REVIEW ARTICLE

Is Rodent Virus Contamination of Monoclonal Antibody Preparations for Use in Human Therapy a Hazard?

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Since the discovery of monoclonal antibodies (Köhler & Milstein, 1975) much attention has been devoted to the development of reagents which would be of use in the detection and treatment of diseases, such as cancer and graft-versus-host disease resulting from human bone marrow allografts. This means that such antibodies are and will increasingly be used either in vivo in patients (Mathieu et al., 1984) or that cells from patients will be exposed to such antibodies for cytotoxic separation procedures prior to replacement into patients (Waldmann et al., 1984). As most large-scale productions of monoclonal antibodies are carried out using ascites preparations induced in either mice or rats, a question which needs careful consideration is whether rodent viruses which contaminate many colonies of laboratory animals and may contaminate hybridomas, will be present in such therapeutically used materials and, if so, whether they constitute an additional pathological threat to the patients undergoing therapy. This is of particular relevance in cases where immunosuppression is used in the treatment regime. To clarify this possibility, we need to answer the following questions.

1. What naturally occurring rodent viruses are known to contaminate rat and mouse colonies, and may therefore find their way into ascites fluids?
2. Is the pathogenesis of any of these viral infections, in their natural host, such that they are likely to be present in ascites preparations?
3. Which of the rodent viruses are known to cause zoonotic disease (i.e. are known to be transmissible from animal to man) under conditions of normal contact between animal and man?
4. Which rodent viruses might be pathogenic for humans when administered by a route such as intravenous injection? This is particularly important, as a virus which might not be able to infect cells of the mucosal surfaces, normally the first site of contact under natural conditions of exposure to viruses excreted by animals, might gain access to other populations of cells for which it is pathogenic by administration directly into the bloodstream.
5. Can rodent viruses infect and damage separated host cells such as bone marrow during in vitro cytotoxic therapy, and would they then be likely to set up an infection in these which could continue when they were returned to a human subject?
6. Can we assume that rodent viruses which have at present been found to replicate only in rodent cell lines or rodents do not constitute a risk to therapy in humans?
7. Even if there is a possibility of such viruses being present in antibody preparations, can they be removed by the procedures used to fractionate antibodies from fluids prior to use?

Prevalence of rodent virus infections in breeding and user colonies

Over the years many results have been reported documenting the serological testing of rodent colonies in the U.S.A. (Parker et al., 1966; Trentin et al., 1966) and the U.K. (Carthew et al., 1978; Gannon & Carthew, 1980) for a wide variety of rodent viruses. It is clear from the serological screening of mice that has been done in the U.S.A. that antibodies to Sendai virus, mouse hepatitis virus, reovirus type 3, pneumonia virus of mice, Thelser's mouse encephalomyelitis virus (GD VII) and polyoma virus were particularly commonly detected, while mouse adenovirus and newborn mouse pneumonia virus (K virus) were less so (Parker et al., 1966).
Monitoring of animal facilities in the U.K. showed a similar prevalence of Sendai virus, pneumonia virus of mice, mouse hepatitis virus (type 1) (Carthew, 1978), Kilham rat virus and the rat coronaviruses (Gannon & Carthew, 1980).

In addition to these general serological data, we must also consider the numerous individual reports of enzootic or epizootic outbreaks of rodent diseases which have been reported. One virus in particular, lymphocytic choriomeningitis virus, has caused outbreaks of disease in humans which were attributed to contact with infected hamsters in the U.S.A. (Baum et al., 1966; Biggar et al., 1977) and Germany (Ackermann, 1976). Ectromelia virus (which is not zoonotic) has also caused severe outbreaks of nationwide disease in mice in the U.S.A. (Held, 1981) and the U.K. (Carthew et al., 1977). The outbreaks of ectromelia in the U.S.A. have been traced back to mice imported from Czechoslovakia (where it seems to be endemic in mouse colonies) which were brought into the U.S.A. via England (Whitney et al., 1981). Sendai virus has caused many outbreaks of disease even in barrier-maintained mouse colonies (Bhatt & Jonas, 1974; Parker et al., 1964; Burek et al., 1977), although the disease can exist in a subclinical form with little evidence of illness (Fujiwara et al., 1976; Parker & Reynolds, 1968). Mouse hepatitis virus has been well documented worldwide as causing severe outbreaks of hepatitis and enteritis (Gledhill et al., 1955; Rowe et al., 1963; Carthew, 1977; Broderson et al., 1976; Hierholzer et al., 1979; Ishida & Fujiwara, 1979).

