Meetings

Molecular Biology of Human Milk

Viruslike particles are observed in some human milks examined by electron microscopy. In addition, human milk particles having the same buoyant density range as known RNA tumor viruses have been shown to contain RNA-dependent DNA polymerase (reverse transcriptase) and also 70*S* RNA —characteristics common to all known oncogenic RNA viruses (oncornaviruses). Many questions concerning these particles are being studied, such as whether the particles have an etiological role in human breast cancer, whether their presence correlates with the risk of getting breast cancer, and whether they share any other characteristics with known oncornaviruses. The inhibitory factors in human milk which cause destruction of added known oncornaviruses are also under scrutiny. A meeting was convened at the Institute for Medical Research (IMR), Camden, New Jersey, on 11 December 1972 to foster communication between 12 research groups studying the particles in human milk.

Polyadenylate [poly(A)] sequences about 200 nucleotides long have been found in the 70*S* RNA of oncornaviruses. D. Colcher, J. Schlom, and S. Spiegelman (Columbia University) opened the meeting by reporting their studies—done in collaboration with D. Gillespie of the National Cancer Institute (NCI)—to detect poly(A) sequences in human milk particles. They used radioactive polyuridylate [poly(U)] for hybridization against RNA isolated from human milk particles. Of 25 human milks examined, 24 percent were found to contain poly(A) sequences. Size determination for the poly(A)·poly(U) complex on 10 percent polyacrylamide gels (done for two samples) revealed a homogeneous poly(A) sequence about 200 nucleotides long. The poly(A) assay can be used to quantitate RNA tumor virus and viruslike particles from human milk.

H. Chopra and P. Ebert (NCI) reported their electron microscopic and reverse transcriptase studies with human milks. They could not detect any particle resembling type B murine mammary tumor virus (MuMTV) in 460 milks examined, but 50 milks were found to contain type C–like particles. Further, there was no correlation between the detection of viruslike particles and family history of breast cancer in the milk donors. Ebert and Chopra examined 40 milk samples by the technique of simultaneous detection for reverse transcriptase and 70*S* RNA in human milk particles. By this technique, 77 percent of the milks were found to be positive.

B. Gerwin (NCI) reported that no enzyme like those of the oncogenic viruses can be purified from human milk. A polydeoxythymidylate [poly(dT)] synthetase is found which elutes at a KCl concentration of 0.55 to 0.65*M*. This enzyme from human milk has not been found in several human cell lines examined. The presence of this enzyme does not correlate with positive endogenous reactions (in which no synthetic template is added) or with simultaneous detection reactions, or with positive family history of breast cancer.

An inhibitory factor (or factors) in human milk interferes with reverse transcriptase activity of oncornaviruses, as determined by measuring the recovery of reverse transcriptase activity when the viruses are mixed with human milk. The nature of the inhibitor (or inhibitors) was discussed at the meeting. N. H. Sarkar (IMR) found considerable morphological destruction of MuMTV added to some human milks. There was also a loss of reverse transcriptase activity. He found that the fat fraction of human milk contains maximum inhibitory activity. In contrast, W. Feller (Georgetown University), using avian myeloblastosis virus (AMV), found the majority of the inhibitory activity in the high-speed pellet fraction of human milk.

J. McCormick and M. Rich (Michigan Cancer Foundation, Detroit) reported that ribonuclease present in human milk contaminates the particle preparation and interferes with the simultaneous detection test for reverse transcriptase, so that when Rauscher leukemia virus was added to human milk the reverse transcriptase activity of the virus was markedly inhibited. Thus, negative results for reverse transcriptase in human milk by the simultaneous test do not necessarily indicate absence of virus, and the results of this method as presently carried out in human milk cannot be expected to correlate with other indications of the presence of a virus.

J. Charney (IMR) and N. H. Sarkar reported on the infectivity titers of MuMTV after the virus was mixed with whole human milk and with the skim and cream fractions. Of the initial infectivity, less than 10^{-4} was left after virus was mixed with cream; the corresponding values were 10^{-3} for whole milk and 10^{-2} for skim milk. There was a high correlation between the loss of titer and the destruction of B particles as seen in the electron microscope. Although ribonuclease may be a factor in the loss of reverse transcriptase caused by many human milks, there must be another factor that acts on the whole virion and destroys the infectivity. Sarkar pointed out that ribonuclease has no effect on whole virions or on nucleoid (viral core) preparations.

A. Dion and A. Vaidya (IMR) described the problems encountered in carrying out correlative studies between the detection of viruslike particles by electron microscopy and the presence of reverse transcriptase in human milks. In addition to the presence of inhibitory factors, it is possible that cryptic viruses without the enzymatic activity, as well as low concentrations of particles with high polymerase activity, might also be causing these problems. On the basis of his studies with MuMTV, Dion recommended the following procedures to circumvent these problems and to increase the sensitivity of the reverse transcriptase assay: (a) banding of the particles in density gradients; (b) prior incubation of the particles at 37°C for 10 minutes after the disruption; and (c) addition of oligo(dT) (about ten nucleotides long) to the reaction mixture. By using these techniques, 72 percent of the milk samples were found to be posi-

tive for the presence of reverse transcriptase and DNA product bound to 70*S* or 35*S* RNA.

M. Apple (University of California, San Francisco) described a technique for aspirating fluids from nonlactating human breasts. Apple reported his studies in which synthetic templates were used to detect DNA polymerase in the fluids from nonlactating breasts. A specified level of enzyme activity was found in 41 of 116 women without family history of breast cancer, 8 of 9 women who themselves had undergone mastectomy, and 13 of 17 women with family history of breast cancer.

P. Roy-Burman (University of Southern California) has been testing for reverse transcriptase activity in milk samples from wild mice trapped from three areas. Electron microscopy of breast biopsies, breast tumors, and the banded milk particles of these mice showed both type C and type B particles. Both types of milk particles contained reverse transcriptase activity, and antibodies against reverse transcriptase from murine leukemia virus (MuLV) inhibited the enzyme of the type C virions from these milks. Roy-Burman also reported that reverse transcriptase activity of added RD-114 virus (a candidate human oncornavirus) disappeared completely, as determined by the simultaneous detection technique, in 11 of 17 human milk samples. In his laboratory, 8 of 43 randomly selected selected human milk samples were found to contain reverse transcriptase and 70*S* or 35*S* RNA.

D. Gillespie (NCI) reported that 70*S* RNA containing homogeneous poly(A) sequences about 200 nucleotides long was found in human leukemia cells. Gillespie also reported that DNA synthesized with RNA templates from four different strains of MuLV failed to cross-hybridize against the RNA's isolated from these strains of viruses.

The outcome of the meeting can be summarized as follows:

1) Reverse transcriptase and 60*S* to 70*S* RNA are being found in human milk particles having a buoyant density of 1.16 to 1.19 g/ml by all groups making such studies.

2) The particles isolated from some human milks were found to contain poly(A) sequences in their RNA molecules.

3) Some human milks mixed with MuMTV caused severe loss of infectivity and morphological destruction of the virions. There was also a decrease in reverse transcriptase activities of

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-