MAJOR ARTICLE

# Prophylaxis and Therapy for Chikungunya Virus Infection

**Thérèse Couderc,[1,3] Nassirah Khandoudi,[5] Marc Grandadam,[2,6] Catherine Visse,[5] Nicolas Gangneux,[1,3] Sébastien Bagot,[5] Jean-François Prost,[5] and Marc Lecuit[1,3,4]**

[1]Groupe Microorganismes et Barrières de l'Hôte and [2]Unité Interactions Moléculaires Flavivirus-Hôtes, Centre National de Référence des Arbovirus, Institut Pasteur, [3]Inserm, Avenir U604, and [4]Université Paris Descartes, Hôpital Necker-Enfants Malades, Centre d'Infectiologie Necker-Pasteur, Assistance Publique–Hôpitaux de Paris, Paris, [5]Laboratoire Français du Fractionnement et des Biotechnologies, Les Ulis, and [6]Service de Santé des Armées, Laboratoire de Virologie, Institut de Médecine Tropicale, Marseille, France

**(See the editorial commentary by Michault and Staikowsky on pages 489–91)**

***Background.*** Chikungunya virus (CHIKV) is a recently reemerged arbovirus responsible for a massive outbreak of infection in the Indian Ocean region and India that has a very significant potential to spread globally because of the worldwide distribution of its mosquito vectors. CHIKV induces a usually self-limited disease in humans that is characterized by fever, arthralgia, myalgia, and rash; however, cases of severe CHIKV infection have recently been described, particularly in adults with underlying condition and neonates born to viremic mothers.

***Methods.*** Human polyvalent immunoglobulins were purified from plasma samples obtained from donors in the convalescent phase of CHIKV infection, and the preventive and curative effects of these immunoglobulins were investigated in 2 mouse models of CHIKV infection that we developed.

***Results.*** CHIKV immunoglobulins contain anti-CHIKV antibodies and exhibit a high in vitro neutralizing activity and a powerful prophylactic and therapeutic efficacy against CHIKV infection in vivo, including in the neonate.

***Conclusions.*** Administration of CHIKV immunoglobulins may constitute a safe and efficacious prevention strategy and treatment for individuals exposed to CHIKV who are at risk of severe infection, such as neonates born to viremic mothers and adults with underlying conditions. These results provide a proof-of-concept for purifying human immunoglobulins from plasma samples from patients in the convalescent phase of an emerging infectious disease for which neither prevention nor treatment is available.

Chikungunya is a mosquito-borne disease caused by Chikungunya virus (CHIKV), a member of the Togaviridae family and *Alphavirus* genus. CHIKV was first isolated in 1953, during an epidemic of polyarthralgia in Tanzania [1]. In 2005, CHIKV emerged in the Indian Ocean region and caused a massive outbreak of infection [2], notably in La Réunion Island, where more than one-third of the inhabitants were infected; 246 of these inhabitants developed severe disease, and 213 died [3–7]. A vast CHIKV infection outbreak also emerged in India in 2006, with an estimated 1.4 million cases [8–10]. Of note, CHIKV has a very significant potential to spread globally because of the worldwide distribution of its mosquito vectors [11, 12], as exemplified by its recent emergence in Italy [13].

CHIKV is maintained in nature by uninterrupted cycles of transmission between mosquito vectors and so far unidentified vertebrate hosts [14]. Classically, CHIKV infection is characterized by fever, polyarthralgia, and myalgia, frequently associated with rash. The joint symptoms, often debilitating, usually resolve within 7 days to a few weeks, but relapsing arthralgia is frequent [15]. During the outbreak on La Réunion Island, previously unreported severe forms of infection caused by CHIKV were observed that were characterized by the occurrence of encephalopathy, notably in elderly patients and adult patients with underlying conditions [16]. Mother-to-child transmission of the virus

Received 13 January 2009; accepted 25 February 2009; electronically published 2 July 2009.

Potential conflicts of interest: none reported.

Financial support: Agence Nationale de la Recherche (MIE 2006), Inserm (Avenir program), Institut Pasteur (PTR 201), and Laboratoire Français du Fractionnement et des Biotechnologies (expertise contract).

Presented in part: 48th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, October 2008 (oral presentation V1848).

Reprints or correspondence: Dr. Marc Lecuit, Groupe Microorganismes et Barrières de l'Hôte, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris, France (mlecuit@pasteur.fr).

**The Journal of Infectious Diseases 2009; 200:516–23**

0022-1899/2009/20004-0006$15.00
DOI: 10.1086/600381

was also reported; vertical transmission was observed in ~50% of the neonates born to viremic mothers [17]. In half of infected neonates, a severe infection developed, with encephalopathy being the most common manifestation (90%) [17, 18].

To study the pathophysiology of Chikungunya, we developed mouse models of CHIKV infection [19]. We showed that, whereas wild type (WT) adult mice do not develop a systemic infection after CHIKV intradermal inoculation, WT mouse neonates are susceptible to CHIKV, and that neonatal disease severity is age dependent. In contrast to WT adult mice, mice with a partially abrogated type-I interferon (IFN) pathway (IFN-$\alpha/\beta R^{+/-}$ mice) develop a mild disease that closely mimicks benign human CHIKV infection. In contrast, adult mice with a totally abrogated type-I IFN pathway (IFN-$\alpha/\beta R^{-/-}$ mice) develop severe infection. In these mouse models, infection leads to viremia, and CHIKV is recovered from skeletal muscles, joints, and skin—a tissue tropism matching the symptoms observed in humans and similar to that observed in biopsy samples from human patients [19]. In adult IFN-$\alpha/\beta R^{-/-}$ mice and in neonates, severe CHIKV disease is associated with high viral load in peripheral tissues and dissemination to the central nervous system (CNS) [19]. In agreement with these results, CHIKV and anti-CHIKV immunoglobulin (Ig) M have been detected in the cerebrospinal fluid of human neonates and adult patients with encephalopathy [20].

To date, there is no effective treatment for CHIKV infection. Human polyclonal antibody preparation and viro-inactivated hyperimmune serum are commonly used for the treatment of human viral infections [21–23]. Administration of human Ig against West Nile virus (an arbovirus of the Flaviviridae family) improves neurological virus-associated disease in humans [24, 25], and passive immunization with convalescent serum from animals infected with alphaviruses has had a protective effect in animal models [26, 27].

