory diseases or their role as host cells for propagation of virus.

Liu (12) was one of the first investigators to demonstrate viral antigens in alveolar macrophages. He employed fluorescent-antibody techniques to identify the viral antigens present within the cells in nasal smears and sections of lungs obtained from ferrets infected with influenza virus.

In a study on the histopathogenesis of mousepox virus in mice, Roberts (19) observed that alveolar macrophages were among the first cells to contain viral antigens after aerosol infections. He also demonstrated that reticuloendothelial cells of the liver were important in the uptake of viruses present in the vascular compartment.

Gresser (4) was probably the first investigator to report that human white blood cells from venous blood had the capacity to produce interferon. His findings suggested that cells of the RES might contribute to the host’s defense mechanisms against viral infections by producing interferon.

Several investigators (1, 6, 7, 10, 17, 22, 23) have attempted to define the possible role of interferon in protecting against and in effecting recovery from viral infections. Collectively, their findings suggest that interferon may be an important factor of immunity during the early phases of viral infection, before antibody immunity becomes expressed.

The present study was initiated to investigate the possible production of interferon by virus-infected alveolar macrophages as well as the feasibility of passively immunizing macrophages in vitro with alveolar macrophage-produced interferon.

MATERIALS AND METHODS

Animals. New Zealand white rabbits weighing between 1.8 and 2.2 kg were utilized as the source of alveolar macrophages. They were killed by injecting air into the marginal ear veins.

Procurement of alveolar macrophages. Alveolar macrophages were obtained from rabbit lungs by a slight modification of the procedure described by Myrvik, Leake, and Fariss (16). The rabbit lungs were removed with the trachea clamped off with a hemostat. The cells were flushed from the lungs by injecting and withdrawing Hanks’ balanced salt solution at a point below the hemostat.

Cell cultures. Alveolar macrophages were maintained as suspension cultures in Medium 199 in Earle’s base (BBL) supplemented with 1.1 g of NaH2PO4 per liter and 20% heat-inactivated calf serum (56 C for 30 min). This “spinner modified” medium contained 100 units of penicillin and 100 μg of streptomycin per ml.

HEP-2 cells, obtained from William S. Jordan, Jr., University of Virginia School of Medicine, were grown as monolayers in Medium 199 supplemented with 10% heat-inactivated calf serum (Medium 199–5% Ca). No antibiotics were added to this growth medium.

All cells were maintained at pH 7.4 to 7.5 in humidified 5% 21% CO2 in air at 35 to 36 C.

Viruses. Rabbitpox virus (Utrecht strain), obtained from the American Type Culture Collection, was propagated in monolayers of HEP-2 cells maintained in Medium 199 supplemented with 5% heat-inactivated calf serum (Medium 199–5% Ca).
Parainfluenza-3 virus, obtained from Clayton Wheeler, University of North Carolina School of Medicine, was propagated in monolayers of HEp-2 cells maintained in Medium 199 supplemented with 5% heat-inactivated chicken serum (Medium 199–5% Ch).

Virus inocula were prepared by freezing and thawing infected cell suspensions three times. The virus-containing supernatant fluids were stored in 1-ml samples at −70°C.

Rabbitpox virus was assayed in HEp-2 cell monolayers by a modification of the procedure described by Postlethwaite (18) for plating vaccinia virus without an agar overlay. The cells were grown for 24 hr in 60-mm plastic petri dishes (Falcon Plastic Co., Los Angeles, Calif.). The growth medium was removed, and 0.5 ml of inoculum, diluted in Joklik’s (9) modification of Puck’s saline A (13), was added to duplicate 35-mm dishes at 35°C. A 4-ml amount of a fluid overlay (Medium 199–5% Ca) was added. After 18 to 24 hr of incubation, this medium was replaced with Medium 199 containing 2% heat-inactivated calf serum. At 36 to 48 hr after inoculation, the medium was removed and the plates were stained for approximately 30 sec with a 1% aqueous solution of crystal violet, washed with tap water, and dried. The technique of reducing the serum content after 18 to 24 hr of incubation reduced the growth rate of the cells, which maintained the cell sheet as a monolayer. The initial 5% concentration of serum was necessary to obtain optimal plaque development.

Parainfluenza-3 virus was assayed in tubes of HEp-2 cells by modification of the hemadsorption technique (21). Half-log dilutions of the virus were made in Hanks’ balanced salt solution (BSS), pH 7.4 to 7.6, and 0.2 ml of inoculum was added to each of two or three cell cultures. The virus was allowed to adsorb for 1 hr at 35 to 36°C, after which 1 ml of maintenance medium (Medium 199-5% Ch) was added to each tube. The culture fluids were changed after 24 hr of incubation. At 48 to 72 hr after incubation, 0.2 ml of 0.4% guinea pig red blood cells in 0.14 M NaCl was added to each tube. The cultures were incubated at 4°C for 30 min, washed with 5 ml of Hanks’ BSS, and observed microscopically for hemadsorption and cytopathogenic effect (CPE).

