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The Pulmonary Host Defense System: of The Icos-Ligand B7-H2 Expression on Alveolar Epithelial Cells

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ABSTRACT

The mechanism of immune defense against pathogens in the lung has so far been poorly understood. Here, we show that human type II alveolar epithelial cells play a key role in defense via interactions between B7 homolog (B7h), also known as ICOS ligand, and its receptor ICOS expressed on activated T cells. The A549 alveolar type II cell line abundantly expresses B7-H2, CD40 and B7-1, but not B7-2 or hGL50. Tumor necrosis factor (TNF)-α significantly induced B7-H2 and CD40 expression by A549 cells, but had no effect on B7-1 or B7-2 expression. TNF-α-deficient mice exhibited low B7-H2 expression on alveolar epithelial cells in comparison with wild-type mice. Co-culture of TNF-α pre-stimulated A549 cells with CD4+ T cells promoted CD154 expression, CD4+ T cell proliferation and cytokine production, especially interferon (IFN)-α. Monocyte-derived TNF-α in combination with IFN-α and LPS markedly induced B7-H2 expression in A549 cells. This study thus identifies a unique co-stimulatory pathway via alveolar epithelial type II cells that preferentially affects CD4+ T helper cell function, implying that alveolar epithelial type II cells play a crucial role in innate immunity in the lung by regulating IFN-α-synthesis via B7-H2/ICOS interactions.

Keywords : B7-H2, Alveolar epithelial cell, CD4+ T cell, ICOS, IFN-γ

Introduction:

Bronchial and lung epithelial cells are in direct contact with the ambient environment; they form the first barrier of defense against inhaled pathogens and external challenges. Growing scientific evidence suggests that bronchial epithelium also functions in the regulation of the immune response through the production of cytokines and chemokines.1 Innate host defense, involving both cellular and humoral mediators, is a prominent function of the human airway. Pulmonary surfactant proteins A and D play dual roles in pulmonary homeostasis by both determining the structure of alveolar lipids and mediating the innate host defense system of the lung. In addition, cellular mediators of innate immunity include dendritic cells (DC), natural killer (NK) cells, CD8+ cytotoxic T cells and macrophages, while humoral mediators of innate immunity consist of epithelial fluid lining the airways. T cell co-stimulatory molecules, i.e. ICOS ligand, is an important co-stimulatory molecule on activated antigen presenting cells (APC), and binds to its receptor ICOS on T cell.2 Subtractive hybridization identified the human B7h as an ICOS ligand; furthermore, hGL50, B7-H2 and AB014553 have been isolated as three splice variants of human B7h.2-11 In the case of humans, resting B cells, DC, monocytes/macrophages, endothelial cells, renal tubular epithelial cells and muscle cells constitutively express hGL50, which contrasts the relatively low expression of B7-1 and B7-2.2,4,6,12,13 Human GL50 is expressed in low amounts on monocytes and is up-regulated by IFN-γ, but not TNF-α, LPS or CD40 ligation in monocytes.9,10 B7-H2 transcripts are widely distributed, and hGL50 variants are specifically transcribed with certain lymphoid tissue.11 In our present report, research findings demonstrated that B7-H2 expressed on human alveolar type II epithelial cell line A549 and mouse alveolar type II epithelial cell, and the communication of A549 cells and CD4+ T cells through the interaction of B7-H2 with ICOS markedly promoted proliferation and cytokine production, especially of IFN-γ by CD4+ T cells. Although the pulmonary host defense system is widely studied, the biological functions of alveolar epithelial cells, with lymphocytes that promote and regulate pulmonary host defense system in vivo remain poorly understood. Current study identified a unique co-stimulatory pathway mediated by type II alveolar epithelial cells that preferentially affects CD4+ T helper cell function, implying that alveolar epithelial type II cells play a crucial role in innate immunity in the lung by regulating IFN-γ synthesis via B7-H2/ICOS interaction.14

Experimental outline:

