



Review

Are NKT Cells Essential for Endotoxic Shock?

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Abstract. Endotoxic shock is a major health threat caused by Gram-negative bacteria and their unique cell wall component, lipopolysaccharide, which induces exaggerated production of proinflammatory cytokines. Although macrophages play a central role in the pathogenesis of endotoxic shock, natural killer (NK)¹⁺ cells are also involved in this mechanism. NK¹⁺ cells comprise two major populations, namely NK cells and NKT cells. It remains, however, elusive whether NK cells, NKT cells or both are involved in the induction of endotoxic shock. This review will focus on the relative contribution of these NK¹⁺ cells to the pathogenesis of endotoxic shock.

Key words: endotoxin shock; NK cell; NKT cell; IFN- γ ; lipopolysaccharide.

Introduction

Bacterial sepsis is a major health threat affecting one million patients in the United States and Europe⁴⁴. The resulting septic shock is caused by an exaggerated systemic cytokine response to bacterial components which, in the vast majority of cases, comprise Gram-negative bacteria and their characteristic cell wall component, lipopolysaccharide (LPS)²¹. The uncontrolled production of proinflammatory cytokines causes various pathophysiological reactions, including fever, hypotension, and multiple organ failure, all of which ultimately form the septic shock syndrome, often with fatal outcome⁵. Macrophages probably play a central role in mediating the biological effects of LPS (Fig. 1), because adoptive transfer of macrophages from endotoxin-susceptible mice renders endotoxin-resistant mice sensitive to the toxin¹⁹. LPS binds to plasma proteins

such as LPS-binding protein (LBP), and the LPS-LBP complexes interact with CD14 expressed on macrophages⁴⁶, leading to release of various proinflammatory cytokines, such as tumor necrosis factor (TNF)- α , interleukin (IL)-1, IL-6, and IL-12^{22, 23} (Fig. 1).

Although TNF- α plays a central role in the pathogenesis of endotoxic shock^{4, 5, 21–23, 36, 38, 39, 42}, IFN- γ is also involved in this mechanism^{6, 9, 22–24, 26, 36} (Fig. 1). Because natural killer (NK) cells are potent interferon (IFN)- γ -producers⁴⁵, these cells have been considered to participate in the pathogenesis of endotoxic shock²⁵. The contribution of NK cells to endotoxic shock is heavily predicated on the data obtained from the *in vivo* depletion of NK¹⁺ cells with monoclonal antibody (mAb) against NK1.1. Yet it appears that the NK1 marker is expressed not only on NK cells, but also on some subsets of T cells (designated as NKT cells)³. It is therefore possible that NKT cell functions have been

Abbreviations used: β 2m – β 2-microglobulin, D-GalN – D-galactosamine, IFN – interferon, IL – interleukin, LBP – lipopolysaccharide-binding protein, LPS – lipopolysaccharide, MHC – major histocompatibility complex, mAb – monoclonal antibody, NK – natural killer, TCR – T cell receptor, TNF – tumor necrosis factor, WT – wild-type.

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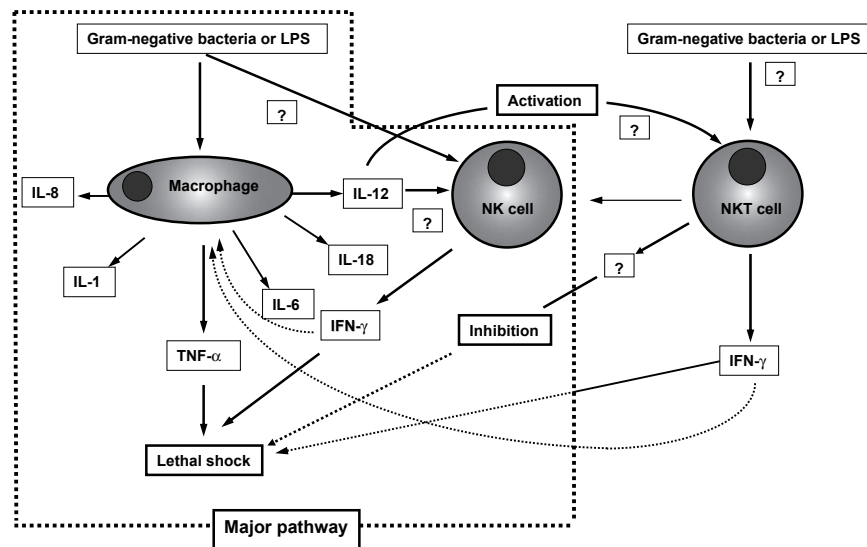


