Memoranda/Mémorandums

Maintenance and distribution of transgenic mice susceptible to human viruses: Memorandum from a WHO meeting*

This Memorandum discusses the use of transgenic mice in poliovirus research and the potential risks to public health. General and specific recommendations are given concerning the maintenance, containment and transport of transgenic animals which are susceptible to pathogenic human viruses, with special attention to transgenic mice susceptible to polioviruses.

Research leading to the development of improved methods for the prevention and control of serious human viral diseases is often limited by the absence of readily available animal models that could faithfully mimic human interactions with the viral agent. Examples of viral diseases where research efforts have been restricted by the lack of such animal models include the acquired immunodeficiency syndrome (AIDS), all forms of viral hepatitis, and poliomyelitis, where in each case only certain non-human primate species have been shown to be susceptible to infection. These non-human primates are expensive to acquire and maintain, and they are often limited in availability, or even endangered with respect to the survival of their species.

New technologies have emerged over the past decade which may allow the replacement of these non-human primate models for research on human diseases with novel transgenic animals which express selected human genes. Typically, the foreign gene (transgene) is incorporated into the chromoso-

* This Memorandum is based on the report of a WHO Consulta-
tion on the Maintenance and Distribution of Transgenic Mice Susceptible to Human Viruses, held in Geneva on 18-19 November 1992. The participants at the Meeting were: H.J. Hedrich and W. Heine (Hanover, Germany); S. Lemon (Chairman) (North Carolina, USA); M. Martin (Bethesda, MD, USA); A. Nomoto (Tokyo, Japan); T. Nomura (Kawasaki, Japan); and V. Racaneli-

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by the year 2000. In addition, the replacement of primates by transgenic mice in biomedical research has important ethical implications. The saving of primates is a most welcome contribution to applied animal protection.

At a theoretical level, however, the development of transgenic mice which are permissive for the replication of pathogenic human viruses may pose special public health hazards. In the case of poliovirus receptor-positive transgenic mice in particular, the unique opportunities for development of better disease control measures far outweigh any theoretical risks these animal lines may pose. Nonetheless, careful consideration must be given to these potential hazards, and measures must be taken to ensure that they are eliminated to the greatest possible extent.

**Use of transgenic animals**

**Potential risks to public health**

The risks posed by transgenic animals that have been constructed to support the replication of pathogenic human viruses include (1) the possibility that infected, laboratory-maintained transgenic animals may shed and transmit a pathogenic virus to susceptible humans, or (2) possibility that the transgenic animals might escape from the laboratory and that the transgene might become established within wild animal populations, potentially leading to the creation of a new animal reservoir for a pathogenic human virus.

In evaluating these risks, the epidemiological characteristics of a particular virus and the conditions under which naturally infected humans may transmit the virus to others need to be carefully considered. For example, were transgenic mice expressing the poliovirus receptor capable of shedding substantial quantities of this virus into the environment, as infected humans do, there would be a significant risk of transmission, as this virus is relatively stable in the environment. However, in making comparisons between the epidemiology of human infections and the potential transmissibility of virus from infected transgenic animals, attention also must be paid to the possibility that expression of the transgene may alter the host cell tropism of the virus, and possibly change its transmission patterns. Thus, in order to arrive at an informed estimate of the infectious risks posed by such animals, it is essential to understand the pathobiology of the relevant agent in the transgenic animal, to carry out quantitative studies of susceptibility by different routes of infection, and to determine the source and magnitude of any virus released into the environment by infected animals.

Similar considerations hold for the risks posed by the potential establishment of the transgene in the wild animal gene pool. If transgenic animals were capable of efficient transmission of the infectious agent, establishment of the gene for the human poliovirus receptor in the wild mouse population could have potentially serious consequences. In particular, the presence of a wild animal reservoir for poliovirus could have an adverse impact on current efforts to achieve global eradication of wild-type polioviruses.

**Transgenic mice susceptible to poliovirus infection**

Transgenic mice expressing the human cellular receptor for poliovirus (TgPVR) have been developed independently in the laboratories of Dr Akio Nomoto in Japan and Dr Vincent Racaniello in the USA.

