Infection of laboratory workers with hantavirus acquired from immunocytomas propagated in laboratory rats

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Summary
Hantavirus has been isolated in cell culture from rat immunocytomas used and stored at a research laboratory in the U.K. where there was evidence of a laboratory-acquired infection leading to haemorrhagic fever with renal syndrome. Both transplantation into LOU/M/Wsl rats and storage of passaged immunocytomas at $-70^\circ C$ over a period of 8–10 years had not eliminated the virus. The isolates were identified as Hantavirus by means of serum obtained from patients with hantavirus infection as well as polyclonal serum derived from laboratory animals. This paper identifies a potential source of hantavirus infection in laboratories. The importing of rats, rat immunocytomas and anti-immunocytoma serum in relation to the potential risks of laboratory-acquired hantavirus infection is discussed.

Introduction
Haemorrhagic fever with renal syndrome (HFRS) is a collective name for a viral infection of human beings and which has various clinical manifestations.\textsuperscript{1,2} The severe form of the disease with acute renal failure and haemorrhagic features has been reported as Korean haemorrhagic fever (KHF) in Korea; as epidemic haemorrhagic fever (EHF) in Japan and China; and as nephrosonephritis or haemorrhagic fever with renal syndrome in the U.S.S.R. A mild non-haemorrhagic nephropathy has been described as nephropathia epidemica (NE) in Scandinavia and the European part of the U.S.S.R.

In 1976 a virus named Hantaan was isolated from an Asian fieldmouse (\textit{Apodemus agrarius corea}) trapped in Korea.\textsuperscript{3} This virus was considered to be the aetiological agent of KHF. Since Hantaan virus has been shown to grow in a human lung carcinoma cell line (A\textsubscript{549})\textsuperscript{4} and in Vero E\textsubscript{6} cells\textsuperscript{5} many other related isolates have been discovered which now form the newly proposed genus \textit{Hantavirus}\textsuperscript{6} of the family Bunyaviridae.\textsuperscript{7} Between 1981 and 1983 some 67 strains of \textit{Hantavirus} were isolated in various parts of China from rodent and human sources. In addition, other hantaviruses have been isolated throughout the world from patients with KHF in Korea\textsuperscript{8} and EHF in China.\textsuperscript{9} Many \textit{Hantavirus} strains have been obtained from urban rats in Korea, Japan and China,\textsuperscript{10} wharf rats in the U.S.A. and South America,\textsuperscript{11} laboratory rats in Japan,\textsuperscript{12} and mice in Korea, China, U.S.A., Sweden and the European parts of the U.S.S.R.\textsuperscript{13}

Epidemiological studies have recognised hantavirus infections to be an
asymptomatic infection of wild rodents (rats, mice and voles) in many parts of the world. Recent examples of infection among urban and laboratory rats suggest the movement of animals carrying hantavirus to centres of human population. Many recorded cases of hantavirus infection among laboratory personnel in contact with rodents have alerted the scientific community to the potential risks of working with these laboratory animals.\textsuperscript{14-17}

During our studies of hantavirus infection of laboratory personnel at the Institute of Cancer Research (ICR), U.K., material from rats was examined in an effort to determine the source of the virus.

\textbf{Materials and methods}

\textbf{Cells}

Vero E6 cells obtained from Dr J. McCormick, CDC Atlanta, U.S.A., were used for virus isolation. The cells were grown in Eagles MEM with Hank’s salts supplemented with 10\% foetal calf serum, 200 mmol glutamine, 1\% non-essential amino acids, 100 mg streptomycin and 100,000 IU/l penicillin.

\textbf{Standard hantavirus}

The prototype \textit{Hantavirus} (76/118) was received from the American Type Culture Collection (ATCC) and bore the number ATCC 938. This virus was originally isolated from Apodemus mouse lung and passaged five times in A549 cells followed by 16 passages in Vero E6 cells. The \textit{Hantavirus} strain CG18/20 was kindly donated by Dr G. van der Groen (Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium). It had been initially isolated from \textit{Clethrionomys glareolus} captured in the western part of the U.S.S.R. and adapted to E6 cells. On receipt, both viruses were maintained and passaged in E6 cells.

