Toward a Vaccine against Argentine Hemorrhagic Fever

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A vaccine against Argentine hemorrhagic fever, the "mal de los rastrojos" of the pampas, has been a dream of physicians and scientists involved with the disease since its recognition in the 1950s. Several killed and live immunogens have been produced and tested in pursuit of this goal, none of which has proved suitable for widespread human use. Recently, a new live-attenuated Junin virus vaccine, Candid #1, was developed through a cooperative international effort. Testing conducted to date indicates that this vaccine holds considerable promise.

Argentina hemorrhagic fever (AHF) is a clinically severe, rodent-borne zoonosis restricted in nature to the fertile pampas of north-central Argentina. The causative agent, Junin virus, is an arenavirus that induces chronic infections among reservoir rodents of the family Cricetidae and is transmitted to man via fomites and aerosols of contaminated excreta. The region's rural inhabitants and agricultural workers constitute the population at greatest risk (1). Cases of AHF, typically fatal among 5% to 30% of those afflicted and generally more severe in older persons, are recognized throughout the year; but the vast majority of the illnesses occur during a well-defined epidemic season that lasts from February through July and coincides with the annual grain harvests.

AHF epidemiology indicates an expanding disease with changing incidence. When the disease first was recognized in the 1950s, its endemic area was limited to a region 16,000 km² within the province of Buenos Aires (1). Since then it has spread to involve three adjacent provinces; it currently covers an area nearly eight times that originally described (2), and appears destined to continue extending outward in a northeasterly direction (3).

During the 1960s and 1970s, epidemic outbreaks exceeding 1,000 cases per year were frequent. Since 1979, however, the annual incidence has rarely exceeded 400 cases (4; J. I. Maiztegui, unpublished data). One reason is that the continually advancing disease front has created "historic" areas where AHF has been seen for 10 years or more. While cases continue to occur in such regions, the annual disease incidence is much lower than that seen in more recently affected regions (2). Curiously, this phenomenon occurs despite a continued population susceptibility that exceeds 95% (5).
HISTORY OF VACCINE DEVELOPMENT

Inactivated Vaccines

The need for a widely available vaccine to protect against AHF has long been recognized. Initial efforts focused on formalin-inactivated mouse brain preparations (6, 7) that induced neutralizing antibodies and protected mice and guinea pigs against virulent Junin challenge (Table 1). One such product was inoculated into more than 15,000 persons between 1959 and 1962, although its efficacy was never assessed (8). However, the need to administer 3–4 weekly doses with or without a “booster,” together with concerns about the virus substrate (i.e., mouse brain), severely limited these vaccines’ potential for general use. Recently, some of these deficiencies were addressed through production of a cell culture-derived, formalin-inactivated immunogen. Neutralizing antibodies were induced successfully with this product; however, vaccinated animals failed to resist challenge with a virulent Junin virus strain (9).

Additional inactivation strategies have included exposure of Junin virus to photoactive dyes (10, 11), ultraviolet irradiation (12), heating, and acetone (13). Though at least one resulting product has been administered to humans in limited studies (Table 1), all such products have proved of limited utility. A subunit immunogen composed of purified G38 envelope glycoprotein emulsified in Freund’s complete adjuvant has induced neutralizing antibodies in rabbits and has protected guinea pigs against virulent Junin virus challenge (14). While this approach has shown some limited promise, additional studies are still needed to assess its potential for human vaccination.

Live Vaccines

The alternative to virus inactivation and classical vaccine development involves production of a live-attenuated immunogen. As Table 2 indicates, a number of such immunogens, derived from both heterologous and homologous viruses, have been produced and tested.

Heterologous live vaccines. Viruses comprising the family Arenaviridae fall serologically into two groups: the so-called “Old World” (lymphocytic choriomeningitis, Lassa, Mopeia, Mobala, and Ippy) and “New World” (Machupo, Junin, Tacaribe, Amapari, Parana, Latino, Tamiami, Pichinde, and Flexal) arenaviruses.

The type-member of the “New World” complex, Tacaribe virus (15), is closely

<table>
<thead>
<tr>
<th>Table 1. Candidate Argentine hemorrhagic fever vaccines (inactivated).</th>
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<tbody>
<tr>
<td><strong>Type</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Formalin-killed</td>
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<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td>Photo-inactivated</td>
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<tr>
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<td>Subunit</td>
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*Bromsted seropositives.*
Table 2. Candidate Argentine hemorrhagic fever vaccines (live).