In Dr Nomoto’s laboratory, the PVR gene was isolated from Hela cells and used to establish four transgenic lines. Three lines (ICR-TgPVR Nom1, ICR-TgPVR Nom5, ICR-TgPVR Nom21) were derived from mouse strain ICR and one line (C57BL/10-TgPVR Nom8) was derived from C57L/10 mice. The sensitivity to poliovirus infection was estimated to follow the order: Tg1 > Tg8 > Tg21 > Tg5.Northern-blot hybridization revealed that the levels of poliovirus RNA in the brain and spinal cord correlated with poliovirus susceptibility of individual mouse lines. The Tg Nom1, Tg Nom8 and Tg Nom21 lines develop paralysis after inoculation with virulent polioviruses by the intraspinal, intracerebral, intraperitoneal, intravenous and oral routes. With all routes of inoculation, poliovirus enters the central nervous system (CNS) where it replicates in neurons. Doses of 10^2 PFU of the virulent strain Mahoney of poliovirus type 1 inoculated intracerebrally cause death of TgPVR mice, while a dose of 10^6 PFU of the Sabin vaccine strain of poliovirus type 1 was required to cause paralysis and death of TgPVR mice. Similar observations were obtained when Sabin vaccine strains of polioviruses type 2 and 3 were compared with virulent poliovirus strains Lansing type 2 and Leon type 3, respectively. Compared with other routes of inoculation to Tg Nom1 mouse line which require 10^3–10^4 PFU of virulent type 1 poliovirus strain Mahoney, approximately 10^7 PFU is needed for induction of paralysis by the oral route of infection, whereas about 10^{4.5} PFU is needed for induction of paralysis and death by the oral route of infection with the Sabin strain of poliovirus type 1. Thus TgPVR mice show different sensitivities to virulent and attenuated strains of poliovirus after oral inoculation as well as by other routes of infection.

To determine whether poliovirus replicates in the gut of TgPVR mice, the animals were fed light-sensitive poliovirus type 1 strain Mahoney or strain.
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Sabin. Light-resistant virus was identified in the faeces, indicating that the virus replicated in the transgenic animals. Some TgPVR mice excreted up to 1000 PFU daily in the faeces, until death occurred. Other TgPVR mice excreted virus only during the first two days after inoculation. These results suggest that poliovirus replicates at low levels in the gut of TgPVR mice. Surprisingly, continuous excretion of wild-type Mahoney strain of poliovirus type 1 was also observed in one non-transgenic control mouse. Further studies are required to determine whether this virus is a mutant selected for its ability to replicate in the mouse alimentary tract.

Oral infection of TgPVR mice with 10⁷ PFU of poliovirus type 1 strain Sabin did not elicit neutralizing antibodies. Only a low level of antibodies was elicited after a booster immunization with the same dose of this vaccine strain. In some mice, infection with 3 x 10⁶ PFU induced low levels of antibodies in both TgPVR and non-transgenic mice. This level of antibodies, however, did not protect against challenge with poliovirus type 1 strain Mahoney. These results indicate that poliovirus replication in the alimentary tract of TgPVR mice is not sufficient to induce protection against challenge with virulent poliovirus.

The ability of poliovirus to spread within colonies of TgPVR mice was determined by placing orally-infected and uninfected transgenic mice in the same cage. Observation of seven mixed cages suggested that poliovirus is not efficiently transferred from infected to uninfected mice, probably because of the small amount of poliovirus excreted, and the low efficiency of oral infection.

TgPVR mice were also established in the laboratory of Dr Vincent Racaniello using PVR genomic DNA from Hela cells. TgPVR mice with different numbers of the PVR gene were isolated. In general, transgene copy numbers did not affect susceptibility to infection, although mice with the highest copy number (n=70) were resistant to infection. TgPVR mice developed poliomyelitis after inoculation with virulent polioviruses by intracerebral, intraperitoneal, intramuscular and intravenous routes. No paralysis was observed after intracerebral inoculation with high levels of the three Sabin vaccine strains of poliovirus. In contrast, between 4 x 10³ and 1 x 10⁵ PFU of virulent strains (such as poliovirus type 1 strain Mahoney, poliovirus type 2 strain Lansing or poliovirus 3 strain Leon) caused paralysis in 50% of mice. Despite widespread expression of PVR mRNA in transgenic mouse tissues, poliovirus replication largely occurred in the brain, spinal cord, and skeletal muscle. These lines of TgPVR mice were also susceptible to infection by the oral route, although development of disease by this route of infection was strongly age-dependent; 1-day-old mice were 100% susceptible after infection with 10⁶ PFU of virulent poliovirus type 1 strain Mahoney, while 7–14-day-old mice were refractory to oral infection.

