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A single, low dose of a cGMP recombinant BCG vaccine elicits protective T cell immunity against the human respiratory syncytial virus infection and prevents lung pathology in mice

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ABSTRACT

Human respiratory syncytial virus (hRSV) is a major health burden worldwide, causing the majority of hospitalizations in children under two years old due to bronchiolitis and pneumonia. HRSV causes year-to-year outbreaks of disease, which also affects the elderly and immunocompromised adults. Furthermore, both hRSV morbidity and epidemics are explained by a consistently high rate of re-infections that take place throughout the patient life. Although significant efforts have been invested worldwide, currently there are no licensed vaccines to prevent hRSV infection. Here, we describe that a recombinant Bacillus Calmette-Guerin (BCG) vaccine expressing the nucleoprotein (N) of hRSV formulated under current good manufacture practices (cGMP rBCG-N-hRSV) confers protective immunity to the virus in mice. Our results show that a single dose of the GMP rBCG-N-hRSV vaccine retains its capacity to protect mice against a challenge with a disease-causing infection of 1×10^7 plaque-forming units (PFUs) of the hRSV A2 clinical strain 13018-8. Compared to unimmunized infected controls, vaccinated mice displayed reduced weight loss and less infiltration of neutrophils within the airways, as well as reduced viral loads in bronchoalveolar lavages, parameters that are characteristic of hRSV infection in mice. Also, *ex vivo* re-stimulation of splenic T cells at 28 days post-immunization activated a repertoire of T cells secreting IFN-γ and IL-17, which further suggest that the rBCG-N-hRSV vaccine induced a mixed, $CD8^+$ and $CD4^+$ T cell response capable of both restraining viral spread and preventing damage of the lungs. All these features support the notion that rBCG-N-hRSV is a promising candidate vaccine to be used in humans to prevent the disease caused by hRSV in the susceptible population.

1. Introduction

For more than fifty years, the human respiratory syncytial virus (hRSV) has been recognized as the single most important cause of infant hospitalizations due to acute lower respiratory tract infections, and also an important pathogen in the elderly and the immunocompromised individuals [1–5]. As such, hRSV has a worldwide impact in the public health and the economy of both developing and developed countries [6–8]. Furthermore, despite significant resources have been invested in researching hRSV biology, there is still no licensed vaccine for the prophylaxis of hRSV disease in children and other susceptible population [9].

HRSV circulates in the community establishing seasonal outbreaks of disease, which are based on repetitive events of infections that can occur even during the same outbreak [1,2]. Reinfections are thought to derive from the poor induction of adaptive immunological memory after the resolution of naturally acquired infections. Indeed, the identification of a scarce population of circulating B cells capable of secreting neutralizing

Abbreviations: BAL, bronchoalveolar lavage; BCG, Bacillus Calmette et Guerin; CFUs, colony-forming units; cGMP, current Good Manufacturing Practices; hRSV, human respiratory syncytial virus; FI-hRSV, formalin-inactivated hRSV; HI-hRSV, heat-inactivated hRSV; FACS, fluorescence activated cell sorting.

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antibodies against the virus [10], as well as the demonstration that adults remain susceptible to few infectious hRSV particles [11], support the notion of a limited acquired immunity to hRSV infections throughout lifetime. Moreover, this is further supported by independent studies demonstrating the deleterious effects of hRSV infection over the capacity of DCs to activate and expand naïve T cells [12,13]. Furthermore, we have recently shown that compared to memory cells, naïve $CD4^+$ T cells have an increased susceptibility to the inhibition delivered by the hRSV nucleoprotein [14], suggesting that (1) this mechanism may be instrumental for impairing acquired immunity to the virus, and (2) in order to promote strong antiviral immunity, a good vaccine candidate must elicit specific memory T cell responses able to overcome the restrictions imposed by hRSV over naïve T cell activation.

Memory $CD4^+$ T cells are instrumental in the host defense against invading viruses [15]. Activated memory cells differentiate into helper T cells (T_H) that among others, exert direct perforin-mediated cytotoxicity and promote the activation of other key immune cells required for virus clearance, including DCs, macrophages, $CD8^+$ CTLs, and B cells [15]. In the context of hRSV natural infections, it is known that human lungs display aberrant T cell responses described as two possible scenarios: a poor infiltration of $CD8^+$ CTLs [16], or conversely, by a more marked infiltration of $CD4^+$ T cells [17]. In mice, hRSV infection elicits a T_H2 immune response, which is poor antiviral and highly pro-inflammatory. Cytokines secreted by T_H2 cells mediate the recruitment of a variety of inflammatory cells, including neutrophils, monocytes, $CD11b^+$-inflammatory DCs, plasmacytoid DCs and eosinophils into the bronchoalveolar spaces of humans and mice (known as bronchopneumonia), and into the tissue interstitium generating thickening of the alveolar walls (known as interstitial pneumonia) [18–21].

