Protective Efficacy of a Live Attenuated Vaccine against Argentine Hemorrhagic Fever

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Argentine hemorrhagic fever (AHF), caused by the arenavirus Junin, is a major public health problem among agricultural workers in Argentina. A prospective, randomized, double-blind, placebo-controlled, efficacy trial of Candid 1, a live attenuated Junin virus vaccine, was conducted over two consecutive epidemic seasons among 6500 male agricultural workers in the AHF-endemic region. Twenty-three men developed laboratory-confirmed AHF during the study; 22 received placebo and 1 received vaccine (vaccine efficacy 95%; 95% confidence interval [CI], 82%–99%). Three additional subjects in each group developed laboratory-confirmed Junin virus infection associated with mild illnesses that did not fulfill the clinical case definition for AHF, yielding a protective efficacy for prevention of any illness associated with Junin virus infection of 84% (95% CI, 60%–94%). No serious adverse events were attributed to vaccination. Candid 1, the first vaccine for the prevention of illness caused by an arenavirus, is safe and highly efficacious.

Arenaviruses are important causes of hemorrhagic fever syndromes across the globe [1]. Lassa, Machupo (Bolivian hemorrhagic fever), Guanarito (Venezuelan hemorrhagic fever), and Sabia viruses have caused outbreaks of severe natural or laboratory-acquired disease. Lymphocytic choriomeningitis virus, the prototype of the family, is an important cause of benign nonspecific febrile illness and aseptic meningitis worldwide but may cause lethal infections as well.

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Written informed consent was obtained from all patients or their parents. Human experimentation guidelines of the authors' institutions were followed in the conduct of this clinical research. The study protocol was approved by the Comité de Evaluación Ética de Investigaciones Biomédicas de INEVH, the Subsecretaría de Regulación y Control of the Ministerio de Salud y Acción Social (Argentina), the Human Subjects Research Review Board of the Office of the US Army Surgeon General, the Ethical Review Committee of the Pan American Health Organization/World Health Organization, and the Committee on Human Research of the Johns Hopkins University School of Hygiene and Public Health.

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Argentine hemorrhagic fever (AHF) is an acute arenavirus infection caused by Junin virus. AHF has been a major public health problem in a well-defined AHF-endemic area of north-central Argentina, with 100–800 cases diagnosed each year [2, 3]. Most infections occur in 15- to 60-year-old male agricultural workers during an epidemic season that peaks in coincidence with the annual grain harvests (March–June); disease is recognized year-round among both male and female workers of all age groups, however. Patients with severe AHF develop extensive hemorrhagic manifestations, shock, and seizures. The case-fatality ratio among untreated patients has been 15%–30%. Treatment with plasma from patients who have recovered from AHF reduces the mortality of acute disease to $\leqslant$1%, but a late neurologic syndrome of unknown etiology has been observed in 10% of survivors [4]. The rodent *Calomys musculinus* is the principal reservoir of Junin virus; human infection most often follows inhalation of infectious rodent secretions during farming activities [5, 6].

No vaccines have been available for the prevention of illness due to arenaviruses. Following years of unsuccessful attempts [7], a new attenuated Junin virus vaccine candidate, called Candid 1, was found to be safe and efficacious against lethal Junin virus challenge in guinea pigs and rhesus macaques [8–10]. Subsequent phase I and II human studies indicated that the vaccine was also safe and immunogenic in volunteers. In this report, we describe our experience with this vaccine in an efficacy trial conducted in the AHF-endemic area.

Methods

Study site. We conducted a prospective, randomized, double-blind, placebo-controlled, efficacy study of Candid 1 in a 41-county

region of southern Santa Fé Province, Argentina, where AHF is endemic. Individual counties were chosen on the basis of epidemiologic studies that demonstrated a high AHF incidence relative to that of surrounding localities. The study area was located near the coordinating institution, the Instituto Nacional de Estudios sobre Virosis Hemorrágicas (INEVH), in Pergamino, Buenos Aires Province, where patients with AHF have been treated for >20 years. The annual incidence of AHF cases among males in the study area was 2.2/1000. The at-risk population from which recruitment took place was estimated to be 21,000.

