INTRODUCTION

Rift Valley fever (RVF) is an arthropod-borne zoonotic disease caused by RVF virus (RVFV), a member of the genus Phlebovirus of the family Bunyaviridae (Elliott et al., 2000). RVFV is maintained in the environment in an enzootic vertebrate–mosquito cycle. The Aedes and Culex mosquito genera are its main vectors even though other arthropods may play a role in its spread (Fontenille et al., 1998; Gerdes, 2004).

RVFV causes significant morbidity and mortality among sheep, goat, cattle and humans. Mortality is extremely high in young animals, causing sweeping abortion storms and mortality ratios of approximately 100% among neonatal animals and of 10–20% among adult ruminant livestock (especially sheep and cattle) (Coetzer, 1977, 1982; Easterday, 1965). The disease in humans is generally mild, however, in 1–2% of affected individuals, RVFV infections can progress to more severe disease including fulminant hepatitis, encephalitis, retinitis, blindness or a hemorrhagic syndrome (Al-Hazmi et al., 2002) where it causes recurrent outbreaks of large socio-economic impact. Introduction of RVFV in non-endemic areas such as Europe would have devastating consequences (Mandell & Flick, 2010).

In the face of expansion of the disease, currently, there are no RVFV vaccines approved for general use in humans. Several vaccines have been developed for use in livestock with either a lack of efficacy or generating substantial side effects especially in pregnant animals (Caplen et al., 1985; Smithburn, 1949; Vialat et al., 1997). In the last decade, progress has been made in the development of novel experimental vaccines (Ikegami & Makino, 2009). Since the development of an attenuated vaccine based on the Smithburn strain (Smithburn, 1949), several alternative attenuated vaccines have been studied: (i) clone 13 that carries a natural large deletion in the gene encoding the non-structural NSs protein (Dungu et al., 2010; Vialat et al., 2000); (ii) the mutagenized MP 12 (Morrill et al., 1997; Saluzzo & Smith, 1990); (iii) VLP (virus-like particle)-based vaccines (de Boer et al., 2010; Mandell et al., 2010; Näslund et al., 2009); (iv) nucleocapsid subunit vaccines (Jansen Van Vuren et al., 2010, 2011); and (v) viral vector-based vaccines (Heise et al., 2009; Holman et al., 2009; Kortezaas et al., 2010a, 2010b; Papin et al., 2011; Soi et al., 2010; Wallace & Viljoen, 2005; Wallace et al., 2006).

The medium (M) RNA segment of the RVFV genome encodes the viral glycoproteins Gn and Gc that are the main targets of protective immunity (Collett et al., 1985; Kakach et al., 1988). When expressed by a recombinant
vaccinia virus, they induce neutralizing antibodies and protective immunity to RVF in mice (Collett et al., 1987) and baboons (Papin et al., 2011). Attenuated strains of lumpy skin disease virus (LSDV) and sheep pox virus (SPV), both members of the genus Capripoxvirus have been used effectively as vaccines for many years (Kitching et al., 1987). Moreover, they have also been developed as vectors for multivalent recombinant vaccines against livestock diseases, such as rinderpest, Peste des Petits ruminants and RVF (Berhè et al., 2003; Diallo et al., 2002; Ngichabe et al., 1997, 1999; Romero et al., 1993; 1994; Soi et al., 2010; Wallace & Viljoen, 2005; Wallace et al., 2006).

