

Expression and function of Toll-like receptors on dendritic cells and other antigen presenting cells from non-human primates

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Abstract

Antigen presenting cells (APCs), especially dendritic cells (DCs), play a crucial role in immune responses against infections by sensing microbial invasion through Toll-like receptors (TLRs). In this regard, TLR ligands are attractive candidates for use in humans and animal models as vaccine adjuvants. So far, no studies have been performed on TLR expression in non-human primates such as rhesus macaques. Therefore, we studied the TLR expression patterns in different subsets of APC in rhesus macaques and compared them to similar APC subsets in human. Also, expression was compared with corresponding DC subsets from different organs from mice. Here we show by semi-quantitative RT-PCR, that blood DC subsets of rhesus macaque expressed the same sets of TLRs as those of human but substantially differed from mouse DC subsets. Macaque myeloid DCs (MDCs) expressed TLR3, 4, 7 and 8 whereas macaque plasmacytoid DCs (PDCs) expressed only TLR7 and 9. Additionally, TLR expression patterns in macaque monocyte-derived dendritic cells (mo-DCs) (i.e., TLR3, 4, 8 and 9), monocytes (i.e., TLR4, 7, and 8) and B cells (i.e., TLR4, 7, 8, and 9) were also similar to their human counterparts. However, the responsiveness of macaque APCs to certain TLR ligands partially differed from that of human in terms of phenotype differentiation and cytokine production. Strikingly, in contrast to human mo-DCs, no IL-12p70 production was observed when macaque mo-DCs were stimulated with TLR ligands. In addition, CD40 and CD86 phenotypic responses to TLR8 ligand (poly U) in mo-DCs of macaque were higher than that of human. Despite these functional differences, our results provide important information for a rational design of animal models in evaluating TLR ligands as adjuvant *in vivo*.

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1. Introduction

In a vaccine setting for human use, optimal stimulation of the broadest immune responses may occur after targeting antigen presenting cells (APCs), especially dendritic cells (DCs). In addition to delivery of antigens, the efficacy of a vaccine can be improved by adjuvants that stimulate APCs (Pulendran, 2004). In this

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regard, DCs and other APCs can be activated through various Toll-like receptors (TLRs) (Akira et al., 2001; Iwasaki and Medzhitov, 2004). TLRs are pathogen-recognition receptors that recognize pathogens via pathogen-specific molecular patterns (PAMPs) (Takeda et al., 2003). Several ligands for TLRs have been identified, including lipoproteins and peptidoglycans for TLR2, double-stranded RNA of viral origin for TLR3, LPS from Gram negative bacteria for TLR4, flagellin, a protein found in bacterial flagella, for TLR5, single-stranded RNA and synthetic imidazoquinoline compounds for TLR7 and 8, and unmethylated CpG motifs found in microbial DNA for TLR9 (Iwasaki and Medzhitov, 2004). Detailed knowledge of TLR expression on APCs and subsequent functional consequences of TLR ligation will be useful for the development of adjuvant formulations.

The human immune system contains different types of APCs, including monocytes/macrophages, B cells, and two distinct subsets of DCs: plasmacytoid DCs (PDCs) and myeloid DCs (MDCs) (Banchereau and Steinman, 1998; Colonna et al., 2002). All APCs exhibit distinct expression patterns of TLRs, and may therefore be equipped to confront different pathogens and to dictate different immune responses. In humans, B cells and PDCs are the only immune cells that are known to express TLR9 and be activated by CpG oligodeoxynucleotide (ODN) (Krug et al., 2001), whereas TLR7 and 8 are found on B cells, PDCs and MDCs and on monocytes (Hornung et al., 2002; Jarrossay et al., 2001; Kadowaki et al., 2001; Krug et al., 2001). However, in mice, a commonly used animal model for testing novel adjuvant formulations, there are important differences in the TLR expression patterns on APCs as compared to humans. In mice, TLR9 is broadly expressed on all major DC subtypes (PDCs and MDCs) as well as in B cells, macrophages and monocytes (Edwards et al., 2003; Hemmi et al., 2000). Moreover, TLR8 does not appear to be functional in mice (Edwards et al., 2003). Overall, these differences affect the validity of mouse models for testing adjuvants, making direct extrapolation from mouse data to human difficult.

Based on their close relationship to humans, non-human primates such as rhesus macaques have proven to be valuable as animal models for testing vaccines and immunization strategies (Hu et al., 2005; Vitral et al., 1998). Moreover, macaque and human DCs exhibit comparable biology, with more recent work confirming the presence of MDCs and PDCs in circulation in macaques (Coates et al., 2003; Pichyangkul et al., 2004; Teleshova et al., 2004). Several groups have used TLR ligands as adjuvants in macaques, including immuniza-

tion of the antigen together with TLR3 (Ichinohe et al., 2005), TLR7/8 (Wille-Reece et al., 2005b), or TLR9 ligands (Klinman et al., 2004). However, so far, it is not known if a similar set of TLRs is present on APCs from human and macaque. Our aim was to establish whether macaque DC subsets and other APCs express TLR patterns similar to human and to study the functionality of the TLRs expressed on macaque APCs. We demonstrate here that macaque APCs express the same TLRs as compared to their human counterparts. However, the responsiveness of macaque APCs to certain TLR ligands activation partially differed from that of human concerning phenotype differentiations and cytokine productions.

2. Materials and methods

2.1. Antibodies and reagents

Directly conjugated monoclonal antibodies (mAbs) against CD45R(B220), CD20, CD11c, CD86, lineage markers (CD3, CD4, CD16, CD56 and CD20; Lin), CD83, and HLA-DR were purchased from BD Biosciences (San Jose, CA). PE-labeled mAbs against CD123 was purchased from BD PharMingen (San Diego, CA). PE-labeled mAbs against CD1c (BDCA-1) and the CD1c (BDCA-1) dendritic cell isolation kit (non-human primate) were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Polyinosinic-polycytidylic acid (poly(I:C)), ultrapure *Escherichia coli* K12 LPS, imiquimod (R837), and single-stranded poly U oligonucleotide complexed with LyoVecTM (ssPolyU/LyoVec) were purchased from InvivoGen (San Diego, USA). CpG ODN 2006 (TCGTGCGTTTGTGCGTTTGTGCGTT) and non-CpG ODN 2041 (CTGGTCTTTCTGGTTTTTTTCTGG) were obtained from Coley Pharmaceutical Group (Wellesley, MA).

2.2. Purification of macaque and human dendritic cell subpopulations

Peripheral venous blood was obtained from healthy human donors and adult rhesus monkeys (*Macaca mulatta*) maintained in accordance with guidelines of the Institutional Animal Care and Use Committee. Peripheral blood mononuclear cells (PBMCs) were obtained by centrifugation using Histopaque-1077 (Sigma-Aldrich, St. Louis, MO) and T cells were removed by rosetting with neuraminidase-treated sheep red blood cells (Pichyangkul et al., 2004), in case of cell isolation by fluorescence-activated cell sorting (FACS).

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