<table>
<thead>
<tr>
<th>Type</th>
<th>Strain used</th>
<th>Passage historya</th>
<th>Laboratory animal experience</th>
<th>Human experience</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Immunogenic</td>
<td>Protective</td>
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<tr>
<td>Heterologous</td>
<td>Tacaribe</td>
<td>Strain 11573</td>
<td>Yes (18–26)</td>
<td>Yes (18–26)</td>
</tr>
<tr>
<td></td>
<td>XIO</td>
<td>GP&lt;sub&gt;2&lt;/sub&gt;, MB&lt;sub&gt;19&lt;/sub&gt; (43)</td>
<td>Yes (43, 44)</td>
<td>Yes (43, 44)</td>
</tr>
<tr>
<td></td>
<td>XJ Cl 3</td>
<td>GP&lt;sub&gt;2&lt;/sub&gt;, MB&lt;sub&gt;14&lt;/sub&gt;</td>
<td>Yes (32)</td>
<td>Yes (32)</td>
</tr>
<tr>
<td>Homologous</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>RMK&lt;sub&gt;4&lt;/sub&gt;, HB&lt;sub&gt;1&lt;/sub&gt;, MA-111&lt;sub&gt;3&lt;/sub&gt;, MB&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Candid #1</td>
<td>GP&lt;sub&gt;2&lt;/sub&gt;, MB&lt;sub&gt;44&lt;/sub&gt;, FRL&lt;sub&gt;19&lt;/sub&gt;</td>
<td>Yes (50, 52, 53)</td>
<td>Yes (50, 52, 53)</td>
</tr>
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</table>

<sup>a</sup>GP = guinea pig; MB = mouse brain; RMK = rhesus kidney cell culture; HB = hamster brain; MA-111 = rabbit kidney cell line; FRL = fetal rhesus lung cell culture. Subscripts indicate passage numbers.

<sup>b</sup>Portions of passage history uncertain: lineage reconstructed from written documents provided by Dr. N. Wiebenga and (31) (see text).

Heterologous live vaccines. A fortuitous observation at the Rockefeller Foundation Virus Laboratories provided the key to developing a live-attenuated homologous vaccine. Investigators there noted that the prototype XJ strain of Junin virus seemed to lose its pathogenicity for guinea pigs after about 40 passages through newborn mouse brain (29)—although this was thought initially to be due to host resistance rather than viral attenuation (30). Subsequently, a virus pool was created by Wiebenga at the U.S. National Institutes of Health by passing a plaque-purified isolate (purified in primary rhesus monkey kidney cells) from mouse brain passage 14 of XJ into suckling hamster brain. This strain, designated XJ Clone 3 (N. Wiebenga, written communication), was passed further in continuous MA-104 and MA-111 cells, one passage (from MA-111 cells) being further passed in suckling mouse brain and evaluated as a prospective vaccine (31).

XJ Clone 3 proved attenuated and immunogenic after intraperitoneal inoculation of guinea pigs, and was found to protect animals against virulent virus challenge (32). Its safety for man then was evaluated in seven individuals involved in Junin virus research. Mild, transitory clinical reactions occurred among the volunteer group (33), and neutralizing antibodies were elicited in all (34).

On the basis of these encouraging results, XJ Clone 3 was administered to an additional 629 Argentine volunteers in the 1968–1970 period. Clinical reactions...
were recorded in 75.6% of 213 intensively studied individuals: 44% developed fever, often accompanied by asthenia, headache, retro-ocular pain, and myalgia which appeared 3–10 days after immunization and resolved spontaneously within 3 days; 29.1% developed asthenia, headache with retro-ocular pain, and myalgia unaccompanied by fever that lasted 1 to 4 days; and 2.4% developed a local reaction at the site of inoculation that disappeared within 48 hours. The remaining volunteers (24.4%) remained asymptomatic (35). Out of 57 individuals given hematologic analyses, 29 (50.9%) displayed mild leukopenia, 5 (8.8%) mildly decreased platelet counts, and 11 (19.2%) both; no alterations were found in the remaining 12 (21.1%) (31, 35). Neutralizing antibodies were found in 97% of 165 individuals examined 1–3 months after vaccination (31). Follow-up studies conducted 7–9 years later on 267 of these volunteers revealed no long-term side effects; 153 of 165 (90%) retained measurable neutralizing antibodies against Junin virus (31).

Because of uncertainties in the passage history of XJ Clone 3 (its previous passage through heteroploid cells and mouse brain substrates), administration of the vaccine was discontinued in 1971 (17). Further characterization of this product continued, however, and additional passages of the prototype XJ strain were evaluated for attenuation.

It was clear that XJ Clone 3 was significantly attenuated for guinea pigs when compared with naturally occurring Junin virus strains (36). Nevertheless, residual peripheral virulence, neurotropism, and neurovirulence could be demonstrated in these animals and in primates (37–39). Moreover, by using sensitive techniques—such as cocultivation and immunoperoxidase staining for antigens—persistence of the virus in guinea pig tissues could be demonstrated for up to three months (39–41), and antigen could be found in primate organs over one year after infection (42).

Another attenuated Junin virus strain, derived from prototype XJ and designated XJO, was maintained in newborn mouse brain and not passaged in cell culture (43). Although XJO reportedly conferred complete protection against virulent Junin virus challenge for guinea pigs, it also exhibited persistence, neurotropism, and neurovirulence similar to those exhibited by XJ Clone 3 (41, 43–47).

