Mouse Sperm can Horizontally Transmit Type C Viruses

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SUMMARY

Washed, non-viable sperm from AKR and NZB mice contain infectious murine type C viruses (MuLV), whereas similarly treated sperm from Balb/c and probably C57Bl/6 mice do not. Interaction of washed, viable AKR, Balb/c and NZB sperm with tissue culture cells leads to a transfer of infectious MuLV to these cells. The most likely mechanism is infection by virus closely associated with the sperm. Another possible mechanism is the introduction of proviral DNA. These experiments indicate that type C viruses can be found with mouse sperm. They suggest sperm could horizontally transmit these MuLV to animals during copulation and to the germ line of mice during penetration of ova.

Studies involving embryo transplants and specific genetic crosses have established that murine type C viruses (MuLV) can be endogenous to the species and inherited through the germ cell (Gross, 1970; Barnes et al. 1972; Rowe, 1973). These endogenous MuLV have been classified descriptively by their host range. The ecotropic infect and grow in mouse and rat cells. The xenotropic can only productively infect cells heterologous to the species. They cannot exogenously infect mouse cells (Levy, 1973, 1974). The ecotropic can be further subdivided into N- and B-tropic viruses depending on their preference for growth in N- or B-type mouse cells (Lilly & Pincus, 1973). There is molecular evidence for one to several copies of ecotropic MuLV and six to nine copies of the xenotropic MuLV genome in the mouse chromosome (Gelb et al. 1973; Benveniste & Todaro, 1974; Chattopadhyay et al. 1974). As well as transfer of mouse type C viruses by genetic means, they can also be passed under certain conditions from the mother to the young through the milk (Law & Moloney, 1961; Gross, 1970; Gardner et al. 1979).

Since it is known that somatic cells can take up either viable or heat-killed spermatozoa (Bendich et al. 1974, 1976; Higgins et al. 1975), we wished to determine whether horizontal transmission of virus information can be achieved by this means. Experiments were designed to establish whether mouse sperm could introduce a type C virus into cells which normally do not harbour the virus. The results would indicate whether type C viruses could be transferred to eggs via infectious proviral DNA which might be present in the sperm head or via infectious particles associated with the viable sperm (Bentvelzen, 1974; Bendich et al. 1976).

For our studies, spermatozoa were isolated from the vas deferens of AKR, Balb/c, C57Bl/6 and NZB/J mice under sterile conditions and washed three times with Hanks' balanced salt solution. In most experiments, the sperm were semi-purified by sedimentation through sucrose to remove virus particles present in the seminal fluid. For these studies, the sperm were diluted with a 1:3 vol. (w/w) of 60% sucrose in 0.01 M-tris-HCl pH 7.5. The mixture was then layered on 28 ml of the same sucrose solution and centrifuged at 4 °C in a SW25.1 rotor for 45 min at 25000 g (Calvin et al. 1973). The pellet was resuspended in Hanks' solution and spermatozoa were counted after appropriate dilution. Aliquots were used for incubation with tissue culture cells and for scanning electron microscopy (EM) studies. Moreover, samples were seeded into culture dishes to determine whether the pellet contained co-sedimenting somatic mouse cells. These cultures receiving the spermatozoa were incubated for several weeks. In all the experiments, somatic cells were never observed.
Table 1. Recovery of type C viruses from mouse sperm preparations*

<table>
<thead>
<tr>
<th>Source of sperm (mouse strain)</th>
<th>Virus detected by EM</th>
<th>Somatic cells detected in culture</th>
<th>Virus recovered from frozen sperm</th>
<th>Virus recovered from cells incubated with viable sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR</td>
<td>0/4</td>
<td>0/4</td>
<td>4/4</td>
<td>4/4 N-tropic AKR-MuLV from NIH-ME and rat embryo cells</td>
</tr>
<tr>
<td>Balb/c</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>1/3 N-tropic AKR-MuLV after IdUrd treatment of NIH-ME</td>
</tr>
<tr>
<td>NZB/J</td>
<td>0/2</td>
<td>0/2</td>
<td>0/1</td>
<td>2/2 X-tropic MuLV</td>
</tr>
</tbody>
</table>

* Procedures employed are described in text. Each number corresponds to an individual experiment.
† Virus was not detected in the sperm preparation recovered after sucrose density-gradient centrifugation. In one experiment the frozen NZB sperm preparation thawed so that it could not be tested.

Aliquots containing approx. $10^7$ viable sperm were also frozen away at $-70^\circ$C for subsequent virus assay. Since freezing kills the sperm, these samples monitored infectious virus associated with the spermatozoa. For scanning EM, spermatozoa were fixed in 2.5% glutaraldehyde, dehydrated in ethanol, freeze-dried, coated with gold-palladium and evaporated using a Denton evaporation coater and viewed with a JEOL JSM-35 scanning electron microscope. In these studies, approx. 200 spermatozoa were examined and no virus-like particles could be detected.

