Interferon Induction by the Psittacosis Agent in Guinea Pig Leukocyte Cultures

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High titers of interferon were induced by the psittacosis agent in guinea pig leukocyte cultures. Optimal yields of interferon were produced from freshly prepared guinea pig leukocyte cultures containing $4 \times 10^6$ cells per ml inoculated with a psittacosis/cell multiplicity of 1.0 and incubated at 35 C for 24 hr. Leukocytes obtained from 2- to 4-month-old guinea pigs produced 20 times more interferon than leukocytes from 3- to 4-week-old animals. The biological activity of interferon and the kinetics of its production from psittacosis-infected guinea pig leukocyte cultures were similar to those reported for virus-induced leukocyte interferons.

The psittacosis - lymphogranuloma - trachoma group of agents are obligate intracellular parasites that possess certain developmental and biochemical properties in common with bacteria and rickettsiae that clearly separate them from typical viruses (13). This group is now included in the list of nonviral agents known to induce or to be inhibited by interferon (7, 11, 12). Although one member of the group, the psittacosis agent, has been reported to be susceptible to virus-induced interferon (9, 16), its ability to induce interferon has not been demonstrated. This report describes the induction and optimal conditions requisite for the production of interferon by the psittacosis agent in guinea pig leukocyte cultures.

MATERIALS AND METHODS

Cell line and cultivation. Guinea pig lung (GPL) cells were used for assay of interferon activity. Cells were grown in 250-ml plastic tissue culture flasks (Falcon Plastic Div. of B-D Laboratories, Inc., Los Angeles, Calif.). Nutrient medium consisted of basal medium Eagle (BME) with Earle's basal salt solution (BSS) containing 0.5% lactalbumin hydrolysate, 10% fetal calf serum, 50 μg of streptomycin, and 75 μg of kanamycin per ml. Cells were maintained in BME and 5% fetal calf serum.

For interferon assay, GPL cells were cultivated on circular cover slips (15-mm diameter) inserted in flat-bottomed glass vials (18 by 100 mm). A 1-ml amount of cell suspension containing $10^4$ to $3 \times 10^6$ cells was introduced onto cover slips that were then incubated at 35 C for 24 hr, or until complete monolayers were formed. Cover slip cell cultures were washed twice with 2 ml of maintenance medium before addition of interferon preparations or virus inoculum.

Leukocyte cultures. Blood obtained from guinea pigs by cardiac puncture was put into sterile plastic-capped tubes (17 by 64 mm) containing phenol-free heparin (1 mg/10 ml of blood). To promote sedimentation of red blood cells, 0.2 ml of Phytohemagglutinin M (Difco), rehydrated with phosphate-buffered saline (pH 7.1), was mixed with each 10 ml of heparinized blood. The tubes were kept at room temperature (23 C) for 30 min and then centrifuged lightly (25 $X$ g) for 2 min to further sediment the red blood cells. The leukocyte-rich plasma was drawn off, pooled, and centrifuged (375 $X$ g) for 10 min; the plasma supernatant fluid was discarded. The resulting pellet was resuspended, washed twice, and finally suspended in 10 ml of GPL cell nutrient medium.

Psittacosis agent and virus. A suspension of the Borg strain of psittacosis agent was prepared from infected McCoy cells and contained $10^3$ cell-infecting units (CIU) per ml (5). The Asibi strain of yellow fever virus was obtained from an infected rhesus monkey in the form of infectious plasma (3). The plasma contained $3 \times 10^6$ CIU of virus per ml. Virus and agent suspensions were distributed in 1-ml portions into glass vials and stored at $-60$ C.

