Molecular Immunology 91 (2017) 57–64

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Distinct pattern of immune tolerance in dendritic cells treated with lipopolysaccharide or lipoteichoic acid

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ARTICLE INFO

Keywords:
Bone marrow-derived dendritic cells
Tolerance
Lipoteichoic acid
Lipopolysaccharide
Pro-inflammatory cytokines

ABSTRACT

Cytokine induction is often critical for the host defense during acute immune responses while, if not tightly regulated, it may cause an immunological pathology coincident with tissue damage. Despite the fact that gram-positive bacterial infection has become increasingly prevalent, immune modulation induced by lipoteichoic acid (LTA), the major cell wall component of gram-positive bacteria has not been studied thoroughly at the cellular level. In the current study, tolerance induction in mouse bone marrow-derived dendritic cells (BMDCs) treated with single or repeated stimulation of *Staphylococcus aureus* LTA was compared with those of *Escherichia coli* lipopolysaccharide (LPS). The results showed that repeated LTA stimulation significantly suppressed pro-inflammatory cytokine (TNF-α and IL-6) production in BMDCs, comparable to that of LPS, but with less extent, down-regulated IL-10 and enhanced the inhibitory molecule, LAG-3-associated protein (LAP). Furthermore, we observed a sustained expression of unique negative regulators, Toll interacting protein (TOLLIP) and Indoleamine 2,3-dioxygenase (IDO), in BMDCs treated with LTA.

A transient hyporesponsiveness period appeared when DCs were treated repeatedly with LTA or LPS showing a distinctive pattern. Intriguingly, LPS exposure induced cross tolerance to LTA while LTA exposure did not to LPS, implicating that a distinct signaling components are involved in response to LTA. Collectively, a distinct immune regulation appeared to be responsible for the LPS- and LTA-induced tolerance on cytokine production, expression of surface markers and intracellular proteins.

1. Introduction

Activation of the toll-like receptor (TLR) signaling pathway is indispensable for host protection against pathogens. On the contrary, uncontrolled and excessive production of pro-inflammatory cytokines can lead to systemic inflammation and potentially deleterious consequences including the systemic inflammatory response syndrome, multi-organ dysfunction syndrome, shock and even death.

Although composition of cell wall structure and expression profile of virulence factors are different, recent clinical dogma determines that similar therapeutic protocols are still applied for both gram-positive and gram-negative septic patients (Surbatovic et al., 2015). However, several evidences suggest that different inflammatory patterns including cytokine profiles at early stage between gram-positive and gram-negative septic patients are clearly different (Surbatovic et al., 2015; Carlet et al., 2008).

Thus, the pathogenesis seems to be quite different. Lipoteichoic acid (LTA) is a cell-wall component of gram-positive bacteria, which shares some pathophysiological properties with lipopolysaccharide (LPS) (Sriskandan and Cohen, 1999). In addition, LTA synergizes with peptidoglycan (PepG), known to be responsible for an excessive production of NO, TNF-α, and IFN-γ (De Kimpe et al., 1995).

Abbreviations: LTA, lipoteichoic acid; BMDCs, bone marrow-derived dendritic cells; LPS, lipopolysaccharide; LAP, LAG-3-associated protein; TOLLIP, toll interacting protein; IDO, indoleamine 2,3-dioxygenase; PepG, peptidoglycan; BLP, bacterial lipoproteins; ET, endotoxin tolerance; *E. coli*, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; IRAK, IL-1R associated kinase; SHP-1, src homology 2 domain phosphotyrosine phosphatase 1

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http://dx.doi.org/10.1016/j.molimm.2017.08.023
Received 1 July 2017; Received in revised form 20 August 2017; Accepted 23 August 2017

