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Toll-like receptor (TLR)2 and TLR3 synergy and cross-inhibition in murine myeloid dendritic cells

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Abstract

Toll-like receptors (TLRs) play an important role in the innate recognition of pathogens by dendritic cells (DCs) and in the induction of immune responses. Few studies have been devoted to address the impact of TLR2 (a fully MyD88-dependent receptor) and TLR3 (a fully TRIF-dependent receptor) co-activation on DC functions, especially in the mouse system. Using canonical agonists, we show that TLR2 acts in concert with TLR3 to induce the synthesis of inflammatory cytokines (TNF-α, IL-6), of some IL-12 family members (IL-12p40, IL-12p23, IL-27p28) and of the Notch ligand Delta-4 by mouse DCs. In contrast, TLR2 interferes with the TLR3-induced expression of type I interferon stimulated genes (MIG/CXCL9, IP-10/CXCL10, GARG39) and IL-12p35. We also report that TLR2 cooperates with TLR3 to enhance the DC-mediated production of IFN-γ by Natural Killer cells and by conventional Ag-specific T lymphocytes. To conclude, our data support the existence of TLR2 and TLR3 synergy and cross-inhibition in DCs that could be important to strengthen immune responses during infection.

Keywords: Dendritic cell; Toll-like receptor; Cooperation; Inhibition; IL-12; ISG; Th1/Th2 response

1. Introduction

Mammalian Toll-like receptors (TLRs) represent important innate receptors able to distinguish distinct pathogen-associated molecular patterns from viruses, bacteria, fungi and parasites (for reviews, refs. [1–3]). Signalling through TLRs can be broadly categorized in two pathways linked to the downstream activation of nuclear factor (NF)-κB, mitogen-activated protein kinases and interferon (IFN) regulatory factors: the myeloid differentiation factor 88 (MyD88)-dependent pathway (exclusively utilized by TLR2, TLR5, TLR7, TLR8 and TLR9) and the Toll/IL-1 receptor-domain-containing adaptor inducing IFN-β-dependent (TRIF)-dependent pathway (exclusively utilized by TLR3) [3]. There is evidence that both pathways share intermediaries such as TNFR-associated factor 6 and NF-κB [4]. Notably, TLR4 exploits both pathways to signal and in particular to induce the expression of inflammatory (mainly MyD88-dependent) and IFN-stimulated genes (ISGs) (mainly TRIF-dependent).

During infection, the activation of TLRs in dendritic cells (DCs) has a pivotal functions in their maturation, a process

Abbreviations: TLR, Toll-like receptor; NF, nuclear factor; IFN, interferon; MyD88, myeloid differentiation factor 88; TRIF, Toll/IL-1 receptor-domain-containing adaptor inducing IFN-β-dependent; ISG, IFN-stimulated gene; DCs, dendritic cells; p(I:C), polyinosine-polycytidylic acid; WT, wild type; Ebi3, Ebstein-Barr-Virus-induced gene 3; Garg39, glucocorticoid-attenuated response gene 39; OVA, ovalbumin; NK, Natural Killer.

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important to trigger and control the strength and the quality of the innate and acquired immune responses (for reviews, refs. [5–7]). Although individual TLR activation in DCs generally allows them to acquire a Th1-promoting activity, some reports have also shown that, in some cases, the impact of TLR engagement on the nature of the immune response might be more flexible than initially thought [8–13]. Moreover, pathogens are generally recognized by multiple TLRs and this can greatly influence the maturation process of DCs as well as the innate/acquired responses they initiate (for review, ref. [14]). Using selective agonists, it was reported that MyD88-dependent TLRs cooperate with each others and in particular with TRIF-associated TLRs to promote the synthesis of inflammatory cytokines (TNF-α, IL-6, IL-12) [15–20]. However, the impact of their combined activation on ISG synthesis is more controversial and may dependent on the nature of the TLR agonists used and on the cell types utilized (human vs. mouse cells, DCs vs. macrophages). To investigate this more in depth, and since TLR cooperation that involves the dual activation of TLR2 and TLR3 might be relevant during infection (i.e. leishmaniasis, schistosomiasis) [21–23], we studied TLR2/TLR3 cross-talk in mouse DCs.

