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Candid No. 1 Argentine Hemorrhagic Fever Vaccine Protects against Lethal Junin Virus Challenge in Rhesus Macaques

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Summary

The protective efficacy of Candid No. 1, a live-attenuated vaccine against Argentine hemorrhagic fever (AHF), was evaluated in non-human primates. Twenty rhesus macaques immunized 3 months previously with graded doses of Candid No. 1 (16–127,000 PFU), as well as 4 placebo-inoculated controls, were challenged with 4.41 $\log_{10}$ PFU of virulent P3790 strain Junin virus. All controls developed severe clinical disease; 3 of 4 died. In contrast, all vaccinated animals were fully protected; none developed any signs of AHF during a 105-day follow-up period. Viremia and virus shedding were readily detected in all placebo-vaccinated controls, while virus could be recovered only once (by amplification) from throat swabs of 2 Candid No. 1 vaccinees on day 21. Vigorous secondary-type neutralizing and immunofluorescent antibody responses were seen in most vaccinees that had received 3 $\log_{10}$ PFU Candid No. 1 or fewer; all others, including those receiving 127,200 PFU, maintained relatively stable titers during follow-up. Candid No. 1 was highly immunogenic and fully protective against lethal Junin virus challenge in rhesus macaques, even at extremely low (16 PFU) vaccine doses.

Key Words
Junin virus
Candid No. 1
Argentine hemorrhagic fever
Viral hemorrhagic fever
Vaccine
Animal model
Arenavirus

Introduction

Argentine hemorrhagic fever (AHF) is a severe and potentially lethal disease cause by Junin virus. Since the disease was initially described in western Buenos Aires province in 1953 [1], more than 22,000 cases have been reported, and the zone of endemnicity has expanded to encompass over 120,000 km^2 in portions of 4 provinces [2, 3]. While the disease can be treated successfully by administering immune plasma [4], risks attendant to the use of

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human blood products, together with uncertainties in diagnosis and access to medical care, dictate a need for control through primary preventive measures.

Efforts to develop a safe, effective vaccine against AHF have been going on since shortly after characterization of the causative agent [reviewed in Barrera Oro and McKee, [5]. While a variety of candidate immunogens have been produced to date, all have ultimately proved unacceptable for human use.

Recently, a live-attenuated vaccine candidate was developed from progeny of prototype XJ strain Junin virus [6]. Serial log dilutions of this vaccine, Candid No. 1, have been inoculated subcutaneously into rhesus macaques, with no detectable adverse effect on any physical, biochemical or hematological parameter measured [7]. The present report documents the protective efficacy of Candid No. 1 in macaques after parenteral challenge with a highly virulent, wild-type strain of Junin virus.

Materials and Methods

Virus Strains

Candid No. 1 vaccine was derived by clonal selection (pseudo single-burst technique) and fetal rhesus lung cell culture expansion from the 44th suckling mouse brain passage of prototype XJ strain Junin virus. As previously reported [6], this vaccine has been found to be significantly more attenuated for newborn mice and guinea pigs than the earlier generation human vaccine, XJ Clone 3. Moreover, Candid No. 1 is phenotypically stable after in vitro passage and protects guinea pigs against lethal challenge with virulent Junin and Machupo virus strains. Preliminary neurovirulence testing indicated that Candid No. 1 was safe by intracerebral inoculation of rhesus macaques.

The challenge Junin virus strain, P3790, was selected for its reproducible 75–100% mortality in rhesus macaques [8]. Other naturally occurring Junin strains have been shown to be less consistent in producing severe clinical illness in this model [K. McKee, unpublished observations]. The P3790 strain of Junin virus (referred to as Espindola in previous publications) was recoverd from the blood of a human who died with 'hemorrhagic' AHF. After isolation in MRC-5 (human diploid lung) cells, the virus was passaged twice more in the same cell line to provide a pool containing 4.4 log$_{10}$ PFU/ml for inoculation into animals.

