

**Interferon-Like Virus-Inhibitor Induced in Human Leukocytes by
Phytohemagglutinin**



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in the absence of any other salts. Riklis reported a small amount of a thymine photoproduct with similar chromatographic properties in lyophilized DNA which had been irradiated in the dry state (7). One of the photoproducts (a), appearing in small amount, chromatographs in the region of the uracil-thymine dimer (II), but other evidence indicates that the photoproduct may not be this dimer. Smith (12) reported finding small amounts of thymine-containing photoproducts chromatographing in the region of our photoproducts (b and c) in DNA from *E. coli* irradiated in vivo and in an irradiated solution of polydeoxyadenylate-thymidylate.

The absence of thymine dimers in the DNA of irradiated spores is sufficient to explain their resistance to ultraviolet irradiation. However, the appearance of large amounts of unidentified photoproducts implies either that such products do not interfere with DNA synthesis or that the cells have a very efficient repair mechanism for dealing with the photoproducts. Certain data (13) indicate that the photoproducts do not remain in the DNA of spores during differentiation into vegetative cells. Our data show that during sporulation the physical state of the DNA within the cell is changed from that found either in vegetative cells or in solution, because normal thymine dimers are not found in irradiated spores.

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Interferon-Like Virus-Inhibitor Induced in Human Leukocytes by Phytohemagglutinin

Abstract. *Phytohemagglutinin*, an extract of the kidney bean, *Phaseolus vulgaris*, induces in human leukocyte cultures an inhibitor of the cytopathic effects of Sindbis virus. The physicochemical and biological properties of this virus-inhibitor are similar to those of interferon induced by Newcastle disease virus, except for an instability at pH 2 and 10 and at 56°C.

During attempts to culture peripheral leukocytes from the blood of persons with and without viral infections, phytohemagglutinin (PHA), an extract of the kidney bean (*Phaseolus vulgaris*) which agglutinates red blood cells in the preparation of cultures, induced synthesis, in leukocytes cultured from normal individuals, of a virus-inhibitor with interferon-like properties. Interferon production in virus-infected cultures of human leukocytes has been previously reported (1, 2).

In this report the properties of the phytohemagglutinin-induced virus-inhibitor are described and compared with interferon induced in white blood cells by infection with Newcastle disease virus (NDV).

Cells were grown in Eagle's minimum essential medium supplemented with tryptose phosphate broth (4 percent) and fetal calf serum (10 percent). The concentration of sodium bicarbonate was 1.75 g/liter, and all cell-culture vessels were gassed with 5 percent CO₂ in air before incubation at 37°C. White blood cells were obtained from the venous blood of normal adults; the blood was placed in tubes containing phenol-free heparin (0.5 ml for each 15 ml blood). The tubes were incubated at 37°C for 50 to 60 minutes and then centrifuged at room temperature at 800 rev/min for 2 minutes. The supernatant leukocyte-rich plasma was then recentrifuged at 1600 rev/min for 8 minutes, and the pellet was suspended in growth medium by gentle pipetting. The leukocytes were counted in a hemocytometer, and the cells were diluted

in growth medium to a final concentration of 2 × 10⁶ cells per milliliter. Screw-cap culture tubes were each planted with 2 ml of the cell suspension, placed in a roller drum, and incubated at 37°C.

Three types of cell cultures were used for the interferon assay: (i) cells derived from a human fetal lung, grown in continuous culture, and used between the 10th and 25th passage; (ii) the BHK 21 clone 13 (C13) an established cell line derived from baby hamster kidneys (3); (iii) the 'L' cell strain of mouse fibroblasts derived from normal mouse skin (4).

Phytohemagglutinin P (5-ml bottles, Difco Co.) was dissolved in phosphate-buffered saline (100 ml) at room temperature and passed through a filter with a pore size of 600 mμ. The filtrate was stored at 4°C.

The specimens to be tested for interferon were diluted in growth medium, and 1 ml of each dilution was added to 1-day-old cultures of human fetal lung cells grown in incomplete monolayers in screw-cap tubes. After 20-hour incubation at 37°C, the cultures were washed once with 4 ml of phosphate-buffered saline. One milliliter of warm Eagle's medium was added to each tube, and then 1 ml of cold growth medium containing 5000 tissue culture infective doses (TCID₅₀) of Sindbis virus (Egypt AR 339 strain) was inoculated. This amount of virus produces gross cytopathic effects in cultures in 24 to 30 hours. Cultures were considered to be protected when there was less than 10-percent cytopathic effect at a time when control cultures exhibited more than 75-percent cytopathic effect.

Interferon titers are expressed as reciprocals of the highest dilution of the specimen, 1 ml of which protected cultures against challenge with Sindbis virus. No specimen was tested at less than 1:10 dilution.

To prepare Newcastle disease virus interferon white blood cell cultures were inoculated with 10⁸ EID₅₀ (egg infective doses) of virus (Hickman strain) and incubated for 48 hours. The media were then collected, and all infective virus was completely neutralized with hyperimmune guinea pig antiserum to the virus (5). The inhibitory property of this preparation could be attributed to interferon on the basis of (i) no reduction in the virus inhibitory titer of the supernatant after centrifugation at 105,000g for 3 hours; (ii) no reduction in inhibitory titer on acidifi-

cation to pH 2 for 24 hours; and (iii) complete loss of inhibitory activity on treatment with crystalline trypsin (0.2 mg/ml) for 1 hour at 37°C. The titers of Newcastle disease virus-induced interferon were approximately 10⁴ culture-protecting units per milliliter.