Enrollment of volunteers. Between May and July 1988, an extensive public relations campaign incorporating video, audio, and print media was conducted throughout the vaccine target region. In each of the 41 selected localities, meetings were held with local governmental authorities, medical and paramedical personnel, and the population at large. Formal vaccine committees were established in each locality to facilitate the recruitment, enrollment, and follow-up of volunteers.

Individuals were considered to be eligible if they were healthy, male, 15–64 years old, resided or worked in a rural agricultural area of the 41-county area, had no history of AHF, had normal baseline blood values (white blood cell [WBC] and differential counts, platelet count, hematocrit, serum glucose level, serum creatinine level, and serum aspartate aminotransferase levels), were negative on screening for human immunodeficiency virus infection, and had no known allergies to vaccine components. Volunteers with chronic disease states associated with altered immunity (e.g., malignancy, autoimmune disorders, asplenia) were not allowed to participate. Females were excluded because of the unknown effect of Candid 1 on pregnancy and their lower (by at least 4-fold) risk of AHF. Potential participants were screened by use of a clinical history and physical examination, and a blood sample was taken for Junin virus antibody measurement. Records were independently reviewed at INEVH to establish final volunteer eligibility. Eligible volunteers were randomized to receive either placebo or vaccine in blocks of 6 persons (3 vaccine, 3 placebo) within each of the 41 counties to insure a similar distribution of vaccine and placebo recipients by county because of the highly focal nature of the disease. The randomization code was stored at the US Army Medical Research Institute of Infectious Diseases (USAMRIID) and was not available to either the Argentine or the US study investigators.

Inoculations began on 3 October 1988 and were halted in January 1989 due to the impending onset of the AHF epidemic season, which usually begins in late February or early March. Additional participants were recruited during a second round of immunization that occurred 11 November to 16 December 1989. A single 1-mL dose of either vaccine diluent or 40,000 pfu of Candid 1 was injected into the deltoid muscle. Volunteers were instructed to go to a local designated clinic to see their primary care physicians if they developed fever, malaise, or other troublesome symptoms at any time during the study.

Following inoculation, volunteers were evaluated before and after each AHF epidemic season. Volunteers enrolled during the first round of inoculations were followed for two seasons, and those inoculated during the second round were followed for one season. At each visit, any history of illness possibly consistent with AHF or hospital admission (or both) was recorded, and a blood sample was obtained for testing of neutralizing antibody to Junin virus.

Evaluation of possible adverse events and AHF. A passive case detection system for possible adverse events associated with inoculation and episodes of AHF was in place throughout the study. Volunteers were given a card with a brief description of the study, telephone numbers of the investigators, and signs and symptoms of AHF. Local physicians had a list of study volunteers and were instructed to refer volunteers with adverse events or suspected AHF to INEVH for evaluation. At INEVH, volunteers were evaluated and admitted to the hospital for observation or, if the illness was not felt to be AHF, treated as outpatients. Adverse events occurring within 2 weeks of inoculation and deaths were recorded and analyzed independently as to whether there was likely causality between the event and participation in the study.

Volunteers hospitalized for suspected AHF were evaluated daily for clinical status: WBC and platelet counts and blood urea nitrogen and serum aspartate aminotransferase levels were monitored daily. Blood for Junin virus serology was collected every third day. Blood for virus isolation was obtained during the first 3 days of hospitalization. Patients with petechiae or thrombocytopenia and other signs suggestive of AHF were treated with immune plasma. The clinicians caring for the patients recorded their final diagnoses without knowing the results of the virologic studies or the final clinical case definition of AHF to be used for the calculation of vaccine efficacy.