Infectivity Assays

Materials for quantitative virus titration were assayed by counting plaques on Vero cell monolayers [9]. Each sample then was passed blindly into 3 T-25 flasks for a single cycle of amplification, and the supernatant fluid cultured after 1 week by plaque assay to recover virus present below the limits of detection for the direct plaque assay.

Serology

Neutralizing antibodies were measured by using a constant virus, serum dilution technique on Vero cells [10]. Endpoints were recorded as the highest dilutions yielding a reduction in plaque counts of $\geq 80\%$ relative to intratest controls. Virus strains used in the assays were XJ Clone 3, P3790, and Candid No. 1.

Immunofluorescent antibodies were measured by a modification of the method of Peters et al. [11].

Experimental Conditions

Twenty-four healthy adult *Macaca mulatta*, which previously had been vaccinated with graded doses of Candid No. 1 or saline, served as experimental subjects. Animals were housed individually in stainless steel cages with collapsible backs. Diet consisted of monkey chow (Ralston Purina Co., St. Louis, Mo.) and water ad libitum.

The macaques used in this experiment previously had been assigned randomly to 1 of 5 experimental groups [7]. Five animals in each of 4 vaccine groups received Candid No. 1 subcutaneously in doses of 16, 318, 6,360, or 127,200 PFU. The 4 remaining animals received an equivalent volume of saline as a placebo. On day 106 after vaccination, each animal was inoculated intramuscularly with 1 ml of P3790 Junin virus. All animal manipulations during and after virulent virus challenge were performed under BL-4 level containment conditions [8]. Cages containing control animals (those receiving saline placebo during the immunization phase) were segregated from the others by means of a portable laminar flow cubicle (Bioclean, Hazelton Systems, Aberdeen, Md.).

Macaques were observed at least once daily throughout the study for signs of clinical illness. Objective disease parameters were measured under sedation (ketamine hydrochloride, 7 mg/kg/dose) thrice weekly for 4 weeks, weekly for 2 additional weeks, then again on days 56 and 105. Objective parameters included

physical examination, body weight, viremia, virus shedding from the oropharynx, and serology.

Blood specimens were obtained by saphenous or femoral venipuncture with a 21- or 23-gauge butterfly needle. Venipuncture sites were cleansed with 70% ethanol before needle insertion. Blood and throat swabs were processed for virus isolation and antibody determinations [8]. All animals were killed when in extremis, or at the termination of the study, by exsanguination under heavy sedation.

Results

Clinical Disease

Beginning 10–17 days after challenge with P3790 Junin virus, all animals that previously had received saline in lieu of vaccine developed clinical illness. Physical findings included weight loss, dehydration, facial flushing and puffiness, macular and petechial rashes of the face, axillae, and upper trunk, lip ulcers, and mucous membrane hemorrhage. Three of the four animals died or were killed because they were in extremis (21, 21, and 26 days after inoculation). The 4th macaque underwent a spontaneous but very slow recovery, gradually regaining strength and appetite over the subsequent 2 months.

In contrast, none of the vaccinated animals became ill. Appetites were maintained throughout the observation period, and hemorrhagic phenomena (puffiness, rash, bleeding, etc.) did not occur. With the exception of 2 single measurements in animals from the lowest and highest vaccine dose groups (both of which probably represented measurement error), no weight loss exceeding 10% of the pre-challenge value was observed in any vaccinated subject. Mean weight loss by vaccine group ranged from 3.1 (6,360 PFU/dose) to 5.4% (16 PFU/dose). Among unvaccinated controls, maximum weight loss ranged from 21 to 38% of baseline.

Virus Isolation

Virus could not be recovered by direct plaquing (or blind passage for amplification) of serum from any vaccinated macaque on any day after challenge. In contrast, viremia was detectable from day 7 until death in 3 of 4 unvaccinated controls (fig. 1). Virus titers increased over time, and peaked (5.43–6.78 $\log_{10}$ PFU) in specimens obtained immediately before death. Samples from the 4th (surviving) control were positive by blind passage on days 7–14 after challenge, but by direct plaquing only once (day 10).