Leukocyte interferon. Flat-bottomed glass vials containing 1 ml of freshly prepared leukocyte suspensions were inoculated with psittacosis agent in 0.2-ml volumes. Centrifugal force ($500 \times g$, 15 min) was employed to sediment the leukocytes and to promote efficient attachment of the psittacosis inoculum onto leukocytes. The tubes were then incubated at 35 C for 24 hr. After incubation, culture fluids were collected, pooled, and centrifuged at 105,000 $X$ g for 90 min. The upper two-thirds of the supernatant fluids were removed, dialyzed against HCl-KCl buffer (pH 2.0) at 4 C for 24 hr, and then against two changes of Earle's BSS (pH 7.1) at 4 C for 24 hr. The fluids were stored at $+4$ or $-60$ C until assayed for interferon activity.

Interferon characterization. The biological activity of psittacosis-induced interferon met the usual criteria for classification as interferon. The substance was trypsin-sensitive, nonsedimentable at 105,000 $X$ g,
nondialyzable, inactive in cell cultures of species other than guinea pigs, not virus-specific, and nonvirucidal. As reported for other serum and leukocyte interferons (17), the psittacosis-induced interferon demonstrated heat- and acid-labile fractions. A portion of interferon activity was lost upon heating at 56 °C for 1 hr or treatment at pH 2.0 for 24 hr.

**Interferon assay.** Preparations were assayed for interferon activity by the procedure based on the 50% reduction of yellow fever virus fluorescent cell counts (9). The assays were usually carried out in quadruplicate. The reciprocal of the interferon dilution that reduced the number of fluorescent cells to 50% of the controls served as the measure of potency of interferon preparations. The 50% reduction value was derived by plotting probit transformations of reduction percentages against corresponding interferon dilutions. Controls consisted of fluids from uninfected leukocyte cultures prepared in the same manner as fluids from infected cultures and showed no inhibitory activity to challenge virus.

**RESULTS**

**Effect of agent/cell multiplicity and leukocyte concentration on interferon production.** Maximal interferon production was determined by adding different doses of psittacosis agent to freshly cultured leukocyte suspensions containing different cell concentrations. The psittacosis agent doses, in 0.2-ml volumes, were employed at agent/cell multiplicities ranging from 0.01 to 100. Leukocyte concentrations in twofold increments ranged from 0.25 \( \times \) \( 10^6 \) to 16 \( \times \) \( 10^6 \) cells per ml. Interferon activity was determined after 24 hr of incubation at 35 °C. There was no significant interferon production at the lower cell concentrations and agent doses (Table 1). Maximal interferon activity was attained when 4 \( \times \) \( 10^6 \) leukocytes per ml were exposed to an agent/cell multiplicity of 1.0. At higher cell concentrations and agent doses, interferon production decreased.

<table>
<thead>
<tr>
<th>Guinea pig leukocyte concen</th>
<th>Psittacosis agent multiplicity</th>
<th>0.01</th>
<th>0.1</th>
<th>1.0</th>
<th>10</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>(X ( 10^4 ) cells/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0*</td>
<td>700</td>
<td>4,800</td>
<td>700</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>1,400</td>
<td>18,600</td>
<td>1,600</td>
<td>146</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>2,400</td>
<td>45,000</td>
<td>560</td>
<td>680</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1,000</td>
<td>2,600</td>
<td>460</td>
<td>156</td>
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</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>180</td>
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</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>22</td>
<td>136</td>
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</tr>
<tr>
<td>0.25</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>240</td>
<td>360</td>
<td></td>
</tr>
</tbody>
</table>

* Interferon units per ml.

**Effect of temperature on interferon production.** To determine the influence of temperature on the production of psittacosis-induced interferon in guinea pig leukocyte cultures, four groups, each consisting of six psittacosis-infected leukocyte cultures, were incubated in a water bath at 25, 32, 35, or 40 °C for 24 hr. Culture fluids from each group were then pooled and assayed for interferon. Maximal yields of 11,000 units of interferon were produced at 35 °C (Fig. 1). The yields of interferon decreased markedly below this temperature; 2,500 and 580 units of interferon per ml were produced at 32 and 25 °C, respectively. Cultures incubated at 40 °C produced less than 10 units of interferon per ml of preparation.