Because of the likely future expansion of the area of transmission of CHIKV, it is now critical to develop prophylactic and therapeutic strategies, especially for individuals at risk of severe disease, such as exposed neonates and infected adults with underlying conditions. Here, we show that human Ig purified from plasma samples from donors in the convalescent phase of CHIKV infection (hereafter referred to as CHIKVIg) exhibits a potent preventive and curative effect against CHIKV infection in mouse models of CHIKV. Because the CHIKVIg production process is used for producing purified polyvalent Ig commercialized under the brand Tégéline, it is possible to use CHIKVIg in humans for prevention and treatment, especially in individuals at risk of severe CHIKV disease.

## MATERIALS AND METHODS

***Virus.*** The CHIKV-21 isolate was obtained from a serum sample from a male newborn with CHIKV-associated encephalopathy during the 2005–2006 outbreak of CHIKV infection in La Réunion and was amplified on C6/36 cells [6]. Titers of virus stocks were determined by Vero cell plaque assay and were expressed as plaque-forming units (PFU) per mL.

***Plasma collection.*** For the immunoreactivity study, human plasma samples collected by Etablissement Français du Sang were obtained from donors ($n = 80$) who were selected with the following inclusion criteria: (1) a reported clinical episode of febrile arthralgia evocative of CHIKV infection during the outbreak in La Réunion, (2) the absence of chronic joint or muscle symptoms to exclude patients with chronic joint or muscle disease, and (3) at least a 6-month delay between the end of acute disease and obtainment of samples. Exclusion criteria were those requested by European regulations, which include a serologic examination positive for human hepatitis A, B, and C viruses; human immunodeficiency virus types 1 and 2; human T cell leukemia virus types 1 and 2; and parvovirus B19.

For the manufacturing of CHIKVIg, plasma samples ($n = 583$) were collected by La Réunion regional Etablissement Français du Sang from donors in the convalescent phase of CHIKV infection. Inclusion criteria were the same as those used in the aforementioned immunoreactivity study, in addition to positivity for anti-CHIKV antibodies by enzyme-linked immunosorbent assay (ELISA). Exclusion criteria were having a sample that was reverse-transcriptase polymerase chain reaction positive for CHIKV [28], in addition to the criteria of the aforementioned preliminary study.

***Ig purification.*** Normal human Ig for intravenous administration (Tégéline) is a highly purified preparation obtained from plasma donors registered in France since 1996 [29]. Although Tégéline is obtained from plasma donors in continental France, where CHIKV is absent, after the outbreak in La Réunion, the Etablissement Français du Sang implemented measures to prevent CHIKV transmission by transfusion in continental France, and all symptomatic travelers returning from areas where CHIKV circulates are excluded from donation for 2 weeks. Tégéline exhibits no immunoreactivity against CHIKV, both in IgG-ELISA and neutralization assays. CHIKVIg was purified after the Tégéline manufacturing process from a pool of 583 plasma samples from donors vaccinated against CHIKV and from plasma for fractionation that was used by the Laboratoire Français du Fractionnement et des Biotechnologies to manufacture plasma-derived therapeutic products, including human Ig and coagulation factors. In brief, Tégéline and CHIKVIg were prepared from plasma by fractionation and purification steps, including filtrations. The purification process results in a 50-g/L human IgG preparation purified at 97%.

***ELISA and virus neutralization assay.*** All plasma samples were tested for antibodies specific for CHIKV by an IgG sandwich method, as described elsewhere [30]. Plasma samples were

**Table 1. Immunoreactivity against Chikungunya Virus (CHIKV) of Human Plasma Obtained from Donors in the Convalescent Phase of CHIKV Infection and of CHIKV Immunoglobulin (CHIKVIg)**

| Test | Immunoreactivity, titer | | | | |
|---|---|---|---|---|---|
| | Plasma A | Plasma B | Plasma C | Normal Ig | CHIKVIg |
| ELISA | <500 | 2000 | 500 | <500 | 800 |
| $PRNT_{80}$ | <20 | 40 | 320 | <20 | 40 |

**NOTE.** ELISA, enzyme-linked immunosorbent assay; $PRNT_{80}$, last dilution for which at least 80% of CHIKV plaque reduction was obtained.

tested in parallel with CHIKV antigen and with a control (i.e., mock-infected Vero E6 cells). The results were considered to be positive if the optical density (OD) ratio (OD of CHIKV antigen divided by the OD of control antigen) was >3. The CHIKV neutralizing antibody titer was determined using a plaque-reduction assay and was expressed as the highest dilution inducing at least 80% plaque reduction.

***Mouse experiments.*** IFN-$\alpha/\beta R^{-/-}$ 129s/v mice were provided by F. Tangy from M. Aguet [31], and 8–9-day-old C57BL/6 mice (B6) were obtained from Charles River laboratories (France). Mice were handled in accordance with the Institut Pasteur guidelines for animal husbandries and were kept in level 3 isolators. Mice were inoculated by the intradermal route in the ventral thorax with 50 $\mu$L (for adult mice) or 30 $\mu$L (for neonates) of a viral suspension diluted with phosphate-buffered saline (PBS). For passive transfer of human immune plasma A, B, and C or CHIKVIg, adult mice and mouse neonates were intraperitoneally injected with 0.5 mL and 0.2 mL, respectively, of the indicated doses immediately or at different times after CHIKV inoculation. The maximal practical dose was 830 mg/kg for adult mice and 2000 mg/kg for mouse neonates. Differences in outcome were assessed by comparing Kaplan-Meier survival curves with use of the log rank test.

For determining viral load in tissue and serum samples, tissue samples were homogenized, and virus titers were determined on Vero cells by tissue cytopathic infectious dose 50 (TCID50). Viral titers in tissue and serum samples were expressed as TCID50/g or TCID50/mL, respectively.