No virus could be recoverd from throat swabs by direct plaque assay from any vaccinated animal on any day after challenge. However, samples from 1 animal in each of the 2 highest vaccine dose groups yielded Junin virus by serial passage (amplification) of oropharyngeal cultures on day 21 after challenge. Although no effort was made to characterize these viruses, they are virtually certain to have been the challenge, rather than the vaccine, strain of Junin. Oropharyngeal swabs were obtained from all animals thrice weekly for 4 weeks following vaccination, then monthly until challenge on day 106. Only 1 sample from 1 macaque yielded virus during this period (on day 10) [6]. Following challenge, animals were again studied thrice weekly. It is extremely unlikely that vaccine virus would appear suddenly on day 21 after challenge (day 127 after vaccination) without its having been found earlier.

As was the case for viremia, virus could be isolated readily from the oropharynx of all unvaccinated controls (fig. 2). Initial isolations were made on the 7th day after challenge, with peak titers (3.2–5.0 $\log_{10}$ PFU) occurring on days 14–21.

Serology

Neutralizing antibodies were measured against 3 Junin virus strains (XJ Clone 3, Candid No. 1, and P3790). Titers were uniformly

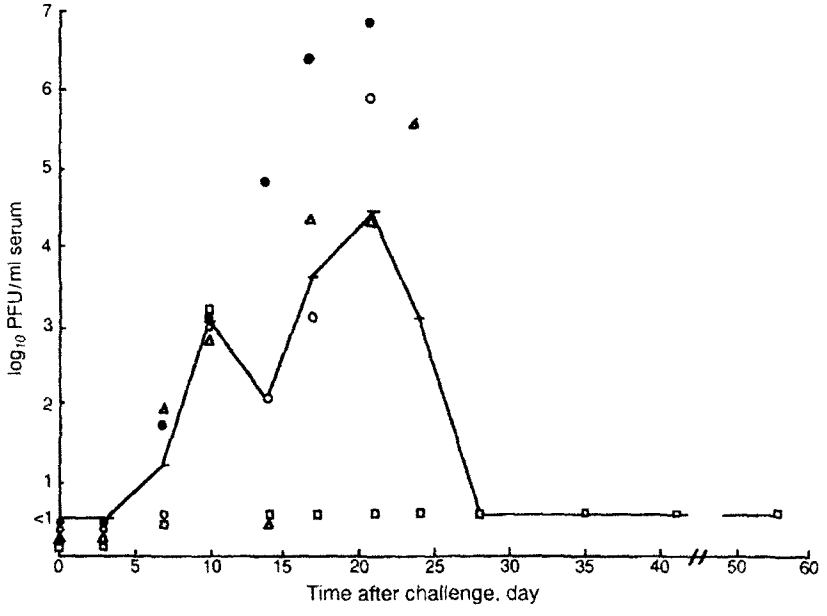

Fig. 1. Viremia among unvaccinated (saline placebo-inoculated) controls. Symbols represent individual animals. Solid line connects geometric mean titers (GMT). No serum viremia was detected in any vaccinated macaque.

