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Chikungunya virus-like particles are more immunogenic in a lethal AG129 mouse model compared to glycoprotein E1 or E2 subunits

Stefan W. Metz[a], Byron E. Martina[b], Petra van den Doel[b], Corinne Geertsema[a], Albert D. Osterhaus[b], Just M. Vlak[a], Gorben P. Pijlman[a,*]

[a] Laboratory of Virology, Wageningen University, Wageningen, The Netherlands
[b] Department of Virology, Erasmus MC, Rotterdam, The Netherlands

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ABSTRACT

Chikungunya virus (CHIKV) causes acute illness characterized by fever and long-lasting arthritic symptoms. The need for a safe and effective vaccine against CHIKV infections is on the rise due to on-going vector spread and increasing severity of clinical complications. Here we report the results of a comparative vaccination-challenge experiment in mice using three different vaccine candidates produced in insect cells by recombinant baculoviruses: (i) secreted (s)E1 and (ii) sE2 CHIKV glycoprotein subunits (2 μg/immunization), and (iii) CHIKV virus-like particles (VLPs) (1 μg E2 equivalent/immunization). These experiments show that vaccination with two subsequent administrations of 1 μg of Matrix M adjuvanted CHIKV VLPs completely protected AG129 mice from lethal CHIKV challenge. Vaccination with E1 and E2 subunits provided partial protection, with half of the mice surviving but with significantly lower neutralizing antibody titres as compared to the VLP vaccinated mice. This study provides evidence that even a modest neutralizing antibody response is sufficient to protect mice from CHIKV infections. Neutralization was the prominent correlate of protection. In addition, CHIKV VLPs provide a superior immune response and protection against CHIKV-induced disease in mice as compared to individual CHIKV-sE1 and -sE2 subunits.

1. Introduction

Chikungunya virus (CHIKV), the etiological agent of chikungunya fever, has infected millions of people in Africa and Asia, since it re-emerged in Central Africa in 2004 [1] and continued to spread in the Indian Ocean region. Recent outbreaks in Italy (2007) [2] and France (2010) [3] exemplify the threat of CHIKV transmission in non-tropical regions including Europe. CHIKV was historically transmitted by *Aedes aegypti*, but recently adapted to a novel transmission vector, the Asian Tiger mosquito *Ae. albopictus* [4]. This vector switch increases the risk of further dissemination of the virus in temperate regions (e.g. Europe) or to invade and cause so-called 'virgin-soil' epidemics on continents with no history of CHIKV circulation, such as North America [5,6]. Research efforts on the development of a safe and efficacious CHIKV vaccine have not yet resulted in a commercially available product. However, the use of CHIKV-subunit proteins [7], engineered viral vectors [8,9] and virus-like particles (VLPs) [10,11] has shown promising results. Large-scale production was not achieved, therefore the baculovirus-insect cell expression system was explored to generate a CHIKV vaccine candidate [12]. Our previous work described effective expression and purification of secreted CHIKV-sE1 and -sE2 subunits [13] and the production of an effective CHIKV VLP vaccine [11] in insect cells using recombinant baculoviruses. Single-shot vaccination with a dose as low as 1 μg/immunization of these VLPs induced complete protection against CHIKV-induced viraemia and foot swelling in mice [11], but the effectiveness of the VLPs has not been compared to immunization with their subunit counterparts, E1 and E2.

The current study describes a comparison of the insect cell-derived CHIKV-subunit and VLP vaccine candidates in a lethal IFN-α/β and -γ receptor null (AG129) mouse model for CHIKV vaccine-challenge studies [14]. In this mouse model, mice were immunized twice with subunits or VLPs that were formulated in Matrix M (Isconova) adjuvant. These mice were subsequently challenged with a lethal dose of the CHIKV-S27 isolate. Determination of neutralizing antibody titres prior challenge and monitoring of mouse survival and viral RNA titres in brain tissue upon

* Corresponding author. Tel.: +31 317 484498; fax: +31 317 484820.
E-mail address: gorben.pijlman@wur.nl (G.P. Pijlman).

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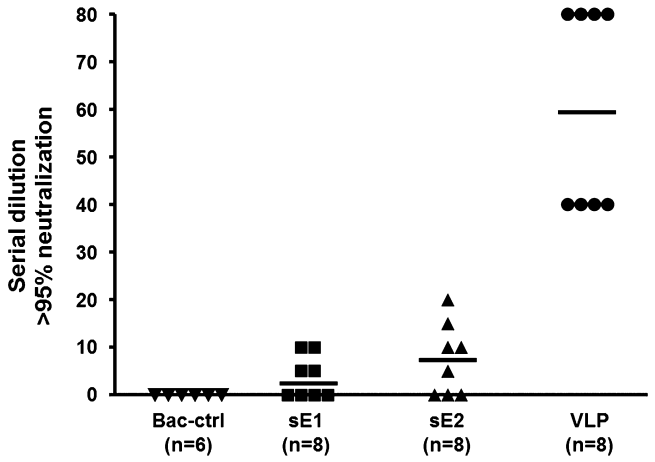

Fig. 1. CHIKV neutralizing antibody titres after immunization. Sera of immunized AG129 mice (twice with 2 μg purified sE1, sE2 [13] or 1 μg VLPs [11], adjuvanted with 5 μg Matrix M) were tested for their neutralizing ability. Neutralization was based upon suppression of CHIKV-induced CPE in Vero cells. sE1, subunit E1; sE2, subunit E2; VLP, virus-like particle; Bac-ctrl, negative control. The number of mice per group is indicated, average titres are indicated with a line.