Several animal models of RVFV infection have been described to better understand the nature of key pathogenesis’ processes applicable to the development and testing of vaccines and therapeutic agents (Ross et al., 2012). RVFV is virulent in a number of species, including rodents, livestock species (sheep, goats and cattle) and non-human primates (NHP), with clinical signs ranging from absent or mild reactions to fever and death in the most susceptible animals. The existing NHP model for RVF utilizes an intravenous exposure route in rhesus macaques (Macaca mulatta). However, severe disease in these animals is infrequent and large cohorts are needed to observe significant morbidity and mortality. The common mara mosets (Callithrix jacchus) are more susceptible to RVFV than rhesus macaques and experienced higher morbidity, mortality, viraemia, and marked aberrations in haematological and biochemical values displaying signs of haemorrhagic manifestations and neurological impairment (Smith et al., 2012). Ethical issues regarding the use of primates and the limitations to their access could, however, be considered as major drawbacks for their use as RVF models. In contrast, hamsters and rats are highly susceptible to RVFV infection and commonly handled in experimental animal facilities (Findlay, 1932; Findlay & Daubney, 1931; Peters & Linthicum, 1994). Nonetheless, a variation in the susceptibility was demonstrated to be linked to a dominant gene in the rat model (Anderson et al., 1987, 1991; Ritter et al., 2000). The hamster model has relied mainly on experimental infection with the Punta Toro bunyavirus (Anderson et al., 1990; Fisher et al., 2003) with only hepatitis and no encephalitis as the dominant pathological feature. In addition, gerbils infected with RVFV reportedly develop uniformly fatal encephalitis in the absence of significant extraneural lesions (Anderson et al., 1988). Mice, prime organisms of choice for modelling human disease are known to be highly susceptible to infection with RVFV by subcutaneous (s.c.) or intraperitoneal (i.p.) injection leading to fulminant hepatitis and a late-developing encephalitis (Findlay & Daubney 1931; Digoutte & Peters, 1989; Mims, 1956). However, an influence of genetic factors could not be demonstrated in a large survey of 34 classical inbred laboratory mouse strains (Peters & Anderson 1981). Recently, the MBT/Pas inbred strain of mice was shown to exhibit an extreme susceptibility to experimental infection with the virulent strain RVFV ZH548. A failure to induce, in due course, a complete innate immune response to RVFV was proposed to be instrumental in their susceptibility to RVF (do Valle et al., 2010). This defect seems to be selective, since MBT/Pas mice were shown to be resistant to infection with either West Nile virus or influenza virus, in conditions where classical inbred mouse strains die (do Valle et al., 2010).

The present investigation aimed at demonstrating that the MBT/Pas mouse model is suitable for the evaluation of the protective ability of vaccine candidates. Two vaccines were tested: the naturally attenuated clone 13 and a recombinant LSDV vectored vaccine candidate, expressing RVFV glycoproteins.

RESULTS

Reproduction of RVF pathology in inbred MBT/Pas mice

To evaluate the susceptibility and pathology induced by RVFV in wild-derived MBT/Pas mice, 6-week-old females were inoculated via the i.p. route with 100 p.f.u. of the virulent strain ZH548, a human isolate from the Egyptian outbreak in 1977–1978 (El-Akkad, 1978). Mortality was monitored daily for 2 weeks. An extreme susceptibility to RVFV was observed. Indeed, all MBT/Pas mice died within 4 days post-infection.

Expression of RVFV glycoproteins and characterization of the rCpoxNSmGn recombinant virus

The expression of RVFV proteins in lamb testis (LT) cells (OA3.Ts) infected with the recombinant rCpoxNSmGn was detected by immunofluorescence assay (IFA) (Fig. 1). A negative control consisted of OA3.Ts cells infected with the wild-type KS-1 strain. Recombinant poxviruses were then generated and selected in the presence of the Escherichia coli xanthine-guanine phosphoribosyltransferase gene (gpt) selection medium containing mycophenolic acid, as described previously (Romero et al., 1993). A PCR assay performed directly on DNA extracted from the viral supernatant and targeting the thymidine kinase (TK) gene (Perrin et al., 2007) failed to detect the KS-1 parental virus, thus confirming the purity of the recombinant viruses.

Survival of inbred MBT/Pas mice conferred by the two vaccines

To evaluate the immunogenicity of the rCpoxNSmGn and clone 13 vaccines and their ability to protect against RVF, two independent inbred MBT/mice experiments were carried out and the survival/mortality rates and the virus replication after virulent RVFV challenge were evaluated. In the first experiment, two groups of 12 mice inoculated at day 0 via the i.p. route with either clone 13 or culture
medium were challenged 31 days later with the virulent ZH548 strain. In each group, four animals were sacrificed at days 28 and 32 and their spleens were removed for analysis of cell-mediated immunity. Therefore, survival after challenge was observed in the four remaining mice. In the negative controls that received the culture medium, three of the four mice died, respectively, at days 3, 4 and 5, whereas all four mice of the clone 13 group were still alive at day 14 post-challenge. RVFV neutralizing antibody titres were measured by the RVFV neutralization test (VNT) assay. High antibody responses to RVFV were detected at day 28 post-immunization with titres higher than 1:80 in all 12 animals inoculated with clone 13.