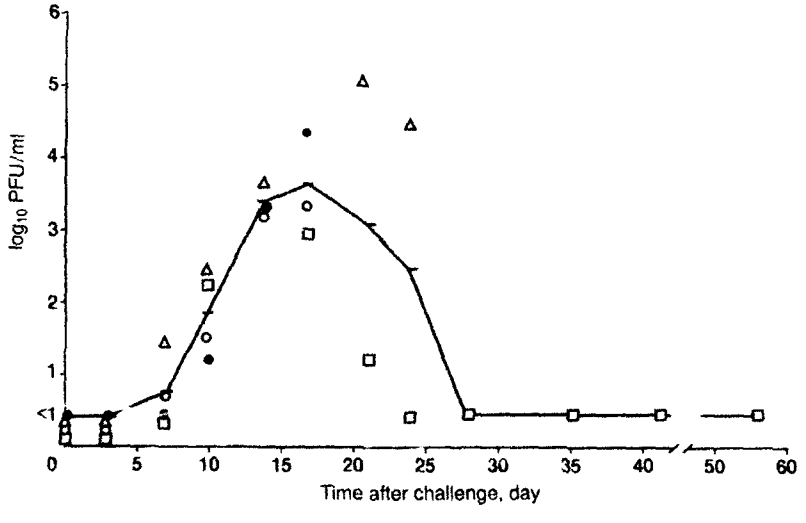

Fig. 2. Virus recovery from oropharyngeal swabs of unvaccinated (saline placebo-inoculated) controls. Symbols represent individual animals. Solid line connects geometric mean titers (GMT). Virus also was recovered (by amplification only) from throat swabs of 2 Candid No. 1-vaccinated macaques on day 21 (see text).

Table 1. Junin virus-neutralizing antibodies at day of challenge

Vaccine dose PFU	Rhesus No.	Neutralizing Antibody Against		
		Candid No. 1	XJ C13	P3790
127,200	18081	1,024	2,048	64
	B6973	1,024	256	64
	632B	4,096	1,024	256
	F2	256	256	16
	354A	64	16	<16
6,360	M376	<16	<16	<16
	9C94	256	32	<16
	R392	16	16	<16
	T359	256	256	16
	OB27	64	256	16
318	M95	64	128	16
	T259R	64	64	16
	P658	4,096	1,024	64
	M326	16	<16	<16
	18422	<16	<16	<16
16	P728	32	<16	<16
	626A	1,024	256	64
	M377	64	64	<16
	9E10	64	32	<16
	M371	64	<16	<16
Saline	C63	<8	<8	<8
	775	<8	<8	<8
	18193	<8	<8	<8
	M374	<8	<8	<8

Note: Values represent reciprocal serum dilutions.

lower when virulent P3790 virus was used when compared with the two attenuated strains. Titers generally could be measured at higher serum dilutions with Candid No. 1 than with XJ Clone 3. All but 2 animals (in the groups receiving 6,360 and 318 PFU of Candid No. 1 vaccine) had detectable anti-Junin antibodies on challenge day 0 when Candid No. 1 was used in the neutralization test. When XJ Clone 3 was used, antibodies were below the limits of detection in animals from each of the 3 lowest vaccine dose groups, and antibodies wer detected only at the initial dilution (1:16) in 2 additional animals (table 1). Therefore, responses among vaccine groups were compared on the basis of Candid No. 1-neutralized sera.

Neutralizing antibody titers immediately before challenge ranged from <1:16 to 1:4,096. The highest pre-challenge neutralizing antibody titers occurred in the group that had received the highest vaccine dose (127,200 PFU) 105 days previously (GMT = 1:588). Antibody responses tended to follow 2 patterns in vaccinees: most animals (4 of 5 in the 16-PFU group, 4 of 5 in the 318-PFU group, 5 of 5 in the 6,360-PFU group, and 3 of 5 in the 127,200-PFU group) displayed a secondary- or booster-type response following challenge. The remainder

Table 2. Representative data from Candid No. 1-vaccinated macaques challenged with virulent Junin virus

