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Depletion of Inflammatory Dendritic Cells with Anti-CD209 Conjugated to Saporin Toxin

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Abstract

Monocytes rapidly infiltrate inflamed tissues and differentiate into CD209⁺ inflammatory dendritic cells (DCs) that promote robust immunity or, if unregulated, inflammatory disease. Previous studies in experimental animal models indicate that inflammatory DC depletion through systemic elimination of their monocyte precursors with clodronate-loaded liposomes ameliorates the development of psoriasis and other diseases. However, translation of systemic monocyte depletion strategies is difficult due to the importance of monocytes during homeostasis and infection clearance. Here, we describe a strategy that avoids the monocyte intermediates to deplete inflammatory DCs through antibody-loaded toxin. Mice with an abundance of inflammatory DCs as a consequence of lipopolysaccharide exposure were treated with anti-CD209 antibody conjugated to saporin, a potent ribosome inactivator. The results demonstrate depletion of CD209⁺ DCs. This strategy could prove useful for the targeted reduction of inflammatory DCs in disease.

Keywords

monocytes; inflammatory dendritic cells; CD209; antibody-conjugated toxin; saporin

INTRODUCTION

Comprising approximately 10% of circulating leukocytes in humans, monocytes encompass a critical component of both innate and adaptive immunity [1]. During steady state conditions, resident or “non-inflammatory” monocytes replenish tissue macrophages that are essential during wound healing and the clearance of dead or dying cells [2, 3]. During inflammatory responses, monocytes rapidly infiltrate inflamed tissue through CCR2- and CCR5-binding chemokines [4, 5]. Upon arrival, monocytes mediate defense against pathogens through the utilization of evolutionarily conserved pattern recognition receptors (PRRs) that recognize and engulf pathogens [6–8]. Inflammatory monocytes also readily differentiate into DCs capable of stimulating local immunity or transiting to the draining lymph nodes to stimulate the adaptive immune system[9–12].

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Monocytes are essential for the regulation of tissue homeostasis and the control of several pathogens including *Listeria monocytogenes*, *Toxoplasma gondii* and *Cryptococcus neoformans* [6, 13–16]. However, the accumulation of monocyte-derived inflammatory DCs can play an important role in the pathogenesis and persistence of certain inflammatory diseases such as psoriasis and Crohn's disease [17–21]. As such, broad strategies have been employed to reduce or eliminate monocyte infiltration and subsequent formation of inflammatory DCs. While some of these strategies, such as CCR2 inhibition [22–24] or depletion of phagocytes with clodronate-loaded liposomes [19, 25, 26], have been effective in murine models, they suffer from widespread immune suppression and lack of efficacy in clinical trials [27, 28]. Thus, a new generation of therapeutics is required that more specifically target inflammatory DCs.

Recent studies indicate that human and murine inflammatory DCs express CD209 following their differentiation from monocytes [11, 20, 21, 29]. As such, we decided to conjugate monoclonal CD209 antibody to the saporin toxin, which is a ribosome inactivating protein that mediates cell death through inhibition of protein synthesis [30]. Saporin is an interesting candidate for targeted cell depletion as it is unable to enter human cells in the absence of a transport protein such as CD209, which mediates phagocytosis upon ligation [31, 32].

MATERIALS AND METHODS

Mice

C56BL/6 mice were purchased from Jackson Labs. All mice were housed in an American Association for the Accreditation of Laboratory Animal Care-accredited animal facility and maintained in specific pathogen-free conditions.

Inflammatory DC Formation and Toxin Administration

Six-week-old C56BL/6 mice were injected intravenously with 10 µg of lipopolysaccharide (LPS) (Sigma) to induce inflammatory DC formation and 10 µg of fluorescently conjugated anti-CD209 (eBioscience, Clone 5H10) or isotype control antibody (eBioscience) to label monocyte-derived inflammatory DCs as described previously [11]. Six hours post injection, mice were injected intravenously with biotinylated anti-CD209 (eBioscience) conjugated to streptavidin-saporin (Advanced Targeting Systems), biotinylated isotype control antibody (eBioscience) conjugated to streptavidin-saporin (Advanced Targeting Systems) or biotinylated anti-CD209 (eBioscience) conjugated to streptavidin-alexa 647 (eBioscience). After 12 hours, the inguinal and brachial lymph nodes were extracted and digested for 30 minutes at 37°C with 20 U/mL type IV collagenase (Worthington) in RPMI media (Gibco) supplemented with 100 U/mL penicillin, 100 g/mL streptomycin, 2mM L-glutamine and 10% fetal calf serum prior to the creation of single-cell suspensions via mechanical dissociation.