Case definitions for AHF. Clinical and laboratory case definitions were based on a retrospective case-control study of previous laboratory-confirmed AHF cases and were developed before the code was broken [11]. A definite clinical case was a volunteer with suspected AHF who had a WBC count of $<2500/mm^3$ and a platelet count of $<100,000/mm^3$. This definition had a sensitivity of 87% and a specificity of 88% for patients with laboratory-confirmed AHF (4-fold rise in Junin virus neutralizing antibody between acute and convalescent specimens) in the retrospective study. Participants with illnesses not meeting the definite case definition who had platelet counts of $<120,000/mm^3$ and one or more of axillary petechiae, gingival bleeding, the late neurologic syndrome, or a WBC count of $<2500\ /mm^3$ were classified as having a possible clinical case. This definition had a sensitivity and specificity of 90% and 71%, respectively, in the retrospective study.

Volunteers with either a 4-fold rise in antibody titer or the isolation of Junin virus from plasma were classified as having laboratory-confirmed Junin virus infection. The classification of volunteers according to the clinical definitions outlined above was done by computer analyses of coded clinical data and was made independently of the results of the serologic or virologic studies. Case definitions were developed independently before the individual clinical and laboratory data from volunteers were examined.

Two primary end points were evaluated: definite or possible clinical case of AHF with laboratory-documented Junin virus infection and laboratory-documented infection with Junin virus, independent of clinical findings. Volunteers presenting with a clinical complaint by 31 July 1990 were included in the efficacy analyses. The clinical and laboratory data from every symptomatic volunteer evaluated for possible infection with Junin virus were reviewed, and each volunteer was classified according to the above case definitions.

Vaccine preparation. Details of the preparation and testing of Candid 1 vaccine are contained in the US Food and Drug Administration's Investigational New Drug Application #2257. In brief, Candid #1 was derived from a documented descendent of the prototype XJ strain Junin virus, originally isolated from a fatal case of AHF. Following 44 newborn mouse brain passages beyond the initial guinea pig isolation and passage (GPp2MBp44), the candidate vaccine strain was cloned by single-burst selection and passed in certified fetal rhesus lung cells (FRLp19; Barrera Oro J, Eddy G, unpublished data) [7, 8, 12]. The vaccine was then extensively tested to ensure freedom from contamination by adventitious agents. Candid 1 was found to be significantly more attenuated in newborn mice and guinea pigs than an earlier generation Junin virus vaccine (XJ clone 3) [8] and appeared to be less able to replicate in macaques than wild-type Junin virus [9, 10].

In initial human trials, Candid 1 was found to be safe and highly immunogenic [13, 14]. Among 83 volunteers inoculated at USAMRIID in cohorts of various size using a variety of dosage and inoculation routes, the vaccine was found to be well tolerated clinically during follow-up periods ranging from 1 month to 2 years. Fourteen Argentine volunteers were inoculated concurrently at INEVH, with similar findings. Laboratory parameters were extensively assessed, including routine hematologic measurements; urinalyses; coagulation studies; measurements of muscle enzymes; amylase, hepatic, and renal function assays; serum levels of immunoglobulins, complement, and interferon; and T lymphocytes and subsets; no significant abnormalities were detected. Virus isolation attempts from throat, plasma, peripheral blood mononuclear cells (PBMC), urine, and semen in 46 vaccinees yielded positive results from PBMC only, in 7 subjects during a window 7–13 days after vaccination; virus could be recovered on only 1 day from 6 of 7 subjects, while the maximum duration of viremia was 3 days. Among nonimmune persons, up to 94% developed a positive response to one or more Junin virus–specific antibody tests (EIA, indirect immunofluorescence assay, and/or neutralizing antibody assay), and 99% developed a Junin virus–specific cellular immune response.

Subsequent (phase II) human trials served to validate phase I findings. Among 82 Argentine volunteers evaluated under a double-blind study design, none of the 55 persons receiving vaccine (30,000 pfu) developed significant clinical, hematologic, biochemical, or urinary abnormalities [15]. Fifteen persons (27%) developed neutralizing antibody by 15 days after inoculation (geometric mean titer [GMT] = 1:17), while 50 (91%) were positive at 30 days (GMT = 1:37), 60 days (GMT = 1:81), and 90 days (GMT = 1:87). At 6 months, 89% were found to have persistent antibody (GMT = 71). Similar findings were obtained among >50 additional volunteers inoculated at USAMRIID ([16], McKee K, Barrera Oro J, unpublished data).