Rhesus No.	Test	Time after challenge, days								
		0	3	7	10	14	21	28	35	56
Control	V Ab[1]	<8	<8	<8	8	32	32			
18103	C Ab[2]	<2	<2	<2	<2	<2	<2			
	S Vir[3]	Neg	Neg	0.8	2.7	0.5	4.3			
	T Vir[4]	Neg	Neg	1.4	2.4	3.6	5.0			
	Wt kg[5]	8.5	8.5	8.2	ND	8.1	7.5	Note: Died day 24,		
Wt gain (loss)		0%	0%	(4%)		(5%)	(12%)	6.7 kg (22%)		
Control	V Ab	<8	<8	<8	8	32	4,096	16,384	4,096	16,384
M374	C Ab	<8	<8	<8	<8	8	64	1,024	1,024	1,024
	S Vir	Neg	Neg	0.5	3.1	0.5	Neg	Neg	Neg	Neg
	T Vir	Neg	Neg	Neg	2.2	3.3	1.2	Neg	Neg	Neg
	Wt kg	12.7	12.8	12.5	12.2	11.7	10.6	9.6	8.8	7.9
Wt gain (loss)		0%	1%	(2%)	(4%)	(8%)	(13%)	(24%)	(31%)	(38%)
Vaccinee	V Ab	4,096	1,024	4,096	4,096	4,096	4,096	1,024	4,096	4,096
632B	C Ab	256	256	256	256	256	256	256	256	1,024
	S Vir	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	T Vir	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Wt kg	12.2	11.7	12.1	12.0	12.0	12.0	12.1	12.1	12.3
Wt gain (loss)		0%	(4%)	(1%)	(2%)	(2%)	(2%)	(1%)	(1%)	1%
Vaccinee	V Ab	16	<16	256	1,024	16,384	16,384	4,096	4,096	4,096
M326	C Ab	<16	<16	16	256	1,024	1,024	1,024	1,024	1,024
	S Vir	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	T Vir	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Wt kg	12.1	11.7	11.7	11.8	11.8	11.6	11.7	11.7	12.0
Wt gain (loss)		0%	(3%)	(3%)	(2%)	(2%)	(4%)	(3%)	(3%)	(1%)
Vaccinee	V Ab	<16	<16	16	16	64	64	1,024	64	64
M376	C Ab	<16	<16	<16	<16	<16	<16	<16	64	<16
	S Vir	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	T Vir	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Wt kg	12.1	12.0	12.0	12.1	11.9	12.0	11.8	12.0	12.2
Wt gain (loss)		0%	(1%)	(1%)	0%	(2%)	(1%)	(3%)	(1%)	1%

[1] Reciprocal neutralizing antibody titer vs. Candid No. 1 (vaccine virus).
[2] Reciprocal neutralizing antibody titer vs. P3790 (challenge virus).
[3] Serum viremia (expressed as $\log_{10}$ PFU/ml.
[4] Oropharyngeal virus recovered (expressed as $\log_{10}$ PFU/ml).
[5] Body weight (in kilograms).

tended to have relatively stable antibody titers which did not vary more than 1–2 dilutions throughout the follow-up period. One animal displayed only a minimal antibody response. Representative serological patterns are provided in table 2.

Among 'boosted' animals, responses typically were noted between 3 and 7 days after challenge. Peak antibody responses occurred at 7–14 days among individual animals. Titers tended to fall slightly between 14 and 42 days, then remained stable (or fell only slightly) for