The second experiment comprised three groups of inbred MBT/Pas mice (12 mice per group), a group inoculated twice 3 weeks apart, with the recombinant rCpoxNSmGn, a negative-control group and a group inoculated with clone 13 as in the first experiment. At days 0 and 21, mice were inoculated with either a suspension of $10^8$ TCID$_{50}$ of the rCpoxNSmGn RVFV recombinant construct or the negative-control recombinant capripox–haemagglutinin of the Peste des Petits ruminants virus (HPPR) vaccine (negative-control group), the suspension being administered via the three routes all together: the intramuscular (i.m.), i.p. and s.c. routes. The third group injected at day 0 intraperitoneally with $10^8$ p.f.u. of clone 13 was used as a positive control. All the animals were challenged at day 32 via the i.p. route with 100 p.f.u. of the RVFV ZH548. In the negative-control group, the four animals died within 5 days post-challenge. RVFV neutralizing antibody titres were measured by the RVFV neutralization test (VNT) assay. High antibody responses to RVFV were detected at day 28 post-immunization with titres higher than 1:80 in all 12 animals inoculated with clone 13.

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**Cell-mediated immunity**

Lymphocyte proliferation was monitored at day 33, 2 days after the challenge in the second experiment. A significant difference was observed for the four animals included in the rCpoxNSmGn RVFV group and for the four animals of the clone 13 group compared with the irrelevant recombinant capripox–HPPR control group (Fig. 2) for both subpopulations CD4 (Fig. 2a) and CD8 (Fig. 2b) (Mann–Whitney U test; $P<0.05$).

**DISCUSSION**

The changes following globalization, climate change and the opening of previously closed ecosystems have considerably modified the pattern of endemic or enzootic infectious diseases and contributed to the emergence of new agents that are pathogenic for humans and domestic animals (Lovejoy, 2008; Morand & Guégan, 2008). In this context, vaccination undoubtedly remains the most cost-effective means of prevention and control by improving health and protecting public health, and even eradicating these infections. A comprehensive review identified 1415 species of infectious organisms known to be pathogenic to humans, including 217 viruses and prions (Cleaveland et al., 2001; Pastoret, 2009). Out of these, 868 (61 %) were classified as zoonotic and 175 pathogenic species were considered to be associated with emerging diseases. Of 175 emerging pathogens of this group, 132 (75 %) were zoonotic (Slingenbergh et al., 2004) including RVFV. Several animal models of RVFV infection have been developed in order to reproduce natural infection in an experimentally controlled system. The wild-derived MBT/Pas inbred strain has already been demonstrated as a suitable model for the study of RVFV pathology (do Valle et al., 2010). The inability of the MBT/Pas cells to limit virus production is the result of several defects in the early and late phases of the interferon (IFN) response. However, MBT/Pas mice are able to produce type I IFNs and to respond to them (do Valle et al., 2010). This is in contrast with IFN (a and b) receptor 1 (Ifnar1$^{-/-}$)-deficient mice that lack responsiveness to type I IFNs (Calvo-Pinilla et al., 2009; Lorenzo et al., 2010; Boshra et al., 2011). The innate immune response defects of MBT/Pas mice result in a failure to control the spread of the fast-growing RVFV in cultured cells, and are likely to contribute to the early death of RVFV-infected MBT/Pas mice. Despite, this selective impairment, MBT/Pas mice are able to elicit an immune response as shown by their resistance to other viruses (do Valle et al., 2010). Therefore, they are a good model to assess the protective immune response to virus infections, in particular against RVFV.

**Fig. 1.** OA3.Ts cells infected with the recombinant RVFV virus rCpoxNSmGn. (a) Negative control, cells infected with the wild-type KS1. (b) Transient expression with the positive control pEGFP-C1 (Clontech). (c) Transient expression with the rCpoxNSmGn 48 h after infection ×10.
Recently, the efficacy and safety of the naturally attenuated RVFV clone 13 vaccine requiring a single injection have been described (Dungu et al., 2010; von Teichman et al., 2011). Development of recombinant vaccines expressing conserved protective antigens and improving their presentation to the immune system in contrast to natural infection would reduce the number of injections required and still provide a cost-effective product. The aim of the present study was to test both clone 13 and RVFV-recombinant capripoxviruses (CPVs) as vaccines. The Gn and Gc viral glycoproteins were selected because of previous studies, with the objective of including the most immunogenic and/or conserved RVFV proteins (Kakach et al., 1988; Collett et al., 1985). Recent studies demonstrated a significant protective immune response with different strains of poxvirus via a cell-mediated mechanism (Wallace & Viljoen, 2005; Wallace et al., 2006; Soi et al., 2010; Papin et al., 2011). The expression of RVFV proteins by the recombinant pox vector was confirmed by IFA using the appropriate antisera. RVFV antibody and cell-mediated immune responses to this recombinant were evaluated in vivo via two animal experiments to evaluate the immunogenicity and protective immunity conferred against a virulent RVFV challenge.

