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Molecular adjuvant IL-33 enhances the potency of a DNA vaccine in a lethal challenge model

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ABSTRACT

Identifying new molecular adjuvants that elicit effective vaccine-induced $CD8^+$ T cell immunity may be critical for the elimination of many challenging diseases including Tuberculosis, HIV and cancer. Here, we report that co-administration of molecular adjuvant IL-33 during vaccination enhanced the magnitude and function of antigen (Ag)-specific $CD8^+$ T cells against a model Ag, LCMV NP target protein. These enhanced responses were characterized by higher frequencies of Ag-specific, polyfunctional $CD8^+$ T cells exhibiting cytotoxic characteristics. Importantly, these cells were capable of robust expansion upon Ag-specific restimulation *in vivo* and conferred remarkable protection against a high dose lethal LCMV challenge. In addition, we demonstrate the ability of IL-33 to amplifying the frequency of Ag-specific $KLRG1^+$ effector $CD8^+$ T cells. These data show that IL-33 is a promising immunoadjuvant at improving T cell immunity in a vaccine setting and suggest further development and understanding of this molecular adjuvant for strategies against many obstinate infectious diseases and cancer.

1. Introduction

There is still a great need for effective vaccines against many chronic infectious including HIV, HCV, Tuberculosis and malaria. For these pathogens, it is known that T cell-mediated immunity is critical in either controlling, preventing or delaying the onset of disease [1]. Thus, a crucial step in vaccine development for these infections requires producing cytotoxic T_H1 versus humoral T_H2 responses. Currently, licensed vaccines such as inactivated and recombinant protein or non-live vaccines predominately drive humoral immune responses [2]. An overall shortcoming of these vaccines, especially non-live vaccines, is their inability to generate both effective T_H1 and $CD8^+$ T cell immunity, thus hindering their beneficial role in limiting or preventing diseases that require adaptive cellular immune responses [2,3]. One way to improve the quality of immune responses during vaccination is to incorporate immunoadjuvants, which have been shown to help increase their T_H1 immune potency [2]. However, it has been a challenge to discover immunoadjuvants that can amplify the induction of $CD8^+$ T cell responses. Notably, IL-33 has emerged as a proinflammtory cytokine capable of promoting both potent T_H1 and cytotoxic $CD8^+$ T cell immunity [4–6]. Therefore, IL-33 has great potential to act as a potent molecular adjuvant in vaccines designed to boost $CD8^+$ T cell immune responses.

IL-33 is a member of the IL-1 cytokine family, which is released by necrotic cells or activated innate immune cells during trauma or infection [6,7]. Therefore, it is considered to serve as the first line of defense against pathogens, by providing an endogenous "danger signal" that triggers inflammation and promotes cell-mediated immune response. Originally studied in the context of T_H2 immunity associated with inflammatory disorders [6,7], evidence has begun to unveil IL-33's unappreciated ability to induce T_H1 and $CD8^+$ T cell-mediated immunity [4–6]. We have recently reported that IL-33 can act as novel immunoadjuvant to induce both potent T_H1 and effective $CD8^+$ T cell responses in an anti-tumor DNA vaccine [5]. Here we expanded the scope of these initial studies to evaluate the capacity of IL-33 to serve as a vaccine adjuvant to enhance and modulate cell-mediated responses against various models of infection that require $CD8^+$ T responses.

In the present study, we use the well-studied lymphocytic choriomeningitis virus (LCMV) model to investigate IL-33's ability to facilitate the induction of antiviral and protective immunity and further elucidate its biological function on memory $CD8^+$ T cell expansion and differentiation in a vaccine setting. We

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hypothesize that IL-33 would have the capacity to improve the efficacy of DNA vaccines against a viral challenge, providing optimal effector function and protection. Here we show that the administration of IL-33 coadministered with a DNA vaccine against LCMV induces robust antigen-specific IFN-γ responses, enhances antigen (Ag)-specific polyfunctional $CD8^+$ T cell immune responses, increases the cytotoxic phenotype of the $CD8^+$ T cells, and provides substantial protective immunity against a high-dose lethal LCMV challenge. We also hypothesized that inclusion of IL-33 can significantly amplify and expand the Ag-specific effector memory $CD8^+$ T cell responses. Furthermore, we provide evidence of IL-33's ability to also enhance cell-mediated immune responses when co-delivered with an HIV DNA vaccine. These findings significantly highlight the important role of IL-33 as a potential future vaccine adjuvant with applicability in the treatment of a variety of chronic viral diseases that require potent T_H1-type immunity for their prevention or control.