Despite susceptibility of young TgPVR mice to oral infection, no replication was detected in the alimentary tract after inoculation with large quantities of poliovirus. After oral administration of virus to 1-day-old TgPVR mice, infectious virus was detected in the faeces for 1–2 days after inoculation, but not thereafter. In addition, viral RNA replication in cells of the intestine was not detected by in situ hybridization. In all the TgPVR lines examined, PVR mRNA expression in the intestine was very low. It is therefore likely that these lines of TgPVR mice do not support poliovirus replication in the gut owing to low levels of receptor expression. The mechanism by which orally administered poliovirus induces paralysis in 1-day-old animals remains to be determined.

After intramuscular inoculation of poliovirus in TgPVR mice, the virus replicates in skeletal muscle cells and spreads to the spinal cord by axonal pathways. Intramuscular inoculation may provide another means of assessing the attenuation of poliovirus vaccine strains.

In summary, the lines of TgPVR mice isolated in two different laboratories are susceptible to poliovirus infection with polioviruses by a variety of inoculation routes. While the resulting disease is clinically and histopathologically similar to human poliomyelitis, the mouse model differs from humans in that alimentary tract replication of orally administered poliovirus is either inefficient or does not occur. TgPVR mice demonstrate profound differences in terms of their susceptibility to neurovirulent (wild-type) and attenuated (vaccine) strains of poliovirus, and they thus offer the means of developing new and improved procedures for testing the safety of vaccines. Lack of substantial replication in the alimentary tract should not affect the value of TgPVR mice in poliovirus vaccine safety testing.

Estimate of the risk to public health posed by PVR transgenic mice

Although further experiments are needed to define better the natural history of poliovirus infections in TgPVR transgenic mice, it was considered very unlikely that escape of these transgenic mice to the wild would result in the establishment of a new animal reservoir for poliovirus. Nevertheless, given the present state of knowledge, this possibility cannot be completely excluded for the following reasons:

1. During breeding of TgPVR mice, the human poliovirus receptor (PVR) is transmitted in a Mendelian dominant mode of inheritance of the PVR gene.
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(2) TgPVR mouse strains may breed readily with other strains of mice.

(3) TgPVR mice have no lethal mutations that would prevent their spread in the wild.

(4) TgPVR mice are susceptible to oral infection when challenged with high titres of poliovirus.

(5) Limited faecal excretion has been observed in some lines of TgPVR mice.

(6) Because virus titres in the central nervous system and muscle tissue of infected TgPVR mice are high, infection might be transmitted to other mice by biting and cannibalism.

(7) Wild poliovirus may be isolated from human sewage, in some cases at titres up to 10^7, and exposure of mice to human sewage may occur in the wild.

(8) Adaptation of poliovirus to more efficient replication in mice is possible with passage of the virus.

(9) Some TgPVR mouse lines do not appear to mount an efficient antibody response to poliovirus following infection, making the detection of infected animals difficult.

On the other hand, several lines of evidence argue strongly against the possibility that poliovirus receptor-positive transgenic mice could serve as a reservoir for the virus in the wild, as described below.

1. The quantities of virus shed in the faeces of infected transgenic mice created in Dr Nomoto's laboratory are 10 000 to 1 000 000 times less than that required for infection and paralysis in the mice by the oral route. No faecal shedding has been noted from infected transgenic animals created in Dr Racaniello's laboratory. Further experiments are required to better define differences in the amount of virus required to establish infection (rather than induce paralysis) in nontransgenic and transgenic mice.

2. In one experiment, a normal nontransgenic mouse was shown to be infected following oral challenge with a high-titrated poliovirus inoculum. This mouse shed virus for a number of days. Despite this observation, there is no evidence that normal mice act as reservoirs for poliovirus in nature.

3. Limited studies completed thus far indicate that poliovirus infected transgenic mice developed in Dr Nomoto's laboratory do not efficiently transmit virus to uninfected, transgenic cage-mates, but in some cases poliovirus transmission occurs from infected to uninfected TgPVR mice. Further studies are required, however, to rule out a low level of transmission.

4. Infected TgPVR mice do not shed poliovirus in the urine.

While the risk posed by escape of poliovirus-receptor transgenic mice thus appears to be very low, it is essential that practices be adopted which will minimize the possibility of escape of these animals, and yet will not interfere with the further development of these very useful animal models for studies of poliovirus pathogenicity, vaccine safety and poliovirus field surveillance.

Recommendations

General recommendations concerning the maintenance, containment and transport of transgenic animals which are susceptible to pathogenic human viruses (Table 1)

1. For each line of transgenic animals, which is shown to be uniquely susceptible to a pathogenic human virus, detailed studies should be conducted to determine the natural history of the virus infection in the transgenic animal, including the routes by which the animal is susceptible to infection, the inoculum size required for infection, and the nature and extent of virus shedding by infected animals.