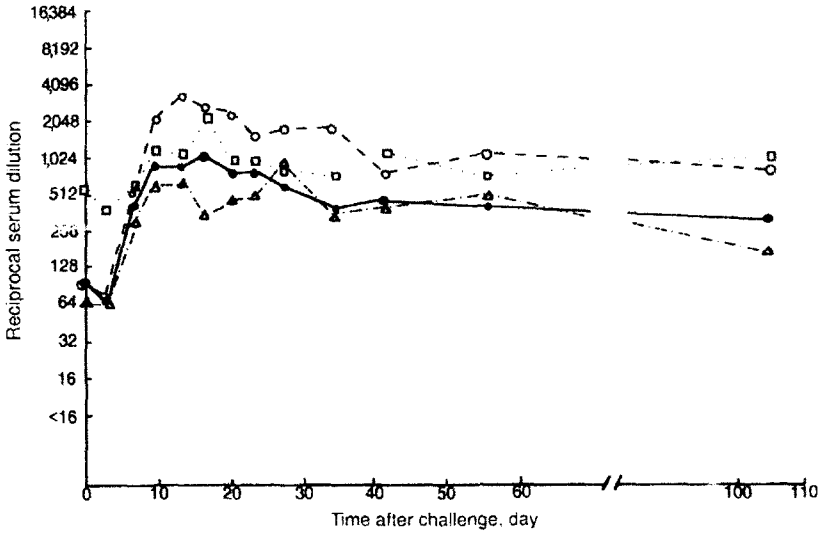

Fig. 3. Geometric mean neutralizing antibody titers in Candid No. 1 vaccinated rhesus macaques challenged on day 105 after immunization with virulent P3790 strain Junin virus. Groups immunized with: ● = 16 PFU; ○ = 318 PFU; △ = 6,360 PFU; □ = 127,200 PFU. Antibody responses in control (unimmunized) animals not shown (see text).

the duration of the 105-day follow-up period (fig. 3). Highest levels after challenge were seen in animals that had received 318 (GMT = 1:3,104) and 127,200 (GMT = 1:2,048) PFU. While the lowest peak GMT occurred in the group receiving 6,360 PFU, depressed values were attributable in large part to a single animal whose titer never exceeded 1:128.

Neutralizing antibodies against Candid No. 1 were detected only once (at 1:16) in the 2 placebo-vaccinated controls that died on day 21. Antibodies were detected at low serum dilutions in the other 2 controls, peaking at 1:32 in the animal that died on day 26, and at 1:16,384 on day 28 in the single control survivor.

Antibodies were detected by indirect immunofluorescence before challenge (day 0) in all animals vaccinated with 127,200 PFU and 6,360 PFU, in 3 of 5 vaccinated with 318 PFU, and in 1 of 3 measured in the group vaccinated with 16 PFU. Among macaques vaccinated with the highest dose of Candid No. 1 (127,200 PFU), all showed little or no 'booster' response; rather, antibody titers remained unchanged, or increased sluggishly only 2- to 4-fold, on days 7–10. In contrast, 13 of the 15 animals in the other dose groups displayed vigorous secondary-type responses that tended to occur more quickly (by day 3 after challenge) in those subjects with the lowest (or undetectable) titers at day 0. The pattern of this response was nearly identical to that seen for neutralizing antibodies. Immunofluorescent antibodies were undetectable in 2 of 3 control (placebo-vaccinated) animals before death, but reached a titer

of 1:16,384 in the 3. Antibody appeared on day 10 after challenge in the control survivor, and peaked at 1:32,768 on day 35.

Discussion

This study was designed to assess in rhesus macaques the protective efficacy of Candid No. 1, a candidate live-attenuated AHF vaccine, against lethal challenge with virulent Junin virus. A secondary objective was to establish an appropriate immunizing dose of Candid No. 1 with which to proceed to clinical trials in humans. The first goal of the study clearly was met; Candid No. 1 completely protected against illness and death in animals vaccinated 3 months previously. Surprisingly, we were unable to establish a minimum immunizing dose. Complete protection was afforded in all macaques, despite their having been vaccinated over a 4 $\log_{10}$ dose range (16–127,200 PFU).

The dose of virulent Junin virus for this experiment, 4 $\log_{10}$ PFU, was chosen to provide a significant, but not overwhelming, challenge to vaccinated animals. This dose approximated that expected to be received by humans following natural exposure to chronically infected *Calomys* spp. (4.2–5.1 $\log_{10}$ PFU/ml) [12]. As expected, all unvaccinated controls became severely ill, and 3 of 4 died. Clinical findings in this group were consistent with those previously described for rhesus macaques experimentally infected with the P3790 Junin virus strain [8, 10]. This syndrome closely mimicks the signs of hemorrhagic AHF seen in humans [13, 14]. In contrast, no vaccinated animal became ill. Careful daily observation failed to demonstrate any constitutional, hemorrhagic, or neurologic signs at any time following challenge. These results are consistent with other studies in guinea pigs and rhesus macaques which failed to show systemic or central nervous system changes following inoculation with Candid No. 1 by peripheral, intracerebral, intraspinal, or combined routes [J. Barrera Oro, unpublished observations].