The most commonly occurring virus of rats responsible for clinical disease (besides Sendai virus) is the sialodacryoadenitis coronavirus which causes outbreaks of mumps-like salivary gland swelling with porphyrin accumulation in the eyes due to damage of the Harderian gland (Jonas et al., 1969; Carthew & Slinger, 1981; Utsumi et al., 1978). More disturbingly, Hantaan virus has been found to be the cause of serious outbreaks of human disease in Belgium (Desmyter et al., 1983) and the U.K. (Lloyd et al., 1984) where Hantaan virus-infected rats were the source of disease. Since these rats were used to propagate rat myeloma cell lines and prepare ascites, the contamination of both cells and ascites with Hantaan virus is a real possibility which is currently being investigated (G. Lloyd, personal communication).

Recently, a rotavirus-like agent has been isolated from suckling rats suffering from diarrhoea (Vonderfecht et al., 1984). This virus has been shown by serological testing and examination of the nucleic acid to be distinct from the well-characterized group of rotaviruses associated with diarrhoea in simian, bovine and murine species, referred to as group A rotaviruses, and is closely related to the porcine group B rotavirus (Chen et al., 1985). It has been shown also to cause gastroenteritis in humans and at least one such infection was caused by contact with rats experimentally infected with this virus (Eiden et al., 1985). Virus isolated from human cases was also found to cause diarrhoea in suckling rats after oral administration. It is also thought that the virus may be the same (inferred by a similar RNA migration pattern) as the virus responsible for an epidemic of gastroenteritis in China (Hung et al., 1984). The evidence would seem to suggest that this new rotavirus-like agent is a zoonotic virus.

Viruses which could possibly be present in ascites preparations of monoclonal antibodies

To estimate the possibility of any naturally occurring rodent viruses contaminating monoclonal antibody ascites preparations, the pathogenesis of each infection should be considered in detail. Obviously, viruses of a predominantly hepatotropic nature will be shed into the peritoneal cavity during the acute phase (or chronic phase) of infection and may contaminate ascites fluids. Also, any infection which produces a viraemia could also contaminate ascites fluids due to bleeding during the collection of ascites. Table 1 summarizes the known natural pathogenicity of viruses of rats and mice. It should be borne in mind that for many experimental infections with rodent viruses, unnatural routes of infection have been used after which these viruses may exhibit different tissue tropisms. These have not been included for that reason. Included in the table are also the existing data on the contamination of transplantable mouse tumours which will also give a valuable idea of the ease with which murine viruses can be found in tumour tissues or cells. Collins & Parker (1972) found that 69% of the murine leukaemia and transplantable tumour specimens they tested were contaminated with murine viruses. Over one-half of the tumours tested (total 465) were contaminated with lactate
dehydrogenase-elevating virus, while nearly one-third were contaminated with minute virus of mice. Both of these cause naturally occurring inapparent infections in mice. A smaller proportion were also contaminated with polyoma virus, mouse hepatitis virus, Sendai virus, lymphocytic choriomeningitis virus and reovirus type 3 in descending order of frequency.

It should also be remembered that when athymic or nude mice or rats are used for hybridoma cell ascites production, many of the natural virus diseases are chronic infections in these immunoincompetent rodents. Sendai virus is persistent in both nude mice (Ward et al., 1976; Iwai et al., 1979) and nude rats (Carthew & Sparrow, 1980a) as is pneumonia virus of mice (Carthew & Sparrow, 1980b), mouse hepatitis virus (Ward et al., 1976; Tamura et al., 1977; Carthew, 1981) and murine cytomegalovirus (Carthew, 1982). The problems associated with these chronic viral infections in nude mice can be overcome by the maintenance of such animals in isolators where they remain virus-free provided they were derived initially by hysterectomy or embryo transfer (Carthew et al., 1983, 1985).

