MuMTV, AMV, and MuLV when these viruses were mixed with human milks. This decrease could be correlated with the amount of ribonuclease found in the milk.

4) Unclassified viruslike particles as well as C-type and occasionally B-type particles are found in human milk samples, which also contain a virulytic factor or factors in varying amounts. Correlation between family history of breast cancer and the presence of viruslike particles or reverse transcriptase in human milk samples is poor. Methods of preserving and separating the various types of particles are rapidly improving.

5) The elution profile of a poly(dT) synthetase from human milk particles on phosphocellulose columns was found to be strikingly different from that of known oncornaviruses.

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Interferon

The interferon system has been recognized as a major defensive response of the host, whether cell or animal, to infection by viruses and possibly by other agents. Apart from its theoretical aspects, interferon is potentially important in human chemotherapy, both in virus and tumor treatment. The current status of interferon research was summarized in a recent international workshop which was held in Williamsburg, Virginia, under the auspices of the Antiviral Substances Program of the National Institute of Allergy and Infectious Diseases.

The first session was devoted to control mechanisms in interferon induction. Studies with metabolic inhibitors have suggested that interferon production by the cell may be controlled in the following way. Messenger RNA (mRNA) for the interferon molecule (a protein) is not expressed in unstimulated cells because of the presence of a repressor protein that binds to the interferon mRNA. When a cell is "induced" to make interferon, the repressor is inactivated, or perhaps its production is stopped, so that the interferon mRNA can be translated. Under some conditions the amount of interferon produced is actually increased in the presence of metabolic inhibitors; one proposed explanation is that the interferon mRNA continues to be produced and translated while the repressor protein is not. Some investigators think that other control mechanisms are likely to operate during transcription.

The event in virus replication that triggers the production of interferon remains only partially defined. Double-stranded forms of RNA are in general better inducers of interferon than are single-stranded forms, and this has been interpreted by some to indicate that the double-stranded nucleic acids formed during virus replication are the triggering entities. However, the fact that under some conditions with Chikungunya virus one can get more than normal amounts of interferon in the absence of any detectable viral RNA synthesis, either single- or double-stranded, would suggest that some activity of the input virion may be the initiating process. Other data suggest that function of virion-bound polymerase may be important in induction by Newcastle disease virus, while with reovirus viral assembly may be responsible.

From studies with derivatives of the synthetic inducer polyinosinic acid · polycytidylic acid [poly(I) · poly(C)] it was concluded that: (i) an unblocked 2′-hydroxy group is needed for activity, and (ii) modifying the structure so as to alter toxicity and activity did not alter the therapeutic index. The possible importance of the cell membrane in interferon induction by poly(I) · poly(C) is becoming increasingly apparent. In regard to stimuli of microbial origin, two materials were discussed: it has been determined that the essential moiety of the lipopolysaccharide interferon stimulator from endotoxin is the lipid A fraction; a soluble protein from *Escherichia coli* was also described as a potent inducer of interferon.

Included in the second session was a discussion on the molecular mechanism of the antiviral action of interferon. Most investigators agreed that cells initially treated with interferon show a selective defect in the translation of a viral genome. This selection defect has been demonstrated both in whole cells and with cell-free systems. The translation of viral RNA may be inhibited to a much greater extent if the cells that furnish the cell fractions are first treated with interferon and then infected with virus as compared to interferon treatment alone. The factors responsible for the decreased translation are in the cell sap and on the ribosomes. Data were presented indi-

cating that in the normally infected cell there are proteins that bind to mRNA's of both viral and cellular type, but that in cells exposed to interferon the binding of viral RNA to cell protein is greatly decreased. It is thus possible that inhibition of translation is attributable to the modification of the protein factors that bind to mRNA prior to translation. When fractions from cells are exposed to very large amounts of interferon, translation of hemoglobin 9*S* mRNA is also inhibited, but translation of endogenous and synthetic mRNA is only slightly affected. However, the above-mentioned investigations are being conducted with interferons of varying degrees of purity, and hence preparation of pure interferon for biochemical and biological studies is necessary.