For the current study, vials of Candid 1 were transported to Argentina on dry ice, stored at −40°C at INEVH, and transported and stored at the field inoculation sites on dry ice. Placebo vials looked identical to the vaccine vials but contained only vaccine diluent. Virus was titrated at both USAMRIID and INEVH in pooled aliquots from residual vaccine vials returned from inoculation sites while the trial was ongoing. Titers ranged from 31,000 to 64,000 pfu/mL (mean = 48,000 pfu/mL), demonstrating stability and site-to-site consistency.

Junin virus antibody measurements. Serum neutralizing antibodies were measured using a complement-enhanced plaque-reduction neutralization test, as previously described [17]. A serologic response to inoculation was defined as a ⩾4-fold rise in neutralizing antibody titer between pre- and postinoculation sera. A serologic response to an illness was defined as a ⩾4-fold rise in neutralizing antibody titer between acute and convalescent sera run in the same test and confirmed by repeat testing. Serologic testing was conducted after the vaccine trial was completed by laboratory personnel who were unaware of vaccine or placebo assignment.

Virus isolation. Junin virus isolation was attempted from both PBMC and plasma from subjects with suspected AHF. PBMC were cocultivated with VERO cell monolayers in duplicate to facilitate virus recovery [18]. Infected cells from the monolayer were identified by immunofluorescence using a fluorescein isothiocyanate–conjugated rabbit anti–Junin virus antiserum. Culture supernatant fluids were assayed directly by plaquing on VERO cells. All samples were passaged blindly through four cycles (unless interrupted by contamination) before being considered negative for the presence of virus. The same methods were used for the isolation of Junin virus from plasma.

Statistical analysis. Vaccine efficacy (percentage) was calculated using the formula [(attack rate in the placebo group)−(attack rate in the vaccine group)]/(attack rate in the placebo group) × 100%. Attack rates were calculated per 10,000 person-seasons of exposure. The 2-tailed exact binomial test was used to compare the attack rates per person-season exposure [19]. Fisher's exact or χ^2 tests were used to compare rates, and *t* tests were used for continuous measurements.

Results

Group comparisons. A total of 7450 of 8144 persons who volunteered for this study met the eligibility criteria, of whom 6500 were enrolled during the two recruitment periods. Reasons for exclusion included underlying medical conditions, a history of AHF, and residence outside of the study localities. When the study code was broken, it was revealed that 3255 persons had received vaccine and 3245 had received placebo (table 1). Of these, 5927 were enrolled during the first round of inoculations, and 573 were enrolled during the second round.

Table 1. Baseline characteristics of volunteers for Candid 1 Junin vaccine protective efficacy study.

Characteristic	Study group: Vaccine (3255)	Study group: Placebo (3245)
No. (%) enrolled during 1st round	2968 (91.2)	2959 (91.2)
No. (%) enrolled during 2nd round	287 (8.8)	286 (8.8)
Mean age, years	37.3 ± 11.9 (SD)	36.7 ± 11.7
Person-seasons exposure	6223	6204
No. (%) seropositive before inoculation	88 (2.7)	81 (2.5)

NOTE. None of the differences was statistically significant.

There was no difference in the distribution of vaccine and placebo recipients by county ($P = 1.00$).

Preinoculation sera from 88 (2.7%) of the vaccine group and 81 (2.5%) of the placebo group were found to have Junin virus antibody titers of ⩾1:4, ($P = .60$). Among these seropositive volunteers, 52 (59.1%) vaccine recipients versus 60 (74.1%) placebo recipients had a baseline antibody titer of ⩾1:256 ($P = .05$). No AHF cases were subsequently observed among these seropositive persons. Baseline Junin virus antibody titers were not available for 178 volunteers, 88 (2.8%) in the vaccine group and 90 (2.9%) in the placebo group ($P = .86$); these persons were included in all analyses.

