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Recombinant rubella vectors elicit SIV Gag-specific T cell responses with cytotoxic potential in rhesus macaques

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ABSTRACT

Live-attenuated rubella vaccine strain RA27/3 has been demonstrated to be safe and immunogenic in millions of children. The vaccine strain was used to insert SIV *gag* sequences and the resulting rubella vectors were tested in rhesus macaques alone and together with SIV *gag* DNA in different vaccine prime-boost combinations. We previously reported that such rubella vectors induce robust and durable SIV-specific humoral immune responses in macaques. Here, we report that recombinant rubella vectors elicit robust *de novo* SIV-specific cellular immune responses detectable for >10 months even after a single vaccination. The antigen-specific responses induced by the rubella vector include central and effector memory $CD4^+$ and $CD8^+$ T cells with cytotoxic potential. Rubella vectors can be administered repeatedly even after vaccination with the rubella vaccine strain RA27/3. Vaccine regimens including rubella vector and SIV *gag* DNA in different prime-boost combinations resulted in robust long-lasting cellular responses with significant increase of cellular responses upon boost. Rubella vectors provide a potent platform for inducing HIV-specific immunity that can be combined with DNA in a prime-boost regimen to elicit durable cellular immunity.

1. Introduction

Several viral vectors are being pursued as potential vehicles to express HIV antigens including CMV, measles, mumps, rubella, adenovirus, vaccinia, yellow fever, VSV and varicella [1–26]. The live-attenuated rubella vaccine strain RA27/3 has a proven track record for safety and immunogenicity efficacy [27–29] with a single dose shown to induce humoral immunity and life-long protection against rubella infection. Rubella vaccine can also boost previously immunized persons, even in the presence of pre-existing rubella immunity [30]. The generation of rubella vectors carrying HIV genes has the potential to elicit durable immunity. The challenging task is the generation of viable recombinant rubella carrying immunogens of the required length. We previously demonstrated that rubella vaccine strain RA27/3 [31] can accommodate insertions up to ~300 amino acids of SIV Gag at the structural insertion site located between the envelope E2 and E1 [18,21]. Gene expression at this site is controlled by the strong subgenomic promoter, which assures efficient expression of the insert.

Using recombinant rubella vectors in macaques, we reported the induction of robust SIV/HIV-specific humoral responses [21], which could be boosted upon re-exposure to the vector, indicating the

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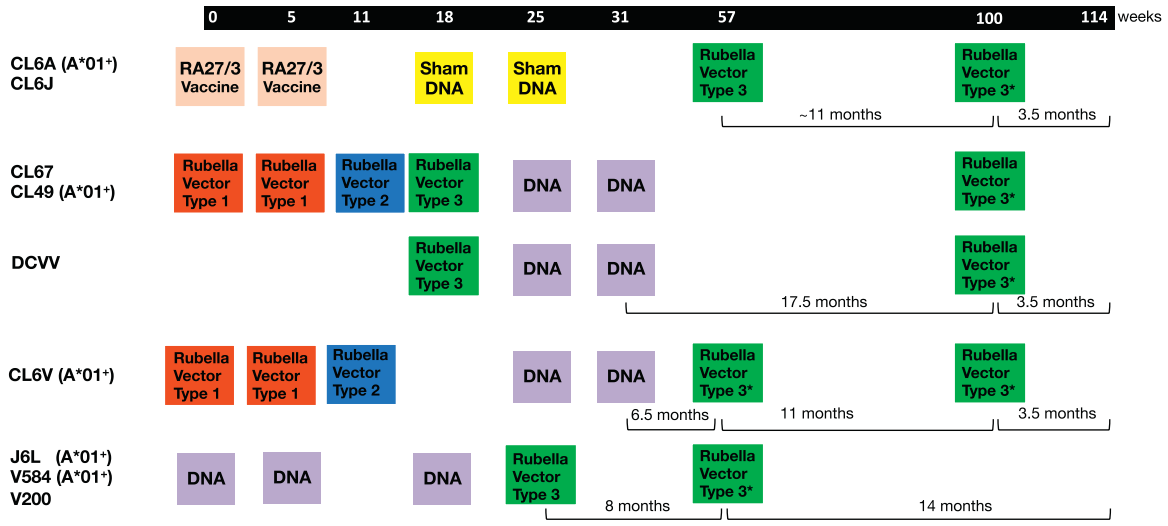

Fig. 1. *Vaccination protocols.* The cartoon is an adaptation from Virnik et al. [19] and details the vaccination timeline used for this Indian rhesus macaque study. Animals CL6A and CL6J had received RA27/3 rubella vaccine strains and sham DNA. Macaques CL67, CL49, CL6V had received rubella types 1 and 2 which were "no takes" and did not induce antibodies to rubella and also did not induce Gag-specific T cell immunity. The animals received sequential vaccinations with DNA and rubella vector type 3 or type 3*, referred to as rubella vector herein, expressing SIV Gag. The animals were analyzed for Gag-specific IFN-γ T cell responses, and macaques positive for the MamuA*01 haplotype were also analyzed for Gag CM9 tetramer responses.

development of memory B cells. Different prime-boost combinations including recombinant rubella vectors and DNA elicited even higher antibody responses with extended longevity (>9 months), indicating that the rubella-induced responses could be further augmented.