Fig. 1. Participation of NK1⁺ cells in high-dose LPS-induced shock. Macrophages produce a wide variety of proinflammatory cytokines such as TNF- α , IL-1, IL-6, IL-8, and IL-12 following high-dose LPS challenge. Although TNF- α plays a central role in high-dose LPS-induced lethal shock, IFN- γ secreted from NK cells also participates in this mechanism. Although NKT cells secrete IFN- γ upon LPS stimulation, it seems likely that the contribution of this cell population is minor

mistaken for NK cell-mediated functions. In this review, I will focus on the relative contribution of NK cells and NKT cells to the pathogenesis of endotoxic shock.

Broad Classification of NK1⁺ Cells

NK1⁺ cells segregate into two major populations, namely NK cells and NKT cells. Although these two cell populations share some characteristic features, in various aspects there are marked differences between the two populations. NK cells lack surface expression of the T cell receptor (TCR) and they develop independently of classical major histocompatibility complex (MHC) class Ia, nonclassical MHC class Ib and CD1²⁹. In contrast, the vast majority of NKT cells (designated as classical NKT cells) express an invariant TCR V α /J α combination, comprising V α 14/J α 281 in the mouse, and the homologous chains V α 24/J α Q in man³, and they develop in a CD1d-dependent fashion^{3, 7, 12, 32, 35, 40}. Functionally, classical NKT cells secrete large quantities of both IFN- γ and IL-4 upon stimulation, whereas NK cells produce IFN- γ , but not IL-4^{1, 2, 11–13, 31, 45, 48}.

High-Dose LPS-Induced Lethal Shock

Although mice are relatively resistant to LPS-induced shock, challenge with high doses of LPS induces several pathophysiological reactions, including fever, hypotension, leukocyte infiltration and inflammation in various organs, resulting in a syndrome resembling septic shock with a high mortality (high-dose LPS-in-

duced shock model)⁴. Although TNF- α plays a central role in the pathogenesis of high-dose LPS-induced shock^{4, 5, 22, 23, 38}, IFN- γ is also involved in this mechanism^{6, 9, 24} (Fig. 1). Because both NK cells and classical NKT cells have been proposed as a critical early source of IFN- γ ^{1, 2, 11–13, 31, 45}, it is possible that both populations participate in the pathogenesis of high-dose LPS-induced shock. We have recently found that 1) β 2-microglobulin (β 2m)^{-/-} mice, which are devoid of classical NKT cells but not NK cells, are more susceptible to high-dose LPS-induced shock than wild-type (WT) mice, 2) serum levels of IFN- γ are markedly higher in β 2m^{-/-} mice than in WT mice following challenge with high doses of LPS, 3) *in vivo* depletion of NK1⁺ cells prevents β 2m^{-/-} mice as well as WT mice from succumbing to the lethal effects of LPS, 4) J α 281^{-/-} mice exclusively lacking classical NKT cells are slightly more susceptible to high-dose LPS-induced lethal shock than their heterogenous littermates, and 5) J α 281^{-/-} mice are rescued from lethal effects of LPS by NK1⁺ cell depletion¹⁶. These findings indicate that NK cells rather than classical NKT cells play a pivotal role in the pathogenesis of high-dose LPS-induced lethal shock. Since considerable numbers of IFN- γ producers are detected among classical NKT cells in WT mice, it cannot formally be excluded that classical NKT cells participate in high-dose LPS-induced shock in normal mice¹⁶. Yet, a pivotal role for classical NKT cells can be excluded, because IFN- γ production in response to high-dose LPS is markedly higher in NK cells than in classical NKT cells^{16, 28} and absolute numbers of NK cells markedly exceed those of classical NKT cells in the body.