Vaccine immunogenicity and reactogenicity. Among the seronegative volunteers, at least 1 serum sample was available within 6 months of inoculation for 2818 vaccine and 2842 placebo recipients. The mean interval between inoculation and the first neutralizing antibody titer measurement was 5 months for both groups. Among vaccine recipients, 91.1% had seroconverted (range, <1:4 to ⩾1:4096); 0.7% of placebo recipients developed antibody during this 5-month period. The geometric mean neutralizing antibody titer among vaccine recipients was 1:152 versus <1:4 for placebo recipients ($P = .0001$). Among the placebo recipients, 18 (0.6%) had a postinoculation antibody titer of ⩾1:256 compared with 1542 (55%) of Candid 1 recipients ($P < .001$). For all volunteers who met the AHF case definition, the initial postinoculation antibody determinations were done before the onset of AHF.

Adverse events. Within the first 2 weeks after inoculation, 36 (1.1%) of the vaccine recipients had one or more reported adverse events, versus 14 (0.4%) of the placebo recipients ($P = .002$; table 2). The only single adverse event that occurred significantly more often in vaccinees than in placebo recipients was headache with constitutional symptoms. The numbers of deaths were similar in both study groups (table 3). One death occurred during the first 30 days after inoculation in a 36-year-old man who had received Candid 1 vaccine. This patient developed gas gangrene of the buttock several days after an intramuscular corticosteroid injection at that site for low back pain using a reusable needle and syringe. He had received Candid 1 in the left deltoid muscle 1 week previously. None of the deaths during the study was judged to be related to immunization.

Table 2. Adverse events occurring within 2 weeks after inoculation with Candid 1 Junin vaccine.

Adverse event	Study group no. (%)		*P*
	Vaccine	Placebo	
Fever	14 (0.43)	7 (0.22)	.13
Headache or myalgias*	11 (0.34)	1 (0.03)	.004
Pharyngitis or bronchitis	4 (0.06)	0 (0.0)	.12
Generalized rash	2 (0.06)	0 (0.0)	.50
Inoculation site pain	1 (0.03)	0 (0.0)	1.00
Gas gangrene†	1 (0.03)	0 (0.0)	1.00
Parotitis	1 (0.03)	0 (0.0)	1.00
Abdominal pain, gastroenteritis, or enterocolitis	2 (0.06)	3 (0.09)	.62
Dizziness	0 (0.0)	1 (0.03)	.50
Varicella	0 (0.0)	1 (0.03)	.50
Appendicitis	0 (0.0)	1 (0.03)	.50
Total	36 (1.1)	14 (0.4)	.002

* With or without 1 of the following symptoms: bone pain, weakness, dizziness, myalgias, arthralgias, nausea or vomiting, and anorexia.

† Fatal adverse event (see text).

Vaccine efficacy. A total of 255 volunteers presented to their primary physicians with complaints. Of these, 94 were referred to INEVH for evaluation of possible AHF, 65 of whom were hospitalized. Laboratory evidence for Junin virus infection was obtained for 29 ill patients; 23 of these met the clinical case definition for AHF. Analysis of the entire study cohort (intent-to-treat analysis) revealed that among the 23 men with AHF, 22 had received placebo (35.5 cases/10,000 person-seasons of follow-up) and 1 had received vaccine (1.6 cases/10,000 person-seasons); vaccine efficacy for protection against AHF was 95% (95% CI, 82%–99%; $P < .001$). Among the 29 men who presented with clinical complaints who were found to have evidence of Junin virus infection, 25 had received placebo (40.3 cases/10,000 person-seasons) and 4 had received vaccine (6.4 cases/10,000 person-seasons), for an efficacy of 84% (95% CI, 60%–94%; $P < .001$) against infection associated with any clinical symptoms. The 25 cases in the placebo group meeting the laboratory case definition occurred among residents of 18 counties; the 4 cases in the vaccinated group (''vaccine failures'') occurred in 4 counties. A secondary analysis, excluding the 169 volunteers found to be seropositive at study entry, yielded identical estimates of vaccine efficacy (data not shown).

