Modulation of TNFSF expression in lymphoid tissue inducer cells by dendritic cells activated with Toll-like receptor ligands

Sinsuk Han, Jihye Koo, Jingyu Bae, Soochan Kim, Song Baik & Mi-Yeon Kim*

Department of Bioinformatics and Life Science, The College of Natural Science, Soongsil University, Seoul 156-743, Korea

INTRODUCTION

Toll-like receptors (TLRs) are type I transmembrane signal receptor proteins that recognize specific components derived from pathogens (1, 2). TLRs are mainly expressed on cells of the innate immune system, including dendritic cells (DCs) and macrophages. Ten TLRs have been identified (TLR1 to TLR10) in human and 12 (TLR1 to TLR13) in mouse, each of which recognizes structural components conserved among pathogens (1, 3-5). For example, TLR2 recognizes zymosan, which is a yeast cell wall component, and TLR4 recognizes lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria (6, 7). Recognition of the structural components of pathogens initiates signal transduction, leading to production of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, and upregulation of costimulatory molecules for the development of adaptive immune responses (1, 2).

Lymphoid tissue inducer (LTI) cells play important roles in secondary lymphoid tissue development (8-10) and adaptive immune responses by providing survival signals to memory CD4 T cells through OX40 and CD30 (11-13). OX40 and CD30 are tumor necrosis factor receptor superfamily (TNFRSF) members and are expressed on activated CD4 T cells. Their ligands, OX40-ligand (OX40L) and CD30-ligand (CD30L), are tumor necrosis factor superfamily (TNFSF) members and are constitutively expressed on LTI cells. Engagement of OX40 and CD30 with their ligands on LTI cells results in the upregulation of anti-apoptotic molecules in CD4 T cells and increased cell survival leading to generation of memory responses (11-13). TRANCE (TNF-related activation-induced cytokine) is another TNFSF member that is expressed on LTI cells and is known to be a key molecule for peripheral lymph node development (14). Its receptor, RANK (receptor activator of NF-κB), is expressed on DCs, and signals through RANK provide DC survival and homeostasis (15). Recent studies have shown that LTI cells constitutively express IL-23 and rapidly upregulate IL-17 secretion after in vivo challenge with zymosan (16), suggesting that they play a role in innate immune responses. In line with this study, two groups reported that LTI-like cells produce IL-17 and IL-22 in stimulation with flagellin, a TLR5 ligand (17), and Pam3CSK, a TLR2 ligand, in an indirect manner (18).

In this study, we analyzed the expression of TLRs on LTI cells and report the modulation of OX40L and CD30L in the presence of the TLR4 ligand LPS or TLR2 ligand zymosan. In addition, we demonstrate that coculture with DCs upregulated the expression of TNF ligands on LTI cells.

RESULTS

Expression of TLRs on LTI cells, DCs, B cells, and macrophages

To detect whether or not LTI cells express TLRs, the mRNA expression of myeloid differentiation primary response gene 88 (MyD88), TLR2, TLR4, and TLR9 was examined (Fig. 1A). Since MyD88 is an adapter protein used by all TLRs except TLR3, its expression was measured and detected in all of the cells. LTI cells showed the highest expression of MyD88 compared to DCs, B cells, and macrophages, indicating that LTI cells express TLRs.
cells possessed the TLR signaling pathway. Since DCs, B cells, and macrophages are antigen-presenting cells, they express various types of TLRs, as observed in this study, specifically TLR2, TLR4, and TLR9. In comparison, LTI cells expressed TLR2 and TLR4 but not TLR9.

Although LTI cells express TLR4 mRNA, their response to LPS is not definite since they do not express CD14, which promotes LPS binding to TLR4 (2). Therefore, the protein expression of LTI cells was examined by flow cytometry (Fig. 1B). The expression levels of TLR2 and TLR4 on LTI cells were 42.1% and 8.0%, respectively, whereas the expression levels on antigen-presenting cells were approximately two times higher than those on LTI cells. The expression levels of TLR2 and TLR4 on DCs were 74.0% and 17.8%, those on B cells were 95.4% and 30.7%, and those on macrophages were 94.9% and 37.4%, respectively.

Effects of zymosan and LPS on TNFSF expression on LTI cells

To determine the effects of ligands for TLR2 (zymosan) and TLR4 (LPS) on LTI cells, splenocytes were stimulated with zymosan or LPS, and TNFSF protein expression on LTI cells was analyzed. LTI cells cultured with zymosan upregulated the expression of OX40L (from 40.3% to 63.3%), CD30L (from 61.9% to 80.5%), and TRANCE (from 78.0% to 84.4%) (Fig. 2A). Surprisingly, LTI cells cultured with LPS upregulated TNFSF expression to a level comparable or slightly higher than those cultured with zymosan; OX40L+ cells were 68.9%, CD30L+ cells were 82.5%, and TRANCE+ cells were 84.3%. The same results were observed in an in vivo experiment (Fig. 2B). LTI cells isolated from zymosan- or LPS-injected mice showed higher expression of OX40L, CD30L, and TRANCE molecules than those isolated from PBS-injected control mice.

However, when purified LTI cells were stimulated with LPS or zymosan, the cells did not upregulate TNFSF expression (data not shown). In addition, the number of LTI cells in the presence or absence of TLR ligands did not change (Fig. 3), whereas those of DCs, B cells, and macrophages increased after activation with TLR ligands, except for that of DCs stimulated with zymosan.

Fig. 1. TLR expression on LTI cells, DCs, B cells, and macrophages. (A) mRNA expression of MyD88, TLR2, TLR4, and TLR9. Relative mRNA expression of genes was normalized to β-actin signals. The results show the average expression of three separate experiments. (B) Flow cytometric analysis of TLR2 and TLR4 expression on LTI cells, DCs, B cells, and macrophages. Numbers above bracketed lines in histograms indicate the percent of positive staining for each Ab. Filled histograms show isotype-matched control Abs. Data are representative of five experiments.

Fig. 2. Effects of zymosan and LPS on TNFSF expression on LTI cells. (A) Flow cytometry analysis of OX40L, CD30L, and TRANCE expression on LTI cells cultured with zymosan (50 μg/ml) or LPS (1 μg/ml) in vitro. Numbers above bracketed lines in histograms indicate the percent of positive staining for each Ab. Filled histograms show isotype-matched control Abs. Results are representative of seven separate experiments. (B) Flow cytometry analysis of surface expression of LTI cells in vivo. Mice were injected intraperitoneally with zymosan (150 μg), LPS (5 μg), or PBS as a control. Three hours after zymosan injection or 18 hours after LPS injection, the spleens were taken and analyzed for the expression of OX40L, CD30L, and TRANCE on LTI cells. Filled histograms show isotype-matched control Abs. Results are representative of three separate experiments.