## RESULTS

***Immunoreactivity to CHIKV of human plasma samples from donors in the convalescent phase of CHIKV infection.*** In the first step, the presence of anti-CHIKV Ig was assessed in 80 regular blood donors from La Réunion who reported a history of clinical manifestations evocative of CHIKV infection during the outbreak in La Réunion. Plasma samples were collected from September through October 2006. The delay between the clinical episode and donation was at least 6 months. In addition to mandatory viral screenings (see Methods), all plasma samples were controlled negative for CHIKV RNA by reverse-transcriptase polymerase chain reaction. In a subgroup of 80 donors, 71 (88.7%) had anti-CHIKVIg detected by sandwich ELISA at the time of plasma sample obtainment. Of the 71 plasma samples positive by ELISA, 85.9% displayed an in vitro neutralization activity, with titers of 20–160, whereas 4.2% displayed high neutralization titers (>320), and 9.9% exhibited no neutralization activity (<20) (figure 1*A*). To determine whether ELISA and neutralization titers correlated, 27 randomly selected samples were assayed. ELISA titers in plasma samples were 500–2000 and did not correlate with neutralization titers (figure 1*B*).

In the second step, we selected 583 blood donors who re-

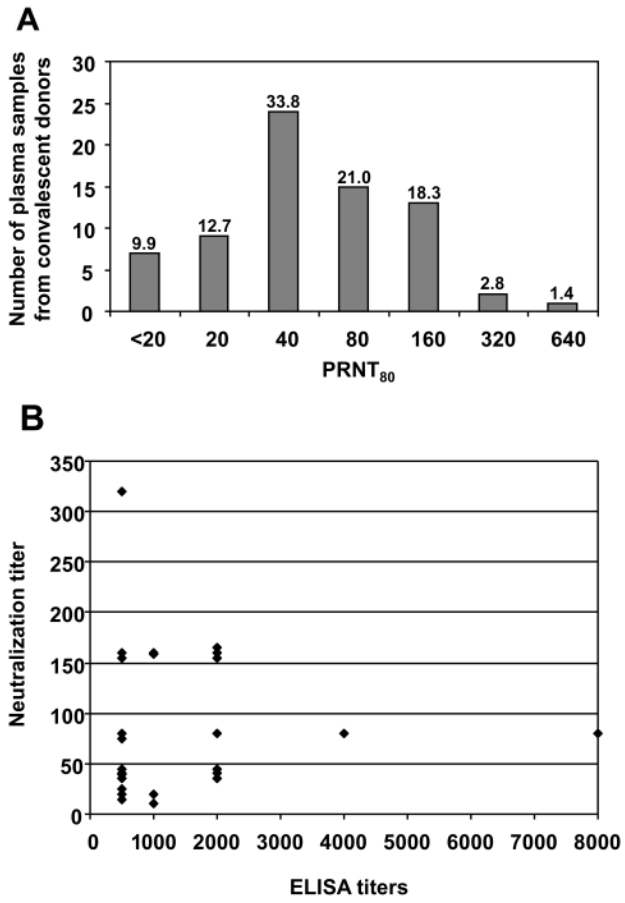


**Figure 1.** Immunoreactivity against Chikungunya virus (CHIKV) of human plasma samples obtained from donors in the convalescent phase of CHIKV infection. The titer of CHIKV neutralizing antibodies was determined by a standard neutralization assay, and the neutralizing titers are expressed as the inverse of the last dilution for which at least 80% of CHIKV plaque reduction was obtained ($PRNT_{80}$). *A,* Neutralizing titers of 71 plasma samples positive for CHIKV by enzyme-linked immunosorbent assay (ELISA). The number above each bar corresponds to the percentage of samples positive for CHIKV at the indicated titer. *B,* Neutralizing titers and ELISA titers in 27 plasma samples randomly chosen among the 71 plasma samples positive for CHIKV by ELISA. Titers in ELISA are expressed as the inverse of the last dilution of plasma for which a positive ratio of optical density has been obtained.