Estimation of viral CPE in alveolar macrophages. Viral cytopathogenic effect in alveolar macrophage preparations was estimated by determining the percentage of the cells which did not exclude trypsin blue (14).

Interferon assay. For virus challenge, extracts of virus-infected and uninoculated control fluids, which were to be assayed for the presence of viral inhibitor, were adjusted to equal amounts of virus and spun in a modified medium. Alveolar macrophages were suspended in the diluted preparations at a cell concentration of 400,000 cells per milliliter. The viral suspensions were incubated at 35 to 36°C for 18 hr in a CO2 incubator. The spinner flasks were incubated at 35 to 36°C for 24 hr in a CO2 incubator. After centrifugation at 200 × g for 10 min, the cell pellets were resuspended in half the original volume of spinner medium. Rabbitpox virus inoculum was added to the cell suspension at a multiplicity of approximately 0.1 plaque-forming unit (PFU) per cell, and the cell concentration was adjusted to 400,000 cells per milliliter with additional spinner medium. The viral inoculum was allowed to adsorb for 3 hr in a CO2 incubator. The cell suspension was then centrifuged at 200 × g for 10 min, and the cell pellet was resuspended in spinner medium at the original cell concentration. Samples for viral titrations were removed at desired intervals and stored at −70°C. At each sampling interval, cell counts and the percentage of viable cells were determined. A virus control was incubated for 18 hr in fresh spinner medium rather than interferon or control fluid before inoculation with rabbitpox virus. An uninfected cell control was treated as above except that rabbitpox virus was omitted.

In the assay for plaque reduction, the samples removed at the various intervals were assayed for infectious virus in HEp-2 cell monolayers as described in Materials and Methods. A 75% reduction in the number of plaques in the test system, as compared with the number of plaques in the control system, was considered to be a significant difference.

RESULTS

Growth of rabbitpox virus in alveolar macrophages. Studies were carried out to determine the course of rabbitpox virus infection in rabbit alveolar macrophages in vitro. Suspension cultures of macrophages at a cell concentration of 400,000 cells per milliliter were infected with rabbitpox virus at a multiplicity of approximately 0.1 PFU per cell. Infected and uninfected cultures were incubated at 35 to 36°C in a CO2 incubator for 1 hr. After centrifugation at 200 × g for 10 min, the cells were resuspended in spinner modified medium. Samples were harvested periodically and were assayed for free and cell-associated infectious virus in HEp-2 cells as previously described. An increase in the quantity of cell associated virus was first observed between 9 and 12 hr after infection. An increase in the quantity of virus in the supernatant fluids was observed 12 to 18 hr after addition of virus. (The infected alveolar macrophages in spinner cultures yielded a higher percentage of virus to the supernatant fluid than did infected HEp-2 cell monolayers. This could be due either to the different pattern of CPE production in alveolar macrophages by rabbitpox virus or to mechanical disruption of the cells in the spinner flasks.) Approximately a 10% decrease in cell viability was observed at this interval. Maximal virus production (104 to 5 × 104 PFU per ml) was achieved 24 to 48 hr after infection.

Failure of alveolar macrophages to support the growth of parainfluenza-3 virus. Suspension cultures of macrophages at a cell concentration of 400,000 cells per milliliter were inoculated with
parainfluenza-3 virus to give a range of 0.05 to 1.0 TCID per cell. The procedures for infecting
and sampling were the same as those described for growth of rabbitpox virus. Samples obtained
from the macrophage suspensions were titrated for the presence of parainfluenza-3 virus as de-
scribed in Materials and Methods.

Multiplication of parainfluenza-3 virus in macro-
phages was not observed under the conditions
of these experiments. The macrophage-associated
virus, which was released by freezing and thawing
the cells, decreased in parallel with the free virus
in the supernatant fluid. No infectious virus
could be recovered 6 to 9 hr after the addition of
the viral inoculum to the macrophage cultures.
Paradoxically, extensive CPE developed in
the cultures even though the parainfluenza-3 virus
disappeared and apparently failed to replicate.

In vitro interferon production by macrophages.
Suspension cultures of macrophages were inocu-
lated with parainfluenza-3 virus to give a range of
0.05 to 1.0 TCID per cell. Samples were removed
from virus-infected and uninfected control cul-
tures at 12, 18, 24, 48, and 72 hr after inoculation.
All preparations were frozen and thawed three
times and centrifuged at 820 × g. The super-
натant fluids were then centrifuged at 100,000 × g
for 1 hr at 4 C. These crude preparations were
assayed for interferon activity as described in
Materials and Methods.

Interferon-like activity was detected when either
0.1 or 0.25 TCID was employed; however,
0.05 TCID failed to induce measurable interferon.
Inocula containing 1.0 TCID resulted in early cell
death and no interferon production. The results
indicated that maximal interferon was produced
with 0.1 TCID per cell, and therefore this multi-
plicity was used in subsequent experiments. Sig-
nificant amounts of interferon were consistently
present at 24 and 48 hr (Table 1) after inocula-
tion with parainfluenza-3 virus. Under the exper-
imental conditions employed, a maximum of 99%
reduction in virus yield from interferon-treated
cells was observed. A corresponding reduction
in the cell death of challenged macrophages also
occurred (Table 2).