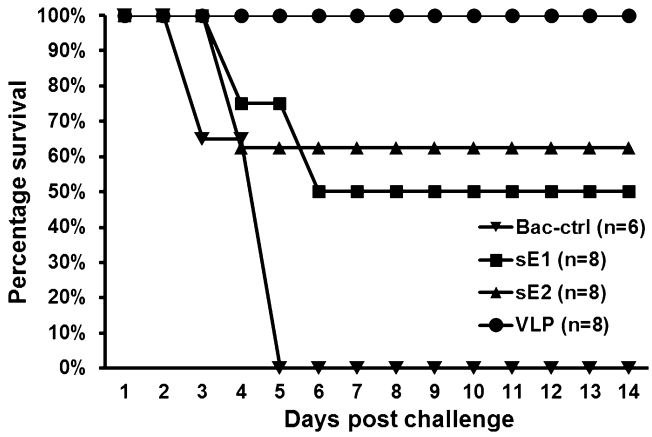

Fig. 2. Survival curves of vaccinated mice upon lethal CHIKV challenge. AG129 mice were immunized twice with purified sE1, sE2 [13] or VLPs [11], adjuvanted with 5 μg Matrix M. Mice were challenged with 1000 $TCID_{50}$ units CHIKV strain S27 six weeks post booster vaccination. Abbreviations as in Fig. 1.

viral challenge indicated that VLPs outperformed the subunits in immunogenicity and conferred complete protection against lethal CHIKV infection.

2. Results

2.1. Vaccination of AG129 mice with CHIKV VLPs and subunits induce neutralizing antibody responses

AG129 mice ($n = 8$/group) were immunized twice with formulations of Matrix M-adjuvanted CHIKV VLPs and their subunit counterparts, CHIKV-sE1 or -sE2, or control ($n = 6$/group). To analyze the neutralizing activity of the mice sera post vaccination but prior challenge, mice sera were subjected to a CHIKV neutralization test. All groups, but not all animals within the groups, vaccinated with CHIKV antigens produced neutralizing antibodies, albeit at different levels (Fig. 1). The CHIKV VLPs induced the highest neutralizing antibody titres and in all of the mice ($n = 8$). The subunits induced significantly lower (Student's T-test, $p < 0.01$) neutralizing titres in 4 out of 8 (sE1) or 5 out of 8 (sE2) mice. Non-responders were only found in the CHIKV-sE1 and -sE2 vaccinated mice. As expected, the control group of mice vaccinated with Bac-ctrl [11] did not produce any neutralizing antibodies against CHIKV.

2.2. Neutralizing antibodies correlate with protection: vaccination with CHIKV VLPs confers complete protection in mice against lethal CHIKV challenge

Six weeks after the second vaccination, all immunized and control animals were challenged intraperitoneally with 1000 $TCID_{50}$ units of CHIKV S27 isolate. As expected, the control group of mice (Bac-ctrl, $n = 6$) reached 100% mortality within 5 days post challenge. In sharp contrast, complete protection against CHIKV-induced mortality was obtained after immunization with VLPs (Fig. 2). Immunization with either CHIKV-sE1 or -sE2 only partially protected mice against lethal CHIKV infection, with 4 out of 8 (50%) and 5 out of 8 (62.5%) mice surviving, respectively (Fig. 2). The mice that died from infection were those that did not develop neutralizing antibodies. These results demonstrate (i) that the presence of neutralizing antibodies correlates with protection and (ii) that a (low) neutralizing antibody response is sufficient to protect mice from lethal CHIKV challenge.

2.3. E1- or E2-specific antibody responses are poor predictors for protective immunity

To clarify whether animals that did not produce neutralizing antibodies and consequently succumbed to infection, produced other types of antibodies, or that they were simply non-responders, Western analyses were performed on mice sera. Neither the sE1 subunit itself nor the E1 protein within the VLPs were detected by sera from sE1-vaccinated animals (Fig. 3A), despite the fact that 4 out of 8 of these mice did produce neutralizing antibodies (Fig. 1) and survived lethal CHIKV challenge. In contrast, 6 out of 8 mice immunized with sE2 produced antibodies that specifically recognized sE2 subunit as well as E2 protein within the VLPs (Fig. 3B). However, the presence of E2-specific antibodies did not entirely overlap with the presence of neutralizing antibodies. For example, mouse #6 produced highly reactive E2-specific antibodies (Fig. 3B) but no neutralizing antibodies (Fig. 1) and succumbed to viral challenge (Fig. 2). Conversely, mouse #8 produced neutralizing antibodies (Fig. 1) but no E2-specific antibodies (Fig. 3B) and survived viral challenge (Fig. 2). These results suggest that there is no correlation between production of antigen-specific antibodies and survival of mice producing neutralizing antibodies. In line with this supposition, antibodies in the sera of VLP-vaccinated animals were only able to detect E2 protein fractions in some cases, but were not able to recognize any E1 protein fractions (Fig. 3C). We conclude that the presence of a neutralizing antibody response but not an antigen-specific immune response is a valid predictor for protective immunity.

2.4. CHIKV RNA quantification in brain tissue

All mice that succumbed from CHIKV challenge in the groups immunized with Bac-ctrl, sE1 and sE2, had large amounts (10^5–10^6 $TCID_{50}$ equiv./g brain) of CHIKV RNA in their brains (Fig. 4). In contrast, minor amounts of CHIKV RNA were detected in the brains of surviving mice immunized with the subunits (Fig. 4). Infectious virus was only isolated from control mice or mice vaccinated with the sE1 and sE2 subunits (data not shown). Significantly less ($p < 0.05$) viral RNA (3 out of 8 mice) or no viral RNA at all (5 out of 8 mice) was detected in the VLP immunized mice (Fig. 4) and no infectious virus could be recovered upon homogenization of the brain material (data not shown). This implies that the modest neutralizing response induced after subunit immunization was enough to protect the mice from lethal challenge, but does not completely