A possible second mechanism of the antiviral action of interferon involves an inhibition of transcription of viral genome by virion-bound polymerase. Studies with intact cells have shown that indeed there is less radioactivity in new early mRNA in interferon-treated cells than in those that were not treated with interferon, under conditions where the radioactive mRNA is probably the product of the action of the virion polymerase. However, several possible explanations other than an inhibition of the polymerase were advanced to explain the decrease, and the question was not resolved.

Interferon preparations exert an antitumor effect in experimental animals either infected with oncogenic viruses, or inoculated with transplantable tumors, or treated with chemical carcinogens. Interferon preparations enhance the activity of sensitized lymphocytes and unsensitized macrophages, and sometimes increase the antibody response to certain antigens. These preparations inhibit the growth of some tumor cells and even of some normal cells in tissue culture. Whether interferon inhibits the multiplication of tumors directly or by enhancing the host's ability to reject the tumor remains to be determined.

A session on methodology dealt with the production, purification, and assay of interferons, and with reference reagents. Considerable efforts have been made to improve methods for producing human interferon, intended either for therapy or as a source from which pure interferon might be made for structural studies. As was mentioned earlier, yields of interferon have been increased by utilizing

metabolic inhibitors during production. Prior treatment of cells with small amounts of interferon also increases the yield of new interferon when these cells are subsequently stimulated. With the use of human diploid cells, one laboratory has been able to produce material having a titer of 10^5 units.

Mouse interferon has been purified to the point of yielding a product with 10^8 reference units per milligram of protein. No comparable degree of purification has been obtained with human interferon. Two factors that contribute to purification difficulties are (i) the low isoelectric point of human interferon, which precludes the use of certain chromatographic procedures that have been useful with other interferons, and (ii) the apparently greater instability of human interferon.

The various forms of interferon from any one animal species vary considerably in size and charge. Some data have been interpreted to suggest that some, though not necessarily all, of the size heterogeneity comes from the view that native interferons are oligomers containing 2, 4, or 8 identical subunits that can be dissociated at low salt concentrations.

Interferon assays still depend on inhibition of virus replication. In one new method the amounts of specific neuraminidase resulting from influenza virus infection are measured, and this forms the basis for another precise and sensitive interferon assay.

Reference standards of interferon are available for chick, mouse, rabbit, and man; there is also a poly(I) · poly(C) reference standard for interferon inducers. These standards may not be totally satisfactory, but they do provide some means of comparison from one laboratory to another.

With respect to the possible role of interferon in the control of disease, evidence presented from experimental animal models indicates that both exogenous interferon and endogenously induced interferon [poly(I) · poly(C) being the inducer most frequently utilized] have been successful in the prophylaxis and early therapy of lytic as well as oncogenic virus infections.

Criteria utilized for selecting model systems for therapy studies include (i) the host must be at risk from further virus replication, (ii) the virus infection must be sensitive to the quantity of interferon applied, (iii) the host must be responsive to the interferon inducer (even though the prior viral infection may have induced a

state of hyporeactivity to further production of interferon) and to interferon, and (iv) interferon, or inducer, must reach the site of infection. Some investigators have found that exogenous interferon is more effective than endogenous; others have found them equally effective. There were also reports of growth inhibitory action against certain protozoa and intracellular shigella. Potentiation by interferon of the toxicity of some double-stranded nucleic acids was also reported.

Problems identified with the potential utilization of exogenous interferon include: the short half-life of exogenously administered interferon in vivo, the low dosages available for therapeutic trials, the difficulty of injection at site of infection in most clinical circumstances, the diffusion gradients between blood and site of infection (such as the blood-brain barrier). Problems associated with inducers [primarily based on data from studies with poly (I) · poly(C)] include: toxicity, possible immunologic alterations, hyporeactivity, and decreased capacity of the infected host to produce interferon. These problems must be considered in planning clinical trials.