Zoonoses and probable human viral pathogens

Two of the laboratory animal viruses listed in Table 1 are known to cause zoonoses, based on reports of outbreaks of disease in humans which were related to contact with infected animals. These are Hantaan virus (Desmyter et al., 1983; Lloyd et al., 1984) and lymphocytic choriomeningitis virus (Baum et al., 1966; Biggar et al., 1977; Ackermann, 1976). On the recent evidence of the transmission of the new rotavirus-like agent from infected suckling rats to an experimenter described by Eiden et al. (1985), this virus would also seem to fall into the category of a zoonotic infection. The question of whether Sendai virus falls into this category is still a matter of some debate. The history of the isolation of this virus and the question of its infecting humans has been reviewed extensively by Ishida & Homma (1978).

There is no doubt that Sendai virus was endemic in the colony of mice originally used for attempting isolations from human lung tissues, and that this has led to much confusion about whether the isolates were of human or mouse origin. However, there were isolates obtained which used eggs rather than mice (Gerngross, 1957), but these have been questioned because of the ease with which Sendai virus can contaminate a laboratory engaged in such experimental studies (Chanock et al., 1963). The problem does not seem to have been resolved by reciprocal cross-neutralization testing of the human (HA2) strain of parainfluenza virus type 1 and Sendai virus (Cook et al., 1959) with sera collected in outbreaks in the U.S.S.R. In summary, it can be said that there is no example of parainfluenza virus type 1 unequivocally isolated from man which has been shown to be serologically identical with Sendai virus and also pathogenic for rodents. Sendai virus has been found to be experimentally communicable to rhesus and cynomolgus monkeys after intranasal instillation. Although no clinical symptoms were observed the monkeys seroconverted, while after intracerebral injection of Sendai virus into monkeys, clinical symptoms of raised temperature, weakness, tremors and ataxia were noted. One monkey died after 12 days with extensive consolidation of the lungs, while another showed haemorrhagic pneumonia at autopsy (Jensen et al., 1955). Although Sendai virus was possibly not isolated from humans originally, and may not be naturally transmissible between mice and humans, there is no doubt that it is pathogenic for human cells (Northrop & Walker, 1965) and also in human organ cultures of the trachea (Tyrrell & Hoorn, 1965) and should therefore be considered a hazard in monoclonal antibody preparations used in humans especially where administration is by the intravenous route.

It has also been reported that both H1 (Toolan) virus and Kilham rat virus replicate in human B lymphocytes in culture and should thus be considered as possible human pathogens (Bass & Hetrick, 1975). The intravenous administration of monoclonal antibodies contaminated with Kilham rat virus, which is particularly prevalent in rats (Gannon & Carthew, 1980) would allow this virus direct access to a susceptible cell type in humans. Also, the use of monoclonal antibodies contaminated with this virus for in vitro selective cytotoxicity to bone marrow cells could lead to attachment of virus to cells and replication once they are replaced in a patient under treatment. This could be particularly dangerous for patients who are immunocompromised by the nature of their existing condition, or where they are also under immunosuppression.
Table 1. Summary of the pathogenicity of rodent viruses during natural infections in rats and mice and their stability to heat treatment