The clinical diagnoses for the 29 volunteers with laboratory evidence of Junin virus infection are shown in table 4. All patients were admitted to the hospital except 2 vaccinated cases diagnosed with fever of unknown etiology. There was one death due to AHF among the study volunteers: a 41-year-old person who received placebo on 27 November 1989. The patient was admitted to the hospital on 24 May 1990 with severe hemorrhagic AHF and had Junin virus isolated from plasma. There was no difference in the number of vaccine and placebo recipients not infected with Junin virus who sought care for the evaluation of an acute illness (table 5).

Discussion

Candid 1 vaccine was highly effective in preventing both AHF and other illnesses associated with laboratory evidence of Junin virus infection. As with other viral vaccines [20], protection against the most serious manifestations was somewhat better than protection against all illnesses associated with Junin virus infection. This suggests that Candid 1 attenuates the

Table 3. Causes of death among Candid 1 Junin vaccine efficacy study volunteers.

Cause of death	Study group	
	Vaccine	Placebo
Accidental trauma	3	3
Malignancy	3*	1†
Ischemic cardiovascular disease	2	1
Severe Argentine hemorrhagic fever	0	1
Gas gangrene	1	0
Cardiac arrest after cholecystectomy	1	0
Suicide	1	0
Acute alcohol intoxication	0	1
Hantavirus pulmonary syndrome	0	1‡
Cerebrovascular accident	0	1
Total	11 (0.4%)	9 (0.3%)

NOTE. No difference in frequency of deaths between vaccine and placebo groups ($P = .66$).

* One each with pancreatic, esophageal, and renal cell carcinoma.

† Esophageal carcinoma.

‡ Diagnosed retrospectively in 1993 by serology (EIA) and immunohistochemistry.

severity of Junin virus disease, most likely due to a reduction in viral replication [9].

Included in the efficacy analyses were 178 volunteers (<3%) with unknown baseline serologic status. Excluding these volunteers did not alter the estimates of vaccine efficacy. The likelihood that these volunteers were seropositive at baseline was low, and they were equally distributed between the vaccine and placebo groups.

The efficacy of Candid 1 for protection against any Junin virus infections may have been lower had we been able to test all volunteers for seroconversion at the end of the follow-up period. We believe that an analysis of subjects who presented with illness provides the most clinically meaningful measure of protection. The investigators who classified the volunteers

Table 4. Clinical diagnoses among patients with Junin virus infection who were enrolled in Candid 1 vaccine efficacy study.

Clinical diagnosis	Study group	
	Vaccine	Placebo
Mild AHF	1	18
Moderate AHF	0	3
Severe AHF	0	2
Fever of unknown etiology	2	1
Viral hepatitis	0	1
Mononucleosis	1	0
Total	4	25

NOTE. Mild, moderate, and severe are subjective classifications; disease severity is skewed by impact of passive antibody therapy on Argentine hemorrhagic fever (AHF). AHF clinical classifications were assigned to patients at time of discharge from acute illness while investigators were still blinded.

Table 5. Clinical diagnoses among patients enrolled in Candid 1 Junin vaccine efficacy trial who were referred to INEVH and proven not to have Junin virus infections.

Diagnosis	Study group			
	Vaccine		Placebo	
	Admitted	Not admitted	Admitted	Not admitted
Mild AHF	2 (2)	0	5 (4)	0
Fever of unknown etiology	13 (2)	10	11	8
Delirium tremens	0	0	1 (1)	0
Acute respiratory failure	0	0	1 (1)	0
Viral hepatitis	1	0	1	1
Umbilical hernia repair	0	0	0	1
Herpes stomatitis	0	1	0	1
Rheumatic fever	1	0	0	0
Furuncles	0	0	0	1
Myalgia	0	1	0	0
Headache	1	0	0	1
Dermatosis	0	1	0	0
Brucellosis	0	1	0	0
Varicella	1	0	0	0
Subtotal	19	14	19	13
Total	33		32	