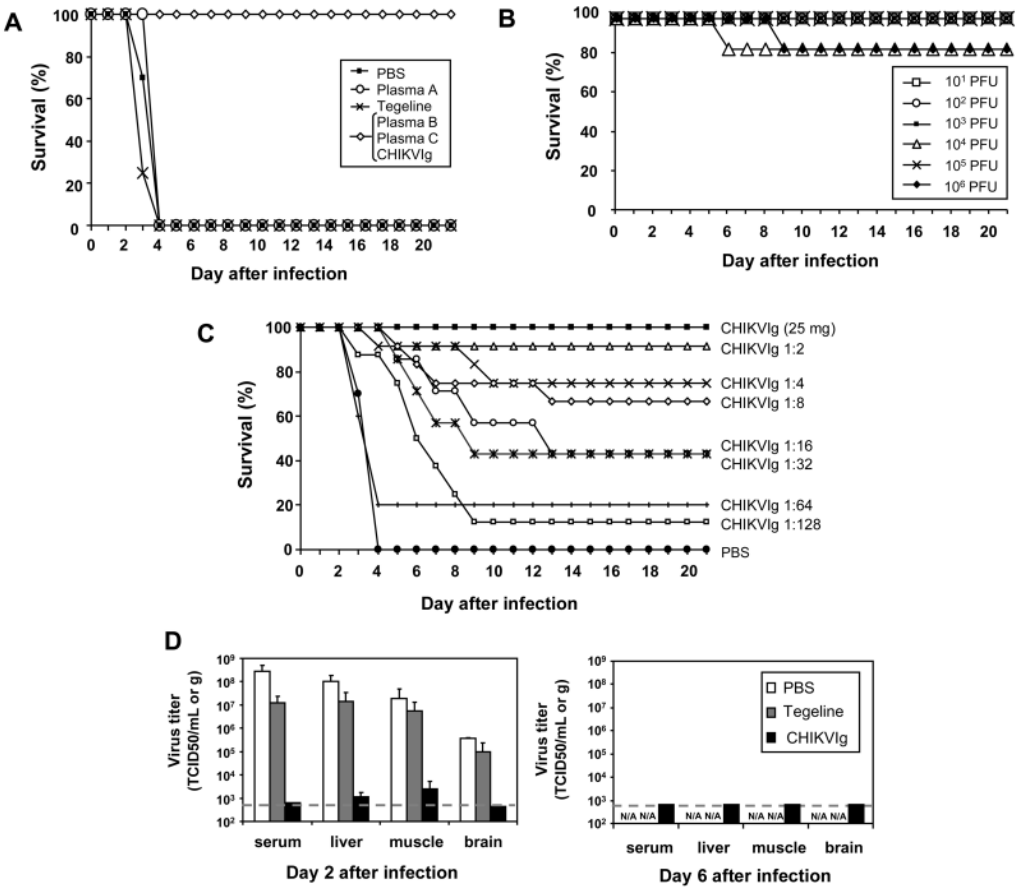


**Figure 2.** Chikungunya virus (CHIKV) infection prophylaxis with human plasma and purified immunoglobulin (Ig) in interferon (IFN)–$\alpha/\beta R^{-/-}$ adult mice. *A,* Passive administration of nonimmune and immune human plasma or human normal Ig and human CHIKVIg to IFN-$\alpha/\beta R^{-/-}$ adult mice infected by intradermal route with 10 plaque-forming units (PFU) of CHIKV. Plasma samples, normal Ig, and CHIKVIg were administrated as a single dose via intraperitoneal route immediately after CHIKV injection. Data correspond to at least 7 mice per condition. Statistical differences in the comparison with the phosphate-buffered saline (PBS) controls were as follows: human plasma A and normal Ig, $P > .9$; human plasma B, human plasma C, and CHIKVIg, $P < .001$. *B,* Passive administration of CHIKVIg to IFN-$\alpha/\beta R^{-/-}$ adult mice infected by intradermal route with 10–$10^6$ PFU of CHIKV. Antibodies were administrated as a single dose via intraperitoneal route immediately after CHIKV injection. Data correspond to 7 mice per condition in mean. Statistical differences were not significant, at $P > .9$. *C,* Passive administration with different amounts of CHIKVIg to IFN- $\alpha/\beta R^{-/-}$ adult mice infected by intradermal route with 10 PFU of CHIKV. Nondiluted CHIKVIg (25 mg; 830 mg/kg) or diluted CHIKVIg (from 1:2 [12.5 mg; 415 mg/kg] to 1:128 [0.195 mg; 6.5 mg/kg]) were administered as a single dose via intraperitoneal route immediately after CHIKV injection. Data correspond to at least 10 mice per condition. Statistical differences in the comparison with PBS controls were as follows: CHIKVIg (25 mg) and CHIKVIg 1:2–1:32, $P < .001$; CHIKVIg 1:64 and 1:128, $P > .9$. *D,* Viral titers in tissue and serum samples from mice inoculated with $10^6$ PFU of CHIKV via the intradermal route and injected with PBS, normal IgG, or CHIKVIg. Mice were sacrificed at the indicated times, and the amount of infectious virus in serum and tissue samples was quantified by tissue cytopathic infectious dose 50 (TCID50). Each data point represents the arithmetic mean ± standard deviation for at least 4 mice. A broken line indicates the detection threshold.

ported a clinical episode of CHIKV infection that was confirmed by serologic examination positive for CHIKV. Human plasma samples obtained from these donors were used to manufacture CHIKVIg, as described in Methods. These purified Ig, obtained in accordance with the validated process used to produce the commercially available human polyvalent Ig for intravenous Tégéline, exhibited immunoreactivity both in ELISA and in neutralization assays, whereas Tégéline, used here as a negative control, exhibited no immunoreactivity against CHIKV (table 1).

***Prophylaxis studies with human immune plasma samples and CHIKVIg in IFN-$\alpha/\beta R^{-/-}$ adult mice.*** We then tested the protection conferred by human immune plasma samples against CHIKV infection. We first evaluated their efficacy to prevent fatal infection in IFN-$\alpha/\beta R^{-/-}$ adult mice. From the 27 human plasma samples selected, we chose 2 samples with different immunoreactivity patterns against CHIKV: plasma sample A (a nonimmune control human sample devoid of anti-CHIKV immunoreactivity by ELISA and neutralization assay), plasma sample B (a sample from a donor in the convalescent

phase of CHIKV infection that had a high ELISA titer but low neutralizing activity), and plasma sample C (a sample that exhibited a low ELISA titer but high neutralizing activity) (table 1).

We first evaluated the innocuousness of these plasma samples by injecting mice intraperitoneally with 0.5 mL of nonimmune (A) or immune plasma samples (B or C). All mice remained alive and healthy (data not shown). We then injected IFN-$\alpha/\beta R^{-/-}$ adult mice with a lethal dose of CHIKV (10 PFU) [19] intradermally with a single dose of 0.5 mL of plasma by intraperitoneal route. Immune plasma with high neutralizing activity (C) or low neutralizing activity (B) completely protected IFN-$\alpha/\beta R^{-/-}$ adult mice against a lethal dose of CHIKV (figure 2*A*). In contrast, administration of nonimmune plasma (A) did not provide protection (figure 2*A*). These data indicate that passive transfer of human immune plasma samples to highly permissive mice is protective against CHIKV infection.

We then tested the protection conferred by CHIKVIg. IFN-$\alpha/\beta R^{-/-}$ adult mice injected with 0.5 mL (25 mg) of CHIKVIg or Tégéline alone remained alive and healthy (data not shown). As observed with human immune plasma, injection of 0.5 mL (25 mg; 830 mg/kg) of CHIKVIg to mice simultaneously inoculated with a lethal dose of CHIKV (10 PFU) protected against lethal infection, whereas injection of the same dose of Tégéline had no effect (figure 2*A*).

We assessed the effect of CHIKVIg against infection with 10–$10^5$-fold higher inoculums of CHIKV (i.e., $10^2$–$10^6$ PFU). As observed with the 10-PFU dose, CHIKVIg protected 100% of mice against infection with $10^3$ PFU and still protected >80% of the animals at higher doses of virus, including the highest dose of $10^6$ PFU (figure 2*B*).

We then investigated the effect of lower doses of CHIKVIg on CHIKV infection. Injection of undiluted CHIKVIg (25 mg; 830 mg/kg) completely protected mice against infection, and injection of an 8-fold lower dose (3.1 mg; 100 mg/kg) protected >60% of mice, whereas treatments with lower doses were less effective (figure 2*C*).