As has been demonstrated previously, viremia and virus shedding in oropharyngeal secretions were prominent in unvaccinated controls [8, 10]. In contrast, virus could not be recovered by direct plaquing or amplification (under fluid overlay) techniques from the blood of any vaccinated macaque, and was detected only once, with difficulty, from the oropharynx of two vaccinees. These findings, together with serologic changes, indicate that prior immunization with Candid No. 1 significantly impeded, but did not completely abiate, virulent virus replication in challenged animals. Recent advances in the molecular characterization of Candid No. 1 hold great promise for the eventual identification of markers for attenuation with which to better characterize virus isolates recovered after vaccination or challenge [15].

Increases in antibody titers were documented for all vaccinated animals (including the 2 macaques with no detectable antibody before challenge). The highest antibody titers before challenge were observed in macaques vaccinated with 127,200 PFU of Candid No. 1. The earliest responses to challenge were observed by immunofluorescence (by day 3), while neutralizing antibodies tended to rise a few days later. While most animals vaccinated with the 3 lowest doses of Candid No. 1 responded to challenge with a vigorous 'boost' in antibodies measurable by neutralization and indirect immunofluorescence, titers in those vaccinated with the highest dose changed very little. Interestingly, peak antibody titers were remarkably similar among vaccine groups (within 4-fold for both neutralizing and immunofluorescent antibodies), and fell only slightly over the duration of the observation period.

The secondary antibody responses observed among vaccinated animals contrast sharply with those occurring after natural or vaccine-

induced primary infection. After primary infection with virulent, wild-type Junin virus, antibodies measured by either neutralization or immunofluorescence often are undetectable as late as 3 weeks after inoculation. After primary vaccine infection, initial responses are not seen earlier than 14–17 days, and peak only after 1–2 months [7].

The mechanism of host defense against virulent Junin virus challenge was not the subject of this work. However, the patterns of neutralizing antibody response observed among vaccinated animals suggest that humoral immunity plays a significant role in protection. In some cases (e.g., rhesus 632B, table 2), preexisting antibody against both Candid No. 1 and challenge virus was associated with solid protection. In others (e.g., rhesus M326, table 2), low or undetectable antibody levels prior to challenge were 'boosted' following inoculation with the virulent strain, again resulting in solid protection against clinical disease. It is of interest that the single surviving control macaque developed neutralizing antibodies against both Candid No. 1 and the challenge virus, while those that died responded poorly or failed altogether to mount antibody responses. The demonstrated relationship between the efficacy of convalescent immune plasma treatment for human AHF and its neutralizing antibody content lends further support to the critical role played by humoral immunity in the recovery and protection from disease [4].

On the other hand, 1 macaque in this experiment was fully protected against virulent Junin virus challenge in the absence of a substantial rise in neutralizing antibodies (rhesus M376, table 2). No measures of cell-mediated immunity were undertaken in this or any other animal in the present study. However, data from human vaccine infections suggest that Junin-specific lymphocyte proliferation responses can be measured in most individuals who fail to develop neutralizing antibodies [R. Kenyon, C.J. Peters, unpublished data]. Taken together, these observations indicate that host defense against this arenavirus is complex. Although protection from clinical disease is associated in most cases with the presence of neutralizing antibodies, a balanced immune response involving humoral, cellular, and perhaps other, as yet undefined, factors appears to function in protecting the host from debilitating illness or death.

In this and previous work [7], we have demonstrated that Candid No. 1 is safe, immunogenic, and protective against parenteral challenge with virulent Junin virus in a well-established primate model for human AHF. Recently, Candid No. 1 was shown to be protective against aerosol challenge with virulent Junin virus as well [16]. Based upon these and numerous additional pre-clinical studies, Candid No. 1 subsequently underwent extensive evaluation in controled human trials, and has been inoculated into more than 70,000 at-risk volunteers in Argentina.

