Legionella pneumophila Infection Up-Regulates Dendritic Cell Toll-Like Receptor 2 (TLR2)/TLR4 Expression and Key Maturation Markers

James Rogers, Amal Hakki, Izabella Perkins, Catherine Newton, Ray Widen, Nicholas Burdash, Thomas Klein, and Herman Friedman*

* Corresponding author. Mailing address: Department of Molecular Medicine, University of South Florida, Tampa, Florida 33612

Department of Molecular Medicine, University of South Florida, Tampa, Florida 33612

Received 12 December 2006/Returned for modification 15 February 2007/Accepted 12 March 2007

Dendritic cells (DCs) have a critical role in linking innate to adaptive immunity, and this transition is regulated by the up-regulation of costimulatory and major histocompatibility complex (MHC) molecules as well as Toll-like receptors. These changes in DCs have been observed to occur following microbial infection, and in the present study, we examined the effect of Legionella pneumophila infection on the expression of these DC markers. We showed that bone marrow-derived DC cultures from BALB/c mice infected with live L. pneumophila resulted in the up-regulation of Toll-like receptors 2 and 4 and the activation of CD40, CD86, and MHC class I/II molecules.

Legionella pneumophila is a ubiquitous intracellular bacillus which can readily infect pulmonary phagocytes, leading to serious and life-threatening pneumonia in humans known as Legionnaires’ disease, with serogroup I causing most cases of Legionnaires’ disease (19). Dendritic cells (DCs) are important phagocytic cells which link innate and acquired immunity (1). While Legionella resides in murine DC endoplasmic reticulum-derived organelles which are morphologically similar to those formed in macrophages, these DCs are able to restrict the growth of L. pneumophila, which may allow these cells ample time to present antigens to lymphocytes mediating immune responses against Legionella infection (15). In contrast to murine DCs, human DCs proficiently support the replication of L. pneumophila; lysis of the DCs does not occur for at least 24 h, allowing the cells time to participate in the transition from innate to adaptive immunity (16).

DCs are present in different stages of maturation in the circulation as well as in lymphoid and nonlymphoid organs (3). Specifically, immature DCs are present primarily in nonlymphoid tissues, where they exert an essential function. After antigen uptake, they migrate to the T-cell zone, where priming of naïve T cells occurs (7). After migration to lymphoid organs, DCs mature and increase their immunostimulatory properties by decreasing their antigen-capturing capacity (5, 6). The functional maturity of DCs reflects the up-regulation of cell surface major histocompatibility complex class I (MHC-I) and MHC-II and costimulatory molecules, especially ligands B7-1 (CD80) and B7-2 (CD86). The high levels of antigen-presenting and costimulatory molecules in a clustered distribution initiate the formation of immunologic synapses, bringing together essential elements such as T-cell receptors and CD28 required for T-cell activation (12). In particular, ligation of CD28 by its natural ligand CD86 induces the polarization of lipid rafts at the cell-CD86 interface of fresh human CD4+ T cells. Even in the absence of peptide–MHC–T-cell receptor ligation, CD86 appears to be necessary in terms of differential activation of T-cell signaling pathways (11). CD40, a member of the tumor necrosis factor receptor family, is also a costimulatory molecule important in the activation of DCs as well as B cells and macrophages (8). In the absence of costimulation by DCs, T cells become anergic (17).