The reticuloendothelial system may be the source of the interferon appearing in response to certain inducers. Studies with the low-molecular-weight inducers, such as Tilorone and perhaps endotoxin, have demonstrated that a glass-adherent cell (presumably a macrophage) obtainable only from lymph nodes or spleen, respond with interferon production in vitro. Studies of interferon stimulation by nonspecific mitogens and Newcastle disease virus, as well as with viral and nonviral antigens reacting with immunologically sensitized cells, have demonstrated that interferon (as well as lymphokines) can be produced by stimulated lymphocytes. The immune-specific interferon response is increased by addition of macrophages to lymphocyte cultures. Gradient separation of lymphocytes suggests that the blastic response occurs in cells other than those producing interferon. Studies with antiserums to the theta factor have implicated thymus-derived lymphocytes in interferon response of mouse lymphocytes to concanavallin A, phytohemagglutinin, and pokeweed mitogen.

The administration of old tuberculin causes the production of circulating interferon by mice infected with BCG (Bacille Calmette-Guérin). This interferon, but not other types of interferon, seems to be closely associated with migration inhibitory factor.

Studies of the effect of interferon on lymphocyte function indicate that relatively high titers of interferon diminish the blastogenic response of lymphocytes stimulated by nonspecific mitogens, and that under certain conditions interferon preparations may potentiate antibody production in mice, while under other conditions no enhancement was noted.

When the use of human interferon in patients was discussed, one investigator reported that daily parenteral administration of up to 3×10^6 units of interferon over periods of up to 1 year produced only some fever and no permanent effects. Diffusion of interferon is known to be retarded by effective blood-brain, blood-eye, and blood-respiratory tract barriers. Poly(I) · poly (C) has also been given to many patients with cancer and neurological disease with no significant metabolic or hematological adverse effects. Rather low levels of circulating interferon were produced by patients receiving poly (I) · poly(C). In one patient (report

from Ottawa) with subacute sclerosing panencephalitis, convulsions apparently were precipitated by administration of intravenous poly(I) · poly(C). Although poly(I) · poly(C) is not oncogenic in hamster or mouse tissue in vitro or in vivo, a strain of rat cells that is highly susceptible to transformation was transformed by poly(I) · poly(C).

Local administration of interferon can prevent cutaneous infection with vaccinia virus in monkeys and in man. Human interferon can retard systemic viral infection of monkeys. In volunteers receiving influenza or rhinovirus, intranasal administration of increasing amounts of interferon gives either no protection or a small protective effect. With even larger doses in monkeys there is significant reduction in the excretion of a parainfluenza virus and of equine rhinovirus. Studies with increased amounts of interferon given intranasally to volunteers are being made. Partial protection against rhinovirus and influenza virus infections and low levels of induced interferon is reported in volunteers given poly(I) · poly(C) as nose drops. Poly(I) · poly (C) administered in eye drops can accelerate healing of herpetic dendrite ulcers; this effect may be due to the influence on corneal cells of interferon induced in the conjunctiva. Tilorone derivatives, which induce interferon in rodents but not in monkeys, prevent viremia in monkeys given Venezuelan equine encephalitis vaccine.

Live infectious bovine rhinotracheitis virus vaccine, which induces interferon if given into the respiratory tract of calves, dramatically reduces symtoms of and subsequent death from respiratory disease occurring in calves kept in feed lots. Rhinovirus infection of man interferes with the take of a live influenza vaccine, and prior injection of rubella vaccine prevents respiratory virus infection; but attenuated influenza virus infection does not protect against experimental parainfluenza infection 1 week later.

Poly(I) · poly(C) treatment of rabbit skin protects against tumors induced by Shope fibroma virus. It also protects against experimental rabies and induces the local production of interferon and a greater amount of antibody. This is an example of the multiple actions brought about by chemical inducers.

Interferon preparations and inducers administered to animals limit the growth of tumors or leukemia and prevent metastases. In patients with severe disease (malignancies, leukemia, and subacute, sclerosing panencephalitis) poly(I) · poly(C) treatment induces low levels of interferon and has no effect on the primary disease.

In man, large amounts of interferon have been detected at the sites of certain, but not all, infections. Long-term production of interferon has been observed in a case of congenital rubella infection. Studies to exploit these phenomena for prophylactic or therapeutic purposes are planned or under way.

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