2. Results

2.1. IL-33 elicits protection against a lethal LCMV challenge

The LCMV infection model has been extensively used to understand the role of virus-specific $CD8^+$ T cell responses in the context of vaccine-elicited protection [8–10]. Considering our recent novel finding that IL-33 can act as an immunoadjuvant to induce both anti-viral and anti-tumor $CD8^+$ T cell immunity [5], we used the intracranial (i.c.) LCMV challenge model to further study the protective efficacy of IL-33. Three groups of C57BL/6 mice (B6) ($n = 10$) were vaccinated by electroporation (EP) one time with 10 μg of pLCMV-NP (NP) construct with or without 11 μg of mature IL-33 (mtrIL-33) construct. The empty vector pVAX was used as a negative control. The LCMV NP structural protein is recognized as a critical component and target for protective LCMV immunity since it is not a target for neutralizing antibodies [11]. All animals were challenged 21 days post-vaccination (dpv) with a lethal $20 \times LD_{50}$ dose of LCMV Armstrong (Fig. 1A) [10–13]. Vaccinated animals with NP plus mtrIL-33 showed complete protection while the NP alone group achieved only 60% protection (Fig. 1B). On the other hand, all control pVAX vaccinated animals succumbed to infection. After showing that mice immunized using mtrIL-33 as an adjuvant exhibited 100% survival rate, we next sought to determine whether vaccinated mice with adjuvant could confer protection against an even higher lethal dose of LCMV challenge. Therefore, mice where challenged with a $40 \times LD_{50}$ dose of LCMV Armstrong [11], 21 days post-single vaccination (Fig. 1C). Notably, animals receiving one immunization of NP plus mtrIL-33 yielded a significant 80% protection, while the NP alone group only conferred 10% protection against this highly lethal dose of LCMV (Fig. 1C). These data show that IL-33 elicits protection against a lethal LCMV challenge.