2. For each line of transgenic animals shown to be uniquely susceptible to a pathogenic human virus, a registry should be maintained by the developer of the line which includes detailed information concerning each of the physical locations at which the animals are maintained, or to which they have been shipped. At each institution maintaining or receiving such animals for experimental studies, an accounting system should be established which faithfully records the birth or receipt, as well as the final disposition, of all transgenic animals.

3. Animals should be maintained in clearly designated, locked rooms with well defined, restricted access by laboratory workers and animal handlers. Experiments involving infection of transgenic animals should be carried out in a similarly limited-access room which is physically separated from rooms used for breeding or maintenance purposes.

4. Laboratory practices involving a level of biosafety which is generally accepted as appropriate for work with specific pathogenic human viruses, and generally accepted standards for the care and use of laboratory animals in biomedical investigation should be rigorously enforced in laboratories engaged in the development or maintenance of transgenic animals that have a unique susceptibility to

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Table 1: Methods for maintaining transgenic animals

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<tr>
<th>Class</th>
<th>Definition</th>
<th>Minimum type of containment</th>
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<tbody>
<tr>
<td>I. Animals capable of transmitting human pathogens</td>
<td>Transgenic animals containing a complete potentially infectious viral genome.</td>
<td>The same level of physical containment as is required for that microbial agent.</td>
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<tr>
<td></td>
<td>Transgenic animals containing a gene conferring susceptibility to a human pathogen.</td>
<td>Infected animals should be maintained at a level of containment appropriate for that pathogen. The levels of containment for uninfected animals should be determined on a case-by-case basis depending on the epidemiology of the human pathogen and the likelihood that the transgenic mice can contribute to its transmission. The level for risk group 2 is the minimum recommended level of containment.</td>
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II. Other transgenic animals

As required by local regulations.\(^b\)


\(^b\) If there are no local regulations, refer to the regulations/guidelines given in: WHO/ICLAS Guidelines for breeding and care of laboratory animals (unpublished WHO document, WHO/ZOON./93.169, 1993) and International guiding principles for biomedical research involving animals. Geneva, CIOMS, 1985.

Pathogenic human viruses, and in laboratories which utilize these animals for research purposes. Although containment laboratories and closed or barrier systems are primarily developed to keep infectious material either in or out, these systems also provide the most effective methods for containment of transgenic animals. These systems include controlled access for the scientific staff, and have been well established in laboratory animal science for many years. Requirements for such high levels of containment should be based upon careful scientific review of the potential risks posed by transgenic animals.

Various laws and regulations, both local and international, specify the general care and management required for the maintenance of mice susceptible to human viruses.\(^b\) In some cases, guidelines or regulations concerning Tg mice are also applied.\(^c\) It is especially important that Tg mice susceptible to a human virus be prevented from escaping from an animal facility.

5. Where feasible, all laboratory workers and animal handlers who may potentially be exposed to transgenic animals which are susceptible to a human pathogenic virus should receive protective immunization against the specific agent.

6. Transgenic animals must be prevented from escaping during transport, since in some cases they could serve as a new reservoir for the human virus to which they are susceptible or which they harbour. The general methods for the transport of transgenic mice susceptible to pathogenic viruses are specified in local and international regulations.\(^d\)

\(^b\) Animals Scientific Procedures Act (UK); Animal Welfare Act (USA); Law Concerning the Protection and Control of Animals (Japan); Tier Schutzgesetz (Germany); UFAW Handbook on the care and management of laboratory animals (UFAW, UK); Guide for the care and use of laboratory animals (UFAW, UK); Guide for the care and use of laboratory animals (ILAR, USA); Standards relating to the care and management of experimental animals (Prime Minister's Office, Japan); International guiding principles for biomedical research involving animals (CIOMS); Laws regulating genetic engineering issues in the Federal Republic of Germany; Society for Laboratory Animal Science: Zur Planung und Struktur von Versuchstierbereichen TierexperimentellTätiger Institutionen, 1988; WHO/ICLAS Guidelines for breeding and care of laboratory animals (unpublished WHO document, WHO/ZOON./93.169, 1993).

\(^c\) Genetic manipulation guidelines on work with transgenic animals (Home Office, UK); Guidelines for recombinant DNA experiments (Ministry of Education, Science and Culture, Japan); Cornell directive on the contained use of genetically modified microorganisms (USA); Laws regulating genetic engineering issues in the Federal Republic of Germany.