To evaluate the effect of CHIKVIg on viral amplification and dissemination in tissue, we determined viral load in tissue (liver, skeletal muscle, and CNS) and serum samples from of IFN-$\alpha/\beta R^{-/-}$ mice infected with 10 PFU of CHIKV and injected with CHIKVIg, Tégéline, or PBS on days 2 and 6 after infection. Treatment with CHIKVIg resulted in inhibition of viral amplification in tissue and prevented viremia (figure 2*D*). In contrast, treatment with Tégéline did not alter viral propagation in mice, and high levels of infectious virus were recovered from tissue and serum samples on day 2 after infection (as high as levels observed in mice that received PBS); all mice were dead by day 6 after infection.

***Prophylaxis studies with CHIKVIg in WT mouse neonates.*** To assess the protective efficacy of passive immunotherapy against CHIKV infection in mouse neonates, we determined the efficacy of immune human plasma and of CHIKVIg to prevent CHIKV fatal infection in 8–9-day-old B6 mouse neonates. Similar to findings for IFN-$\alpha/\beta R^{-/-}$ adult mice, intraperitoneal injection of 0.2 mL of nonimmune human plasma (A) and immune plasma (B), as well as CHIKVIg or Tégéline (10 mg; 2000 mg/kg), in mouse neonates had no toxic effect (data not shown). All infected neonates treated with a single dose of immune plasma B or CHIKVIg survived, whereas all mouse neonates treated with PBS, nonimmune plasma (A), or Tégéline that were inoculated intradermally with a lethal dose of CHIKV ($10^6$ PFU) died within 15 days after infection (figure 3*A*).

We then assessed the effect of lower doses of CHIKVIg on CHIKV infection in neonates. Treatment with a single dose of ⩾0.6 mg (dilution, 1:16; 100 mg/kg) of CHIKVIg completely protected against infection (figure 3*B*). Treatment with lower doses was less effective, although injection of 0.1 mg (dilution, 1:64; 31 mg/kg) of CHIKVIg protected ~60% of mouse neonates.

We also evaluated the effect of CHIKVIg on viral dissemination in tissue (liver, muscle, and brain) and serum of mouse neonates after infection with $10^6$ PFU of CHIKV and treatment with CHIKVIg, Tégéline, or PBS on days 3 and 6 after infection. Tissue and serum samples obtained from mouse neonates treated with CHIKVIg demonstrated little or no detectable infectious virus at both day 3 and day 6 after infection, whereas tissue and serum samples from mice treated with Tégéline exhibited high viral loads on day 3 after infection that were maintained in muscle on day 6 after infection (figure 3*C*).

***Therapeutic effect of CHIKVIg in adult and neonatal mice.*** Because of the lack of specific treatment for CHIKV infection that is available to date, we evaluated the therapeutic efficacy of CHIKVIg in IFN-$\alpha/\beta R^{-/-}$ adult mice and in WT mouse neonates. Adult mice and mouse neonates were inoculated with 10 PFU and $10^6$ PFU of CHIKV, respectively, at the time of infection and received a single dose (25 mg and 10 mg, respectively) of CHIKVIg by hours 8, 24, and 48 after infection. The latter time was omitted for IFN-$\alpha/\beta R^{-/-}$ adult mice, because their mean survival time is only 3 days. In contrast to PBS, CHIKVIg injected by hour 8 after infection had a therapeutic effect both in all IFN-$\alpha/\beta R^{-/-}$ adult mice and in all WT mouse neonates, and administration of CHIKVIg by hour 24 after infection protected again at least 50% of mice (figure 4*A* and 4*B*). Treatment of mouse neonates after hour 48 after infection had no significant effect on mortality or mean survival time.

## DISCUSSION

CHIKV is a recently reemerged arbovirus with a high epidemic potential. The fear for a pandemic of CHIKV infection arises

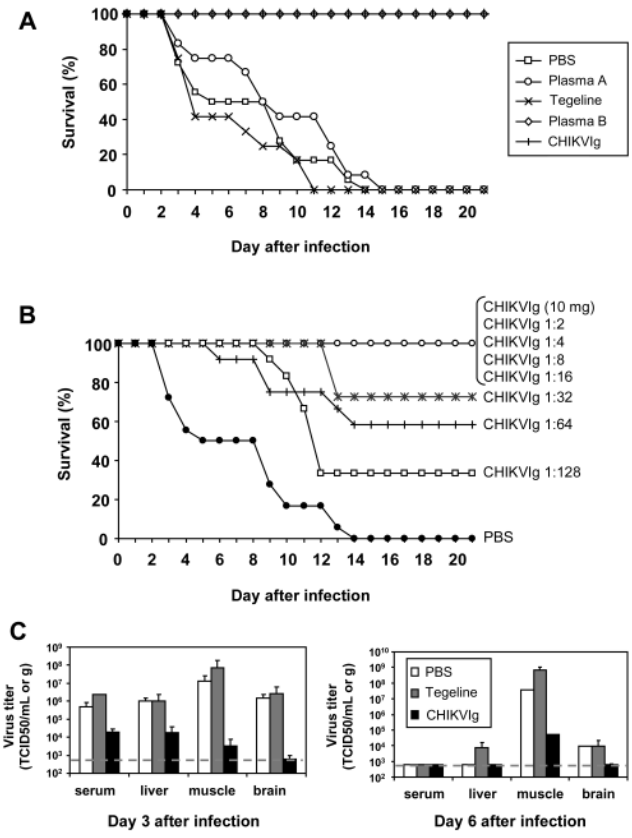


**Figure 3.** Chikungunya virus (CHIKV) infection prophylaxis with human plasma and purified immunoglobulin (Ig) in B6 mouse neonates. *A,* Passive administration of nonimmune and immune human plasma and normal Ig or CHIKV immunoglobulin (CHIKVIg) to 8–9-day-old mice infected by intradermal route with $10^6$ plaque-forming units (PFU) of CHIKV. The indicated plasma and Ig were administrated as a single dose via intraperitoneal route immediately after CHIKV injection. Data reflect at least 12 mice per condition. Statistical differences in the comparison with phosphate-buffered saline (PBS) controls were as follows: human plasma A and normal Ig, $P > .9$; human plasma B and CHIKVIg, $P < .001$. *B,* Passive administration with different amounts of CHIKVIg to 8–9-day-old mice infected by intradermal route with $10^6$ PFU of CHIKV. Nondiluted (10 mg) or diluted (1:2–1:128) CHIKVIg was administrated as a single dose via intraperitoneal route immediately after CHIKV injection. Data correspond to at least 12 mice per condition. $P < .001$ for statistical differences in the comparison of PBS controls with mice treated with CHIKVIg (10 mg) and CHIKVIg 1:2–1:128. *C,* Viral titers in tissue and serum samples from mice inoculated with $10^6$ PFU of CHIKV via the intradermal route and injected with PBS, normal Ig, or CHIKVIg. Mice were sacrificed at the indicated times, and the amount of infectious virus in serum and tissue samples was quantified by tissue cytopathic infectious dose 50 (TCID50). Each data point represents the arithmetic mean ± standard deviation for at least 4 mice. A broken line indicates the detection threshold.