In both animal experiments, the production of RVFV neutralizing antibodies was observed prior to the RVFV challenge in the two groups (clone 13 and rCpoxNSmGn RVFV) compared with the control group receiving the recombinant capripox–HPPR. These results demonstrate the capacity of the inbred MBT/Pas mice to elicit an immune response against the virus and the transgenes expressed by a CPV. These data agree with previous studies showing that the neutralizing humoral response against the RVFV glycoproteins and the cellular immunity induced by the nucleoprotein protect against challenge (Lorenzo et al., 2010; Kortekaas et al., 2010b; Mandell et al., 2010; Jansen van Vuren et al., 2011).

Interestingly, in addition to the production of neutralizing antibodies, a significant and specific increased CD4 and CD8 lymphocyte proliferation against whole inactivated Smithburn RVFV was observed in both vaccinated groups confirming the immunogenicity of the vaccines. This may indicate that the immunity induced by both candidate vaccines (recombinant rCpoxNSmGn RVFV and clone 13) is also cell-mediated and possibly involves CD8 cytotoxic cells.

A protective effect of both vaccines was assessed in the inbred wild-derived MBT/Pas mice. The survival rate varied between 75 and 100% depending on the experiment and the vaccine candidate. The bivalent protection against both RVFV and CPVs challenge renders the vector-based vaccines with a significant advantage, as previously quoted by Soi et al. (2010).

Ideally, it would be interesting to discriminate infected and vaccinated animals (DIVA) through the development of a companion test based on ELISAs using antigens specific to the vaccine or to the wild-type virus. Clone 13 and the rCpoxNSmGn RVFV constructs are convenient candidates to provide a DIVA test since they drive the expression of a few RVFV genes and thus vaccinated animals would lack antibodies to other viral proteins (such as NSs for clone 13 and nucleoprotein for RVFV-Cpox), whereas these antibodies are present in animals recovering from natural infection (Fernandez et al., 2012). One of the potentials of vector-based vaccines such as poxviruses is the ability to include additional immunogenic genes in the construction to protect against diseases occurring in the same

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**Table 1.** RVFV neutralizing antibody responses and protection studies for experiment 2

<table>
<thead>
<tr>
<th>MBT/Pas group</th>
<th>Individual RVFV SN antibody titres at day 0*</th>
<th>Individual RVFV SN antibody titres at day 28*</th>
<th>No. surviving mice/no. challenged mice (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrelevant recombinant capripox–HPPR</td>
<td>&lt;5 (6)†</td>
<td>&lt;5 (6)†</td>
<td>0/4 (0)</td>
</tr>
<tr>
<td>rCpoxNSmGn RVFV</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>3/4 (75)</td>
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<tr>
<td></td>
<td>&lt;5</td>
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<tr>
<td></td>
<td>&lt;5</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Clone 13</td>
<td>&lt;5</td>
<td>160</td>
<td>3/3 (100)</td>
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<tr>
<td></td>
<td>&lt;5</td>
<td>80</td>
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<td></td>
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<td>160</td>
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<tr>
<td></td>
<td>&lt;5</td>
<td>80</td>
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</tbody>
</table>

*Four mice at days 28 and 32 per group were killed for immunological studies.
†Total number of mice.
geographical area. The potential use of Cpox-vectorized recombinant constructs as dual vaccines in hosts other than cattle (for LSDV) is supported by the level of Cpox-neutralizing antibodies obtained in mice. However, challenge experiments are still required in sheep and goats. As the results of these experiments were performed in compliance with French and European regulations on the care and protection of the laboratory animals (accreditation nos B 75 15-01 and B 75 15-07). All protocols included in this study were formally approved by the Institut Pasteur animal welfare veterinarian. Protocols were in compliance with the NIH Animal Welfare Insurance #A5476-01 issued on 02/07/2007.

