B7 Molecules on Dendritic Cells after LPS Stimulation

Subjects: Immunology

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A key aspect of the inflammatory phenomenon is the involvement of costimulatory molecules expressed by antigen-presenting cells (APCs) and their ability to secrete cytokines to set instructions for adaptive immune response and to generate tolerance or inflammation. In a novel integrative approach, the evaluation of the kinetic expression of the membrane and soluble B7 costimulatory molecules CD86, ICOS-L, PDL1, PDL2 was presented, the transcription factor Interferon Regulatory Factor 4 (IRF4), and the cytokines produced by monocyte-derived dendritic cells (Mo-DCs) after challenging them with different concentrations of stimulation with *E. coli* lipopolysaccharide (LPS) for various lengths of time. The evaluation showed that the stimuli concentration and time of exposure to LPS are critical factors in modulating the dynamic expression pattern of membrane and soluble B7 molecules and cytokines.

B7 molecules Dendritic cells Cytokines IRF4 PDL1 PDL2 CD86 ICOSL LPS

1. Introduction

Whether an inflammatory response is evoked depends on different extrinsic and intrinsic characteristics of the antigen and the host, which together define immunogenicity [1]. One key aspect of the inflammatory response is the initiation of the adaptive immune response, which depends on the intertwined interaction between APCs and T cells[2]

Dendritic cells (DCs) are the primary antigen-presenting cells (APCs) due to their ability to sense, capture, process, and present antigens to T cells. The initiation and polarization of adaptative immune responses are well orchestrated by a display of signals, including the expression of membrane-bound costimulatory molecules, soluble costimulatory molecules, and the secretion of cytokines by DCs.[3][4] MHC/peptide–TCR interaction, costimulatory molecules' ligation with receptors, and cytokine production are the main phenomenon occurring in the immunological synapse [5] .

The best known is the B7 costimulatory family, belonging to the immunoglobulin superfamily (IgSF).[6] This family comprises costimulatory molecules that promote activation, polarization, and proliferation in T cells, such as CD86 and ICOS-L (inducible T cell costimulator ligand), and coinhibitory molecules that regulate the tolerance, suppression, and cellular death, such as PDL1 (programmed death ligand 1) and PDL2 (programmed death ligand

2).[7] Interestingly, previous studies have proven that soluble forms of B7 molecules (sB7) can be detected in different tissues and supernatants of cell cultures.[8][9][10][11] This finding improves our understanding of the mechanism of action independently of cell contact interactions. However, it is still unclear whether a costimulatory or coinhibitory profile of sB7 molecules prevails under the steady or activation states of dendritic cells and what signals are conveyed to the T cells.

Critical regulators at the transcriptional level for B7 molecules expression have been reported. For example, CD80, CD86, and ICOS-L transcription factors include NF-kB and PU.1.[12][13][14] Recently, a novel transcription factor, the Interferon Regulatory Factor 4 (IRF4), was reported to be related to the expression of PDL1 and PDL2.[15][16][17]

B7 costimulatory molecules have received attention due to their relevance in clinical conditions such as allergies, autoimmunity, cancer, and transplantation.[18] Nevertheless, little is known about the behavior of these molecules in terms of both counterparts (stimulation and inhibition) in physiological conditions since most *in vitro* studies have evaluated only single molecules and single time points or have focused on samples of patients with specific clinical conditions. [19][20][21]

In addition to the expression of B7 molecules, autocrine-secreted cytokines play a relevant role in the expression of costimulatory molecules. Previous studies have suggested that IL-6 downregulates CD86- and HLA-associated molecules, impairing T-cell activation capacity in DCs;[22] meanwhile, TNF-α favors the expression of these molecules (in a viral context).[23] On the other hand, IFN-γ has been shown to directly regulate PDL1 and PDL2 expression in melanoma cells.[24] However, no studies have evaluated the profiles of cytokine and costimulatory molecules integrally.

This study aimed to explore the dynamics of B7 costimulatory molecules CD86,ICOSL, PDL1, and PDL2 in an integrative way, encompassing the membrane-bound costimulatory molecules, the soluble B7 costimulatory molecules, and the cytokines found in the microenvironment. The present model proposed Mo-DCs as archetype APCs due to their feasibility of obtaining a significant yield from peripheral blood mononuclear cells (PBMNCs), as reported in previous studies [25][26][27]. On the other hand, the use of LPS as a model antigen has been widely explored due to its capacity to activate innate immune cells and induce the expression of costimulatory molecules and cytokine secretion by DCs [28][29].