Phytohemagglutinin (0.1 ml) was added to each 2 ml of white-blood-cell cultures in suspension and incubated at 37°C; at hourly intervals thereafter media and cells were collected and assayed for virus-inhibitory activity. An inhibitor of cytopathic effects of Sindbis virus in lung cells of the human fetus appeared in the white-cell cultures 2 hours after addition of phytohemagglutinin, increased in titer until approximately the 20th hour, and remained at a constant titer thereafter. The titers of the virus inhibitor produced in leukocyte cultures obtained from different individuals varied from 10 to 80 culture-protecting units per milliliter with an occasional culture producing no detectable inhibitor. The virus-inhibitor was present in the media; it was not detected in cells disrupted by high-frequency sound. In white-cell cultures incubated without phytohemagglutinin, virus-inhibitor could not be detected in either the media or cell-disrupted fraction.

The 2-hour incubation period between the addition of phytohemagglutinin and the appearance of virus-inhibitor suggests a succession of intracellular events in the course of inhibitor production. To provide evidence that the virus-inhibitor was synthesized in white blood cells, the following experiments were performed. The white cells were treated with phytohemagglutinin for 30 minutes and then washed five times in growth media to remove all phytohemagglutinin not associated with cells. On incubation at 37°C these cultures synthesized the virus-inhibitor, but at 4°C failed to produce the inhibitor. When phytohemagglutinin was added to cell-free growth media and incubated for 24 hours at 37°C, no virus-inhibitor was produced. Finally, lung cell cultures treated with phytohemagglutinin were as susceptible to the cytopathic effects of Sindbis virus as were untreated cultures. These experiments suggest the intraleukocytic synthesis of virus-inhibitor but do not rule out the possibility that phytohemagglutinin is degraded within white cells into a protein with virus-inhibitory properties.

The possibility of a virus contaminant in the media in which the white

Table 1. Comparison between properties of phytohemagglutinin-induced and Newcastle disease virus-induced virus-inhibitors. Plus (+) indicates greater than a fourfold reduction in titer of inhibitor after treatment. Minus (−) indicates no reduction in titer of inhibitor after treatment.

| Treatment | Effect on virus-inhibitor | |
|--|---------------------------|-----|
| | PHA | NDV |
| Crystalline trypsin (0.1 mg/ml; 1 hr; 37°C) | + | + |
| Ribo- or deoxyribonuclease (0.5 mg/ml; 1 hr; 37°C) | − | − |
| Centrifugation 105,000g, 3 hr (supernatant assay) | − | − |
| 46°C, 1 hr | − | − |
| 56°C, 1 hr | + | − |
| 70°C, 1 hr | + | + |
| pH 1; 24 hr | + | − |
| pH 2; 24 hr | + | − |
| pH 3→9; 24 hr | − | − |
| pH 10; 24 hr | + | − |
| pH 11; 24 hr | + | − |
| Dialyzable | No | No |

cells were cultured was eliminated by the demonstration that heparinized whole blood treated with phytohemagglutinin and incubated at 37°C produced a virus-inhibitor detectable in the plasma. Attempts to isolate a virus from phytohemagglutinin or the phytohemagglutinin-induced virus-inhibitor were unsuccessful, an indication that a virus was probably not introduced from without nor activated from white cells in the course of the experiments.

The properties of the phytohemagglutinin-induced virus-inhibitor were determined and compared (at comparable concentration) with interferon induced by Newcastle disease virus in leukocyte cultures as described above. Both virus-inhibitors are nondialyzable, nonsedimentable proteins (Table 1) which have no direct virus-neutralizing properties but which can exert their virus-inhibitory effects by incubation with cells before inoculation of challenge virus. Further, both proteins inhibit virus multiplication in human cells and not in mouse or hamster kidney cells. In contrast to the virus-induced interferon, however, the phytohemagglutinin-induced inhibitor is unstable at pH 2 and 10 and at 56°C. Thus the phytohemagglutinin-induced virus-inhibitor is an interferon-like substance labile to heat and to extremes of pH.

Phytohemagglutinin stimulates RNA and DNA synthesis in human leukocytes, with subsequent transformation of lymphocytes into blastoid cells, and increases the mitotic rate (6). I now report a further and perhaps related effect by phytohemagglutinin.

Interferon-like substances have been

produced in animals and cell cultures exposed to a variety of other nonviral agents: bacteria and endotoxin (7), an anionic polysaccharide (8) and nucleic acids from animal cells (9), yeast (10), and bacteria (11). The interferon-like substance produced in rabbits in response to endotoxin has properties similar to the phytohemagglutinin-induced virus-inhibitor (12).

The production of different interferons in human leukocyte cultures by phytohemagglutinin and Newcastle disease virus may either reflect the heterogeneous nature of the cell population or be the result of different mechanisms of interferon synthesis in the same cell type. In support of the latter hypothesis are the findings that phytohemagglutinin acts on lymphocytes (6) and that mononuclear cells do produce interferon in response to viral infection (2). Also, Ho has recently reported that the production of virus-induced interferon can be inhibited by actinomycin, whereas production of endotoxin-induced virus-inhibitor cannot; he attributes this phenomenon to different mechanisms of interferon production (13). The phytohemagglutinin-induced virus-inhibitor may, however, be produced in white cells in response to a stimulation of cellular RNA synthesis and may be a feedback mechanism for control of RNA synthesis.

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