IL-4 is involved in anti-inflammatory responses and this cytokine is secreted from classical NKT cells upon

stimulation^{1, 3, 12, 13, 48}. This raises the possibility that classical NKT cells participate in prevention of LPS-induced lethal shock¹⁸. It is conceivable that classical NKT cells play a dual, antagonistic role in septic shock. They could promote shock via IFN- γ and prevent it via IL-4. Yet, the susceptibility of C57BL/6 mice to LPS-induced lethal shock is virtually unaffected by endogenous IL-4 neutralization¹⁶. Therefore, IL-4 secreted from classical NKT cells may be negligible.

In addition to classical NKT cells, various CD1d-independent NKT cells have been described^{10, 14, 17, 47, 49}. Because these nonclassical NKT cells are also potent IFN- γ producers^{14, 17}, it cannot be excluded that these nonclassical NKT cells participate in high-dose LPS-induced shock. However, it is conceivable that these nonclassical NKT cells play a minor role, if any, in high-dose LPS-induced shock, because 1) nonclassical NKT cells are rare in the body and 2) the IFN- γ -producing activities of NK cells are markedly higher than those of nonclassical NKT cells¹⁷.

Low-Dose LPS-Induced Lethal Shock/Liver Injury

D-galactosamine (D-GalN) increases susceptibility of mice to LPS-induced shock by impairing liver metabolism^{20, 30} (low-dose LPS-induced shock model). In contrast to high-dose LPS-induced shock, which induces a systemic disorder including multiple organ failures⁴, the liver is a major target organ of low-dose LPS-induced shock^{20, 30}. Similar to high-dose LPS-induced shock, proinflammatory cytokines, in particular TNF- α from macrophages and IFN- γ from NK1⁺ cells, play a key role in the disease process^{6, 30, 38, 39, 42}. Because classical NKT cells play a crucial role in the induction of liver injury in murine experimental models such as concanavalin A-induced hepatitis^{27, 41, 43}, it is speculated that this cell population participates in low-dose LPS-induced shock/liver injury. However, we have recently found that 1) the susceptibility of TCR $\beta^{-/-}$ and CD1d $^{-/-}$ mice, both of which are devoid of classical NKT cells but not NK cells, to low-dose LPS-induced shock/liver injury is comparable to that of WT mice, 2) the susceptibility of $\beta 2m^{-/-}$ mice to low-dose LPS-induced shock/liver injury is slightly higher than that of other mouse strains and 3) C57BL/6 mice are not rescued from the lethal effects of LPS by *in vivo* depletion of NK1⁺ cells⁵⁰. These findings suggest that neither NK cells nor NKT cells participate in low-dose LPS-induced shock/liver injury.

The Generalized Schwartzman Reaction

The generalized Schwartzman reaction describes a lethal shock syndrome which is induced by consecutive challenge with low doses of LPS. Locally injected LPS causes IFN- γ production by NK1⁺ cells which in turn primes macrophages^{25, 26} (Fig. 2). Upon subsequent exposure to LPS, primed macrophages produce large amounts of TNF- α , which results in acute lethal shock^{25, 26} (Fig. 2). There is controversy over whether NKT cells, NK cells or both are involved in the generalized Schwartzman reaction. Primarily, OGASAWARA et al.³⁴ have reported that $\beta 2m^{-/-}$ mice are far more resistant to the generalized Schwartzman reaction as compared with WT mice and that IFN- γ secreted from classical NKT cells plays a central role in the lethal shock. Subsequently, DIELI et al.⁸ have also concluded that IFN- γ secreted from classical NKT cells rather than NK cells plays a central role in the generalized Schwartzman reaction by experiments using J α 281 $^{-/-}$ mice. The dispensable role of NK cells in the generalized Schwartzman reaction has also been reported by others²⁸. These findings raise the possibility that classical NKT cells play a crucial role in the generalized Schwartzman reaction. Yet, we have recently found that 1) $\beta 2m^{-/-}$ mice are more susceptible to the lethal consequences of the generalized Schwartzman reaction than WT mice, 2) J α 281 $^{-/-}$ mice are slightly more susceptible to the generalized Schwartzman reaction than their heterozygous littermates, and 3) the susceptibility of J α 281 $^{-/-}$ mice to the generalized Schwartzman reaction is reduced by NK1⁺ cell depletion¹⁶. These findings argue against a central role of classical NKT cells in the generalized Schwartzman reaction. Thus, it still re-