**METHODS**

**Mice, viruses and cells.** The MBT/Pas inbred strain was derived from the Mus musculus musculus wild progenitors trapped by F. Bonhomme near General Toshevo in Bulgaria in 1980. The colony has been propagated by sib-matings at the Institut Pasteur (Bonhomme & Guenet, 1996). Animals were housed in animal facilities accredited by the French Ministry of Agriculture to perform experiments on live mice, in compliance with French and European regulations on the care and protection of the laboratory animals (accreditation nos B 75 15-01 and B 75 15-07). All protocols included in this study were formally approved by the Institut Pasteur animal welfare veterinarian. Protocols were performed in compliance with the NIH Animal Welfare Insurance #A5476-01 issued on 02/07/2007.

The CPV strain KS1 used for vector construction was derived from a virulent field sheep pox isolate from Kenya (0240 isolate) after two passages in LT cells, two passages in BHK-21 (baby hamster kidney, ATCC #CCL-10) cells and four passages in lamb kidney cells, and has since been shown to be a strain of LSDV used as a live-attenuated vaccine for sheep pox and goat pox in Kenya (Davies, 1976, 1981; Kitching et al., 1987, 1989). CPV was propagated in LT cells (OA3.Ts, ATCC CRL6546) in Dulbecco’s modified Eagle’s medium (DMEM; Gibco) supplemented with 10% FBS as described previously (Perrin et al., 2007).

The RVFV ZH548 strain was isolated from a human case in Egypt (El-Akkad, 1978) and propagated in Vero cells using DMEM (Gibco) containing 10% FBS, 2 mM l-glutamine. Virus-containing medium was harvested when the cytopathic effect (CPE) exceeded 75%, and the viral infectivity titre was determined by limiting dilution (Reed & Muench, 1938).

The Smithburn vaccine was obtained from OBP.

**Generation of the recombinant CPV.** The complete sequence of the M genome segment of RVFV (ZH548 strain) was cloned into plasmid pTVT7R at the sites BbsI (Vialat et al., 1997; Johnson et al., 2000) including the non-structural protein (NSm) and the two glycoproteins (Gn and Gc) (GenBank accession no. Q380200). The Gn contains three neutralizing epitopes inducing protection against a lethal RVFV challenge (Keegan & Collett, 1986; Schmaljohn et al., 1989). Consequently, the NSm/Gn gene was cloned into pKSCATpSGPT, a shuttle plasmid designed from a pBluescript II KS backbone for homologous recombination in the CPV vector (Berhé et al., 2003) under the control of the early/late synthetic promoter (PS) from vaccinia virus (generous gift from Dr Drillien, University of Strasbourg, France). The E. coli xanthine-guanine phosphoribosyltransferase gene (Ecogpt), also under control of the PS promoter, but in the opposite orientation, was used as a dominant selectable marker to isolate the recombinants (Falkner & Moss, 1988; Boyle & Coupar, 1988). The insertion site in the CPV genome was the viral TK gene (Dubbs & Kit, 1964; Boyle et al., 1985; Wallace & Viljoen, 2005). The plasmid pKSCATPSGPT-NSmGn amplified in E. coli Top10 competent cells (Stratagene) was purified under endotoxin-free conditions using the Endofree Plasmid Maxi kit (Qiagen) following the manufacturer’s instructions.

The recombinant pox virus was then generated, as illustrated in Fig. 3, by homologous recombination after transfection of the plasmid pKSCATPSGPT-NSmGn in OA3.Ts infected with KS-1 at an m.o.i. of 0.1 as described previously (Berhé et al., 2003), but using Lipofectamine 2000 (Invitrogen) in place of Lipofectamine. After several rounds of limiting dilution with GPT selection, standard PCR techniques (using primers pairs) were performed to confirm the presence of rCpoxNSmGn and the absence of the wild-type CPV KS-1.

**Vaccination and challenge of mice.** Two sets of experiments were carried out in inbred MBT/Pas mice: the first set aimed at testing the live-attenuated clone 13 as a vaccine, while the second set aimed at testing the recombinant capripox RVF vaccine, rCpoxNSmGn. In
both sets of experiments, the animals were challenged with the virulent strain ZH548.