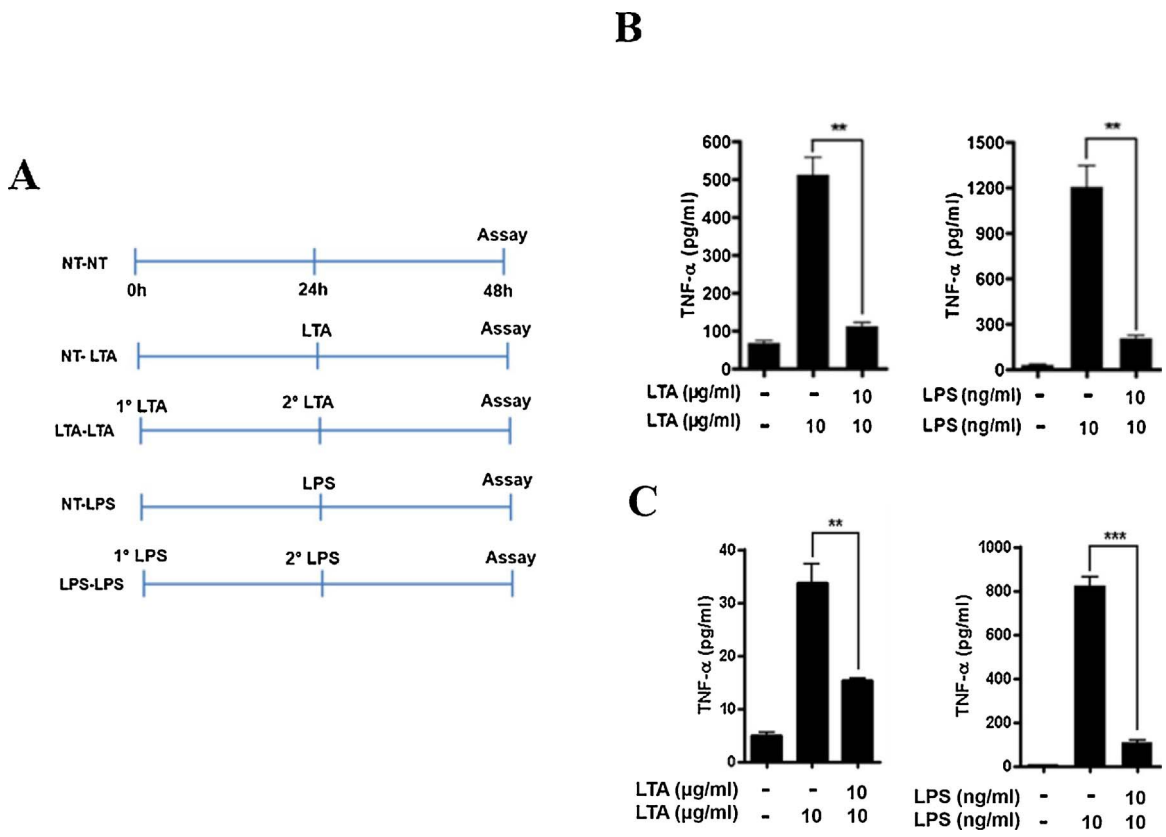

Fig. 1. TNF-α production in DCs re-stimulated with LTA or LPS. (A) Schematic diagram of experimental approach (NT-NT; no treatment, NT-LTA or LPS; single stimulation of LTA or LPS, LTA-LTA; repeated stimulation of LTA, LPS-LPS; repeated stimulation of LPS). (B) TNF-α production was measured in the culture supernatants in BMDCs with repeated stimulation of LTA (10 μg/ml) (LTA-LTA) or LPS (10 ng/ml) (LPS-LPS). (C) TNF-α expression in $CD11c^+$ splenic DCs with repeated stimulation of LTA or LPS. The mice were injected i.p. with saline, LTA (20 μg/ml) or LPS (400 ng/ml). 24 h later, $CD11c^+$ cells were isolated from spleen with magnetic conjugated anti-CD11c antibody. DCs were treated with LTA (10 μg/ml) (LTA-LTA) or LPS (10 ng/ml) (LPS-LPS), respectively for 24 h. Then the supernatants were collected and TNF-α production was measured by using ELISA. $^{**}P < 0.01$, $^{***}P < 0.001$.