\textbf{Standard antisera to hantavirus}

Convalescent-phase human sera from confirmed hantavirus patients was supplied by Dr J. Dalrymple (Fort Detrick, U.S.A.). Mouse, rabbit and non-human primate anti-hantavirus sera (76/118 and CG18/20) were raised in our own laboratory.

\textbf{Standard reovirus antisera}

Rabbit antisera raised against \textit{Reovirus} strains 1, 2 and 3 were supplied by the Standards Laboratory, Central Public Health Laboratory, Colindale.

\textbf{Sero-survey of laboratory personnel}

Serum samples were collected from several persons working in a cancer research institute and screened against hantavirus isolates by an indirect immunofluorescent antibody (IFA) test. The groups of people studied are described in Table I.

\textbf{Imported sera from LOU/M/Wsl rats}

In addition to immunocytomas being available, LOU rat sera from Belgium (dated 1974–1977) were also received by various U.K. laboratories (Table II)
Table I Detection of antibodies against different hantavirus antigens in sera from personnel associated with a laboratory-acquired hantavirus infection at the Institute of Cancer Research, Sutton, U.K.

<table>
<thead>
<tr>
<th>Personnel*</th>
<th>Area employed</th>
<th>Immunofluorescence titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>W. U.S.S.R.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CG 18/20</td>
</tr>
<tr>
<td>Group A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worked with</td>
<td>A.P.</td>
<td>Animal Unit</td>
</tr>
<tr>
<td>LOU/M/Wsl</td>
<td>S.B.</td>
<td>Animal Unit</td>
</tr>
<tr>
<td>rats and</td>
<td>R.B.</td>
<td>Animal Unit</td>
</tr>
<tr>
<td>immunocytomas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did not work with</td>
<td>S.D.</td>
<td>Animal Unit</td>
</tr>
<tr>
<td>LOU rats or</td>
<td>S.E.</td>
<td>Animal Unit</td>
</tr>
<tr>
<td>immunocytomas</td>
<td>G.B.</td>
<td>Animal Unit</td>
</tr>
<tr>
<td></td>
<td>E.J.</td>
<td>Animal Unit</td>
</tr>
<tr>
<td></td>
<td>L.G.</td>
<td>Animal Unit</td>
</tr>
<tr>
<td>Group C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worked with</td>
<td>J.P.</td>
<td>Laboratory</td>
</tr>
<tr>
<td>rat immunocytomas</td>
<td>C.D.</td>
<td>Laboratory</td>
</tr>
<tr>
<td>but not rats</td>
<td>J.S.‡</td>
<td>Laboratory</td>
</tr>
<tr>
<td></td>
<td>S.H.</td>
<td>Laboratory</td>
</tr>
<tr>
<td>Group D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worked in</td>
<td>I.M.</td>
<td>Laboratory</td>
</tr>
<tr>
<td>laboratory</td>
<td>C.B.A.</td>
<td>Laboratory</td>
</tr>
<tr>
<td>building but not with LOU rats or tissue</td>
<td>D.D.</td>
<td>Laboratory</td>
</tr>
<tr>
<td>Group E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed since 1981</td>
<td>K.D.</td>
<td>Laboratory</td>
</tr>
<tr>
<td></td>
<td>S.P.</td>
<td>and/or</td>
</tr>
<tr>
<td></td>
<td>P.H.</td>
<td>Animal Unit</td>
</tr>
<tr>
<td></td>
<td>C.H.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P.D.</td>
<td></td>
</tr>
</tbody>
</table>

*All sera were taken in 1984 from personnel who were present in the building at the time of the laboratory-acquired infection with the exception of Group E.
† Result similar with U.K. isolates H96, H98 and H99.
‡ Main laboratory worker involved with immunocytoma processing.

and examined by IFA tests against Hantavirus strain 76–118 (Korea), CG 18/20 (U.S.S.R.) and our own U.K. isolates. The sera were derived from LOU/Wsl rats which had been implanted with various immunocytomas (Table II).