**Kinetics of interferon production in leukocyte cultures obtained from young and adult guinea pigs.** Experiments were performed to determine the rate and time of peak interferon production in leukocyte cultures from young (3- to 4-week-old) and adult (2- to 4-month-old) guinea pigs. Freshly prepared leukocyte cultures containing 4 \( \times \) \( 10^6 \) cells per ml were infected with psittacosis agent at a multiplicity of 1.0 and incubated at 35 °C. At varied intervals, from 0 to 72 hr, culture fluids from four to six vials were pooled.

![FIG. 1. Effect of temperature on interferon production in guinea pig leukocyte cultures (4 \( \times \) \( 10^6 \) cells/ml) infected with the psittacosis agent at multiplicity 1.0.](http://iai.asm.org)
INTERFERON INDUCTION

DISCUSSION

Although inhibition of maturation of the psittacosis agent in cell cultures by interferon has been reported (16), to our knowledge, this present study demonstrates for the first time the induction of interferon by the psittacosis agent. It was shown previously that the psittacosis agent interferes with the replication of yellow fever virus in cell culture (4), but no attempt was made at that time to detect the presence of interferon. In view of the current findings, the mechanism of interference in the aforementioned study may have been related to the induction of interferon by the psittacosis agent.

That human as well as bovine and mouse leukocytes can be induced to produce interferon in vitro by viral agents has been extensively documented (1, 2, 8, 18). Although not included in this report, preliminary tests showed that high titers of interferon were also produced from human leukocytes by the psittacosis agent. In general, conditions for optimal production of interferon by psittacosis-infected guinea pig leukocytes were similar to those described for virus-induced interferon in human leukocytes (18); however, the amount produced by the former was approximately twofold greater than the latter. The finding that leukocyte cultures derived from younger guinea pigs produced less interferon than those obtained from older animals appears to confirm the observation of others on the lower capacity of cells from young animals to produce interferon (6, 15). The kinetics of interferon production by the psittacosis agent and the properties of the resultant inhibitor were similar to those described for virus-induced leukocyte interferons (8, 10, 17). These cursory observations suggest that similar mechanisms may be involved in the production of interferon from leukocytes by both the psittacosis agent and viral inducers.

Whether the role of interferon can be related to latency or attenuation of infection, often noted in natural and experimental infections with this group of agents (14), remains unknown. The induction of interferon by the psittacosis agent in leukocytes demonstrated in this study offers a suitable in vitro system for exploring this problem.

ACKNOWLEDGMENT

We gratefully acknowledge the excellent technical assistance of John W. Freed.

LITERATURE CITED

2. Glasgow, L. A. 1965. Leukocytes and interferon in the host response to viral infections. I. Mouse leukocytes and leuko-

Fig. 2. Kinetics of interferon production with the psittacosis agent in leukocyte cultures derived from young and adult guinea pigs. Symbols: (●) 2- to 4-month-old guinea pigs; (○) 3- to 4-week-old guinea pigs.

processed by ultracentrifugation and dialysis, and then stored at 4°C until assayed for interferon content. Results revealed a significant difference in the amount and rate of interferon production in leukocyte cultures from young and adult guinea pigs (Fig. 2). The amount of interferon produced in the cells from older animals was 20 times that from cells of young animals. The rate of interferon synthesis in leukocytes from adults was also more rapid than in cells from younger animals. Interferon was detected in culture fluids within 2 hr after agent adsorption. Maximal synthesis occurred at a linear rate between 2 and 10 hr after induction and reached a peak titer of 43,000 interferon units per ml at 24 hr. No further increase of interferon production was detected after this time.

In leukocyte cultures from young animals, interferon production was not detected until 4 hr after agent adsorption. Interferon synthesis also proceeded at a linear rate but was less rapid than that noted with cells from adult donors. In contrast to the rate of synthesis in cells from adult animals, which had synthesized three-fourths of the total amount of interferon in 10 hr, less than one-tenth of the total amount had been produced in cells from young animals in the same period of time. The peak titer of interferon attained at 24 hr was 2,100 units per ml of sample.