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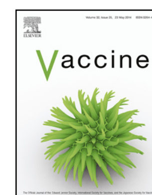

Fc receptor-targeting of immunogen as a strategy for enhanced antigen loading, vaccination, and protection using intranasally administered antigen-pulsed dendritic cells

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ABSTRACT

Dendritic cells (DCs) play a critical role in the generation of adaptive immunity *via* the efficient capture, processing, and presentation of antigen (Ag) to naïve T cells. Administration of Ag-pulsed DCs is also an effective strategy for enhancing immunity to tumors and infectious disease organisms. Studies have also demonstrated that targeting Ags to Fcγ receptors (FcγR) on Ag presenting cells can enhance humoral and cellular immunity *in vitro* and *in vivo*. Specifically, our studies using a *Francisella tularensis* (*Ft*) infectious disease vaccine model have demonstrated that targeting immunogens to FcγR *via* intranasal (i.n.) administration of monoclonal antibody (mAb)-inactivated *Ft* (i*Ft*) immune complexes (ICs) enhances protection against *Ft* challenge. *Ft* is the causative agent of tularemia, a debilitating disease of humans and other mammals and a category A biothreat agent for which there is no approved vaccine. Therefore, using i*Ft* Ag as a model immunogen, we sought to determine if *ex vivo* targeting of i*Ft* to FcγR on DCs would enhance the potency of i.n. administered i*Ft*-pulsed DCs. In this study, bone marrow-derived DCs (BMDCs) were pulsed *ex vivo* with i*Ft* or mAb-i*Ft* ICs. Intranasal administration of mAb-i*Ft*-pulsed BMDCs enhanced humoral and cellular immune responses, as well as protection against *Ft* live vaccine strain (LVS) challenge. Increased protection correlated with increased i*Ft* loading on the BMDC surface as a consequence of FcγR-targeting. However, the inhibitory FcγRIIB had no impact on this enhancement. In conclusion, targeting Ag *ex vivo* to FcγR on DCs provides a method for enhanced Ag loading of DCs *ex vivo*, thereby reducing the amount of Ag required, while also avoiding the inhibitory impact of FcγRIIB. Thus, this represents a simple and less invasive strategy for increasing the potency of *ex vivo*-pulsed DC vaccines against chronic infectious diseases and cancer.

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1. Introduction

Dendritic cells (DCs) play a central role in generating immunity to infection [1]. Specifically, DCs are highly efficient at taking up, processing, and presenting antigens (Ags) to naïve T cells [1]. This has lead to studies focused on DC-based vaccines against cancer and infectious diseases including HIV-1 and influenza [2–6]. Furthermore, numerous studies, including our own, have demonstrated that targeting Ag to Fcγ receptors (FcγRs) on Ag presenting cells (APCs), can enhance humoral and cellular immunity and protection against infectious diseases and cancer [6–12]. Consistent with these observations, engagement of FcγRs can induce DC maturation, a key event required for Ag processing and presentation to T cells [13,14]. In this regard, studies from our laboratory have demonstrated that inactivated *Francisella tularensis* (i*Ft*) Ag can induce enhanced protection against *Ft* challenge, when targeted to FcγRs as mAb-i*Ft* complexes (ICs) administered intranasally (i.n.). The latter involves enhanced Ag binding to DCs, DC maturation, Ag processing/presentation by DCs, and i*Ft* trafficking to lymphoid tissues [7,12,14]. Thus, we hypothesized that the ability of *ex vivo* Ag-pulsed DCs to induce immunity/protection could be significantly enhanced by pulsing DCs with FcγR-targeted Ag administered i.n. Importantly, while parenteral immunization generally utilizes needle injection and is not optimal for stimulating mucosal immunity, i.n. immunization is less invasive and stimulates strong parenteral

Abbreviations: DCs, dendritic cells; BMDCs, bone marrow-derived dendritic cells; mAb, monoclonal antibody; *Ft*, *F. tularensis*; i*Ft*, inactivated *Ft*; IC, immune complex; Ag, antigen; Ab, antibody; FcγR, Fcγ receptors; APCs, Ag presenting cells; i.n., intranasal; BALF, bronchial alveolar lavage fluid; MLN, mediastinal lymph node.