from its possible dissemination by rapid long-distance travels and its transmissibility by urban mosquito vectors [32]. In humans, CHIKV produces a sudden debilitating disease because of its incapacitating joint symptoms. Moreover, CHIKV infection is a cause of neuropathology, particularly in elderly persons and neonates and can be fatal in these patients. There is currently neither specific treatment nor vaccine available for CHIKV infection. In this context, the development of prophylactic and therapeutic strategies for CHIKV infection is a priority.

During the course of CHIKV infection in humans, an antibody response develops in the early stages, but its persistence over time is not fully established [33]. Follow-up of healthy volunteers vaccinated with an attenuated CHIKV strain has provided evidence of persistence of significant antibodies with in vitro neutralizing activity after 12 months [33, 34]. In the present study, we provide evidence that, similar to what was observed in individuals vaccinated with an attenuated CHIKV strain, naturally infected individuals still have circulating neu-

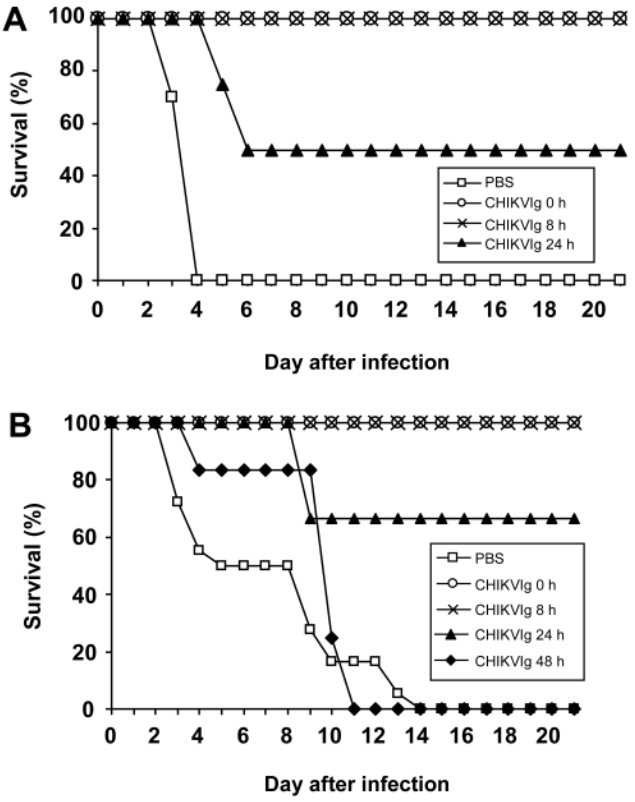


**Figure 4.** Therapeutic activity of Chikungunya virus (CHIKV) immunoglobulin (Ig) in IFN-$\alpha/\beta R^{-/-}$ adult mice and B6 mouse neonates. *A,* A single dose (25 mg) of CHIKVIg was administrated via intraperitoneal route immediately after CHIKV injection (0 h) or at the indicated hours after intradermal administration of 10 plaque-forming units (PFU) of CHIKV in IFN-$\alpha/\beta R^{-/-}$ adult mice. Data correspond to at least 7 mice per condition. $P < .001$ for statistical differences in the comparison with phosphate-buffered saline (PBS) controls. *B,* A single dose (10 mg) of CHIKVIg was administrated via intraperitoneal route immediately after CHIKV injection (0 h) or at the indicated hours after intradermal administration of $10^6$ PFU of CHIKV in B6 mouse neonates. Data correspond to 12 mice per condition in mean. Statistical differences in the comparison with PBS controls were as follows: CHIKVIg 0 h and 8 h after infection, $P < .001$; CHIKVIg 24 h and 48 h after infection, $P < .005$.

tralizing antibodies up to 12 months after acute CHIKV infection. In our study, the detection rate of anti-CHIKV antibodies by ELISA in 80% of donors results from the clinical criteria used for selecting the donors of the immunoreactivity study. Thus, in the context of a high prevalence of CHIKV infection (e.g., ~33% of the Réunionese population) and a selection of plasma donors in the early postepidemic period, purified anti-CHIKVIg (exhibiting in vitro and in vivo neutralizing activities) can be obtained by applying the classical procedure used to produce the purified polyvalent Ig Tégéline to plasma samples from donors selected only on the basis of clinical criteria. This approach demonstrates the possibility to easily recruit donors and produce massive amounts of CHIKVIg in the context of a vast epidemic.