**Immunofluorescence staining**

The IFA test procedure as outlined by McCormick and colleagues\(^5\) was followed. Standard positive and negative sera were included. The fluorescent conjugate was supplied by Miles Laboratories Ltd.
Table II Detection of antibodies against different hantavirus antigens in LOU/Wsl rat sera originally imported from Belgium by three U.K. laboratories using the indirect immunofluorescence assay

<table>
<thead>
<tr>
<th>Belgian sera from rats with implanted immunocytomas</th>
<th>IF antibody titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>West U.S.S.R. Korea U.K.</td>
</tr>
<tr>
<td></td>
<td>CG 18/20 76/118 H95 H96 H97 H98</td>
</tr>
<tr>
<td>IR22 O,B</td>
<td>&lt; 8 1024 2048 1024 N.D. 1024</td>
</tr>
<tr>
<td>IR31 S</td>
<td>128 1024 2048 1024 1024 1024</td>
</tr>
<tr>
<td>IR33 S</td>
<td>256 2048 512 1024 512 1024</td>
</tr>
<tr>
<td>IR162 O,B,S</td>
<td>&lt; 8 2048 1024 2048 1024 1024</td>
</tr>
<tr>
<td>IR202 B,S</td>
<td>&lt; 8 512 256 128 64 128</td>
</tr>
<tr>
<td>IR314 O</td>
<td>&lt; 8 512 256 256 128 256</td>
</tr>
<tr>
<td>IR418 B</td>
<td>&lt; 8 256 128 256 64 256</td>
</tr>
<tr>
<td>IR461 S</td>
<td>&lt; 8 512 1024 512 128 512</td>
</tr>
<tr>
<td>IR1060 B</td>
<td>&lt; 64 1024 512 N.D. 1024 N.D.</td>
</tr>
</tbody>
</table>

* O, Oxford University Department of Pathology; B, Bristol University, Department of Veterinary Medicine; S, Institute of Cancer Research, Sutton.
N.D., Not done.

Specimens for virus isolation
A number of rat immunocytomas (Table I) which had been stored at -70 °C were examined. These tumour cells were of varying passages and were dated between April 1977 and February 1983. The original immunocytomas had been received from Belgium in 1974 and were derived from the LOU/Wsl rat strains.

The immunocytomas had been passaged in colonies of LOU/M/Wsl rats also obtained from Belgium. Later transplantations of immunocytomas were achieved by means of the same rat strain derived from commercial sources in the U.K.

Virus isolation
The solid immunocytomas were homogenised in a small quantity of EMEM with the aid of a blender run for 2 min within a Class III safety cabinet. The homogenate was clarified by centrifuging at 1000 r.p.m. for 10 min in a Denley centrifuge having a rotor with sealed buckets. Two procedures were adopted for isolating the virus. Firstly, 0.25 ml supernatant from the clarified homogenate was inoculated into a 25 cm² flask containing a monolayer of E6 cells. After adsorption for 1 h at 37 °C, 4.75 ml growth medium were added. Secondly, 0.5 ml homogenate was mixed with 4.5 ml of a suspension of E6 cells (5 x 10⁵ ml), introduced into a 25 cm² flask and incubated at 37 °C. After 48 h the medium was changed. Further changes of medium were made according to the condition of the cells. The presence of antigens was detected by means of IFA tests.
Laboratory-acquired hantavirus infection

Results

Laboratory-acquired hantavirus infection

Between January and July 1977 four people who worked in an animal suite housing LOU/M/Wsl rats developed symptoms similar to those of hantavirus infection. These persons had been exposed to the rats from April 1975, the time when they were introduced from the University of Louvain, Belgium. From early 1976, rat immunocytomas also were introduced from Louvain for passage into the LOU/M/Wsl strain of rat, the procedures being performed by the same persons. The work with rats and immunocytomas was done within a small confined animal unit containing a rat operating theatre and with an adjacent post-mortem (PM) room in which the four persons worked for long periods. Unwanted rat tissue and carcasses were placed in an automated macerator (situated in the PM room) before disposal into the domestic sewerage system. The macerator did not have any safety lock or seal on the lid and was frequently opened mid-cycle to add further animal tissue. The ventilation flowed from input vents in the ceiling over the macerator and toward the exit vent situated by the door. This, coupled with back-tracking of macerator waste into the operating theatre sink, could have resulted in the generation and emission of aerosols into the immediate vicinity, including corridors and other rooms of the animal unit. Such practices that may have created an aerosol have since ceased.