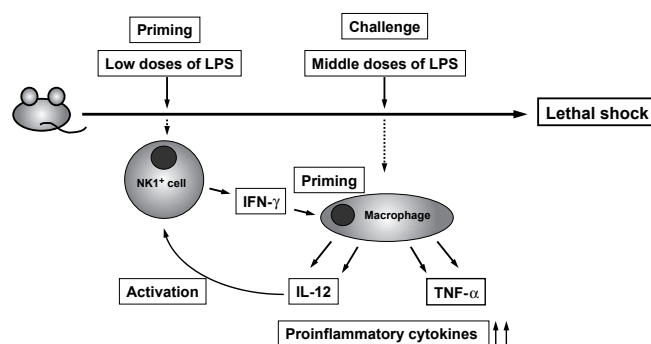


Fig. 2. The generalized Schwartzman reaction. Consecutive challenge with low doses of LPS induces lethal shock. First challenge with low doses of LPS prime NK cells, leading to secretion of IFN- γ . After secondary challenge with middle doses of LPS, primed macrophages secrete large amounts of proinflammatory cytokines, resulting in lethal shock

mains elusive to what extent these NK1⁺ cells participate in the generalized Shwartzman reaction.

Possible Tools for Studies on the *in Vivo* Role of NK Cells and NKT Cells in Endotoxic Shock, and Their Controversial Points

NK cells had been recognized as a significant and pivotal component in host immune responses against endotoxic shock. However, in recent years evidence has been growing that NKT cells rather than NK cells play a crucial role in the induction of endotoxic shock. Since genes which exclusively affect NK cell development have not been identified so far, anti-NK1.1 mAb and anti-asialo GM1 Ab have been employed to discriminate NK cells from NKT cells *in vivo*. Yet, we have reported that some NKT cells express asialo GM1 on their surface and thus are depleted by *in vivo* administration of anti-asialo GM1 Ab^{15,33}, and that NK cells in the liver, but not in the spleen, are not depleted by anti-asialo GM1 Ab at the doses used in papers published so far¹⁵ (Table 1). Moreover, we have recently found that considerable numbers of V α 14⁺ T cells are not depleted by anti-NK1.1 mAb treatment (EMOTO et al., submitted for publication; Table 1). These findings argue against these Abs as reliable tools for *in vivo* depletion of these NK1⁺ cells. Recent studies employ various knockout mice which are devoid of classical NKT cells to analyze the role of classical NKT cells in endotoxic shock. However, conflicting results on the role of NK cells and NKT cells in endotoxic shock have been presented. Although the discrepancy could be due to the use of different experimental systems, it is possible that the discrepancy is caused by the different genetic background. Indeed, it has been suggested that backcross onto a certain strain of mice influences characteristic features of gene knockout mutants³⁷. Consistent with this, we found that 129 mice, which are generally employed for generating gene disruption mutants, crossed onto C57BL/6 are more resistant to LPS-induced lethal shock than C57BL/6 mice (EMOTO, unpublished observation). In addition, an influence of body weight on resistance to LPS-induced shock is also possible.

Concluding Remarks

Conflicting data on the role of NK cells and NKT cells in endotoxic shock have been reported by different groups so far. It is tempting to assume that endotoxic shock is not caused by a single cell type, and that different cell populations participate in the shock in a hierarchical order. Depending on the shock model employed and the type of knockout mice used for analysis, the importance of distinct cell population for pathology seems to vary. Thus, it should be emphasized that observations obtained with gene-disruption mutants and with Ab must be carefully interpreted and the role of NK cells and NKT cells must be re-evaluated.

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Table 1. Susceptibility of NK cells and classical NKT cells to anti-NK1.1 mAb or anti-asialo GM1 Ab treatment

Treatment	NK cell		Classical NKT cell (TCRV α 14 ⁺ cell)	
	spleen	liver	NK1 ⁺ cell	NK1 ⁻ cell
Anti-NK1.1 mAb	susceptible	susceptible	susceptible	resistant
Anti-asialo GM1 Ab	susceptible	resistant	resistant ($\approx$ 95%)	resistant ($\approx$ 95%)

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