Brief Communication

Effect of Human Milk on the Mouse Mammary Tumor Virus

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SUMMARY

Mouse mammary tumor virus when mixed with whole, skimmed, or cream fractions of some human milks and incubated at 37° for 18 hr resulted in degradation of the particle morphology and decreased infectivity and reverse transcriptase activity of the virions. The cream fraction was more effective in the physical, biochemical, and biological degradation of the virions than the whole or skimmed milk, but the nature of the degrading component(s) has not been determined. The significance of the destructive effect of human milk upon mouse mammary tumor virus in relation to our search for the putative human breast cancer virus particles and reverse transcriptase activity in human milk isolates has been discussed.

INTRODUCTION

The composition of milk differs greatly from one species to another and even among individuals of the same species. These differences have been made especially apparent during the search for viruses associated with breast cancer (3–8). MuMTV2 is relatively stable in mouse milk but seems to be unstable when mixed with some human milks; the morphological degradation and the loss of reverse transcriptase activity of MuMTV, has been mentioned earlier (6,8). Of the methods of detection of MuMTV, we found that the bioactivity assay is more sensitive than the electron microscopic findings of particles or the reverse transcriptase assay. This paper reports a correlative study between the extent of MuMTV virion degradation, the loss of infectivity, and the reduction in RNA-dependent DNA polymerase caused by some human milks.

MATERIALS AND METHODS

Skim milk from high-mammary-tumor strain (incidence 97%) RIII/Haag mice was diluted 1:10 with: Sample A, PBS; Sample B, whole human milk; Sample C, a skim milk fraction prepared from an equal amount of the same milk used in Sample B; and Sample D, a cream fraction obtained during the preparation of Sample C which was reconstituted to the original milk volume by dispersion in PBS. Each of these preparations was incubated at 37° for 18 hr, and a portion of each was titrated for infectivity in C57BL/Haag mice (1). The remaining portions were centrifuged for 30 min at 25,000 rpm in an SW27 rotor at 4°, and each pellet was resuspended in 0.1 ml PBS. Specimens negatively stained with sodium phosphotungstate, pH 7.0, were examined in the electron microscope for the condition of the virions (4).

To test the effect of human milk on the morphology and reverse transcriptase activity of RIII milk virions, 0.4-ml aliquots of the same sample of RIII skim milk (approximately 10^12 B particles/ml) were diluted 1:10 with PBS (control) or with samples of human milk, and the mixtures were incubated at 37° for 18 hr. All but one (H806) of the human milk samples, in this particular experiment, were skimmed by dilution with an equal volume of 0.15 M EDTA (pH 7.4) and centrifugation at 12,000 X g for 15 min. In the case of H806, 0.1 volume of RIII skim milk was added directly to whole human milk. Duplicate samples were prepared for both electron microscopy and reverse transcriptase assays. After incubation at 37° for 18 hr, each sample was centrifuged at 12,000 X g for 15 min after the addition of 6 ml 0.01 M Tris:0.15 M NaCl:0.002 M EDTA buffer at pH 8.3 and 10 ml 0.15 M EDTA. Virions in the skim milk fractions were then pelleted at 25,000 rpm (SW 27 rotor) for 30 min. The ratio of intact to damaged MuMTV virions was estimated by electron microscopy. For the analysis of RNA-templated DNA polymerase activity, the high-speed pellet was resuspended in 0.01 M Tris:0.02 M phosphate buffer, pH 7.4, containing 0.15 M NaCl.

RESULTS

The control portion of the RIII milk contained typical B
Table 1

Effect of human milk on MuMTV infectivity

<table>
<thead>
<tr>
<th>No. of mice infected/no. of mice at risk at following dilutions</th>
<th>10⁻³</th>
<th>10⁻⁴</th>
<th>10⁻⁵</th>
<th>10⁻⁴</th>
<th>10⁻⁷</th>
<th>Approximate 50% infective dose in 0.1 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. PBS (control)</td>
<td>10/15 (67)%</td>
<td>12/15 (80)</td>
<td>7/13 (54)</td>
<td>7/14 (50)</td>
<td>5/14 (36)</td>
<td>&gt;10⁻⁶</td>
</tr>
<tr>
<td>B. Whole human milk</td>
<td>3/13 (23)</td>
<td>1/15 (7)</td>
<td>0/10 (0)</td>
<td>2/14 (14)</td>
<td>1/14 (7)</td>
<td>&lt;10⁻³</td>
</tr>
<tr>
<td>C. Skim milk fraction from Sample B</td>
<td>9/14 (64)</td>
<td>1/14 (7)</td>
<td>0/14 (0)</td>
<td>2/13 (15)</td>
<td>10⁻³</td>
<td></td>
</tr>
<tr>
<td>D. Cream fraction from Sample B</td>
<td>1/13 (7)</td>
<td>1/12 (8)</td>
<td>0/16 (0)</td>
<td>0/14 (0)</td>
<td>0/12 (0)</td>
<td>&lt;10⁻³</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of mice.

