REPORT OF A MEETING ON
HANTAVIRUS VACCINE DEVELOPMENT

Helsinki, Finland
31 May 1995

WORLD HEALTH ORGANIZATION
Bacterial, Viral Diseases and Immunology
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1. INTRODUCTION

During the past 15 years, understanding of haemorrhagic fever with renal syndrome (HFRS) has greatly increased. Prior to the mid-1970s the clinical disease was known, but its cause remained illusive. With the isolation of Hantaan virus by Ho Wang Lee and his colleagues, a new era began which led to a better understanding of the clinical disease, its epidemiology, and the viruses which are now known to cause a complex of related diseases. For example, several distinct yet antigenically and genetically similar viruses are capable of causing HFRS. Indeed, this group of viruses not only causes classic HFRS, but other closely related viruses cause a completely distinct disease characterized by acute pulmonary distress, now called hantavirus pulmonary syndrome.

With the isolation of Hantaan virus, work began almost immediately to develop a protective vaccine for HFRS. Today, that work has progressed markedly, so that several inactivated vaccines are now undergoing human efficacy trials in China, and another is commercially available in Korea. In addition, candidate engineered vaccines have been developed using the knowledge gained from molecular analysis of the hantaviruses and the tools available from the biotechnological revolution. Thus, the field of vaccine development for HFRS and related hantaviruses is moving ahead on several fronts. In an attempt to foster collaborations and better understanding of the current status of the field, the World Health Organization hosted a meeting of international experts working in hantavirus vaccine development to share their preliminary results and plan future activities. The meeting was held immediately prior to the Third International Conference on HFRS and Hantaviruses held in Helsinki, Finland, thereby allowing participants to attend both the Congress and this special satellite meeting.

2. SUMMARY OF GLOBAL BURDEN OF DISEASE DUE TO HANTA VIRAL INFECTIONS

Asia. The greatest number of cases of hantaviral infections occur in Asia, with HFRS well documented in Korea, China, and the Asian regions of the former Soviet Union. Human disease is caused by at least two distinct hantaviruses - Hantaan virus and Seoul virus. Other serologically related hantaviruses have been documented from the region, and while their role as human pathogens is currently undetermined, they clearly are less significant than either Hantaan or Seoul virus.

In China, HFRS has been recognized at least since 1931 in the northeast provinces, and since 1955 it appears to have spread to other regions of the country. In recent years, 40,000 to 100,000 cases were documented annually, and 28 of 30 provinces are now endemic for the disease. Incidence rates have risen in the last two decades, and a national surveillance system has been in operation since 1984. Between 1950 and 1994, over 1 million HFRS cases were documented, with more than 42,000 deaths. The overall mortality rate for the entire country for this period was 3.85%. Table 1 summarizes the morbidity and mortality rates for HFRS documented in China from 1931 to 1994. Approximately half of the HFRS patients are due to Seoul virus infection.
Table 1

Morbidity and Fatality Rates of HFRS in China from 1931 to 1994

<table>
<thead>
<tr>
<th>Period</th>
<th>No. of cases</th>
<th>Morbidity (1/100,000)</th>
<th>No. of deaths</th>
<th>Fatality rates (%)</th>
</tr>
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<tr>
<td>1931-42</td>
<td>10,378 (reported)</td>
<td>*</td>
<td>3,047</td>
<td>29.4</td>
</tr>
<tr>
<td>1950-59</td>
<td>3,568 (registered)</td>
<td>0.02-0.03</td>
<td>297</td>
<td>8.3</td>
</tr>
<tr>
<td>1960-69</td>
<td>23,764</td>
<td>0.10-0.30</td>
<td>3,587</td>
<td>15.1</td>
</tr>
<tr>
<td>1970-79</td>
<td>143,949</td>
<td>0.40-2.19</td>
<td>12,471</td>
<td>8.7</td>
</tr>
<tr>
<td>1980-89</td>
<td>696,074</td>
<td>3.12-11.08</td>
<td>22,867</td>
<td>3.3</td>
</tr>
<tr>
<td>1990-94</td>
<td>241,857</td>
<td>3.66-4.95</td>
<td>4,809</td>
<td>2.0</td>
</tr>
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</table>

* undetermined

In the Republic of Korea, slightly more than 1,000 patients were hospitalized with HFRS during 1994, but only 34 of them were Korean soldiers. This represents a significant decrease from previous years, when over 100 soldiers were hospitalized annually. The decrease is purportedly due to the mass vaccination of Korean soldiers with a recently developed, commercially available vaccine to Hantaan virus (see below).