Flow Cytometry

Single cell suspensions were incubated with anti-CD16/32 mAb (eBioscience) to block Fc receptors prior to staining cells with a panel of mAbs against CD3, CD11b, CD11c, CD19,

CD40, DX5, GR1 and MHC II (I-A^b). Cells were washed, labeled with DAPI (Invitrogen) and analyzed on a BD LSR II. FACS plots were generated by FlowJo(Treestar).

Statistical analysis

An unpaired students T test (two-tailed) with 95% confidence interval was utilized to analyze all experimental data. $P < 0.05$ was considered significant.

RESULTS

Antibody-conjugated toxins deplete inflammatory DCs *in vivo*

To investigate the potential of anti-CD209 antibody conjugated to saporin toxin to deplete inflammatory DCs *in vivo*, mice were injected intravenously with LPS and fluorescently conjugated anti-CD209 to elicit and label inflammatory DCs, respectively [11, 29]. After six hours, mice were injected with PBS, biotinylated anti-CD209 conjugated to streptavidin-saporin (CD209-toxin) or biotinylated isotype control antibody conjugated to streptavidin-saporin (iso-toxin). Lymph nodes were processed after 12 hours and assessed by flow cytometry. The results indicate that inflammatory DCs, defined as CD209⁺ myeloid DCs (lineage⁻ MHC II⁺ CD11c⁺ CD11b⁺ GR1⁻), were markedly depleted in a small cohort of mice following administration of CD209-toxin (Figure 1A). Subsequent experiments in larger cohorts of mice confirmed these results (Figure 1B). To control for the potential of reduced labeling efficiency of inflammatory DCs in the CD209-toxin condition, mice were also injected with biotinylated CD209 conjugated to streptavidin-alexa 647 (CD209-Ax647) 6 hours after injection of LPS and fluorescently conjugated anti-CD209. The results indicate that the depletion was specific as the frequencies of CD209⁺ DCs were similar between the CD209-Ax647 and iso-toxin conditions (data not shown).

DISCUSSION

Monocytes readily infiltrate inflamed tissues and differentiate into inflammatory DCs that are critical for the clearance of infection [9, 10]. However, uncontrolled accumulation of inflammatory DCs can promote and sustain inflammation associated tissue damage, and subsequent depletion of inflammatory DCs through the elimination of monocytes ameliorates disease [19, 25]. In an effort to avoid systemic immunosuppression, we have investigated several methods capable of specifically depleting or inhibiting the formation of inflammatory DCs without eliminating their monocyte precursors. The data presented in this report indicate that saporin-conjugated anti-CD209 antibody efficiently depletes inflammatory DCs *in vivo*. Conjugation of CD209 to other toxins should also demonstrate significant efficacy at depleting inflammatory DCs *in vivo*. On a cautionary note, CD209 expression has been reported on splenic pre-plasmacytoid DCs [33]. The effects of antibody-toxin depletion methods on other phagocytes such as monocytes, granulocytes and macrophages warrants further investigations, as these cells may take up antigen-antibody complexes nonspecifically.

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Figure 1. CD209 conjugated to saporin toxin depletes inflammatory DCs

C57BL/6 mice were given intravenous injections of LPS and anti-CD209 to elicit and label inflammatory DCs. After 6 hours, PBS, biotinylated anti-CD209 or isotype control antibody conjugated to streptavidin-saporin was administered intravenously. 12 hours later, the inguinal and brachial lymph nodes were processed and stained with DC-associated and lineage markers to identify CD209⁺ inflammatory DCs. A) Gating strategy utilized to identify lineage⁻ MHC II⁺ CD11c⁺ CD11b⁺ GR1⁻ CD209⁺ inflammatory DCs. B) Frequency of CD209⁺ inflammatory DCs among myeloid DCs defined as lineage⁻ MHC II⁺ CD11c⁺ CD11b⁺ cells. Data are from 3 independent experiments and 12 mice (mean and SEM), *p<0.05.