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The Effect of 6-Mercaptopurine on the Synthesis and Action of Interferon (33671)

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Since other antimetabolites which disrupt nucleic acid and protein synthesis also inhibit the synthesis and action of interferon (1–7), it seemed plausible that 6-mercaptopurine (6-MP) may function in a similar fashion. Previous experiments demonstrated that 6-MP inhibits viral multiplication *in vitro* (8). However, infection of weanling mice with herpes simplex virus and simultaneous administration of 6-MP enhanced the synthesis of virus and interferon in the liver and brain, compared to untreated controls, suggesting that 6-MP fails to inhibit the elaboration of interferon (9).

These observations were clarified by study of the effect of 6-MP on both the synthesis and action of interferon *in vitro*. Inactivated chikungunya virus was employed to evoke satisfactory titers of interferon in chicken fetal cells (1). Alteration of the system by 6-MP was evaluated with the inclusion of actinomycin D as an inhibitory control. As anticipated, actinomycin fully inhibited the synthesis and action of interferon while 6-MP failed to alter significantly either phase of interferon biology.

Methods. Cells. Chicken fetal cell cultures were prepared from decapitated 10–11-day-old red rooster fetuses by trypsinization (0.2%) of minced, rinsed tissue and seeding of 16 × 125 mm glass tubes with 10^6 cells. Cells were plated in growth medium (GM) consisting of Eagle's basal amino acid and vitamin supplement in buffered Hanks' balance salt solution (BSS), 90 vol, and bovine serum, 10 vol. Cells were nourished during experiments with a similar maintenance medium (MM), substituting only bovine fetal serum, 2 vol.

Human diploid cells were derived by similar methods from a 6-week-old embryo.[1]

Virus. Chikungunya virus, strain S-27, obtained from American Type Culture Collection as intracerebral passage 173 in infant mice, was passed in the same manner twice more in our laboratory. The virus pool of suspended 10% brain was assayed in chicken fetal cells and yielded a titer of $10^{7.5}$ infectious doses (ID_{50})/ml.

Sindbis virus was obtained as the first mouse brain and subsequent second chicken allantoic fluid passage from Dr. Marcus Jensen. Two additional intracerebral passages were performed in infant mice, first, strain Swiss albino and next, strain C_3H/eB. The virus pool of suspended 5% brain was assayed in chicken fetal cells and produced a titer of $10^{8.8}$ ID_{50}/ml.

Interferon. Chick fetal cell cultures were inoculated with inactivated chikungunya virus (37°, 24 hr) at a multiplicity of 1.0 and culture fluids were harvested after 24 hr of incubation at 35°. Fluids were acidified to pH 2 with 1 *N* HCl and stored for 1 week at 4° before restitution to pH 7.2 with 1 *N* NaOH. Spent media from uninfected chicken fetal cell cultures were also harvested and

[1] Obtained through the Human Tissue Procurement Program, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

treated in a similar manner as control.

For titration of interferon, chicken fetal cells were pretreated overnight with 2-fold dilutions of test fluids in MM, beginning with a dilution of 1:10. Three culture tubes were used for each dilution, adding 1.0 ml/culture. The test fluids were decanted and following a rinse with BSS the cultures were challenged with 100–1000 ID_{50} of Sindbis virus. The titer of interferon in units per milliliter was expressed as the reciprocal of the dilution of test fluid which protected 50% of the combined cell population from the cytopathic effects of Sindbis virus at 48–72 hr. The protective effect of these fluids was trypsin-sensitive and species-specific, consistent with interferon.

Antimetabolites. 6-Mercaptopurine (Purinethol, Burroughs-Wellcome & Company), 150 mg, was dissolved in 1.0 ml of 1 *N* NaOH at 37° for 30 min. Dilutions were made in MM and concentrations of 0.3, 3, and 30 μg/ml of 6-MP were used in these experiments.

Actinomycin D (Dactinomycin, Merck & Co. Inc.), 25 mg, was dissolved in 2.5 ml of MM and further diluted to provide a concentration in the experimental cultures of 0.06 μg/ml. Greater concentrations of actinomycin caused detachment of cells from glass.