In the Asian territory of Russia, nearly 3,000 HFRS cases were documented between 1978 and 1994. Cases occurred in 15 of 29 Asian administrative territories of the country, and comprised 3.6% of all HFRS cases documented throughout the Russian Federation during this period.

**Europe and European Russia.** Four distinct hantaviruses cause human disease in Europe, but the vast majority are due to Puumala virus, etiological agent of nephropathia epidemica. Other hantaviruses present and causing human disease include Hantaan, Seoul, and Dobrava/Belgrade, currently now recognized only in the Balkan region of Europe. In the Russian Federation, 46 of 60 administrative regions suffered over 80,000 cases between 1978 and 1994, and the trend appears to be toward increasing incidence of disease. Puumala virus is responsible for the greatest number of cases, and has now been well documented throughout Scandinavia and most countries of Europe, with significant numbers of cases reported from Norway, Sweden, Finland, Germany, the Netherlands, France and Belgium. The Balkan countries are at the southern border of the distribution of *C. glareolus*, the primary host of Puumala virus; consequently, the incidence of disease due to this virus begins to diminish in this area, but severe HFRS due to Hantaan and Dobrava/Belgrade viruses appears to be more prevalent there than elsewhere in Europe. Overall, the greatest incidence of hantaviral disease is found in the northern regions of Europe, and decreases to the south.
The Americas. Prior to recognition of hantavirus pulmonary syndrome (HPS), human disease was virtually unknown in the Americas, in spite of the well documented presence of Seoul virus in rats in both North and South America. Today, over 100 cases of HPS have been documented in North America, and strong evidence is appearing that a clinically similar disease is present in South America. Although a rare disease, the risk of death following infection appears to be great, with the current case fatality rate being over 50%. Rodent hosts of Sin Nombre and related viruses which cause of HPS in North America are rural in nature, and human infection is limited to individuals exposed either through rural occupation or recreation. As a result, the overall burden of disease is slight.

3. SUMMARY OF CURRENT STATUS OF HANTAVIRUS VACCINE DEVELOPMENT

Inactivated vaccines. Three different hantavirus vaccine preparations have been licensed for production in China, and one in Korea. Vaccines have been made to both Hantaan and Seoul viruses. Three substrates have been used and at present very little difference has been noted in the quality of the final products. These are suckling mouse brain (PMB), Mongolian gerbil kidney cell culture (MGK) and golden hamster kidney (GHK) cell cultures. Chinese vaccines have been examined for quality control by the National Institute for the Control of Pharmaceutical and Biological Products, while the Korean product has been controlled by the National Institute of Health. Examinations include tests to detect residual live virus, serum protein content, total protein content, antigen content, and immunogenicity in laboratory animals. Indications are that suckling mouse brain vaccines contain a greater proportion of nucleoprotein antigen than the cell culture preparations and thus elicit lower neutralizing antibody responses; however, protective efficacy tests in laboratory animals do not show a significant difference between the two preparations. Immunization schedules have been compared and it appears that to elicit the best immune response, three inoculations are required during the primary series, followed by a booster at one year. Long-term maintenance of antibody titres is still being examined, and at present there is no data to indicate whether boosters beyond one year will be required to maintain protective immunity.

Phase 1 and 2 safety tests have been performed on all candidate vaccines and no significant adverse reactions have been found.