The clinical illness observed in these workers ranged from mild to severe. Three of the four were in hospital for 2–3 weeks and all required additional 4–13 weeks of convalescence. All of them had clinical features of classical KHF—an early toxic reaction characterised by fever, myalgia, weakness, petechiae, sore throat, dysphagia and diaphoresis, oliguria, proteinuria, raised blood urea nitrogen, and diuresis with fluctuating electrolyte concentrations and varying degrees of anaemia. All complained of abdominal discomfort together with kidney pain. Two of them developed aneuria which required haemodialysis. The fourth person had a mild attack with an acute phase lasting only 6–7 days and was treated at home.

Serum was not available from the period of illness from any of the patients but three samples of serum obtained during 1984 were tested for antibodies and the results are shown in Table I (Group A). Serum was not available from the fourth patient, a visiting research worker from overseas at the time of the laboratory outbreak, since he had returned home before our study began. Other laboratory personnel who worked during the same period were also studied retrospectively; data from a representative sample within each group are shown in Table I. Within Group B, five of six people found to be seropositive to hantavirus had worked in other parts of the animal unit and had not had any direct contact with the LOU rats or their tissues. The combined serological data of Groups A and B show that nine of the ten workers (90%) associated with the animal unit during the period of the infection had significant titres of hantavirus antibodies. It should be noted that five of these workers did not report any ill effects suggesting the possibility of a laboratory-derived inapparent infection. Studies of other persons working within the laboratory but not with
animals revealed only one with seroconversion, a person in Group C and whose contact was confined to handling immunocytomas removed from rats. Direct contact with the animals was never made because the person concerned was allergic to animal fur. Her only exposure was through homogenising the solid immunocytomas in the laboratory, a procedure which was confined to a small area designed for that purpose. The remaining five workers in the laboratory were not involved with processing immunocytomas. The homogenisation procedure suggested additional risks from aerosol transmission of infection. Group D consisted of 12 laboratory workers based in other parts of the building. Hantavirus antibodies were not found in their sera indicating that hantavirus infection had been confined to those people associated with the animal unit (Groups A and B) or with the laboratory processing of immunocytomas derived from LOU rats (Group C).

In nine persons employed since 1981 (Group E) and working in both the laboratory and the animal unit there was not any evidence of seroconversion to hantavirus. None of these had worked with LOU rats or immunocytomas.

Comparative serological data presented in Table I also show a greater affinity for the Korean and U.K. isolates than for the U.S.S.R. hantavirus.

**Recovery of hantavirus**

The only material studied and stored by the laboratory workers during the period of interest (1975 onwards) consisted of several rat immunocytomas which had been propagated in LOU/M/Wsl rats. Hantavirus could be demonstrated in these immunocytomas (Table III) when primary cultures were maintained for between 15 and 34 days after inoculation. Subsequent
passage of the isolates in E6 cells reduced the period required for isolation of virus to 5–9 days (3rd passage). The antigen was detected in the cytoplasm by IFA tests on day 2 as fine granules of fluorescence increasing in intensity by day 9 to give an average mean viral titre of $10^{6.8}$ pfu/ml.

It will be noted (Table III) that hantavirus was isolated from batches of immunocytomas dated between 1977 and 1983. The passage of these immunocytomas into the LOU rats during that period revealed a perpetuating source of hantavirus within the laboratory since they were still found to be viable even after storage in liquid nitrogen for between 2 and 8 years.

**Serological response of rats originating in Belgium**

The data presented in Table II demonstrate that the imported Belgian sera had IF antibody titres to hantavirus ranging from 256 to 2048 suggesting that these rats had been exposed to hantavirus. Similar serological reactions with sera from laboratory staff and the Belgian LOU rats were apparent when tested against U.K. and Korean isolates.