2.2. IL-33 significantly increases LCMV-specific $CD8^+$ T cell responses

Considering that $CD8^+$ T cell responses are essential for facilitating control against LCMV [8–13], we hypothesized that the IL-33 adjuvanted vaccine induced $CD8^+$ T cells mediated antiviral protection. Thus, to better characterize the protective immune correlates driven by mtrIL-33, groups of mice ($n = 4$–5) were immunized once with NP either with or without mtrIL-33. The magnitude of NP-specific immune responses was measured 21 dpv in response to peptide re-stimulation (2.5 μg/ml) using the immunodominant epitope in the H-2^b background: $D^bNP_{396\text{-}40}$ (NP396) [11]. Compared to NP alone-vaccinated mice, we found that co-immunization with mtrIL-33-encoding plasmid elicited stronger NP-specific T cell responses by greater than 2.5 fold (Fig. 2A); IFN-γ ELISpot counts were ~2500 spot-forming cells [SFCs] per 10^6 splenocytes in the IL-33 vaccinated mice versus ~980 SFC/10^6 spenocytes for the NP alone group. Next, we assessed the phenotypic and functional profile of vaccine-induced $CD8^+$ T cells in response to NP396 peptide re-stimulation (2.5 μg/ml final peptide concentration). The gating strategy for intracellular cytokine flow cytometry analysis is depicted in Supplementary Fig. 1. Twenty-one days after vaccination there was a significant difference among vaccine groups in the frequency of $CD8^+$ T cells producing effector cytokines (Fig. 2B and C). The NP vaccine coadministered with mtrIL-33 elicited a higher percentage of Ag-specific $CD8^+$ T cells producing all three cytokines (Fig. 2B), and a significant number of the $CD8^+$ T cells were polyfunctional (Fig. 2C). Compared with the NP alone vaccinated group, the NP + mtrIL-33 vaccinated group elicited substantially higher frequencies of NP-specific $CD8^+$ T cells producing either IFN-γ alone (NP, 1.3%; NP + mtrIL-33, 2.3%), dual IFN-γ^+TNF-α^+ (NP, 0.76%; NP + mtrIL-33, 1.63%), or triple-positive IFN-γ^+TNF-α^+IL-2^+ (NP, 0.20%; NP + mtrIL-33, 0.43%) in the spleens 21 dpv (Fig. 2C). Collectively, the enhanced Ag-specific $CD8^+$ T cell response induced by IL-33 is indicative of IL-33's ability to provide substantial protection against LCMV challenge. We next characterized the cytotoxic potential of vaccine-induced $CD8^+$ T cells. $CD8^+$ T cells isolated from mice vaccinated with IL-33 showed a significantly higher frequency of antigen-specific (IFN-γ^+CD107a^+: 2.5%) degranulation compared to NP alone-vaccinated mice (IFN-γ^+CD107a^+: 1.2%) (Fig. 2D). We next evaluated the proliferative capacity of the $CD8^+$ T cells by monitoring Cell Trace Violet dilution in splenocytes isolated from mice 21 dpv rechallenged *in vitro* with NP396 peptide re-stimulation. Fig. 2E shows that mtrIL-33 vaccinated mice underwent significantly higher Ag-specific proliferation of $CD8^+$ T cells, being ~2 fold greater than NP control group. Notably, there was an enrichment of effector memory $CD8^+$ T cells ($CD44^+CD62L^-$) in the adjuvant-vaccinated group (Fig. 2F). There was no significant difference in the central memory $CD8^+$ T cell ($CD44^+CD62L^+$) population (data not shown). Taken together, the inclusion of IL-33 elicits robust levels of NP-specific T cell immunity, especially enhancing $CD8^+$ T cell immune responses.

To better understand the biological function of IL-33 on the induction of Ag-specific $CD8^+$ T cells during the course of vaccination, we further characterized IL-33's ability to expand the Ag-specific effector memory $CD8^+$ T cell population. To achieve this goal, we took advantage of the well studied $D^bGP_{33\text{-}41}$ MHC class I tetramer to follow Ag-specific $CD8^+$ T cells as they develop after initial priming. Mice were vaccinated once with a LCMV glycoprotein LCMV-GP (GP) DNA vaccine and the frequency of D^bGP_{33}-specific $CD8^+$ T cells was monitored in the peripheral blood during the course of vaccination either with or without mtrIL-33 (Fig. 4A). Delivery of IL-33 expanded the number of D^bGP_{33} tetramer-specific $CD8^+$ T cells in the peripheral blood (Fig. 3A). In peripheral blood lymphocytes (PBLs), the frequency of GP33-specific $CD8^+$ T cell was significantly 2-fold higher at 18 and 21 dpv compared with the nonadjuvanted group (Fig. 3A). Similarly, the inclusion of IL-33 also increased the number of GP33-specific $CD8^+$ T cells in the spleen 21 dpv (Fig. 3B) and the Ag-specific $CD8^+$ T cells secreting IFN-γ, undergoing degranulation, and expressing the transcription factor T-bet (Fig. 3C–F). Additionally, all mice were boosted with the GP construct alone (21 days after initial immunization) to quantify the Ag-specific recall responses. Compared to control group, the IL-33 vaccinated group significantly increased the Ag-specific $CD8^+$ T cells. Notably, the IL-33 immunized group GP33 tetramer-specific T cells were ~3-fold higher starting 3 days post-boost vaccination (d24) compared to the NP-vaccinated group (Fig. 3A). The significant difference in the amplification of the GP33-specific $CD8^+$ T cells was still observed 10 days after DNA boost (d31). Consistent with Fig. 2, the

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