<table>
<thead>
<tr>
<th>Virus</th>
<th>Species affected</th>
<th>Organs, tissues and fluids from which virus was isolated</th>
<th>Viraemia</th>
<th>Found to be contaminant of transplantable tumours</th>
<th>Inactivation by heat treatment*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectromelia (orthopoxvirus)</td>
<td>Mouse</td>
<td>Liver, spleen, skin</td>
<td>Yes</td>
<td>(Fenner, 1948)</td>
<td>30 min, 55 °C</td>
</tr>
<tr>
<td>Epizootic diarrhoea of infant mice (rotavirus)</td>
<td>Mouse</td>
<td>Intestine, faeces</td>
<td>Not recorded†</td>
<td>(R. E. Shope, personal communication)</td>
<td>15 min, 70 °C</td>
</tr>
<tr>
<td>Encephalomyocarditis (picornavirus)</td>
<td>Mouse</td>
<td>CNS, heart, liver, spleen</td>
<td>Not recorded‡</td>
<td>30 min, 60 °C in 0.1% bovine albumin; &gt;20 min, 96 °C in 20% monkey serum</td>
<td>Survives 2 h at 80 °C</td>
</tr>
<tr>
<td>HI (Toolan parvovirus)</td>
<td>Rat</td>
<td>Lung, liver, intestine</td>
<td>Yes</td>
<td>(Human tumour: Toolan, 1961)</td>
<td>30 min, 56 °C (Lee, 1982)</td>
</tr>
<tr>
<td>Hantaan (bunyavirus)</td>
<td>Rat, mouse</td>
<td>Lung</td>
<td>Yes</td>
<td>(Lee et al., 1982)</td>
<td>Survives 2 h at 80 °C</td>
</tr>
<tr>
<td>Kilham rat (parvovirus)</td>
<td>Rat</td>
<td>Lung</td>
<td>Yes</td>
<td>(Moore &amp; Nicastri, 1965)</td>
<td>40 min, 60 °C</td>
</tr>
<tr>
<td>Lactate dehydrogenase (togavirus)</td>
<td>Mouse</td>
<td>Serum/plasma</td>
<td>Persistent</td>
<td>(Moore &amp; Nicastri, 1965)</td>
<td>60 min, 56 °C</td>
</tr>
<tr>
<td>Lymphocytic choriomeningitis (arenavirus)</td>
<td>Mouse</td>
<td>Liver, brain</td>
<td>Yes</td>
<td>(Notkins et al., 1966); (Kilham &amp; Oliver, 1959)</td>
<td>&gt;95% infectivity inactivated by 60 min, 100 °C (Harris et al., 1974)</td>
</tr>
<tr>
<td>Minute virus (parvovirus)</td>
<td>Mouse, rat</td>
<td>Brain, liver, urine, intestine</td>
<td>Yes</td>
<td>(Kilham &amp; Margolis, 1970)</td>
<td></td>
</tr>
<tr>
<td>Mouse adenovirus (adenovirus)</td>
<td>Mouse</td>
<td>Liver, intestine, (Hashimoto et al., 1966; Hartley &amp; Rowe, 1960)</td>
<td>Yes</td>
<td>(Collins &amp; Parker, 1972)</td>
<td></td>
</tr>
<tr>
<td>Mouse hepatitis (coronavirus)</td>
<td>Mouse</td>
<td>Liver, spleen, intestine</td>
<td>Yes</td>
<td>(Collins &amp; Parker, 1972)</td>
<td></td>
</tr>
<tr>
<td>Murine cytomegalovirus (betaherpesvirus)</td>
<td>Mouse</td>
<td>Salivary gland, liver, kidney, intestine, spleen</td>
<td>Yes</td>
<td>(Collins &amp; Parker, 1972)</td>
<td></td>
</tr>
<tr>
<td>Virus/Gene/Agent</td>
<td>Host(s)</td>
<td>Tissue(s)</td>
<td>Causes Viraemia?</td>
<td>Isolated from Viraemia?</td>
<td>Temperature/Conditions</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>---------------</td>
<td>----------------------------</td>
<td>------------------</td>
<td>------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Murine leukaemia viruses (retrovirus)</td>
<td>Mouse</td>
<td>Lymphatic tissues</td>
<td>Yes</td>
<td>Yes</td>
<td>30 min, 56 °C, Moloney and Rauscher viruses; 30 min, 68 °C, Gross virus</td>
</tr>
<tr>
<td>Newborn mouse pneumonitis (K virus)</td>
<td>Mouse</td>
<td>Lung</td>
<td>Not recorded</td>
<td>No</td>
<td>4.5 h, 70 °C (Collins &amp; Parker, 1972)</td>
</tr>
<tr>
<td>Polyoma (papovavirus)</td>
<td>Mouse</td>
<td>Faeces, urine, saliva</td>
<td>Yes</td>
<td>No</td>
<td>30 min, 70 °C (Collins &amp; Parker, 1972)</td>
</tr>
<tr>
<td>Pneumonia virus of mice (paramyxovirus)</td>
<td>Mouse, rat</td>
<td>Lung</td>
<td>No</td>
<td>No</td>
<td>30 min, 56 °C (Collins &amp; Parker, 1972)</td>
</tr>
<tr>
<td>Rat coronavirus (coronavirus)</td>
<td>Rat</td>
<td>Lung</td>
<td>Not recorded</td>
<td>Not recorded</td>
<td>Undetermined</td>
</tr>
<tr>
<td>Rat rotavirus-like agent (rotavirus)</td>
<td>Rat</td>
<td>Intestine</td>
<td>Not recorded</td>
<td>Not recorded</td>
<td>30 min, 56 °C (No longer transmissible to susceptible animals; Vonderfecht et al., 1984)</td>
</tr>
<tr>
<td>Reovirus 3 (reovirus)</td>
<td>Mouse</td>
<td>Lung, liver, intestine</td>
<td>Yes</td>
<td>Yes</td>
<td>30 min, 60 °C (Nelson, 1964)</td>
</tr>
<tr>
<td>Sendai (paramyxovirus)</td>
<td>Mouse, rat</td>
<td>Lung, saliva (less frequently liver, kidney)</td>
<td>Yes</td>
<td>Yes</td>
<td>30 min, 50 °C (Collins &amp; Parker, 1972) (Hamparian et al., 1963)</td>
</tr>
<tr>
<td>Sialodacryoadenitis (coronavirus)</td>
<td>Rat</td>
<td>Salivary gland</td>
<td>Not recorded</td>
<td>Not recorded</td>
<td>Undetermined</td>
</tr>
<tr>
<td>Theiler's mouse encephalomyelitis GD VII</td>
<td>Mouse</td>
<td>CNS, intestinal mesentery</td>
<td>Not recorded</td>
<td>Not recorded</td>
<td>Half-life at 50 °C, 8.5 min (Gard, 1955)</td>
</tr>
<tr>
<td>Thymic (herpesvirus)</td>
<td>Mouse</td>
<td>Thymus, brain, liver, kidney (Rowe &amp; Capps, 1961)</td>
<td>Yes</td>
<td>Not recorded</td>
<td>30 min, 50 °C</td>
</tr>
</tbody>
</table>