Experiments and Results. Effect of 6-MP on the multiplication of primary chicken fetal cells. Cultures were seeded in replicate with 1.5×10^6 cells and 24 hr later four cultures were removed for assessment of the cell population. Cells were harvested from cultures with trypsin (0.2%) and counted in a hemocytometer. Other cultures were replenished with fresh GM and GM containing 6-MP in final concentrations of 0.3, 3, and 30 μg/ml. At 24, 48, and 96 hr, three cultures from each group were harvested and their cell populations were quantitated. As with previous experiments with heteroploid cells in our laboratory, the chicken fetal cell population of cultures ranged approximately 15% on either side of the means depicted in Fig. 1.

Although 3 μg/ml of 6-MP effectively interrupts the replication of established heteroploid human cells (8), 30 μg/ml was required to alter the multiplication of primary chicken fetal cells.

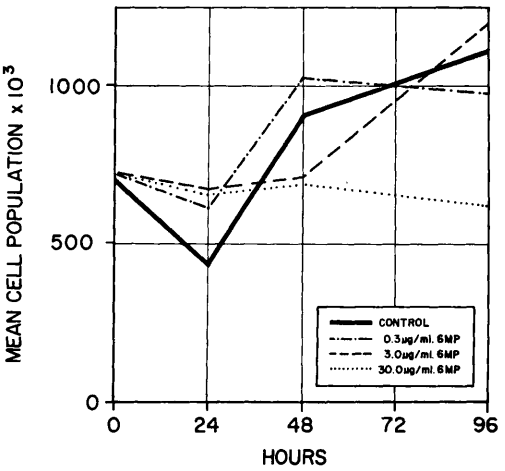

FIG. 1. The effect of 6-mercaptopurine on the multiplication of primary chicken fetal cells: Replicate cell cultures were prepared in 16 × 125 glass tubes, harvested in triplicate with trypsin (0.2%), and counted in a hemocytometer. The control cultures were replenished at zero time with growth medium ($BoFe_{10}E_{90}$). The experimental cultures were fed with growth medium containing 6-MP.

Effect of 6-MP on the synthesis of interferon. Chicken fetal cell cultures were pretreated with actinomycin, 0.06 μg/ml, or 6-MP, 3 and 30 μg/ml, for 4 hr, then rinsed twice with BSS, and inoculated with inactivated chikungunya virus. Chikungunya virus was adsorbed to chicken fetal cells in 0.1-ml volumes for 1 hr at 35°. Following adsorption of virus, 0.9 ml of fresh MM was added to the cultures.

Another group of cultures was pretreated with 6-MP, 3 and 30 μg/ml, for 4 hr. The cultures were rinsed and infected as above and then replenished with the same concentration of antimetabolites for the duration of the experiment, 24 hr. The third group of cultures was pretreated with MM alone, infected, and replenished with MM. These cultures were the interferon controls. The final group of cultures was left uninfected but nourished with MM alone or MM with similar concentrations of antimetabolites as above for the same periods of time. These cultures constituted the spent medium and antimetabolite controls. Three cultures were

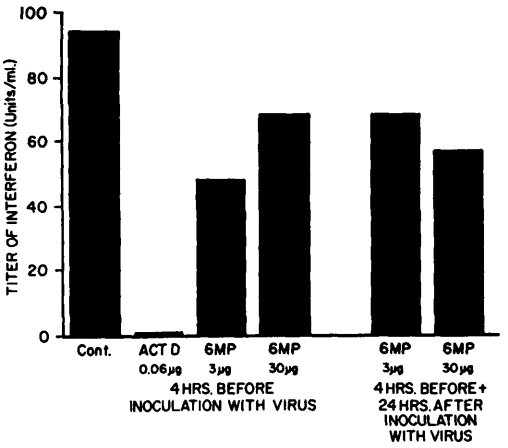

FIG. 2. The effect of 6-mercaptopurine on the synthesis of interferon: Primary chicken fetal cells were treated with 6-MP or actinomycin D for 4 hr prior to interferon-evocative inoculation with heat-inactivated chikungunya virus. Cells were also treated with 6-MP before and after inoculation. The titer of interferon represents the reciprocal of the dilution of test fluid which protected 50% of the combined cell population from the cytopathic effect of Sindbis virus.

used for each experimental manipulation and the fluids from all of these triplicate culture sets were harvested after 24 hr and pooled for assay of interferon.