In the present study, a virulent strain of L. pneumophila, obtained from a case of legionellosis from Tampa General Hospital (Tampa, FL), was cultured on buffered charcoal-yeast extract agar (Difco, Detroit, MI) medium and suspensions were prepared in pyrogen-free saline. DCs were prepared as described previously with several modifications (9). Briefly, bone marrow-derived DCs (BMDCs) were extracted from the femurs and tibias of BALB/c mice and the red blood cells lysed with ammonium chloride potassium bicarbonate lysing buffer to deplete the red blood cells. The cells were plated in six-well plates (10⁶ cells/ml) and cultured overnight in RPMI 1640 medium and 10 ng/ml granulocyte-macrophage colony-stimulating factor (BD, Farmington, San Diego, CA) to generate DCs from bone marrow precursors. Nonadherent cells were removed and the adherent cells incubated with fresh granulocyte-macrophage colony-stimulating factor for an additional 7 to 8 days. During this time, the DCs became nonadherent and were harvested. The cells were typically circular, and about 97% were CD11c⁺, indicating maturation of the DCs was evident. In particular, L. pneumophila infection increased the percentage of CD11c⁺ cells expressing the surface marker CD40 to 71%, compared to 13% for uninfected controls (Fig. 1). The expression of CD86-
FIG. 1. *Legionella* infection up-regulates MHC-I/MHC-II and costimulatory molecule expression on DCs. Flow cytometric dot plots of CD11c and costimulatory/MHC surface molecule expression. DCs were infected at 10 bacteria per cell and cultured at $1 \times 10^6$ cells/ml. Numbers in quadrants reflect percentages rounded to the next greatest whole integer. Results are representative of one of five independent experiments with similar results. Lp, *L. pneumophila*; FL, fluorochrome.
positive cells also increased after *L. pneumophila* infection compared to that for uninfected cells (68% versus 20%). The DCs infected with *L. pneumophila* showed greater MHC-I and -II expression and a larger and more granular appearance, indicative of a more mature phenotype. In particular, *Legionella* infection increased the percentage of CD11c+ cells expressing MHC-I to 80%, compared to 48% for uninfected controls, and increased the percentage of CD11c+ cells expressing MHC-II to 32%, compared to 14% for uninfected cells (Fig. 1). In addition, *L. pneumophila* infection increased the percentage of cells expressing surface TLR2 from 19% in controls to 64% for infected cells after infection of the murine DCs (Fig. 2). Similarly, infected cells induced the expression of the TLR4 surface marker compared to uninfected controls (43% versus 16%) (Fig. 2).

TLRs are expressed on DCs and are known to be involved in downstream effector functions, including the expression of co-stimulatory molecules (1, 18). *L. pneumophila* is a gram-negative pathogen and thus would be expected to activate TLR4, which is a receptor for other bacterial products (13). However, related studies suggest that TLR2, rather than TLR4, plays a prominent role in *L. pneumophila* infection since purified *L. pneumophila* lipopolysaccharides as well as *L. pneumophila*, either viable or formalin killed, are able to activate BMDCs from TLR4-deficient C3H/HeJ mice but fail to activate BMDCs from TLR2 knockout mice (4). In the present study, it was found that infection with viable *Legionella* resulted in marked up-regulation of TLR2 on DCs, and this may be related to TLR4, since microbial stimulation leads to NF-κB activation and the promoter region of the TLR2 gene from NF-κB consensus binding sequences up-regulates gene transcription (14). Inhibition of extracellular signal-related kinase or NF-κB has also been reported to suppress the induction of TLR4 and TLR2 mRNA expression in mouse DCs stimulated with lipopolysaccharide (2).

Contrary to our results, the expression levels of maturation surface markers CD40, CD86, and MHC-II were strikingly lower in DCs from A/J mice infected with live *L. pneumophila* than in noninfected cells (10). The differences between these

FIG. 2. *Legionella* infection up-regulates TLR2/TLR4 surface expression on DCs. DCs were infected at 10 bacteria per cell and cultured at 1 × 10^6 cells/ml. (A) Flow cytometric dot plots of CD11c and TLR2/TLR4 surface molecule expression. Numbers in quadrants reflect percentages rounded to the next greatest whole integer. The results shown are representative of one of three independent experiments with similar results. (B) Bar graphs of the percentage of CD11c+ and TLR2/TLR4 surface molecule expression. Data represent means ± standard deviations from three independent experiments. Asterisks indicate statistically significant differences (*P < 0.05 compared to non-*L. pneumophila*-infected cells). Lp, *L. pneumophila*; FL, fluorochrome.
results and ours may be related to the different strains of mice used. A/J mice are relatively more susceptible to \textit{L. pneumophila} infection, whereas the BALB/c mice used in this study are relatively resistant. The differing results also suggest that co-stimulatory and MHC-II up-regulation of BALB/c DCs may account for the increased resistance to infection with \textit{Legionella} in this mouse strain. Although not examined in the A/J model, TLR up-regulation may serve as an additional important factor in differences between the two strains in susceptibility to \textit{Legionella} infection.

In summary, our results show that \textit{Legionella} infection of DCs can significantly impact costimulatory and MHC molecule as well as TLR surface expression on DCs. These studies show that DCs are susceptible to immune modulation following \textit{L. pneumophila} infection, which is likely important in the transition from innate to adaptive immunity.

\textbf{REFERENCES}


\textit{Editor: A. D. O’Brien}