* Data taken from Andrewes & Pereira (1967) unless otherwise stated.
† Not recorded as causing viraemia in the original or any subsequent studies of the pathogenesis of this virus in rats or mice.
‡ Not recorded in the literature for the reason that no attempts were made to isolate these viruses in the papers examining viral contaminations of transplantable tumours.
as part of their subsequent treatment regime. Although there are no recorded cases of transmission of reovirus 3 from animal to man this virus is so ubiquitous that this has probably occurred without being noticed. The original isolation of reovirus 3 was from a human anal specimen (Rosen et al., 1960) and reovirus 3 has also been isolated from patients with Burkitt's lymphoma (Bell, 1967). Reovirus 3 has been shown to persistently infect human embryonic fibroblasts (Bell & Ross, 1966) and to replicate in monkey cells (Hsiung, 1958). Although the isolation of reovirus 3 from patients with Burkitt's lymphoma may be adventitious and not associated with any pathology or disease state, the possibility of persistent infection with this virus and its exacerbation by immunosuppression would make it an undesirable contaminant in therapeutic treatments. Eencephalomyocarditis virus of mice is said to be indistinguishable serologically from the other strains of this virus (Columbia-SK, MM and Mengo) (Andrewes & Pereira, 1967). The virus, which can be routinely passaged in mice, was originally isolated from a chimpanzee (Helwig & Schmidt, 1945). It has also been isolated from man (Dick et al., 1948) and in a number of cases of patients with central nervous system disease, this virus was isolated (Warren, 1965). Wild rats are thought to be a possible reservoir for encephalomyocarditis virus as antibodies to it are common in their sera (Warren et al., 1949). The virus can be grown in a wide variety of embryonic cells including human and mouse (Andrewes & Pereira, 1967).

Ectromelia, the poxvirus of mice, has not been isolated from any other species; however, this virus replicates in a wide variety of tissue culture systems including chick, mouse and HeLa cells (Porterfield & Allison, 1960). For this reason, it should also be regarded as a possible human pathogen. Mouse adenovirus has been tested and found not to replicate in primary monkey kidney cells, human embryonic skin or muscle cultures (Blackmore, undated); however, it has been shown to be adaptable to human synovial tissue (McCoy) cells (Sharon & Pollard, 1964).

In recent years, the presence of oncavirus-like particles in myeloma cells has attracted much discussion. Oncavirus particles were first identified in myeloma cells in 1970 (Watson et al., 1970) and since this time other authors have found type C oncavirus particles in hybridoma cell lines and discussed the implications of this (Bartal et al., 1982; Rozman et al., 1982; Thornton & Nicholas, 1982). While the expression of endogenous leukaemia viruses occurs spontaneously at high levels only in AKR, C57L and NZB strains of mice, the fact that hybridoma cells derived from BALB/c myeloma cell lines have been found to contain C type oncavirus particles must mean that ascites fluids derived from the in vivo culture of these cells or the supernatants derived from the in vitro culture of hybridoma cells must be regarded as possibly contaminated with retroviruses.