Phase 3 efficacy tests are under way in China on candidate vaccines produced in all three systems. Each evaluation has included unvaccinated control populations at risk of disease. Preliminary results obtained after one season suggest that all candidates appear to protect against natural infection, with rates of protection varying between 95-100%. The Korean product has not been subjected to formal efficacy testing, but has been used to immunize a large segment of the Korean army, which is known to be at risk of hantaviral infection. The absolute number of clinical HFRS cases reported among Korean military forces in 1994 was 34 cases, down from 104 cases reported in 1989.
4. CONCLUSIONS AND RECOMMENDATIONS

After considerable discussion among the participants, the following conclusions and recommendations were agreed upon:

(1) Hantaviruses are significant human health pathogens in many parts of the world. Consequently, the final objective of hantavirus vaccine development efforts should be to produce a polyvalent vaccine that protects against all pathogenic hantaviruses. To attain this objective, additional information is needed regarding cross-protection among the various hantaviruses, especially those newly recognized viruses that cause hantavirus pulmonary syndrome.

(2) Inactivated vaccines for hantaviruses are the most advanced in development, and several observations can now be made:

- Two methods of inactivation have been used, beta propiolactone (BPL) and formalin. Both appear to yield equivalent final products, although BPL appears to retain some surface antigens that may be lost with formalin inactivation. However, BPL may not be available for use in all countries.

- Various dose schedules have been tried and the conclusions reached to date are that a primary series appears to require three doses, but more data are needed regarding antigenic content and the possible influence on the number of doses needed. In addition, it appears that a one year booster will be required for all currently formulated inactivated vaccines. Additional information is required regarding long-term immunity; at present follow-up studies have been limited to only a year. Further information is also needed to determine the level and type of antibody required to maintain lasting immunity.

- Three substrates have been successfully developed for inactivated vaccine candidates, and these vary in cost. The approximate costs for inactivated vaccines produced in China are: Mouse brain vaccine is US $3.60 (30 rmb); Mongolian gerbil is US $3 (25 rmb); and hamster kidney is US $2.40 (20 rmb). The mouse brain substrate does not support growth of Puumala virus, cause of nephropathia epidemica in Europe, to sufficient titre for vaccine production. This virus does, however, grow well in Mongolian gerbil and hamster kidney cell substrates. In addition, there may be quality control issues in some countries that preclude the use of these substrates for vaccine production, such as screening for retroviruses and other adventitious agents. Thus, further evaluation of vaccine substrates should be encouraged.

- Vaccine efficacy should be determined for each candidate prior to widespread distribution or marketing.

- Animal testing for vaccine efficacy appears to offer promising insights into candidate vaccines’ ultimate efficacy, but better animal models are still required.

- Additional information is required to determine the roles played by cytokines, interferons, cell-mediated and mucosal immunity in response to inactivated vaccines.
• Standardized safety testing is required and should include systematic screening for adventitious agents, inactivation procedures, and final vaccine composition.

• There is a need to improve packaging and storage conditions of vaccines, as all candidate inactivated vaccines now appear to be stored as liquids. Thus, lyophilization procedures should be developed and standardized.

• There may be value in considering simultaneous vaccination against other endemic, vaccine-preventable diseases. For example, HFRS and Japanese encephalitis in Asia, and Puumala virus with Tick-borne encephalitis in Europe. Ultimately, candidate HFRS vaccines should be considered for inclusion into routine EPI immunization schedules in high risk areas. Additional information is required to determine the feasibility of these recommendations.

(3) **Recombinant vaccine candidates** are not as well developed as inactivated vaccines, but the group clearly encouraged further development, including expanded human phase 1 and 2 trials of recombinant vaccines.

• Vaccinia vectors have shown promise for use in engineered hantavirus vaccines, but possible problems in vaccinia-immune populations should be addressed. Further, two of the most promising candidate vectors, NYVAC and ALVAC, are apparently not available for commercial development. Care should be taken to ensure that any vaccinia vectors selected for use will ultimately be available for commercial development.

• Adenovirus vectors are in the exploratory phases of investigation and may prove useful as vectors of hantavirus genes. Further investigations of adenovirus vectors should be encouraged.

• Additional studies are required to compare inactivated and recombinant candidate vaccines.

(4) At present, development of **live attenuated candidate vaccines** is hampered by the lack of an adequate animal model to safely reflect attenuation for human disease. Consequently, additional work is needed to identify useful animal models. Additional studies are likewise required into virulence markers.

(5) Additional information is required regarding the optimum route of immunization of candidate hantavirus vaccines.
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