NOTE. INEVH, Instituto Nacional de Estudios sobre Virosis Hemorrágicas; AHF, Argentine hemorrhagic fever. Volunteers meeting definite or possible clinical case definitions for AHF are in parentheses. There are no significant differences between groups.

at the end of the study according to the case definitions could not be fully blinded to the vaccination status of the volunteers because vaccine recipients were more likely to have had measurable neutralizing antibodies at the time of evaluation for suspected AHF. However, the volunteers were evaluated for the clinical component of the case definition before the results of the virologic studies were known, and the case definitions were based on predetermined objective criteria that could not be easily compromised by investigator bias.

All patients hospitalized at INEVH for whom a diagnosis of AHF is suspected on clinical grounds receive immune plasma in conjunction with general supportive therapy. The administration of immune plasma attenuates disease severity and significantly reduces mortality in AHF [4, 21, 22]. It is highly probable that the distribution of AHF severity observed in the study population (table 5) was skewed toward the milder end of the disease spectrum through use of immune plasma. The impact of Candid 1 in preventing severe disease or death would probably be much greater in the absence of ameliorating therapy.

Candid 1 proved to be safe, as only 1% of vaccinated volunteers reported adverse events temporally associated with immunization. Recorded events were mild, although the passive method of reporting of adverse events likely resulted in some underreporting. However, given the excellent access to medical care in this population, continuous interaction between local health care providers and study personnel, the information col-

lected during scheduled visits, and the safety data from phase I and II studies (McKee K, et al., unpublished data), it is unlikely that serious events were missed.

The mechanisms by which Candid 1 induces clinical protection are unknown, but it is likely that both humoral and cellular immunity are important. Both the presence and titer of neutralizing antibody directed against Junin virus are important factors in the success of plasma therapy for AHF [4, 21, 22]. In addition, a correlation has been demonstrated between the appearance of antibodies and clinical improvement in patients with this disease [23]. Serum neutralizing antibodies are directed primarily against viral surface glycoproteins [24]. Purified human immune plasma IgG and the immune $F(ab')_2$ fraction from the same plasma pool in the presence of complement neutralize Junin virus in vitro. Although immune plasma IgG protects guinea pigs from Junin virus infection, the $F(ab')_2$ portion alone does not [25]. In addition, in guinea pigs, resistance to Junin virus parallels the development of virus-specific antibody-dependent cellular cytotoxicity [26]. Anti–Junin virus antibodies also can sensitize cells for complement-mediated lysis [27]. Thus, the role of antibody may be mainly in the elimination of Junin virus–infected cells rather than direct virus neutralization, although the latter mechanism likely plays a role in preventing infection.

Antibody-independent cellular mechanisms may also be important. In rhesus macaques, Candid 1 viremia often disappears up to several weeks before the appearance of measurable antibody [10]. In addition, Junin virus–specific lymphocyte proliferation can be measured in vaccinated individuals who do not develop serum neutralizing antibodies (Kenyon R, Peters CJ, Enria D, et al., unpublished data).

Candid 1 represents the first successful vaccine for prevention of disease caused by an arenavirus. The duration of protection induced by Candid 1 is unknown and is the subject of ongoing investigations. However, 90% of subjects immunized with an unacceptably reactogenic Junin virus vaccine candidate (referred to as XJ clone 3) were found to have detectable neutralizing antibody titers 7–9 years following immunization [28]. A similar proportion of vaccinees in this study was found to have detectable neutralizing antibody 42 months following immunization with Candid 1 [29]. Since the conclusion of this study, >150,000 at-risk persons living or working in the AHF-endemic area have received Candid 1. Ongoing disease surveillance efforts have documented a substantial reduction in reported AHF cases and a shift in demographics toward groups traditionally felt to be at lower overall disease risk (and hence not targeted for vaccination in public health campaigns), suggesting that Candid 1 may be having its intended effect in reducing the incidence of this disease (Enria D, et al., unpublished data).

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