Of particular concern are the xenotropic retroviruses. These only infect foreign hosts and caused concern during human tumour xenograft work in nude mice (Weiss, 1980). There are examples of this type of infection occurring with a xenotropic feline virus, which infected and replicated in a human rhabdomyosarcoma after passage in the brain of a foetal kitten (McAllister et al., 1972; Livingston & Todaro, 1973); as well as this, human tumour xenografts in mice have also been shown to acquire murine xenotropic virus infections (Todaro et al., 1973; Crawford et al., 1979).

If this is possible in human tumour xenografts there would also seem to be the possibility that a murine retrovirus introduced into humans (via ascites fluids or cell supernatants) could cause a similar problem. Further possible dangers associated with xenotropic retroviruses are the production of a variant or recombinant virus more oncogenic or pathogenic than the original (Huebner et al., 1979) or the enhanced expression of cellular oncogenes leading to neoplastic transformation, as occurs with the activation of the c-myc gene by avian leukosis virus (Hayward et al., 1981). For these reasons it would seem to be necessary to add the murine retroviruses to the list of possible human pathogens to be excluded from therapeutic immunoglobulin preparations. The available data on the pathogenicity for humans and primates and replication of viruses in human or primate cell lines are summarized in Table 2.

**Rodent viruses not known to be pathogenic to humans**

To understand the possible pathological significance of the remainder of the rodent viruses it would seem to be reasonable to ask what information is available on their replication in monkey
Table 2. Classification of rodent viruses according to their known pathogenicity for man or ability to replicate in human or monkey cell lines*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Zoonotic disease</th>
<th>Isolation from human specimens or monkeys</th>
<th>Replication in human or monkey cell cultures</th>
<th>Causes disease in monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hantaan</td>
<td>Yes</td>
<td>Yes (human)</td>
<td>Yes (human and monkey)</td>
<td>Yes</td>
</tr>
<tr>
<td>Lymphocytic choriomeningitis</td>
<td>Yes</td>
<td>Yes (human and monkey)</td>
<td>Yes (monkey)</td>
<td>Yes</td>
</tr>
<tr>
<td>Rat rotavirus-like agent</td>
<td>Yes</td>
<td>Claimed (human)</td>
<td>Yes (B lymphocyte)</td>
<td>Yes</td>
</tr>
<tr>
<td>Enocephalomyocarditis</td>
<td>Yes</td>
<td>Yes (human and monkey)</td>
<td>Yes (B lymphocyte)</td>
<td></td>
</tr>
<tr>
<td>Reovirus 3</td>
<td>Yes</td>
<td>Yes (human)</td>
<td>Yes (HeLa cells)</td>
<td></td>
</tr>
<tr>
<td>Sendai</td>
<td></td>
<td></td>
<td>Yes (LLCMK2)</td>
<td></td>
</tr>
<tr>
<td>Kilham rat</td>
<td></td>
<td></td>
<td>Yes (human)</td>
<td></td>
</tr>
<tr>
<td>H1</td>
<td></td>
<td></td>
<td>Yes (human)</td>
<td></td>
</tr>
<tr>
<td>Ectromelia</td>
<td></td>
<td></td>
<td>Yes (Vero cells)</td>
<td></td>
</tr>
<tr>
<td>Epizootic diarrhoea of infant mice</td>
<td></td>
<td></td>
<td>Human tumour xenografts</td>
<td></td>
</tr>
<tr>
<td>Mouse adenovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Murine cytomegalovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia virus of mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retroviruses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* References are included in the text.