2. Results

According to the findings, the researchers propose that B7 costimulatory molecules express differently in response to different antigen concentrations and stimulation times. Interestingly, the researchers observe an increased expression of the coinhibitory molecules PDL2 favored by IRF4 and PDL1 at an early stimulation time and under low antigen concentrations., and a second expression peak at 48h. The researchers also reaffirmed the proposed correlation between IRF4 and PDL2 expression [15][16]. Meanwhile, the costimulatory molecules CD86 and ICOS-L express at late times regardless of the antigen concentration. Similar to previous results, increased production of cytokines TNF-α, IL-6, and IL-10 can be observed at early times of stimulation, which may influence the PDL1 and

PDL2 expression. In contrast, cytokines related to T-cell activation, IFN-γ, and IL-2, had a maximum secretion peak at the most prolonged time evaluated, similarly to the CD86 and ICOS-L expression. Although correlation analysis only showed an association between IFN-γ and IL-2 with both CD86 and PDL1.

Concerning soluble forms of costimulatory molecules, the researchers found sPDL1 and sPDL2 to be the predominant soluble B7 forms in Mo-DC cultures over sCD86 in a 2:1 ratio, regardless of stimuli conditions in the culture. sICOSL was not detected in any of the conditions evaluated.

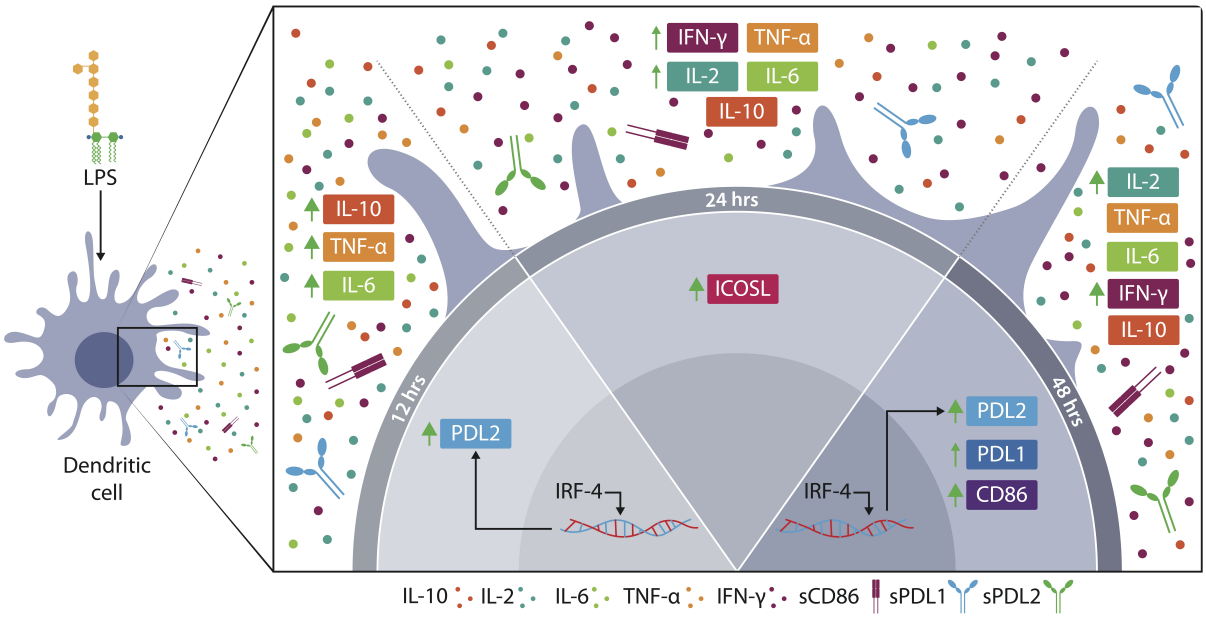

Figure 1. The behavior of B7 costimulatory molecules expression under LPS stimulation. Costimulatory molecules express in a differential fashion after LPS stimulation. Dendritic cells tend to increase PDL2 during the early stages of antigen exposition favored by IRF-4; ICOS-L expression is notable at 24 h. At 48 h, peak expression of molecules CD86 and PDL1 is observed. PDL2 and IRF-4 expression increase again at 48h. Regarding cytokines, TNF-α, IL-6, and IL-10 were found to increase at early stages; meanwhile, IFN-γ and IL-2 had a production peak at 48 h. Finally, soluble costimulatory molecules exhibited constant behavior regardless of the time of exposition or antigen concentration. sPDL1 and sPDL2 had twice the concentration of sCD86.

3. Conclusions

The data showed that Mo-DCs might be predisposed to respond silently and prone to initiating tolerogenic responses unless a threshold antigen concentration and stimulation time are reached, promoting B7 molecule activation and cytokine expression. The findings provide novel insights into dendritic cell function and allow a better understanding of its role in promoting or halting adaptative immune responses. Additionally, the knowledge of transcription factors, cytokines, and costimulatory molecules also provides a framework for future research in various clinical settings and the design of treatment strategies.

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