DNA as vaccine platform has several advantages related to its simplicity, scalability, and possibility of repeated applications due to lack of immunity against the vector (reviewed in [32]). HIV/SIV DNA vaccines administered by the intramuscular route (IM) followed by *in vivo* electroporation (EP) were shown to induce high cellular and humoral immune responses that persisted for >5 years after the last vaccination [33–35]. The potency of these cellular immune responses was demonstrated by their ability to greatly reduce viremia in macaques infected with pathogenic SIV or SHIV, in both preventive and therapeutic vaccine studies [32]. Humoral responses could be significantly augmented by combining DNA vaccine with different boosts (protein, recombinant viral vectors) [32].

In this report, we examined the Gag-specific cellular immune responses from macaques vaccinated with rubella vectors or with DNA and rubella vectors in different prime-boost combinations. We focused on Gag as antigen, because Gag-specific T cell responses were reported to correlate with control of viremia in infected individuals [36–39] and such responses are expected to reduce viremia in both preventive and therapeutic vaccination protocols.

2. Methods

2.1. Cellular immune response analysis in vaccinated macaques

Macaques were sequentially vaccinated *via* the IM route [19] with rubella vectors expressing four T cell epitopes (GY9, TE15, CM9 and ME11; vector type 3) or $p27^{gag}$ and part of $p2^{gag}$ (amino acids 135–391 of Gag; vector type 3*) of SIVmac239 Gag [19] and SIV gag DNAs [40].

Gag-specific cellular immune responses were measured at the day of vaccination and indicated time points thereafter using a Gag peptide pool [33] and $Gag_{181-189}$ CM9 tetramer [41] in MamuA*01^+ macaques as detailed in Supplementary methods.

3. Results

3.1. Sequential vaccination regimens using recombinant rubella vectors and DNA

We tested the ability of recombinant rubella vectors to induce Gag-specific cellular immune responses in macaques. Fig. 1 shows the vaccination regimens, which included rubella vectors and DNA expressing SIV *gag* in different prime-boost regimens. Rubella vector type 3 and type 3* differ by the size of the *gag* insert, comprising 4 epitopes located in the $p19^{gag}$ and $p27^{gag}$ regions (type 3) or the complete $p27^{gag}$ and $p2^{gag}$ (type 3*), and both elicited antibodies against Rubella and SIV Gag in vaccinated macaques [19]. Some of the macaques were recycled from a previous study where they received the rubella vaccine strain RA27/3 (rubella vaccine) or rubella vectors types 1 and 2 which did not replicate *in vivo* or show a vaccine "take" and did not develop humoral [19] or cellular immunity to Gag (not shown).

This report focuses on the induction and characterization of cellular immune responses addressing the following questions: (i) Can rubella vector vaccination induce *de novo* Gag-specific cellular immune responses (5 animals: CL6A, CL6J, DCVV, CL67, CL49)? (ii) Can DNA vaccination boost pre-existing rubella vector induced responses (3 animals: CL67, CL49, DCVV)? (iii) Can rubella vector vaccination boost pre-existing DNA induced responses (7 animals: CL67, CL49, DCVV, CL6V, J6L, V584, V200)? (iv) Can a 2nd rubella vector vaccination boost responses after rubella vector priming (all 9 animals: CL6A, CL6J, CL67, CL49, DCVV, CL6V, J6L, V584, V200)?

3.2. Vaccination with rubella vectors induces Gag-specific T cell responses in macaques

Two macaques (CL6A, CL6J), previously vaccinated with the live-attenuated rubella vaccine strain RA27/3, were immunized one year later, when the anti-rubella antibody titer had slightly declined, with the rubella vector expressing SIV Gag. A single vaccination with rubella vector was able to induce Gag-specific cellular responses in both macaques, reaching up to ~0.2% IFN-γ^+ T cells (Fig. 2A). In CL6A, the responses were mediated by similar levels of $CD4^+$ and $CD8^+$ T cells, while CL6J showed a primarily $CD4^+$ T cell