RNA-DEPENDENT DNA-POLYMERASE ACTIVITY IN HUMAN MILK

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Particles resembling type B mouse mammary tumour virus have been detected by electron microscopy in human milk (Feller and Chopra, 1969; Moore et al., 1969, 1971; Sarkar and Moore, 1972). Their frequency was found to be much higher in women with a family history of breast cancer than in women without such a history (Moore et al., 1971). Schlom, Spiegelman and Moore (1971) were able to correlate the presence of the particles with RNA-dependent DNA-polymerase activity, now known to be associated with oncornaviruses, in the milk. On the other hand, Calafat and Hageman (1973) failed to find either type B or type C-like particles in human milk.

We have applied a simple and reliable test for RNA-dependent DNA-polymerase activity to determine indirectly the presence of oncornavirus particles in milk samples obtained from nursing mothers. The test examined (1) the ability of the pellet obtained by centrifuging milk at 145,000g to incorporate radioactive thymidine triphosphate into DNA in the presence of all four unlabelled deoxyribonucleoside triphosphates, and (2) the sensitivity of this incorporation to inhibition by ribonuclease, as a check on the RNA-dependent nature of the enzyme reaction.

MATERIALS AND METHODS

Ten- to 30-ml samples of milk were collected from mothers at the Grace Maternity Hospital, Halifax, NS., on the 5th or 6th day post-partum. Each sample was taken from a single breast. The women were questioned at the same time about the family history of breast cancer. The migratory nature of the population made it impossible to check the family history from medical records, but any women who were doubtful on this point were excluded from the study.

All samples were tested within 1 h of collection. The method was a modification of the technique of Schlom, Spiegelman and Moore (1972). The milk, diluted with an equal volume of 0-15M EDTA (disodium ethylenediaminetetraacetic acid) was centrifuged in a refrigerated International Centrifuge, model B-20, for 10 min. at 3000g. The clarified fluid between the surface layer and the casein deposit was removed and centrifuged again for 10 min. at 3000g to separate out the final content of lipid. This was carefully discarded, and the sample was incubated at 30°C for 30 min. with trypsin, added to a final concentration of 1 mg per ml. Lima-bean trypsin-inhibitor (Sigma Chemical Co., St Louis, Miss., USA) was then added to a final concentration of 0-5 mg per ml, and the treated material was layered over 10 ml of 20% glycerol and centrifuged at 145,000 g for 60 min. in a model B-60 refrigerated centrifuge. The resulting pellet was suspended in 0-5 ml of 0-01M tris-hydroxymethyl aminomethane (pH 8-3) containing 0-33% (v/v) NP-40 detergent and 0-1M dithiothreitol, and kept at 4°C for 30 min.

The suspension was added to a standard reverse-transcriptase reaction-mixture containing 10 μmole of tris-hydroxymethyl aminomethane (pH 8-3), 1 μmole of magnesium acetate, 12 μmole of sodium chloride, 4 μmole of dithiothreitol, 0-2 μmole each of unlabelled deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytosine triphosphate, deoxythymidine triphosphate and (3H) deoxythymidine triphosphate at a specific activity in the mixture of 4000 c.p.m. per p.mole. After incubation at 37°C for 45 min. the reaction was stopped by addition of 1 ml of 0-08M sodium pyrophosphate, 0-4 mg of Escherichia coli B transfer-RNA and 2 ml of 25% (w/v) trichloracetic acid. The sample was collected on
Family history of breast cancer and breast-nursing history of patients with RNA-dependent DNA-polymerase activity in their milk

<table>
<thead>
<tr>
<th>Patients (case number)</th>
<th>Family history of breast cancer</th>
<th>Whether breast-fed as a child</th>
<th>Number of infants breast-fed by patient</th>
<th>Size of milk sample examined (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>88</td>
<td>None</td>
<td>Not known</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>102</td>
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<td>11</td>
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<td>1</td>
<td>19</td>
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<td>168</td>
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<td>Yes</td>
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<td>30</td>
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<tr>
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<td>Not known</td>
<td>1</td>
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<tr>
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<td>1</td>
<td>8</td>
</tr>
<tr>
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<td>Aunt</td>
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<tr>
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<td>Aunt</td>
<td>No</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>184</td>
<td>Grandmother and aunt</td>
<td>Yes</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

Whatman glass-fibre filters (GF/A), washed with 50 ml of 5% trichloracetic acid and then with 25 ml of 95% ethanol, placed in a vial containing 15 ml of 0.4% BBOT [2,5-bis-(tert-butylbenzoxazolyl) thiophene] in toluene and counted in an Isocap/300 Nuclear Chicago Scintillation Counter with a counting efficiency for tritium of 55%.)

RESULTS

Milk from 137 nursing mothers without a family history of breast cancer was examined, and five samples (3.6%) were found to be positive for RNA-dependent DNA-polymerase activity (table). Among samples from 31 nursing mothers with a family history of breast cancer, six (19.3%) were positive. In no case was there any evidence of incorporation of radioactive thymidine triphosphate in the presence of ribonuclease. The actual size of the milk sample did not influence the amount of enzyme activity; positive results were obtained from samples that ranged in size from 8 to 30 ml (table).

DISCUSSION

Only about 10% of patients in the Grace Maternity Hospital breast feed their infants and approximately 10% of them have a family history of breast cancer. However, even with our restricted numbers, we have been able to show a higher frequency of RNA-dependent DNA-polymerase activity in milk from women with a family history of breast cancer than in milk from women without such a history. Our figure of 3.6% for polymerase activity among women without a family history of breast cancer is similar to the frequency of B-type particles (5%) reported by Moore et al. (1971) in such women, but we detected polymerase activity in only 19.3% of women with a positive family history, compared with their finding of B-type particles in 40–60% of cases. This discrepancy may be due to the instability of the human milk virus, because Moore (1974) was able to show a difference in polymerase activity in "fore" milk (milk stored in the ducts) and "hind" milk (freshly synthesised milk); in our study no attempt was made to separate "fore" and "hind" milk. This could also possibly account for the failure of Calafat and Hageman (1973), in their study of 43 Dutch women, to detect virus in milk samples by electron microscopy. The RNA-dependent DNA-polymerase activity, however, is probably more stable than the morphological integrity of the virus particle (Moore, 1974).
We were unable to obtain reliable nursing histories from the majority of women in the study, and most of the patients with positive polymerase activity were primiparae (table). It was not possible, therefore, to examine reliably for any correlation between enzyme activity in the milk and either the nursing history of the patient herself as a child, or the number of infants she had breast-fed (Borden, 1974).

Because of the promising results obtained from these preliminary studies we plan to investigate larger numbers of women, especially women with a family history of breast cancer, and to use the artificial template poly rC oligo dG(12-18); this has been found to be specific for oncornavirus RNA-dependent DNA polymerase (Moore, 1971). The use of the template may help to overcome the lack of stability of the virus particle and its associated polymerase.

**SUMMARY**

A simple method is described for testing milk specimens from nursing mothers for the presence of RNA-dependent DNA-polymerase activity. Positive results were obtained in five of 137 women (3.6%) without a family history of breast cancer, and in six of 31 women (19.3%) with a family history of breast cancer.

We are indebted to the doctors and the nurses (particularly Mrs J. Elloway and Mrs E. Joudrey) of the Grace Maternity Hospital for excellent co-operation.

**REFERENCES**