The need of a balanced T-cell response to hRSV has been further stressed by the study of the formalin-inactivated hRSV (FI-hRSV)-vaccine-enhanced disease (VED) first reported in children [22,23]. The FI-hRSV-VED was successfully replicated in the mouse model of infection, yielding compelling evidence of the role of suboptimal $CD4^+$ T-cell responses in hRSV pathogenesis [24]. FI-hRSV immunization elicits a T_H2 response that mediates lung damage through the massive recruitment of eosinophils and neutrophils into infected lungs [25]. Importantly, several reports indicate that hRSV-specific $CD8^+$ T cells successfully prevent FI-hRSV-VED, supporting the notion that $CD8^+$ T cells are both important regulators of hRSV pulmonary pathology, and attractive elements for rationale vaccine design [26,27]. Along these lines, we recently demonstrated that a recombinant BCG expressing the hRSV nucleoprotein (N) (herein rBCG-N-hRSV) elicits IFN-γ-secreting $CD4^+$ (T_H1) T cells and $CD8^+$ CTLs that promoted viral clearance and prevented lung damage in vaccinated mice [12,28]. Hence, our results demonstrated that induction of memory T cell responses by rBCG-N-hRSV immunization was an efficient strategy to prevent hRSV lower respiratory tract infections [12,28]. Nevertheless, that initial approximation used extremely high vaccination doses (10^8 CFUs of rBCG-N-hRSV), raising concerns regarding its immunogenicity in infants, which are immunized with BCG at significant lower doses ($1–4 \times 10^5$ CFUs). Here, in order to move forward into the characterization of this promising vaccine for clinical use, we studied the safety and immunogenicity of a low dose of rBCG-N-hRSV formulated under current Good Manufacture Practices (cGMP) standards. Using the mouse model of infection, we observed that a single, human dose of $1–4 \times 10^5$ CFUs of cGMP rBCG-N-hRSV elicited an acquired immunity able to prevent lung damage, while promoting a significant clearance of infectious viral particles from the airways. We also observed that the immunization with rBCG-N-hRSV prevented the CNS alterations caused by hRSV described previously [29]. Moreover, our results suggest that the transfer of as few as 1.5×10^6 hRSV-specific T cells, are sufficient to restrain viral dissemination in the lungs and to protect recipient mice from hRSV respiratory disease. Therefore, the rBCG-N-hRSV vaccine prototype appears as a safe and immunogenic tool for the prophylaxis of hRSV in susceptible individuals.

2. Material and methods

2.1. Mice

BALB/cJ mice were purchased from The Jackson Laboratories (Bar Harbor, ME) and maintained under SPF conditions at the Pontificia Universidad Católica de Chile animal facility. Mice were used according to the Guide for the Care and Use of Laboratory Animals (Eighth edition, 2011).

2.2. Virus preparation & titration

The hRSV serogroup A2 strain 13018-8 (obtained from the Public Health Institute of Chile) was propagated and titrated over HEp-2 cell monolayers (ATCC-CCL-23) as previously described [14]. Heat-inactivation of hRSV (56 °C/30 min) was performed in a thermo block (Thomas Scientific). Supernatants of uninfected HEp-2 cells were prepared in parallel to virus stocks and used in all experiments as mock controls.

2.3. Current good manufacturing practices (cGMPs) BCG strains

The rBCG-N-hRSV (Danish 1331 strain) was produced following cGMP standards at the AERAS Global TB Vaccine Foundation (Rockville, MD USA). As negative control for hRSV immunizations, a non-recombinant, cGMP quality, wild type Danish 1331 BCG strain (herein BCG-WT) was used (Statens Serum Institute, Copenhagen, Denmark).

2.4. Mice immunization and challenge

Six to eight weeks old BALB/cJ mice were immunized by a sub dermal (s.d.) injection of 3×10^5 colony-forming units (CFUs) of wild type BCG (BCG-WT) or rBCG-N-hRSV [28] (no vaccine boost was performed). Twenty-one days after immunization mice were infected intranasally with 1×10^7 PFUs of hRSV A2 clinical strain 13018-8 [28]. To evaluate possible side effects of rBCG-N-hRSV immunization we recorded clinical parameters daily (mouse body weights, injection site appearance) (Fig. 1/Table 1). To evaluate long-term protection, a group of mice was vaccinated as explained above and challenged at day 50th post-immunization with the same strain of hRSV. Following infection, mouse body weights were recorded daily until the end of the experiment at day 7th post-infection.

2.5. Ex vivo T cell stimulation

Twenty-eight days after immunization, spleens from rBCG-N-hRSV and BCG-WT-immunized mice were collected, disaggregated in sterile RPMI 1640 media using a 70-μm cell-strainer, treated for Red Blood Cells (RBC) lysis, and resuspended in fresh, supplemented RPMI 1640 media (10 mM HEPES, 10% FBS, 50 μM 2-mercaptoethanol). Immediately after collection, T cells were incubated for 72 h in the presence of recombinant hRSV nucleoprotein (N; 10 μg/mL), heat-inactivated hRSV (HI-hRSV, at a final multiplicity-of-infection (MOI) of 5 plaque-forming units (PFUs)/-cell), or irrelevant antigens (human metapneumovirus, hMPV, at MOI 5 and mock). Heat inactivation of virions produces the physical disruption of the viral particle and the viral protein denatura-

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