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and mucosal immune responses [15]. Furthermore, recent studies have demonstrated Ag-containing DCs administered i.n., like Ag-containing peripheral DCs, migrate to lymphoid tissues [16,17].

In this report, we demonstrate that immunization of mice i.n. with *ex vivo* FcγR-targeted Ag (mAb-i*Ft*)-pulsed bone marrow-derived DCs (BMDCs) enhances humoral and cellular immune responses and protection against *Ft* challenge. Furthermore, despite the presence of the inhibitory FcγRIIB on DCs [18], protection is not impacted by FcγRIIB. Thus, these studies identify a vaccine strategy, which can be used to increase the potency of DC-based vaccines, as well as simultaneously induce mucosal and peripheral immunity, while bypassing the inhibitory impact of FcγRIIB.

2. Materials and methods

2.1. Cells and reagents

RPMI 1640 medium (CellGro, Manassas, VA) supplemented with 10% FCS (HyClone, Logan, UT), 2 mM L-glutamine, 1 mM sodium pyruvate, 1% MEM nonessential amino acids (CellGro), 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco Life Technologies, Carlsbad, CA), and 50 μM 2-ME (Bio-Rad, Hercules, CA) was used to generate BMDCs. The *Ft*-specific T cell hybridoma (FT256D10) is specific for an *Ft* ribosomal protein-derived peptide, and was provided by Dr. Jeffrey Frelinger (University of North Carolina at Chapel Hill). The T cell hybridoma was maintained in RPMI 1640 medium containing 500 μg/ml of hygromycin B (CellGro). The mouse IgG2a anti-*Ft* LPS mAb used to make mAb-i*Ft* ICs was purchased from Fitzgerald (cat #10-F02, clone M0232621, Acton, MA). Mouse recombinant Flt3 ligand (Flt3L) was obtained from R&D systems (Minneapolis, MN). Abs for flow cytometry: anti-mouse CD11c, CD11b, CD8a, B220, CD3, CD4, MHC class II (I-A/I-E), CD40, CD83, CD80, CD86, DEC205, or IFN-γ, as well as isotype control Abs, were purchased from eBioscience (San Diego, CA). Luminex Bio-Plex assay kits were purchased from Bio-Rad (Hercules, CA).

2.2. Mice

C57BL/6 and FcγRIIB knockout (KO) mice on a C57BL/6 background were purchased from Taconic Laboratories (Hudson, NY). Mice (6–8 weeks of age) were housed in the Animal Resources Facility at Albany Medical College and provided with *ad libitum* water and food during the course of each experiment. Animal studies were reviewed and approved by the Institutional Animal Care and Use Committee utilizing NIH standards.

2.3. i*Ft* (Ag) and mAb-i*Ft* ICs

Inactivated *Ft* LVS (i*Ft*) (immunogen) was prepared as previously described using endotoxin-free PBS [7,14,19]. The mAb-i*Ft* ICs were generated by adding i*Ft* to DCs in tissue culture medium, followed by mAb to a final concentration of 1 μg/ml. Unless otherwise indicated in the figure legend, i*Ft* was used at a ratio of 10 i*Ft*/cell. In all experiments comparing i*Ft* *versus* mAb-i*Ft*, i*Ft* amounts were equalized between i*Ft* and mAb-i*Ft* preparations.

2.4. BMDCs

BMDCs were generated from mouse BM precursor as previously described [14,20]. BMDC phenotype was verified by flow cytometry (>93% CD11c+, high CD11b and B220, very low CD8a).

