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A role for IFNγ in differential superantigen stimulation of conventional versus plasmacytoid DCs ☆

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Abstract

Superantigens (SAgs) are known to play a role in food poisoning, toxic shock syndrome and have been identified as a potential mediator of autoimmunity. Although much is known about the effects of SAgs on T cells, by comparison few studies have investigated how SAgs influence innate immune cells. In particular no study has examined how SAgs affect murine plasmacytoid dendritic cells (pDC). We report that *in vivo* administration of staphylococcal enterotoxin A (SEA) increased the number of pDCs in secondary lymphoid organs, and induced CD86 and CD40 expression. Similar to SEA activation of conventional DCs (cDCs), pDCs relied on T cells, but not on CD40. Nonetheless, pDCs strictly required IFNγ for upregulation of CD86 and CD40, but cDCs did not depend upon IFNγ for activation. Further, even though IFNγ deficient pDCs were not activated by SEA, they were still capable of producing wild-type levels of IFNα in response to CpG oligodeoxynucleotide (ODN). The source of IFNγ for pDC activation was not T cells, nor did pDCs themselves have to synthesize or bind IFNγ, but the presence of IFNγ was essential. After SEA stimulation, IFNγ deficient mice fail to induce expression of the pDC dependent chemokines CXCL9, and demonstrated a defect in recruitment of pDCs to marginal zones of lymphoid organs. Thus, SEA exerts its combined effect on pDC activation, recruitment and chemokine induction through the action of IFNγ. This fundamental dichotomy of the effects of SAgs on pDCs versus cDCs show how a non-PAMP from bacteria, can selectively and indirectly stimulate innate cell subpopulations much in the same way that differential TLR expression influences cells of the innate immune system.

Keywords: Superantigen; Plasmacytoid DC; IFNγ

1. Introduction

A mammalian host recognizes different types of bacterial toxins resulting in profound stimulation of the innate or adaptive immune system. In particular, PAMPs, such as endotoxin, are pathogen byproducts that potently activate the innate immune system by binding pathogen recognition receptors such as TLR and Nod. Many different cells of the innate immune system express these receptors and stimulation through them results in proinflammatory cytokine production and enhanced phagocytic ability [1–4]. In contrast, superantigens (SAgs) like *Staphylococcal aureus* enterotoxins stimulate the adaptive immune system by activating T cells bearing specific TCR Vβ chains. This type of oligo clonal stimulation results in massive T cell expansion and cytokine production [5]. Nevertheless, these seemingly different pathogenic toxins inextricably link the innate and adaptive immune systems, and their combined effects lead to very powerful immune responses.

The effects of SAgs on T cells have been documented for well over a decade [5–7]. They mediate a variety of maladies including toxic shock syndrome, food poisoning, and are implicated in several autoimmune diseases [8–10]. Based on functional and crystallographic structure studies it is established that SAgs bridge MHC class II molecules with the variable portion of the β chain of the TCR. Because this does not require MHC peptide specificity, but only specific TCR Vβ chains, the frequency of stimulated T cells is substantially enhanced over that of an experimental peptide

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MHC ligand. This results in release of proinflammatory cytokines by T cells, and is thought to be a major aspect in the etiology manifested by this type of toxin [11–13]. Perhaps, this emphasis on adaptive immunity has largely dissuaded investigations regarding the influence of SAgs on the innate immune system.

In recent years, however, the effect of SAgs on the innate immune system has been documented. One *in vivo* study showed that staphylococcal enterotoxin B (SEB) stimulated dendritic cells to upregulate maturation or activation markers, but only in a T cell dependent manner [14]. This is in sharp contrast to PAMPs, which do not require T cells to stimulate dendritic cells or macrophages. A second study by our laboratory showed that SEA conditions DCs to secrete heightened cytokine levels in response to challenge with various PAMPs, whereas conditioning with LPS induced endotoxin tolerance [15]. Therefore, SAgs reveal how the adaptive immune system can initiate and condition cells of the innate immune system.

In this report, we define a split mechanism for differential DC activation by SAg. Similar to conventional $CD11c^{hi}$ MHC $II^{+}B220^{-}$ DCs, we found that pDCs increased in number and upregulated CD40 and CD86 in response to SAg. Moreover, activation of pDCs and cDCs were both T cell dependent, but CD40 independent. Nevertheless, in direct contrast to cDCs, pDCs required IFNγ for upregulation of CD86 and CD40. We demonstrated through the use of mixed bone marrow chimeras that pDCs neither needed to synthesize nor bind IFNγ, but the presence of IFNγ was essential for activation of pDCs. The lack of IFNγ, however, did not inhibit pDC synthesis of IFNα in response to TLR9 stimulation, but IFNγ was shown to be essential for production of the pDC-dependent chemokine CXCL9. Finally, we demonstrated that IFNγ was pivotal for the accumulation of pDC into the marginal zones of spleen and LNs. Therefore, bacterial SAg conditioned pDCs in a T cell and IFNγ-dependent manner leading to increased expression of costimulatory molecules, and support for traverse through lymphoid tissue.