In occasional experiments, the extracts ob-
tained from noninfected control macrophage
cultures conferred interferon-like activity on
recipient macrophages. These effects were usually
slight and showed animal to animal variation.
It would not be surprising to find low levels of
interferon-like activity in cells obtained from the
respiratory tract of rabbits encountering micro-
organisms in their natural environment. Accord-
ingly, rabbits having a naturally occurring respira-
tory infection caused by Bordetella bronchiseptica

| Table 1. Interferon production in rabbit
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<td>Time after inoculation with</td>
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<tr>
<td>parainfluenza-3 virus</td>
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<tr>
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<tr>
<td>hr   %</td>
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<td>12   0</td>
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<td>24   97</td>
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<td>48   97</td>
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<td>72   70</td>
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* The data presented in this table represent one

typical experiment from a series of nine

experiments. Alveolar macrophages were inocu-
lated with 0.1 TCID of parainfluenza-3 virus per

cell. At the designated intervals, samples were

removed and assayed for interferon. Alveolar macro-

phages were incubated in the interferon pre-

parations for 18 hr and challenged with 0.1 PFU

of rabbiptox virus per cell. After 24 hr of incuba-
tion, samples were assayed for infectious rabbi-
tox virus in HEp-2 cells. The per cent plaque

reduction was calculated by dividing the differ-
ence between mean control and mean test counts

by control counts times 100.

were studied with respect to interferon-like ac-

activity associated with their alveolar macrophages.

Several experiments of this type failed to reveal

the presence of interferon-like activity.

In view of the report that homologous nucleic

acid can, under certain conditions, induce inter-

feron activity in cells (20), it appeared desirable
to determine whether or not dead alveolar macro-

phages or their products could induce interferon-

like activity. This possibility warranted special

attention, since it was routinely observed that

living macrophages removed small numbers of
dead cells from the cultures. Experiments were

conducted in which suspensions of killed macro-

phages were added to an equal number of living

cells. Incubation was carried out for 24 to 48 hr.

No evidence was obtained suggesting that, under

the conditions of these experiments, interferon-

like activity was induced in living macrophages

by dead alveolar macrophages or their breakdown

products.

Properties of the macrophage interferon. Experi-

ments were performed to determine whether the

inhibitor possessed the properties of the proto-
typic interferon described by Isaacs and Linden-

mann (8). Representative interferon preparations

were subjected to the following tests.

Acid stability and dialyzability. Crude inter-

feron prepared in macrophages was dialyzed at

4 C against 100 to 200 volumes of saline, adjusted
to pH 4 with 1 N HCl, for 18 to 24 hr, and was
back-dialyzed overnight against Hanks' BSS (pH 7.6). The activity of the extracts was not reduced, indicating that the interferon-like inhibitor was nondialyzable and stable at pH 4.

**Extracellular inactivation of virus.** When 100 PFU of rabbitpox virus was incubated with undiluted interferon or control fluid for 1 hr at 37°C, the amount of infectious virus recovered from the two suspensions was the same. Thus, the interferon preparation did not directly inactivate the virus.

*Species specificity.* Primary green grivet monkey kidney cells were employed to determine whether the inhibitor exhibited species specificity. The cells were pretreated for 6 hr with a 1:2 dilution of the inhibitor and challenged with 100 PFU of rabbitpox virus. After 48 hr of incubation, approximately 75% of both the interferon-treated cells and virus control cells underwent cytopathogenesis. Extracts of the monkey kidney cells were assayed in HEp-2 cells. There was no reduction in virus titer in the interferon-treated cells, indicating that the rabbit macrophage interferon did not protect cells of another species against rabbitpox virus.

These data (Table 3) indicate that the interferon induced in alveolar macrophages by parainfluenza-3 virus exhibits the major properties of previously described interferons.

**DISCUSSION**

The review by Mims (15) of the pathogenesis of viral diseases summarized numerous investigations on the interaction of viruses and the RES. In the case of nonpathogenic viruses, cells of the RES probably play an important role in clearance and disposal of viral particles. On the other hand, pathogenic viruses appear to be resistant to this process and possibly succeed in using cells of the RES for replication or as vehicles for transport to other anatomical sites, or both.
lar macrophages produce an interferon after inoculation with parainfluenza-3 virus in vitro. This interferon can passively protect other alveolar macrophages from infection with virulent rabbitpox virus. In this regard, Gresser and Enders (5) demonstrated that a small population of virus-resistant interferon-producing cells interspersed in a large population of susceptible cells altered the course of a sindbis virus infection. Accordingly, interferon produced by a few alveolar macrophages as a result of infection with viruses of low virulence could enhance the resistance of the host by passively immunizing neighboring cells against superinfection with more virulent viruses. The production of interferon could also attenuate or promote recovery from an existing viral respiratory infection. The unique anatomical location of alveolar macrophages, coupled with their almost certain important role in the phagocytosis of microorganisms, makes them prime candidates for interferon production during the initial stages of a viral respiratory infection.

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LITERATURE CITED