cell lines or adaption to human cell lines. Epizootic diarrhoea virus of infant mice is known to replicate in LLCMK2 cells, as this is the method of choice for identification of primary isolates (Woode et al., 1976). Murine cytomegalovirus is normally grown in mouse embryo fibroblasts but will also 'adapt' to growth in rat, hamster, rabbit, sheep, monkey (BSC-1) (Kim & Carp, 1971; Hudson, 1979) and human fibroblast cells (Raynaud et al., 1969). Polyoma virus multiplies in mouse embryo cells and mouse tumours. Despite its well-known oncogenic transforming properties it probably does not constitute a risk to humans, as it has not been shown to transform any human cell lines, nor have any antibodies against the virus been found in humans (Stoker, 1963). However, polyoma virus has been propagated in monkey kidney cells (Stewart et al., 1957) and a hamster strain has been reported to grow in human amnion cells (Dmochowski, 1959). Pneumonia virus of mice has been shown to replicate in African green monkey kidney cells (Vero) (Berthiaume et al., 1974). Most of the other rodent viruses have not been tested for their ability to replicate or cause a cytopathic effect in human or monkey cells, although this may have been attempted but not reported. It is not difficult to understand why this is so, as virologists working with rodent viruses would normally attempt to grow them, after preparation from diseased tissue, in the primary, secondary or continuous cell lines of the species affected and be content with a primary propagation in rodent cell lines, prior to growth in bulk and characterization. Of the remaining rodent viruses in Table 1 the mouse thymic herpesvirus and K virus have not even been successfully propagated in rodent cell lines (Rowe & Capps, 1961). Although 30 different cultures were used in an attempt to grow K virus, only opossum kidney and hamster kidney cells showed any replication of the virus and this was insufficient to warrant further work (Tennant et al., 1966). The commonly used strains of mouse hepatitis virus are very fastidious and can only be grown to high titre in mouse NCTC 1469 cells (Parker et al., 1965) although they will replicate to low titre in mouse L929 cells for diagnostic purposes (Carthew, 1978). Lactate dehydrogenase-elevating virus does not replicate in cells other than mouse macrophages, while minute virus of mice replicates in rat and mouse embryo cultures (Crawford, 1966). The rat coronavirus originally isolated by Parker et al. (1970) only grows in primary rat kidney cell culture (although they do not mention testing monkey or human cells), while the sialodacryoadenitis virus which also grows well in primary rat kidney culture was tested in other cell lines, including rhesus monkey kidney cells, but was not found to grow in any other cells (Bhatt et al., 1972).
Perhaps the pertinent point to bear in mind concerning the rodent viruses not known to infect human or monkey cell cultures is that in all probability no-one has really tried such cell lines as yet (after all, why should they prior to the present day situation?) and that one indicator of the possible human pathological potential of such viruses would be to examine their replicative potential in cultures of human macrophages or other human cell lines, by sensitive procedures such as the immunoperoxidase or immunofluorescence techniques. Human macrophage cultures can be routinely obtained as a by-product from patients undergoing continuous ambulatory peritoneal dialysis (Maddox et al., 1984).

**Removal of contaminating rodent viruses during fractionation of monoclonal antibodies**

One possible approach to the problem of viruses contaminating monoclonal antibody preparations is to use a purification procedure for the preparations which removes all of the viruses which may be present. The often used technique of salt precipitation of antibodies followed by filtration through a 0.22 μm filter would not be stringent enough to remove all viruses. Heat treatment has been recently used to inactivate human T cell leukaemia virus III in factor VIII derived from blood plasma, but because of the extreme stability of some rodent viruses to heat (see Table 1) this is not likely to be a useful general procedure. Chemical treatments such as altering the pH or inactivation using β-propiolactone would also be unsuitable as these would have a detrimental effect on the immunoglobulins in the preparations. Alternatives such as molecular sieving to separate high molecular weight viruses from immunoglobulins are another possibility, while affinity chromatography on columns of anti-rat or anti-mouse antibody would be perhaps the best approach. The efficiency of any such operation can be monitored in the same way as was the purification of Namalwa cell-induced human interferon (Finter & Fantes, 1980). In this case a range of marker viruses, Sendai, Newcastle disease virus and Rous sarcoma virus (along with other viruses and micro-organisms) were deliberately added to the preparations and then the product was assayed for their absence (or inactivation) at the end of the procedure. This ‘spiking’ proved that the purification scheme used was very successful at removing a whole range of possible contaminants from interferon preparations and has the added advantage of also eliminating other micro-organisms (outside of the scope of the present review) which might well be present and which should be taken into consideration. A good separation technique such as affinity chromatography might also have the added advantage of rendering the need for costly routine screening of batches of antibody for all viruses not necessary, as only the ‘spiking’ markers would have to be tested for. This overcomes the one possible criticism of testing for known rodent viruses, which is ‘what about the ones that haven’t yet been discovered?’.

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**REFERENCES**


Review: Rodent viruses as a human health hazard