Acknowledgements

In conducting the research described in this report, the investigators adhered to the 'Guide for the Care and Use of Laboratory Animals' as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

References

1 Arribalzaga RA: Una nueva enfermedad epidemica a germen desconocido: Hipertermia nefrotoxica, leucopenica y enantematica. Dia Medico (Buenos Aires) 1955;27: 1204.

2 Carballal G, Videla CM, Merani MS: Epidemiology of Argentine hemorrhagic fever. Eur J Epidemiol 1988; 4:259–274.

3 Maiztegui J, Feuillade M, Briggiler A: Progressive extension of the endemic area and changing incidence of Argentine hemorrhagic fever. Med Microbiol Immunol 1986;175:149–152.

4 Enria DA, Franco SG, Ambrosio A, Vallejos D, Levis S, Maiztegui J: Current status of the treatment of Argentine hemorrhagic fever. Med Microbiol Immunol 1986;175:173–176.

5 Barrera Oro JG, McKee KT Jr: Toward a vaccine against Argentine hemorrhagic fever. Bull PAHO 1991; 25:118–126.

6 Barrera Oro J, Eddy G: Characteristics of candidate live attenuated Junin virus vaccine (abstract S4-10). 4th Int Conf on Comparative Virology, Banff, Oct 1982.

7 McKee KT Jr, Barrera Oro JG, Kuehne AI, Spisso JA, Mahlandt BG: Safety and immunogenicity of a live-attenuated Junin (Argentine hemorrhagic fever) vaccine in rhesus macaques. Am J Trop Med Hyg, in press.

8 McKee KT Jr, Mahlandt BG, Maiztegui JI, Eddy GA, Peters CJ: Experimental Argentine hemorrhagic fever in rhesus macaques: Viral strain-dependent clinical response. J Infect Dis 1985;152:218–221.

9 McKee KT Jr, Green DE, Mahlandt BG, Bagley LR, Lyerly WH Jr, Peters CJ, Eddy GA: Infection of *Cebus* monkeys with Junin virus. Medicina (Buenos Aires) 1985;37:144–152.

10 McKee KT Jr, Mahlandt BG, Green DE, Peters CJ: Virus-specific factors in experimental Argentine hemorrhagic fever in rhesus macaques. J Med Virol 1987;22:99–111.

11 Peters CJ, Webb PA, Johnson KM: Measurement of antibodies to Machupo virus by the indirect fluorescent technique. Proc Soc Exp Biol Med 1973;142:526–531.

12 Sabattini MS, Contigiani MS: Ecological and biological factors influencing the maintenance of arenaviruses in nature with special reference to the agent of Argentinean haemorrhagic fever (AHF); in Pinheiro FP (ed): Int Symp on Tropical Arboviruses and Hemorrhagic Fevers, Belem, 1982. Rio de Janeiro, Academia Brasilera de Ciencias, 1982.

13 Maiztegui JI: Clinical and epidemiological patterns of Argentine hemorrhagic fever. Bull WHO 1975;52: 567–575.

14 Peters CJ: Arenaviruses; in Belshe R (ed): Textbook of Human Virology, ed 2. St Louis, Mosby Year Book, 1991, pp 541–570.

15 Romanowski V, Ghiringhelli PB, Albarino C, Pibaul M: The glycoprotein precursor gene of the Junin virus vaccine strain. 10th Annu Meet Am Soc Virol, Fort Collins, July 1991.

16 Kenyon RH, McKee K, Barrera Oro J, Ragland D, Crabbs C, Higgins Y: Protective efficacies in rhesus monkeys of Candid 1 strain Junin virus (JV) and Tacaribe virus against aerosol challenge with virulent JV (abstract 136): 38th Annu Meet Am Soc Trop Med Hyg, Honolulu, Dec 1989.