2. Materials and methods

2.1. Reagents and Abs

Pam_3CSK_4 was purchased from EMC Microcollections (Tuebingen, Germany) and polyinosinic:polycytidylic acid (p(I:C)) from Amersham Pharmacia Biotechnologies (Roosendaal, The Netherlands). Ovalbumin (OVA) was obtained from Sigma–Aldrich (Steinheim, Germany). Dynal Mouse CD4 Cell Negative Isolation Kit was from Invitrogen (Cergy-Pontoise, France). The monoclonal Ab against mouse CD3 (unlabelled) was purchased from BD Pharmingen (Le Pont de Claix, France). The neutralizing rat IgG1 directed against the mouse IL-10 receptor was provided by Dr. A. Herbelin (CNRS UMR 8147, Hôpital Necker, Paris). The neutralizing goat IgGs directed against mouse IFNAR1 and IL-12 was from R&D Systems (Abingdon, UK). The isotype control Abs was from Sigma–Aldrich (Steinheim, Germany).

2.2. DC activation assays

DCs (98% MHC class II^+, $CD11c^+$ pure) were generated from the bone marrow of wild type (WT) or TLR-deficient C57BL/6 mice as described previously [21]. DCs (1×10^6 cells/ml) were stimulated with Pam_3CSK_4 (0.5 μg/ml), p(I:C) (1 μg/ml) (or the combination of both) or were left untreated. In some cases, increasing concentrations of Pam_3CSK_4 (0.005, 0.05 and 0.5 μg/ml) and/or p(I:C) (0.01, 0.1 and 1 μg/ml) were used. After 16 h, culture supernatants were collected and IL-12p40, TNF-α, MIG/CXCL9 (R&D Systems) and IFN-β (PBL Biomedical Laboratories, Piscataway, NJ, USA) concentrations were measured by ELISA. Gene expression analysis was performed by real-time quantitative RT-PCR. Total RNA from BM-DCs (1×10^6 cells/ml) were isolated and cDNA were synthesized using standard procedures [24]. cDNAs were used as templates for PCR amplification using the SYBR® Green PCR Master Mix (Molecular Probes, Leiden, The Netherlands) and the ABI PRISM 7700 Sequence Detector (Applied Biosystems, Foster City, CA, USA). Primers specific for *Gapdh*, *Il-12p40*, *Tnf-α*, *Il-23p19*, *Il-27p28*, *Ebstein-Barr-Virus-induced gene 3* (*Ebi3*), *Il12-p35*, *glucocorticoid-attenuated response gene* (*Garg*)*39*, *Ip-10*/*Cxcl10*, *Ifn-β*, *Delta-4* and *Jagged-1* (Table 1) were designed by the Primer Express Program (Applied Biosystems) and used for amplification in triplicate assays. PCR amplification of *Gapdh* was performed to control for sample loading and to allow normalization between samples. ΔCt values were obtained by deducting the raw cycle threshold (Ct values) obtained for *Gapdh* mRNA, the internal standard, from the Ct values obtained for investigated genes. For graphical representation, data are expressed as fold mRNA level increase compared to the expression level in unstimulated cells.

2.3. Preparation of sorted NK cells and DC/NK cell co-culture

For sorting of Natural Killer (NK) cells, liver mononuclear cells were labeled with APC-conjugated anti-CD5 and PE-conjugated anti-NK1.1 Abs. After cell surface labelling, cells were sorted using a FACSAria (BD PharmMingen). Sorted

Table 1
Oligonucleotides for real-time RT-PCR

Genes[a]	Sense	Reverse
Gapdh	TGCCCAGAACATCATCCCTG	TCAGATCCACGACGGACACA
Il-12p40	TCCCCATTCCTACTTCTCCCTC	GGAACGCACCTTTCTGGTTACA
Tnf-α	TCTTCTCATTCCTGCTTGTGGC	CCTAACCCGTCTTGCTTGTGA
Il-23p19	AATCTCTGCATGCTAGCCTGG	GATTCATATGTCCCGCTGGTG
Il-27p28	GGAGGAGGACAAGGAGGAAG	AGGACACTTGGGATGACACC
Ebi3	CAATGCCATGCTTCTCGGTAT	GCCTGTAAGTGGCAATGAAGGA
Il-12p35	CACGCTACCTCCTCTTTTTG	CAGCAGTGCAGGAATAATGTT
Garg39	GCCATTGCGAACTACCGTCT	ACCGCGTCAAGCTTCAGTG
Ip-10/*Cxcl10*	TGGCAGCTGAGGTCTGTAAGG	CCAGAGAGGTTCTCCCGACTG
Inf-β	CAGGTGGATCCTCCACGCT	CATTCAGCTGCTCCAGGAGC
Delta-4	GGAAGTGGACTGTGGTCTGGAC	GAGGATACTTCCCAGGCATGC
Jagged-1	TGTAAGGAAGCGGCGGAAG	GTTGGCTCCGTGTTTCTCGA

[a] Generated from mouse genomic databases by PrimerExpress (Applied Biosystems).