**Experiment 1** Six-week-old MBT/Pas females (12 mice per group) were inoculated i.p. at day 0 with either a suspension of 100 μl containing either 10^6 p.f.u. of clone 13 or culture medium as a negative control. Blood samples were collected at days 0, 21 and 28. Animals were challenged via the i.p. route with a suspension of 100 μl containing either 10^4 p.f.u. of clone 13 or culture medium as a negative control. A recombinant capripox–HPPR vaccine containing 100 p.f.u. of the ZH548 RVFV virulent strain. Serum for antibody detection was collected immediately prior to immunization and at day 28 post-immunization. The spleens of four mice per group were collected for the analysis of cell-mediated immunity.

**IFA test.** To test the expression of RVFV glycoproteins by the recombinant CPV rCpoxNSmGn, monolayers of OA3.Ts cells were infected and fixed after 48 h with cold acetone for 30 min at −20 °C. Polyclonal antibodies against RVFV glycoproteins were added for 30 min at 37 °C at a dilution 1:400. After three PBS washes (Sigma), cells were incubated with a 1:50 dilution of an anti-mouse FITC (Dako) for 30 min at 37 °C. After three PBS final washes, readings were performed using an inverted epifluorescence microscope (Eclipse TE300; Nikon). Positive and negative controls were included in the test.

**Antigen-specific lymphoproliferation assays in mice.** Spleens were removed from vaccinated and challenged mice at day 33 (2 days post-challenge) and cell suspensions were prepared as follows. Spleens were forced through fine wire mesh and splenic erythrocytes were lysed by osmotic treatment (170 mM Tris-buffered saline, 155 mM ammonium chloride solution), followed by three washes in RPMI 1640 (Gibco-BRL). Cell viability was evaluated by trypan blue dye exclusion, and cells were labelled with Cell Trace Carboxyfluorescein Diacetate Succinimidy Ester (CFSE; Molecular Probes). They were then resuspended in RPMI 1640 supplemented with 10% heat-inactivated FBS (SRH BioSciences), 50 μg gentamicin (Scherling-Plough) ml⁻¹, 2 mM glutamine (Seromed), 1% non-essential amino acids (Seromed), 1 mM sodium pyruvate (Sigma) and 5 × 10⁻⁵ M β-mercaptoethanol (Merck) and seeded in round-bottom microtitre plates. UV-inactivated Smithburn RVFV was added at an equivalent of five particles per cell. Negative and positive controls for lymphoproliferation consisted of RPMI and Concanavalin A (ConA, 5 μg ml⁻¹; Sigma), respectively. Lymphocytes were collected after 4 days of incubation at 37 °C and analysed for proliferation by flow cytometry using a FACSsort and the CellQuest 3.01 software package (Becton Dickinson). Forward (FSC) and side (SSC) scatter dot plots were used to exclude dead cells (lower left events) and to estimate lymphoblastic cells (upper-rightward displacement of events). In order to look at the stimulated lymphocyte populations, cells were further PE-labelled with anti-CD4, anti-CD8 antibodies (cat no. 553049 and 553457 respectively; Becton Dickinson) or with an isotype control (cat no. 553457; Becton Dickinson). Lymphoproliferative subpopulation cells were estimated as events with reduced green fluorescence on an FL1 × FSC dot plot and on an FL2 × FSC.

**RVFV antibody detection by VNT.** Duplicates of twofold serial dilutions of sera in a 100 μl volume starting from 1:5 were added to 100 TCID₅₀ of Smithburn RVFV (in a 50 μl volume) into 96-well microtitre plates and incubated for 1 h at 37 °C. Then 1 × 10⁶ Vero cells were added to each well and the plates were incubated at 37 °C with 5% CO₂ for 5–6 days. Titres were expressed as the inverse highest dilutions giving 50% of CPE. Sera with titres higher than 5 were considered to be positive.

**Statistical analysis.** The non-parametric Mann–Whitney U test was used to analyse differences between different T-cell subsets. A value of P<0.05 was considered statistically significant. The non-parametric Kruskal–Wallis test was used to compare the viral titres obtained between the control and RVFV-Cpox or clone 13 groups with P<0.05 was considered significant.

**ACKNOWLEDGEMENTS**

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