Table 2

Effect of human milk on reverse transcriptase activity of RIII MuMTV

<table>
<thead>
<tr>
<th>Human milk</th>
<th>Intact/damaged virions (estimated by electron microscopy)</th>
<th>TTP-³H (pmoles incorporated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H783 (skim)</td>
<td>0.76</td>
<td>0.32</td>
</tr>
<tr>
<td>H785 (skim)</td>
<td>0.72</td>
<td>0.31</td>
</tr>
<tr>
<td>H806 (whole)</td>
<td>0.00</td>
<td>0.13</td>
</tr>
<tr>
<td>H806 (skim)</td>
<td>1.08</td>
<td>0.66</td>
</tr>
<tr>
<td>H808 (skim)</td>
<td>0.97</td>
<td>0.39</td>
</tr>
<tr>
<td>F. D. (skim)</td>
<td>0.75</td>
<td>0.70</td>
</tr>
<tr>
<td>Mouse milk in</td>
<td>2.30</td>
<td>1.05</td>
</tr>
<tr>
<td>PBS (control)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

particles with uninjured spike-covered membranes and many tailed forms (Fig. 1a). Virions in RIII milk incubated with some human milk specimens or human milk fractions were damaged to varying degrees (Fig. 1, b to d). The damage to MuMTV inflicted by human milk seems to be primarily to the viral nucleoid since the particles appear empty. The characteristic head and tail formation does not occur. Most of the damage virions show surface projections, although a number of particles appear to have partially or totally lost the spikes. The effect of cream fraction of the human milk is, however, very drastic. After exposure to cream, no particles with distinct surface spikes were found (Fig. 1d).

The bioactivity of the mouse virus incubated in the whole human milk and in the reconstituted cream was reduced more than 1000-fold when compared with the same mouse milk incubated in PBS (Table 1). The data indicate that this milk was severely destructive to the MuMTV virions and that at least some of the injury was due to the cream fraction of the milk. The effect of the 5 different samples of human milk on the reverse transcriptase activity of RIII milk virions is recorded in Table 2. The decrease in reverse transcriptase activity in the skim milk fractions were then pelleted at 25,000 rpm (SW27 rotor) for 30 min and resuspended in 400 µl 0.01 M Tris (pH 8.3). Aliquots of 50 µl were preincubated at 0°C for 10 min after the addition of 10 µl 1 M dithiothreitol and 3 µl 10% NP-40. The reaction mixture contained (in µmoles/125 µl): Tris-HCl (pH 8.3), 6.25; NaCl, 1.25; MgCl₂, 1.0; and 0.2 µmole of each of the unlabeled deoxyribonucleoside triphosphates and TTP-³H. The specific activity of TTP-³H was 3172 cpm/pmole, and the reaction was terminated after 30 min at 37°C. Labeled product complexed to RNA was analyzed by electrophoresis on polyacrylamide gels.
correlates with the decrease in ratio of the intact to damaged particles. Chart 1 demonstrates the effect of whole and skimmed human milk on the 70 S RNA-templated DNA polymerase reaction of MuMTV. A decreased association of label 70 S RNA was observed when mouse milk-borne virions were diluted with human milk, as compared to the control, which was diluted with PBS.

DISCUSSION

The results reported here indicate that all 3 measures of MuMTV integrity (morphology, infectivity, and reverse transcriptase activity) are adversely affected by exposure to human milk or human milk fractions. The agent primarily destructive to morphology and infectivity has not been characterized. It segregates with the fat fraction of the human milk; either a chemical, enzymatic, or physical mechanism (surface denaturation or surfactant activity) is possible. It would appear that the primary effect upon the virus, as deduced from the morphology, is to render the virion permeable. This may suffice to degrade the virion structure and in turn reduce infectivity to the extent observed. A secondary effect may be due to the RNase(s) present in human milk. If the virion is rendered permeable, permitting nuclease penetration, the endogenous viral RNA template would be degraded and the enzyme (reverse transcriptase) would be lost during the sedimentation step involved in reverse transcriptase assay, resulting in reduced activity. In addition, nuclease contamination of the pelleted virus would further adversely affect the enzyme assay. RNase per se has no effect on the whole virions or on isolated nucleoids (7).

The presence of these degrading components in human milk may account for the difficulty of detection of the putative human mammary tumor agent. In human milk, particles similar in morphology with MuMTV have been observed only occasionally, and what appear to be fragments or morphologically changed particles are often found (5). These latter particles by a degenerative effect of human milk similar to that found for MuMTV in this study. This is in contrast to the mouse milk, in which virions are relatively stable and the infectivity and measurements of reverse transcriptase are easily reproducible.

The destructive effect of human milk is not only confined to the integrity of MuMTV but also seems to inhibit the reverse transcriptase activity of avian myeloblastosis virus (W. F. Feller, personal communication) and Rauscher leukemia virus (J. J. McCormick, personal communication). Furthermore, in a limited study similar to that with human milk, we observed no inhibitory effect of bovine milk on the reverse transcriptase activity of MuMTV.

REFERENCES


Fig. 1. Electron micrographs of negatively stained mouse MuMTV virions (type B particles) after incubation at 37° for 18 hr under various conditions. Electron microscope grids were prepared from resuspensions of high-speed pellets without further purification. a, virions incubated in PBS; some of the B particles show typical head and tail forms with spike-covered membranes; others vary greatly in size and shape; all have spikes. b, virions incubated in whole human milk; all of the particles have been damaged and the cores of most of them have been lost; some of the particles show complete loss of spikes (arrows) while others have retained them in varying state of preservation. c, virions incubated in human skim milk; most of the B particles have been damaged, but in general the degree of damage by skim milk is less than that by whole milk. d, virions incubated with human cream; all B particles appear damaged; no particles with distinct spikes could be found. Cream causes the most severe damage. × 90,000.

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