The titer of interferon in control culture fluids was 94 units/ml (Fig. 2). Actinomycin completely blocked the synthesis of interferon. Although the titers of interferon in cultures treated with 6-MP were uniformly less than that of control cultures, ranging from 47 to 67 units, the end points fell within the limit of the usual 2-fold variation of such an assay. The fluids from spent medium and antimetabolite control cultures were diluted 10-fold and failed to inhibit the cytopathic effect of challenge Sindbis virus.

Effect of 6-MP on the action of interferon. Chicken fetal cells were treated with 6-MP, 3 and 30 μg/ml, or actinomycin, 0.06 μg/ml, or MM for 4 hr. These cultures were rinsed, treated overnight with 2-fold dilutions of a standard chicken interferon preparation, rinsed again, and challenged with 1000 ID_{50} of Sindbis virus. Final protective end points were determined at 72 hr. These studies revealed complete inhibition of the protecting effect of the interferon standard by actinomycin (Fig. 3). The titer of chicken interferon was 188 and 133 units in cultures pretreated with 6-MP. These titers do not differ significantly from the control value of 112 units.

During the same experiment, additional cultures were pretreated with the usual concentrations of 6-MP for 4 hr, but the antimetabolites were left in the liquid medium during the subsequent 24-hr treatment of cultures with dilutions of interferon. The titer of interferon was unaltered in cells treated continuously with both concentrations of 6-MP (Fig. 3).

Discussion. It was anticipated that 6-MP would inhibit the synthesis and the action of interferon. There may have been an inadequate period of cellular exposure to 6-MP before either the induction of interferon by virus or the induction of antiviral protein by interferon. Timing is important because the transcriptive phase of interferon synthesis lasts only a brief 1.5 hr following infection with chikungunya virus *in vitro* (10). It is also apparent that the effect of interferons on polysome function occurs within several hours (11, 12). The antiviral response of cells exposed to interferon is maximal following only 20 min of presumed binding at a superficial cellular site (13).

Previous studies revealed that exposure of

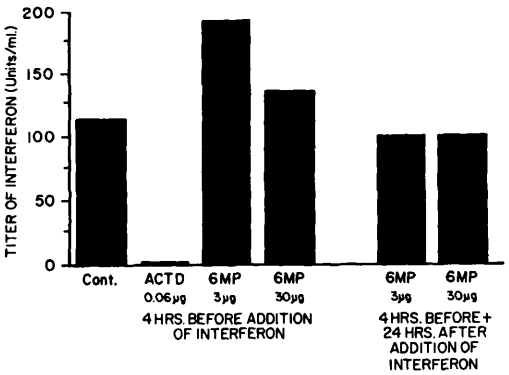

FIG. 3. The effect of 6-mercaptopurine on the action of interferon. Primary chicken fetal cells were treated with antimetabolites prior to and during exposure to dilutions of standard chicken interferon. Challenge Sindbis virus was added to cells following their treatment with antimetabolites and the potency of the interferon preparation was titered.

established heteroploid human cells to 6-MP for only 4 hr diminishes the incorporation of thymidine-^{3}H and uridine-^{3}H (14). Ribosomal RNA synthesis was also reduced in these experiments. So, the 4-hr period of treatment of primary chicken fetal cells *before* addition of evocative chikungunya virus or interferon should have been adequate to sustain a biologic effect.

The immunosuppressive drugs, 6-MP and azathioprine, alter the resistance of the intact host to viral infection (15, 16). Since 6-MP does not inhibit the synthesis or the action of interferon, the fundamental basis for this iatrogenic alteration of the host–virus relationship may not involve the protective response of the infected cell. The most crucial and susceptible component of viral resistance in this circumstance may be lymphocellular delayed hypersensitivity.

Summary. The purine analog, 6-mercaptopurine, failed to inhibit the synthesis and the action of interferon in primary chicken fetal cells. Actinomycin D, included as a positive control, completely blocked the synthesis and the action of interferon. Several aspects of host resistance to viral infection other than interferon are impaired by 6-mercaptopurine and delayed hypersensitivity may be the more important component.

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