The viable sperm preparations were mixed directly for 2 h at 37 °C with cell suspensions of NIH mouse embryo (ME), Fischer rat embryo, mink lung or human foreskin fibroblast (HuF) cells at a ratio of 10 sperm/cell by a technique previously described (Bendich et al. 1974). The number of sperm employed was approx. $5 \times 10^6$. The NIH-ME and rat embryo cells were used since they contain no ecotropic AKR-MuLV genome (Rowe, 1973; Chatto-padhyay et al. 1974). The HuF and mink lung cells were employed because they lack MuLV and are very sensitive for detection of mouse xenotropic virus (Levy, 1975). The cells, after receiving the sperm, were cultivated initially in plastic Petri dishes and then in flasks for up to 10 weeks in attempts to look for expression of ecotropic or xenotropic MuLV. All cultures negative for virus were treated after passage 4 with iododeoxyuridine (IdUrd) (30 #g/ml) for 36 h by standard techniques (Lowy et al. 1971) and overlaid with sensitive mouse or human cells and cultured for another 3 week period. The monolayers were then co-cultivated with the NRK-Harvey line, a non-virus producing MSV-transformed rat cell line (Levy, 1971). Formation of pseudotype murine sarcoma virus (MSV), as assayed by focus formation in rat and human cells, was used as an indication of replicating MuLV (Levy et al. 1975). Moreover, the XC plaque assay was employed to detect infectious ecotropic MuLV (Rowe et al. 1970) and the mink S+L- assay to detect xenotropic MuLV (Peebles, 1975). The tropism of the ecotropic viruses isolated was determined by the XC assay using NIH Swiss and Balb/c ME cells. Virus identification was confirmed by standard neutralization assays using specific antisera for ecotropic and xenotropic virus (Levy et al. 1975).

The result of these studies are presented in Table 1. In four experiments involving AKR mice, N-tropic AKR-MuLV was easily recovered from all frozen sperm preparations inoculated directly onto NIH-ME. AKR sperm collected after sucrose density-gradient centrifugation also contained infectious virus. Ecotropic AKR virus was also recovered from the NIH Swiss and rat embryo cells propagated for 3 weeks after they had been incubated with the four viable AKR sperm preparations. Human cells did not produce any xenotropic
or ecotropic MuLV either after direct inoculation of a thawed AKR sperm preparation on to the cells or by cultivation of the HuF cells after they had been exposed to viable sperm. When the AKR sperm preparation was pretreated with ether, or heated to 80 °C for 10 min, the transfer of infectious virus was eliminated. These treatments do not affect the uptake of sperm by somatic cells (Bendich et al. 1974, 1976) but may alter the biological properties of the sperm.

Spermatozoa removed from Balb/c mice yielded no infectious virus by direct assay of the thawed preparations on mouse and human cell cultures. Cells propagated after incubation with the viable Balb/c sperm also did not release infectious virus. A preparation of sperm from C57Bl/6 gave a similar result, but more studies need to be done with this strain before proving that its sperm is free of infectious MuLV. In one out of three occasions, NIH-ME cells, receiving the viable Balb/c sperm and passed six times, yielded an N-tropic AKR-MuLV after treatment with IdUrd. No B-tropic or xenotropic virus was detected. Also, the same mouse cell line, not treated with IdUrd, released no infectious virus. Since NIH-ME do not contain ecotropic virus, this observation raised the possibility that viable Balb/c sperm penetrated the NIH mouse embryo cell and transferred the proviral DNA of AKR-MuLV into the cell line. Nevertheless, although the sperm preparation by tissue culture analysis was free of any detectable Balb/c cell, we are unable to rule out completely the possibility that a few somatic cells from the vas deferens had been transferred with the sperm fraction to the mouse cells.

Sperm from NZB mice showed a pattern of type C virus association similar to the AKR since the frozen uncentrifuged sperm preparation contained infectious xenotropic virus. However, the sperm collected after sucrose density-gradient centrifugation did not have infectious MuLV. Mink lung and HuF cells exposed to both preparations of viable sperm released xenotropic virus. Conceivably, the infection of these cells by the sucrose density-separated sperm preparation occurred via proviral DNA, but we assume a low level of infectious xenotropic MuLV was closely associated with the centrifuged sperm but was not detectable following freezing and thawing. As noted above, no viable somatic cells were detected in any NZB sperm preparations and scanning electron microscopy did not reveal any budding particles on the representative sperm examined (Table 1).

These experiments indicate that some mouse sperm preparations, even after extensive washing and after centrifugation through sucrose, contain infectious MuLV closely associated with the membrane of the sperm or contained within the sperm head. The studies with Balb/c and NZB sperm also raise the possibility that transfer of proviral DNA to cells not containing the virus may also occur, as suggested by the results with mouse mammary tumour virus (Bentvelzen, 1974). Most importantly, they suggest that MuLV could become incorporated into the mouse germ line not only vertically via the germ cell, but also horizontally during sperm penetration of the ovum. In vitro exposure of pre-implantation mouse embryos to Moloney leukaemia virus has led to efficient integration of this virus into the germ line of mice (Jaenisch, 1976). Likewise, in nature, if MuLV is introduced into the ovum by sperm at the time of fertilization it could conceivably become part of the mouse germ line. This possibility, which requires further study, could explain virus genome integration site differences in offspring of certain genetic crosses. These experiments also indicate that sperm transfer could be responsible for MuLV infection of both mother and offspring by venereal epigenetic means as observed with MuLV in wild mouse strains (Gardner et al. 1979). Furthermore, they might explain germ line integration by new type C viruses which is a rare event in nature (Todaro, 1975). While transfer of virus by mating between heterologous species is highly unlikely, horizontal spread of a xenotropic virus to a species by some other means might eventually lead to contamination of its sperm by this virus. Then, by sperm transfer, integration into the germ line of that species could ultimately occur.

NZB males sire small litters (Bielschowsky & Bielschowsky, 1964) and their sperm
preparations contain large quantities of type C xenotropic viruses (Fernandes et al. 1973; this report). Moreover, a glycoprotein similar to MuLV gp70 has been detected in seminal fluid, glandular epithelium of the testes and on the surface of sperm (Del Villano & Lerner, 1976; Lerner et al. 1976). In addition, reverse transcriptase-like activity has been detected in human sperm heads and cell-free seminal fluid (Witkin et al. 1975). These and other observations cited above suggest that the association of type C viruses with sperm may have a direct influence on normal embryonic development (Levy, 1978).

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REFERENCES


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