2. Materials and methods

2.1. Mice, reagents and in vivo treatments

C57BL/6 mice were purchased from Charles River—National Cancer Institute (Fredrick, MD). $IFN\gamma^{-/-}$, $IFN\gamma R^{-/-}$ and $CD40^{-/-}$ breeder mice were purchased from Jackson Laboratory (Bar Harbor, ME). $TCR\beta\delta^{-/-}$ mice were a kind gift from Dr. T.V. Rajan (Department of Immunology, UConn Health Center). All mice were maintained in the Central Animal Facility at the University of Connecticut Health Center in accordance with federal guidelines.

SEA (staphylococcal enterotoxin A) was purchased from Toxin Technology Inc., (Sarasota, FL). Recombinant Murine Interferon-γ (IFNγ) was purchased from MBL International Corp. (Watertown, MA). Mice received 1 μg of SEA intraperitoneal (i.p.), and all IFNγ treated mice received either 5 or 15 μg of recombinant murine IFNγ i.p.

PE-conjugated and APC-conjugated anti-mPDCA-1 (JF05-1C2.4.1) were purchased from Miltenyi Biotec (Auburn, CA). PE-conjugated anti-CD11c (HL3) and control ratIgG2b, FITC conjugated streptavidin and anti-CD11b (Mac-1), APC conjugated anti-CD11c (HL3), biotin-labeled anti-CD40 (3/23), anti-CD86 (GL1) and control ratIgG2aκ were purchased from BD Biosciences (Mountain View, CA). PE-Cy5.5 conjugated anti-CD45.1 (A20) and anti-CD45.2 (104), PE-conjugated Ly-6G (RB6-8C5) were purchased from eBioscience (San Diego, CA).

2.2. Dendritic cell isolation and flow cytometric analysis

For analyzing the expression of maturation markers and for obtaining actual numbers of DCs, density gradient fractionation of innate APCs was followed as given in Swiggard et al. without DC adherence [16]. Briefly, spleens from mice were crushed mechanically with forceps and treated with 1 ml of a 3.3 mg/ml solution of collagenase D (Roche Diagnostics Corporation, Indianapolis, IN) in MEM 2% fetal bovine serum (FBS, Gibco BRL, Grand Island, NY), 10 mM Hepes (Gibco). Splenic tissue was incubated for 30 min at 37 °C, 5% CO_2. After treatment with 100 μl 0.1 M EDTA for 5 min at room temperature, spleen tissue was disassociated through cell strainers and rinsed with Ca/Mg free BSS. The red blood cells were lysed and cells were resuspended in 2 ml PBS 35% BSA, added to ultra-clear centrifuge tubes (Beckman, Palo Alto, CA) with an additional 2 ml Ca/Mg free BSS layered on top. After centrifugation at 9500*g* for 15 min at 4 °C, cells from the interface were collected and washed with BSS. Cells were resuspended in cell staining buffer (BSS, 3%; FCS, 0.1% NaAz) and counted on a Z1 particle counter (Beckman Coulter, Miami, FL). For DC numbers, red blood cells were lysed and resuspended in cell staining buffer without the gradient centrifugation step.

For flow, cells were resuspended in cell staining buffer and non-specific binding was blocked using a combination of 2.4G2 hybridoma supernatant, 5% heat inactivated normal mouse serum and approximately 10 μg/ml human IgG. Cells were incubated on ice with primary antibodies for approximately 30 min, washed, and resuspended in staining buffer. If a secondary incubation was necessary, cells were washed and resuspended in staining buffer, and incubated on ice with streptavidin-FITC for 30 min. The cells were analyzed on a FACSCalibur flow cytometer and data was analyzed using either CELL Quest software (BD Biosciences, Mountain View, CA) or FlowJo software (Tree Star Inc., Ashland, OR).

2.3. T cell purification and adoptive transfer

Splenocytes from either C57BL/6, IFNγ or $IFN\gamma R^{-/-}$ mice were crushed through a cell strainer and run through a nylon-wool column (PerkinElmer Life and Analytical