A protective efficacy of Ig against alphavirus infections has been previously demonstrated in mice [26, 35, 36]. Here, we evaluated the efficacy of pre- and postexposure protection conferred by CHIKVIg in immunocompromised adult mice and immunocompetent mouse neonates. These mouse models of CHIKV infection exhibit the features characterizing severe human CHIKV infection [19]: in both models, a single dose of CHIKV immune human plasma or CHIKVIg inhibited viral dissemination and abrogated lethality associated with CHIKV when administered at least 8 h after infection, and the level of protection correlates with the amount of antibodies transferred. The fact that the dose of CHIKVIg that protected 100% of animals was higher for adult mice (830 mg/kg) than for mouse neonates (100 mg/kg) likely results from the higher susceptibility of the IFN-$\alpha/\beta R^{-/-}$ mouse model. Indeed, the LD100 in WT mouse neonates is $10^6$ PFU, and it is only 10 PFU in adult IFN-$\alpha/\beta R^{-/-}$ mice; the mean survival time is shorter for adult IFN-$\alpha/\beta R^{-/-}$ mice than for infected mouse neonates [19]. Nevertheless, total protection can be obtained regardless of the mouse IFN-$\alpha/\beta R^{-/-}$ status and the age of animals. Moreover, CHIKVIg administration completely abolishes viral dissemination into serum and the brain and strongly alleviates liver and muscle infection. It is noteworthy that the 100-mg/kg dose of CHIKVIg did not completely inhibit viral replication in muscle of mouse neonates by day 6 after infection, although the 830-mg/kg dose abolished muscle infection in IFN-$\alpha/\beta R^{-/-}$ mice. Indeed, we have previously shown that after intradermal injection, CHIKV is detectable first in liver and then in blood before reaching its peripheral target tissues: the muscles, joints, and skin [19]. In the present study, we found that CHIKVIg strongly restricts liver infection and viremia and totally blocks CHIKV dissemination to the CNS. These data suggest that liver infection and viremia precedes virus spreading to the CNS and that control of liver infection and viremia by anti-CHIKV antibodies is sufficient to prevent CNS infection. Similarly, it has been shown that administration of anti–West Nile virus antibodies in mice infected with West Nile virus prevents CNS infection [37, 38].

Although both human plasma samples from donors in the convalescent phase of infection and CHIKVIg provide protection in mice, they exhibited different in vitro immunoreactivity to CHIKV. Indeed, human plasma B samples and CHIKVIg had low in vitro neutralizing activity, whereas human plasma C samples had high in vitro neutralizing activity. Numerous studies have shown the importance of neutralizing antibodies in recovery and protection from viral infections. In addition to the neutralization of virus-host interactions in vivo, protective antibodies can exert their beneficial effect via other mechanisms, such as antibody-dependent and complement-mediated cellular cytoxicity attributed to Fc$\gamma$R engagement. In this study, antibodies from plasma B samples, which had low neutralizing activity, conferred protection against CHIKV, as was previously shown for Venezuelan equine encephalomyelitis virus, for which Fc$\gamma$R engagement is thought to be essential [39].

It is widely recognized that passive vaccination is an appropriate preventive and therapeutic option for many viral infections in human, including those spread by viral vertical transmission [23]. Indeed, antibody preparations derived from vaccinated human donors have been widely used for prophylaxis and therapy of human viral diseases, especially when no alternative therapy is available. In particular, hyperimmune human IgG is used as a standard prevention therapy of mother-to-child transmission of hepatitis B and varicella-zoster viruses. Polyclonal Ig therapy has also been used with success for parvovirus B19, enterovirus, and West Nile virus infections [23–25]. Recently, severe acute respiratory syndrome coronavirus infections were treated with convalescent patient plasma containing anti-severe acute respiratory syndrome–coronavirus polyclonal antibodies [40, 41], and purification of severe acute respiratory syndrome–coronavirus hyperimmune Ig from pooled convalescent plasma has also been reported [42]. Similar results have been reported for treating influenza A H5N1 infection in humans [43]. Furthermore, vaccine developments have frequently been based on previous reports of protection conferred by passive immunotherapy, thereby suggesting that a vaccine inducing a similar antibody response could be effective.

Altogether, our results suggest that antiviral prevention and therapy with CHIKVIg may constitute an effective medical intervention for humans with a known exposure to CHIKV who are at risk of severe disease. Prophylaxis with CHIKVIg could thus be recommended especially at birth for neonates born to viremic mothers, as well as for exposed heavily immunocompromised patients. Such a tailor-made immunotherapy development should be applicable to other emerging infectious agents for which neither treatment nor prevention is available.

## Acknowledgments

We thank P. Bergeat, Director of the Laboratoire Français du Fractionnement et des Biotechnologies, for his support; A. Michault, for his continuing support and help with this study; Matthew Albert, for critically reading the manuscript; and O. Lortholary, for his support.