**Conclusion**

Epidemiologists from the Soviet Union and Scandinavia have suggested that the route of transmission of hantavirus to human beings is by infective excreta, particularly urine. Hantavirus infection of laboratory personnel in a Moscow Institute was thought to originate from colonies of wild *Clethrionomys glareolus* (vole) via aerosols created in the animal storage area of the laboratory. Reports of Japanese laboratory infections confirm the aerosol route as the means of transmission to human beings from Wistar rats. The present investigation supports this view and demonstrates an additional risk from the removal, processing or transplantation of rat immunocytomas.

Two reports from Belgium and the U.K. have implicated the involvement of LOU/M/Wsl rats in human laboratory infections. Both institutes concerned were involved with studies on tumour immunology. The LOU/Wsl strain of rat consists of albino rats which originated from Wistar stock (derivation uncertain) and which have been inbred since 1956. This inbred stock has shown a high incidence of spontaneous tumours secreting monoclonal immunoglobulins. These tumours are described as ileocaecal immunocytomas in recognition of the site of origin of the tumour. They have the capacity to synthesise monoclonal rat immunoglobulins. These immunocytomas are transplantable in histocompatible animals and their secreting properties are maintained over many passages. The LOU/Wsl strain of rat has been regarded as a suitable model for the study of neoplastic transformation in immunoglobulin-secreting plasmocytomas, this being a valuable experimental model in cancer immunology and in molecular biology.

A suggestion of tumours being involved in the transfer of hantavirus during their transplantation into laboratory rats was made in the Belgian study but characterisation of the tumours and the rats was not recorded. The isolation of a virus was not reported. In a Japanese study, hantavirus was isolated from a histocytoma derived from Fischer rats in which the tumour had been induced chemically by 4-nitroquinoline-N-oxide.
In the present study virus has been isolated directly from rat immunocytomas thereby identifying the source of the U.K. laboratory hantavirus infection at the Institute of Cancer Research, Sutton, U.K. All the immunocytomas and LOU/WsI rats were originally imported from Belgium in 1975. At various times between 1975 and 1985 immunocytomas were transplanted into rats, samples of the tumours being taken and stored at $-70\,^\circ\text{C}$. The data demonstrate that the immunocytomas retained their infectivity during transplantation and storage. Thus a reservoir of hantavirus had been retained unnoticed for 10 years. Studies at present under way also demonstrate that immunocytomas free of hantavirus became positive when introduced into LOU/C rats persistently infected with hantavirus.

Examination of several different samples of rat anti-immunocytoma serum obtained from Belgium by three independent laboratories in the U.K. demonstrated that all were obtained from a source which was contaminated with hantavirus. The rat immunocytomas derived from the same laboratory during 1974–1975 yielded hantavirus. It has not been possible in this study to determine whether the laboratory rats or the original immunocytomas were the primary source of hantavirus. Nevertheless, it is important to note that some batches of immunocytomas derived from LOU/WsI rats have at some stage become infected with hantavirus.

Many of the rat immunocytomas listed are currently being studied in many non-microbiological laboratories. On the basis of the results presented here it would seem important to have a fully documented history of each immunocytoma and to determine its hantavirus status.

Attention must also be drawn to the use of laboratory rats in general because many workers have demonstrated laboratory and wild rats to be potential carriers of hantavirus. Since the rat does not display any observable clinical features and is widely used in many scientific disciplines, it would appear that a screening procedure to define hantavirus-free stocks is needed.

A review of rat immunocytomas and of rat colonies would seem to be increasingly important when one considers the current interest in rat monoclonal antibodies and their application to diagnosis as well as their use in therapy. Development of rat myeloma cell lines enables large amounts of antibody to be derived from ascitic fluid, a pre-requisite for clinical trials. As Bazin has emphasised, the LOU rat model forms the basis of this technology. Moreover it has provided the first rat fusion line (Y3 Ag 1,2,3) and the first rat non-secreting non-patented fusion line (IR 983F).

Although limited studies have not so far found any of the lines of rat fusion cells to be carrying hantavirus, it is conceivable that these lines may be exposed to hantavirus-infected laboratory rats. There is increasing evidence that many laboratory and wild rats are proving to have antibody against hantavirus. This confirms the need for all rat colonies to be screened before use.

References