CANDID #1

Encouraging discussions held in 1976 during the First International Seminar on Hemorrhagic Fevers Produced by Arenaviruses (48) led to a joint effort directed at developing an acceptable live-attenuated Junin virus vaccine against AHF. This was begun as a joint effort in 1979 by the U.S. Army Medical Research Institute of Infectious Diseases and the Argentine Ministry of Health and Social Action, under auspices of the United Nations Development Program and PAHO. The goals were straightforward: identify a virus at least as attenuated as XJ Clone 3 for humans with an acceptable passage history; adapt the virus to replication in certifiable cell substrates; select attenuated variants with minimal residual virulence that are immunogenic and confer protection upon laboratory animals; and develop and test this candidate vaccine in human volunteers under the supervision of U.S., Argentine, and PAHO regulatory agencies.

Two approaches to vaccine development were undertaken concurrently. The first, identification of an already attenuated Junin virus strain that could be used as a parent virus for further modification, provided a direct route to a highly attenuated virus that could be evaluated for
acceptability as a vaccine candidate by appropriate methods. The second entailed isolation and passage in certified cell substrates of a novel, naturally occurring Junin virus strain in order to enrich for attenuated variants. The latter effort yielded viruses with reduced but still significant virulence, and this approach was abandoned.

The development initiative culminated in the production of Candid #1 vaccine. The virus strain involved was derived from a documented (mouse brain and guinea pig passaged) descendant of prototype XJ Junin virus that had been passaged and cloned in certified diploid mammalian cells (Figure 1) (49; J.G. Barrera Oro et al, manuscript in preparation).

Candid #1 is more attenuated for newborn mice and guinea pigs than XJ Clone 3 (50; J.G. Barrera Oro et al, manuscript in preparation). Neuroinvasiveness and neurovirulence in nonhuman primates are minimal, being less than what is exhibited by XJ Clone 3 (51-53). Candid #1 has demonstrated immunogenicity and protective efficacy in both guinea pigs and nonhuman primates (26, 50, 52, 53).

![Figure 1](Figure 1. Passage history of Candid #1 Junin virus vaccine. GPp = guinea pig passage, MBp = newborn mouse brain passage, FRLp = fetal rhesus lung cell culture passage.)

It has also been found to protect macaques against heterologous challenge with virulent Machupo virus, the closely related etiologic agent of Bolivian hemorrhagic fever (54, 55).

Candid #1 has been evaluated for safety and immunogenicity in more than 200 human volunteers from both the U.S. and Argentina (56, 57). To date, no significant adverse reactions related to vaccination have been observed, and more than 95% of the recipients have developed antibodies after immunization. The vaccine’s efficacy is currently being studied by means of a large, double-blind, placebo-controlled field trial in Argentina’s Santa Fe Province (J.I. Maiztegui, K.T. McKee, Jr., personal communication).

**CONCLUSIONS**

The history of vaccination against AHF spans a period of more than 30 years. During that time, a variety of classical approaches to vaccine development have been employed in an attempt to produce a safe and effective human immunogen. Development of the current most promising candidate, Candid #1, has depended upon experience gleaned from years of experimentation in laboratories and clinics on two continents. Specifically, extensive testing of XJ Clone 3 in animal models and humans provided a wealth of scientific information permitting continued modification of the prototype XJ Junin virus strain to ultimately produce Candid #1.

If successful, Candid #1 will become the first vaccine widely employed against an arenavirus. It thus appears that experience gained in the efforts to develop it should have broader applicability to other arenaviruses pathogenic for man, most notably Lassa virus. Recently, vaccine research on Lassa fever prophylaxis has focused upon molecular cloning and vector insertion (58-61). While this excit-
ing new approach holds great promise for vaccine development, it has not yet produced a product suitable for human use. Should the potential of this new technology fail to be realized, the experience gained through Junin virus vaccine development will be of great utility in planning and implementing a classical Lassa vaccine program.

REFERENCES


### Cholera Takes Hold in the Americas

The epidemic of cholera that is currently raging in South America has spread from its initial focus on the Pacific coast of Peru to areas throughout that country and to parts of Chile, Colombia, and Ecuador. From the start of the epidemic in January to 2 May 1991, almost 175,000 cases, of which 1,390 were fatal, had been notified by health authorities in those four countries, as follows: 169,255 cases and 1,244 deaths in Peru, 5,005 cases (1,107 confirmed) and 140 deaths in Ecuador, 189 cases and 5 deaths in Colombia, and 26 cases and 1 death in Chile. In addition, five cases have been reported from Amazonas State in Brazil, and five imported cases have occurred in the United States.

Cholera is an acute bacterial enteric disease, usually of sudden onset, that causes profuse watery stools, vomiting, rapid dehydration, acidosis, and circulatory collapse. Mild cases may be indistinguishable from other types of diarrhea except by fecal cultures. Without treatment, the case-fatality rate of serious cholera can reach 50%. With adequate treatment, mortality is around 1%. Management of the disease focuses on replacing fluids and electrolytes.

The prognosis for the course of the epidemic in the affected countries and in other countries in the Region is guarded, since it is not possible to prevent the spread of cholera from one country to another. The living conditions of the population are a crucial factor in determining the intensity with which cholera epidemics spread.

A comprehensive report on cholera in the Americas will appear in the next issue of the *Bulletin of PAHO*.