## References

1. Robinson MC. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952–53. I. Clinical features. Trans R Soc Trop Med Hyg **1955**; 49:28–32.
2. Enserink M. Infectious diseases: massive outbreak draws fresh attention to little-known virus. Science **2006**; 311:1085.
3. Bonn D. How did Chikungunya reach the Indian Ocean? Lancet Infect Dis **2006**; 6:543.
4. Josseran L, Paquet C, Zehgnoun A, et al. Chikungunya disease outbreak, Reunion Island. Emerg Infect Dis **2006**; 12:1994–5.
5. Bessaud M, Peyrefitte CN, Pastorino BA, et al. Chikungunya virus strains, Reunion Island outbreak. Emerg Infect Dis **2006**; 12:1604–6.
6. Schuffenecker I, Iteman I, Michault A, et al. Genome microevolution of Chikungunya viruses causing the Indian Ocean outbreak. PLoS Med **2006**; 3:e263.
7. Pialoux G, Gauzere BA, Jaureguiberry S, Strobel M. Chikungunya, an epidemic arbovirosis. Lancet Infect Dis **2007**; 7:319–27.
8. Ravi V. Re-emergence of Chikungunya virus in India. Indian J Med Microbiol **2006**; 24:83–4.
9. Saxena SK, Singh M, Mishra N, Lakshmi V. Resurgence of Chikungunya virus in India: an emerging threat. Euro Surveill **2006**; 11:E060810.2.
10. Mavalankar D, Shastri P, Raman P. Chikungunya epidemic in India: a major public-health disaster. Lancet Infect Dis **2007**; 7:306–7.
11. Gratz NG. Emerging and resurging vector-borne diseases. Annu Rev Entomol **1999**; 44:51–75.
12. Charrel RN, de Lamballerie X, Raoult D. Chikungunya outbreaks—the globalization of vectorborne diseases. N Engl J Med **2007**; 356:769–71.
13. Rezza G, Nicoletti L, Angelini R, et al. Infection with Chikungunya virus in Italy: an outbreak in a temperate region. Lancet **2007**; 370:1840–6.
14. Diallo M, Thonnon J, Traore-Lamizana M, Fontenille D. Vectors of Chikungunya virus in Senegal: current data and transmission cycles. Am J Trop Med Hyg **1999**; 60:281–6.
15. Borgherini G, Poubeau P, Jossaume A, et al. Persistent arthralgia associated with Chikungunya virus: a study of 88 adult patients on reunion island. Clin Infect Dis **2008**; 47:469–75.
16. Lemant J, Boisson V, Winer A, et al. Serious acute Chikungunya virus infection requiring intensive care during the Reunion Island outbreak in 2005–2006. Crit Care Med **2008**; 36:2536–41.
17. Gérardin P, Carbonnier M, Bintner M, et al. Severe neonatal encephalopathy and haemorrhagic fever as a complication of materno-fetal transmission of Chikungunya virus. PLoS Medicine **2008**; 5:e60.
18. Lenglet Y, Barau G, Robillard PY, et al. Chikungunya infection in pregnancy: evidence for intrauterine infection in pregnant women and vertical transmission in the parturient: survey of the Reunion Island outbreak [in French]. J Gynecol Obstet Biol Reprod (Paris) **2006**; 35: 578–83.
19. Couderc T, Chrétien F, Schilte C, et al. A mouse model for Chikungunya: young age and inefficient type-I interferon signaling are risk factors for severe disease. PLoS Pathogens **2008**; 4:e29.
20. Laurent P, Le Roux K, Grivard P, et al. Development of a sensitive real-time reverse transcriptase PCR assay with an internal control to detect and quantify Chikungunya virus. Clin Chem **2007**; 53:1408–14.
21. Dunman PM, Nesin M. Passive immunization as prophylaxis: when and where will this work? Curr Opin Pharmacol **2003**; 3:486–96.
22. Keller M, Deveikis A, Cutillar-Garcia M, et al. Pneumococcal and influenza immunization and human immunodeficiency virus load in children. Pediatr Infect Dis J **2000**; 19:613–8.
23. Dessain SK, Adekar SP, Berry JD. Exploring the native human antibody repertoire to create antiviral therapeutics. Curr Top Microbiol Immunol **2008**; 317:155–83.
24. Shimoni Z, Niven MJ, Pitlick S, Bulvik S. Treatment of West Nile virus encephalitis with intravenous immunoglobulin. Emerg Infect Dis **2001**; 7:759.
25. Hamdan A, Green P, Mendelson E, Kramer MR, Pitlik S, Weinberger M. Possible benefit of intravenous immunoglobulin therapy in a lung transplant recipient with West Nile virus encephalitis. Transpl Infect Dis **2002**; 4:160–2.
26. Phillpotts RJ. Immunity to airborne challenge with Venezuelan equine encephalitis virus develops rapidly after immunization with the attenuated vaccine strain TC-83. Vaccine **1999**; 17:2429–35.
27. Stanley J, Cooper SJ, Griffin DE. Monoclonal antibody cure and prophylaxis of lethal Sindbis virus encephalitis in mice. J Virol **1986**; 58: 107–15.
28. Pastorino B, Bessaud M, Grandadam M, Murri S, Tolou HJ, Peyrefitte CN. Development of a TaqMan RT-PCR assay without RNA extraction step for the detection and quantification of African Chikungunya viruses. J Virol Methods **2005**; 124:65–71.
29. Gardi A. Quality control in the production of an immunoglobulin for intravenous use. Blut **1984**; 48:337–44.
30. Peyrefitte CN, Rousset D, Pastorino BA, et al. Chikungunya virus, Cameroon, 2006. Emerg Infect Dis **2007**; 13:768–71.
31. Muller U, Steinhoff U, Reis LF, et al. Functional role of type I and type II interferons in antiviral defense. Science **1994**; 264:1918–21.
32. Chevillon C, Briant L, Renaud F, Devaux C. The Chikungunya threat: an ecological and evolutionary perspective. Trends Microbiol **2008**; 16: 80–8.
33. Edelman R, Tacket CO, Wasserman SS, Bodison SA, Perry JG, Mangiafico JA. Phase II safety and immunogenicity study of live chikungunya virus vaccine TSI-GSD-218. Am J Trop Med Hyg **2000**; 62:681–5.
34. Myers RM, Carey DE. Concurrent isolation from patient of two arboviruses, Chikungunya and dengue type 2. Science **1967**; 157:1307–8.
35. Rabinowitz SG, Adler WH. Host defenses during primary Venezuelan equine encephalomyelitis virus infection in mice. I. Passive transfer of protection with immune serum and immune cells. J Immunol **1973**; 110:1345–53.
36. Boere WA, Benaissa-Trouw BJ, Harmsen M, Kraaijeveld CA, Snippe H. Neutralizing and non-neutralizing monoclonal antibodies to the E2 glycoprotein of Semliki Forest virus can protect mice from lethal encephalitis. J Gen Virol **1983**; 64:1405–8.
37. Engle MJ, Diamond MS. Antibody prophylaxis and therapy against West Nile virus infection in wild-type and immunodeficient mice. J Virol **2003**; 77:12941–9.
38. Ben-Nathan D, Lustig S, Tam G, Robinzon S, Segal S, Rager-Zisman B. Prophylactic and therapeutic efficacy of human intravenous immunoglobulin in treating West Nile virus infection in mice. J Infect Dis **2003**; 188:5–12.
39. Mathews JH, Roehrig JT, Trent DW. Role of complement and the Fc portion of immunoglobulin G in immunity to Venezuelan equine encephalomyelitis virus infection with glycoprotein-specific monoclonal antibodies. J Virol **1985**; 55:594–600.
40. Cheng Y, Wong R, Soo YO, et al. Use of convalescent plasma therapy in SARS patients in Hong Kong. Eur J Clin Microbiol Infect Dis **2005**; 24:44–6.
41. Yeh KM, Chiueh TS, Siu LK, et al. Experience of using convalescent plasma for severe acute respiratory syndrome among healthcare workers in a Taiwan hospital. J Antimicrob Chemother **2005**; 56:919–22.
42. Zhang Z, Xie YW, Hong J, et al. Purification of severe acute respiratory syndrome hyperimmune globulins for intravenous injection from convalescent plasma. Transfusion **2005**; 45:1160–4.
43. Zhou B, Zhong N, Guan Y. Treatment with convalescent plasma for influenza A (H5N1) infection. N Engl J Med **2007**; 357:1450–1.