As most studies on antigen-presenting cells focused on initiating innate immunity and bridging adaptive immunity, the role of dendritic cells (DCs) in sepsis is not extensively explored compared to macrophages. It is well known that preventing apoptosis of sepsis-induced DCs increased survival rate in lethal septic shock (Gautier et al., 2008). Despite of its potential roles in sepsis, most of the studies were performed in macrophages, not DCs.

Numerous studies have been conducted to delineate the pathogenesis of gram-negative sepsis with LPS at the cellular and molecular levels. Down-regulated IL-1R associated kinase (IRAK) 1 activity (Medvedev et al., 2002) and negative regulators that interfere with TLR signaling pathway including A20 (Xiong et al., 2011), SHIP (Sly et al., 2004), SMAD4 (Pan et al., 2010), indoleamine 2,3-dioxygenase (IDO) (Pallotta et al., 2011) and IRAK-M (Kobayashi et al., 2002) are thought to be associated with LPS tolerance.

IDO, a tryptophan catabolizing enzyme, was recognized as an immune checkpoint produced by alternatively activated macrophages and DCs that acting through the activation of SHP-1 targeting IRAK-1. A blockade of IDO protects mouse against LPS-induced endotoxin shock (Jung et al., 2009). TOLLIP is known to shut down MyD88-dependent signaling pathway by the inactivation of IRAK-1 (Zhang and Ghosh, 2002). A20 has ubiquitin editing activity that removes K63-linked polyubiquitin chains from TRAF6 to prevent protein–protein interactions with TLR4 (Vereecke et al., 2009). IRAK-M prevents dissociation of IRAK-1 and IRAK-4 from MyD88 (Kobayashi et al., 2002) thus regulating TLR signaling pathway.

Repeated stimulation of LTA and PepG derived from *S. aureus* has also been reported to suppress pro-inflammatory cytokines such as TNF-α and IL-1β in macrophages from mouse (Lehner et al., 2001; Nakayama et al., 2004) and human (Jacinto et al., 2002). The molecular mechanism of LTA-induced tolerance is largely unknown although the synthetic TLR2 ligands such as Pam3CSK4 (Siedlar et al., 2004), bacterial lipoproteins (BLP) (Li et al., 2012) and PepG (Nahid et al., 2013) have been investigated. IRAK-M is involved in Pam3CSK4-induced tolerance by selectively attenuating p38 activation via stabilizing MAPK phosphatase 1 (MKP-1) (Su et al., 2007). Reduced expression of TLR2 and IRAK-1 are the characteristic of BLP-mediated tolerance for down-regulation of TLR signaling pathway although overexpression of IRAK-1 alone restored the suppressed TNF-α (Li et al., 2012). Here in, we used *S. aureus*-derived LTA to induce tolerance using mouse bone marrow-derived dendritic cells (BMDCs) and compared with those of *Escherichia coli*-derived LPS tolerance.

2. Materials and methods

2.1. Reagents

Highly purified LTA derived from *S. aureus* (ATCC 6538, Manassas, VA, USA) with free of endotoxin was prepared as previously described (Morath et al., 2001). LPS derived from *E. coli* (026:B6) was purchased from Sigma-Aldrich (St. Louis, MO, USA). RPMI-1640 Glutamax medium, fetal bovine serums (FBS), sodium pyruvate, HEPES, 2-mercaptoethanol and gentamycin were purchased from Gibco (Grand Island, NY, USA). Recombinant mouse GM-CSF was purchased from Creagene (Korea). Anti-mouse CD11c-APC, MHC class II-FITC, CD80-PE, CD86-FITC, CD4-PerCP and anti-mouse latency associated peptide (LAP)-PE-Cy7 antibodies were purchased from BD Biosciences (San Diego, CA, USA). Anti-mouse IDO (SantaCruz Biotechnology, CA, USA) and anti-mouse TOLLIP (Abcam, Eugene, OR, USA) polyclonal antibodies were purchased. Accudenz® was purchased from Accurate