2.5. Immunization and challenge

BMDCs (3×10^6 cells/ml) in RPMI 1640 medium were pulsed with medium, i*Ft*, or i*Ft* plus 1 μg/ml anti-*Ft* LPS mAb at 37 °C for 3 h. BMDCs were then washed 3 times in PBS. Each mouse was then anesthetized and administered i.n. either 30 μl of PBS (vehicle) or 3×10^6 pulsed BMDCs. Before and after immunizations, serum was collected and analyzed for the presence of *Ft*-specific Ab. To measure *Ft*-specific Ab in BAL, immunized mice were sacrificed 2 and 4 weeks post-boost. In challenge experiments, immunized mice were infected i.n. 2 and 4 weeks post-boost with 4× LD_{50} of live *Ft* LVS. Survival was monitored for 28 days.

2.6. BMDC maturation and cytokine secretion

BMDCs were cultured overnight in RPMI medium alone, or with i*Ft*, or mAb-i*Ft* ICs. Cells were then harvested, stained, and analyzed for maturation markers (CD40, CD80, CD86, CD205, and MHCII) *via* flow cytometry as previously described [14]. To monitor cytokine secretion, BMDCs were cultured with medium, i*Ft*, or mAb-i*Ft* for 3 days at 37 °C. Culture supernatants were then collected and analyzed for cytokines/chemokines using the Luminex Bio-Plex assay system (Bio-Rad).

2.7. Ag presentation

BMDCs were pulsed overnight at 37 °C with medium, i*Ft*, or mAb-i*Ft* ICs. Cells were then washed twice with medium to remove unbound i*Ft* and adjusted to 2×10^6 cells/ml. In a 96-well plate,

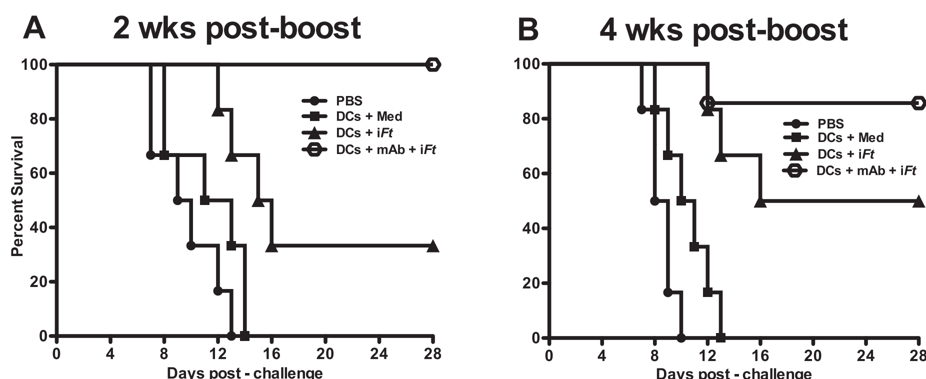

Fig. 1. Mice immunized with mAb-i*Ft*-pulsed BMDCs are better protected against a lethal *Ft* LVS challenge. BMDCs pulsed with medium, i*Ft*, or mAb-i*Ft* (10 i*Ft*/cell) were washed with PBS. Pulsed cells (3×10^6 cells/dose) were then suspended in 30 μl of PBS and administered to C57BL/6 mice ($n = 6$) i.n. as droplets in alternating nares. Mice were primed and boosted with the Ag-pulsed BMDCs on days 0 and 14. Two weeks (A) and 4 weeks (B) post-boost, mice were challenged i.n. with 4× LD_{50} (2×10^4 CFU) of *Ft* LVS in a volume of 20 μl of PBS. Survival was monitored for 28 days post-infection. The vehicle control groups received PBS only. Data presented are from a single experiment containing 6 mice per group. Each figure depicts results from a single experiment. The figure in panel A is representative of four independent experiments. The figure in panel B is representative of two independent experiments. In the case of panel A, the P value for the difference between i*Ft* *versus* PBS is $P = 0.0045$ and for i*Ft* *versus* mAb-i*Ft* 0.018. In the case of panel B, the P value for the difference between i*Ft* *versus* PBS is $P = 0.0006$ and for i*Ft* *versus* mAb-i*Ft* 0.2108.

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