To demonstrate our contention that ICOS-ligand B7-H2, which is expressed on human type II alveolar epithelial cells, plays a role in pulmonary innate immune responses, we performed a series of in vitro experiments, with a human type II alveolar epithelial cell line and *in vivo* experiments, with TNF-α deficient mice. (I) To analyze the expression of B7 family proteins in A549 human type II alveolar epithelial cells and human primary B cells, RT-PCR was performed with primer sets specific for hGL50 or B7-H2. To examine the enhancement of B7-H2 mRNA expression by several stimuli including TNF-α, RT-PCR was performed using A549 cells under various culture conditions (II) To demonstrate that B7-H2 expression on normal type II alveolar epithelial cells significantly involved TNF-α, immunohistochemistry (IHC) with antibody to B7-H2 and RT-PCR using lung tissues obtained from wild type and TNF-α deficient mice was performed. To examine if TNF-α induces the enhancement of B7-H2 expression in vivo, intratracheal challenge of TNF-α deficient mice with TNF-α was performed. (III) The effect of cytokines (IL-2, IL-4, IFN-α, TNF-γ) and bacterial components (CpG, flagellin, LPS) on B7-H2 or other B7 family proteins on A549 cells were examined by FACS analysis (IV) Quantitative analysis on histological sections by Mac scope version 2 5 (Mitani Co, Ltd. Fukui, Japan) was performed to calculate the densities of B7-H2 positive mouse alveolar epithelial cells (V) To examine the inducibility of CD154, the CD40 ligand of ICOS stimulation,CD154 expression on the surface of T cells was examined on a FACS 24 hours after co-culture of activated CD4+T cells with TNF-α stimulated A549 cells. (VI) To investigate whether TNF-α induced NF-κB activation enhances B7-H2 expression in A549 cells, A549 cells were pre-treated with the NF-κB inhibitors MG-132 or NAC for 2 hours before TNF-α stimulation. The effect of MG-132 or NAC on B7-H2 expression was exam-

ined by FACS analysis. (VII) To determine whether B7-H2expressed on A549 cells promotes CD4+ T cell activation, we co-cultured human T cells with A549 cells and measured T cell proliferation and cytokine production (VIII) To examine whether monocyte-derived TNF-α can induce B7-H2 expression on the surface of A549 cells, A549 cells were cultured in the condition-medium derived from LPS/IFN-γ treated monocytes and the expression of B7-H2 on A549 was determined by FACS analysis.

Result and Discussion:

The mechanism of the defense against pathogens in the lung has so far been poorly understood. Here, we show that human type II alveolar epithelial cells play a key role in host defense via interactions between B7-H2, also known as ICOS-ligand, and its receptor ICOS expressed on activated T cells. A549 type II alveolar epithelial cell line abundantly expresses B7-1, but not B7-2 or hGL50 These cells also expressed B7-H2 and CD40 at considerable levels We found that TNF-α significantly augmented B7-H2 and CD40 expression. But had no effect on B7-l and B7-2 expression by A549 cells. TNF-α deficient mice exhibited lower B7-H2 expression on alveolar epithelial cells in comparison with wild type mice. Co-culture of TNF-α pre-stimulated A549 cells with CD4+T cells promoted CD154 expression, proliferation and cytokine especially IFN-γ production. Monocytes-derived TNF-α in combination with IFN-γ and LPS markedly induced B7-H2 expression On A549 cells. This study thus identified a unique co-stimulatory pathway via type II alveolar epithelial cells that preferentially affects T helper cell function, implying that type II alveolar epithelial cells play a crucial role in innate immunity in the lung by regulating IFN-γ synthesis via B7-H2/1COS interactions.

Bronchial epithelial cells may have the capacity to act as APC because they express not only MHC class I and class II molecules, but also co-stimulatory molecules. B7h and B7-1 were found to be constitutively expressed on the human type II alveolar epithelial cell line A549, although the biological function of B7h expression on airway epithelial cells is not yet clearly understood.1 Our findings are in agreement with a recent study that demonstrated a more lymphoid-restricted expression pattern of hGL50, compared to B7-H2 expression in all organs examined.11 A549 cells and other nonprofessional APC, such as endothelial and epithelial cells, display a unique profile of co-stimulatory molecules (B7-H2+, B7-1+, B7-2–), which is different from those of classic APC, such as macrophages (B7-H2+, B7-1+, B7-2+).6,12 Our research findings support our contention that in the course of lung infection, the interaction of type II alveolar epithelial cells with T cells via B7-H2/ICOS association may be an important biological function for the host defense system. It is likely that in response to endotoxin released from bacteria, alveolar macrophages secrete the inflammatory cytokine TNF-α, which up-regulates B7-H2 expression on human type II alveolar epithelial cells. ICOS-expressing T cells presumably interact with human type II alveolar epithelial cells through the association of B7-H2 with ICOS, thereby secreting more cytokines, especially IFN-γ, which finally activate alveolar macrophages to carry out the microbicidal functions.

Conclusion:

This research study shows that a proposed regulatory mechanism of type II alveolar epithelial cells in host defense The pulmonary host defense system is a multitiered immune system Each component of this system is essential in maintaining the sterility of the lung Studies on recently identified antimicrobial functions of lymphocytes and macrophages have greatly advanced our knowledge concerning the mechanisms underlying the microbicidal activates of the airways. Further investigation of these host-pathogen interactions with increase our understanding of pulmonary host defense against infectious disease, and provide direction for the development of novel therapeutic approaches.

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