\(^d\) Live Animal Regulations (IATA); UFAW Handbook on the care and management of laboratory animals (UFAW, UK); Guide for the care and use of laboratory animals (ILAR, USA); Standards relating to the care and management of experimental animals (Prime Minister's Office, Japan); International guiding principles for biomedical research involving animals (CIOMS); WHO/ICLAS Guidelines for breeding and care of laboratory animals (unpublished WHO document, WHO/ZOON./93.169, 1993).
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7. Given that numerous laboratories may wish to have access to transgenic mice which are susceptible to pathogenic human viruses, careful consideration should be given to the possibility of creating a subline of such transgenic mice which contain a lethal mutation with a phenotype which may be nutritionally or otherwise suppressed under normal laboratory conditions, and which would result in rapid death or sterility in the event of escape to the wild. WHO should encourage the development of such a modified poliovirus-receptor positive transgenic mouse line.

Specific recommendations and principles for maintaining and transporting transgenic mice susceptible to polioviruses

1. Because of the potential hazards involved in the maintenance and distribution of TgPVR mice susceptible to poliovirus it is recommended that the maintenance and use of fertile TgPVR mice be restricted to laboratories which meet all the standards outlined in this report and which are able to provide a level of containment which is sufficient to exclude escape of any mice.

2. The breeding of TgPVR mice should be restricted to facilities which meet all the standards outlined in this report, ensuring that TgPVR mice cannot escape into the environment. Breeding facilities should be completely separate from laboratories in which poliovirus or poliovirus-infected animals are handled. If possible, breeding animals should be maintained in SPF (specific pathogen-free) facilities as defined in WHO ICLAS guidelines (WHO/ICLAS Guidelines for breeding and care of laboratory animals (unpublished WHO document, WHO/ZOON./93.169, 1993).

3. TgPVR mice to be used in experimental studies or for assessment of vaccine neurovirulence in institutions which do not meet the standards outlined in this report should be castrated (neutered) before transfer from the breeding laboratory in order to prevent the risk of reproduction of animals inadvertently released during this process. Orchidectomy and ovariectomy are easily performed by experienced technicians. Alternative methods of sterilization of TgPVR mice may also be used, provided that they guarantee sterilization of TgPVR mice without changing their susceptibility to poliovirus.

4. Fertile TgPVR mice should not be shipped to any institution unless the receiving institution provides evidence that the animal maintenance facilities fulfil the requirements detailed in this report.

5. For the containment of fertile TgPVR mice, all the general recommendations (as detailed above) must be met. In addition, special measures should be taken to prevent escape of fertile TgPVR animals from individual cages and racks. In the event that the mice do escape, there must be a second level of containment so that the mice are not able to escape from the animal room. The following measures are recommended:

   - Cages and covers: the cages should be made of hard plastic which is resistant to chemicals and heat. A recessed cover should be used so that the mice cannot damage the edge of the cage by biting. Fasteners should be attached to the covers.
   - Racks: It is recommended that cage racks have sliding doors to prevent mice, which have escaped from the cage, from leaving the rack. These racks should be separately ventilated with clean air.
   - Rodent barriers: Animals should be maintained in designated locked rooms. There should be a double-door entry to the animal room through a screened vestibule. All openings, including drain pipes should be covered with wire mesh. Movable rodent barriers (minimum 50 cm high) should be provided around the doors of animal rooms. It is recommended that large animal rooms be subdivided by movable rodent barriers.

   Mouse traps should be placed in the room and vestibule. All interior walls must be free of cracks and crevices, and they should be easily cleaned. Ventilation ports should be examined to exclude potential routes of escape. Transport of animals between rooms should be in a sealed box. Isolators are very effective in preventing escape of the animals. Negative pressure isolators are also recommended to prevent the spread of infection.

   - Individual TgPVR animals should bear a permanent physical marker.
   - A careful animal accounting system must be in place. Daily records concerning individual animals should be maintained. Cage tags should indicate the number and type of transgenic animals and should be in agreement with the laboratory records.

6. Special measures should be taken to prevent escape during transportation of the animals. The containers used for transporting fertile TgPVR mice should be escape-proof. They must be constructed of materials which are microbiologically impermeable. Impact-resistant shipping containers for transgenic mice are best set up as a box-in-box system. The outer transport container should be padlocked to prohibit release of the animals by unauthorized persons. It should be clearly indicated on the side of the transport containers that the mice are potentially hazardous. Animals should not be shipped until the recipient has verified that the available maintenance facilities fulfil the requirements outlined above.