Available online at www.sciencedirect.com

SCIENCE DIRECT

Immunology Letters 98 (2005) 200–207

Immunology Letters

www.elsevier.com/locate/

Murine TLR2 expression analysis and systemic antagonism by usage of specific monoclonal antibodies

Guangxun Meng[a], Alina Grabiec[a], Mark Rutz[a], Jochen Metzger[b], Peter B. Luppa[b], Hermann Wagner[a], Stefan Bauer[a], Carsten J. Kirschning[a,*]

[a] Institute of Medical Microbiology, Immunology, and Hygiene, Technical University of Munich, Trogerstr. 9, 81675 Munich, Germany
[b] Institute of Clinical Chemistry and Pathobiochemistry, Technical University of Munich, Trogerstr. 9, 81675 Munich, Germany

Received 6 August 2004; received in revised form 16 November 2004; accepted 16 November 2004
Available online 8 December 2004

Abstract

Cellular recognition of immuno-stimulatory microbial products alarming the host immune system upon infection, as well as endogenous molecular patterns representing perturbation of regular homeostasis such as through necrosis of host cells is mediated by innate pattern recognition receptors to which toll-like receptors (TLRs) belong. A variety of agonists has been attributed to TLR2. We raised monoclonal antibodies (mAbs) toward the murine TLR2 extracellular domain (mT2ECD) in order to analyze murine TLR2 expression. Murine macrophages were stained TLR2-specifically with distinct mAbs as shown by flow cytometry, immuno precipitation, and immuno-cytochemical analysis. TLR2-specific murine macrophage activation was inhibited through pre-incubation with a mAb mT2.4 while another mTLR2-specific mAb mT2.7 did not affect cell activation through TLR2. Plasmon resonance based analysis showed inhibition of lipopeptide binding to mT2ECD if complex formation with mT2.4 preceded binding analysis. Systemic induction of IL-6, IL-12p40, and GROα/KC release to the serum upon lipopeptide challenge of mice was inhibited by systemic administration of mT2.4. Furthermore, 120 mg/kg of mT2.4 protected mice from lethal shock-like syndrome in an experimental low-dose model of septic shock. This result validates blockage of cell surface TLR2 for inhibition of immune cell over-activation upon microbial challenge.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Toll-like receptor (TLR) 2; Monoclonal antibody (mAb); Extra cellular domain (ECD); Antagonism

1. Introduction

In response to microbial challenge, first-line immune cells release pro-inflammatory mediators in order to initiate immediate-early host defense [1]. Coordinated activation of the adaptive by the innate immune system whose cells release inflammatory cytokines mediates comprehensive protection upon infection [2]. On the one hand, too low innate immune responsiveness to infection may prevent a sufficient immune defense allowing propagation of infecting microbes in the host. On the other hand, transgression of innate reaction strength beyond an intrinsic limitation can result in eventually fatal shock [3–5].

Within the super-family of pattern recognition receptors (PRRs) which bind exogenous ligands of microbial origin, toll-like receptors (TLRs) belong to a sub-group which mediates respective signals from outside into cells [6]. In contrast, the prototypic *Drosophila* receptor toll binds the endogenous protein spaetzle leading to cellular activation during embryonic development, as well as upon fungal and Gram-positive bacterial infection [7,8]. Identification of human TLR cDNA sequences was the prerequisite for their functional

Abbreviations: TLR, toll-like receptor; ECD, extracellular domain; PRR, pattern recognition receptor; PGN, peptidoglycan; LPS, lipopolysaccharide; LTA, lipoteichoic acid; P_3CSK_4, N-palmitoyl-*S*-(2,3-bis(palmitoyloxy)-(2*R*,*S*)-propyl)-(*R*)-cysteinyl-seryl-(lysyl)3-lysine; MALP-2, mycoplasmal macrophage activating lipoprotein 2; LRR, leucine-rich repeat motif; HEK293, human embryonic kidney cell line; PMA, phorbol 12-myristate 13-acetate; FC, flow cell; SPR, surface plasmon resonance

* Corresponding author. Tel.: +49 89 4140 4132; fax: +49 89 4140 4139.
E-mail address: carsten.kirschning@lrz.tum.de (C.J. Kirschning).

0165-2478/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.imlet.2004.11.015

implication including direct interaction with microbial products such as LPS [1,9–12]. Out of the 10 human TLRs described, only TLR10 remains an orphan receptor whereas specific microbial products have been attributed as ligands to all other TLR family members [13]. The largest variety of agonists has been implied for TLR2 [6]. Lipopeptides and lipoteichoic acid (LTA) aside from other agonists such as LPS and heat shock proteins (HSPs) stand out because properties of synthetic agonists already confirmed the principle of their TLR2-dependency [14–19]. TLR2-mediated host response mechanisms have been implicated in clinically important infections such as staphylococcal, mycobacterial, and pneumococcal infections [20–23]. Expression patterns, as well as regulation of TLR2 have been characterized and differential regulation of murine as compared to human TLR2 has been indicated [10,11,24,25]. Expression levels of TLR2 might be pronounced in immune cells such as macrophages as compared to other cells not primarily attributed to the innate immune system such as endothelial cells [26,27].

Here we describe characterization of 12 distinct TLR2-specific mAbs. All mAbs mediated detection of recombinant murine TLR2 extracellular domain (mTLR2ECD) protein by ELISA. The mAbs mT2.4 and mT2.7 stained overexpressed, as well as endogenous cell surface- and intracellular mTLR2, while mT2.4 but not mT2.7 antagonized specifically mTLR2-mediated cell activation. Systemic application of mT2.4 largely inhibited release of specific cytokines and GROα/KC to the serum and protected mice from lethal effects of challenge with lipopeptide and D-galactosamine.

2. Materials and methods

2.1. Reagents

Synthetic *N*-palmitoyl-*S*-(bis(palmitoyloxy)propyl)cysteinyl-seryl-(lysyl)3-lysine (P_3CSK_4) was purchased (EMC micro collections, Tuebingen, Germany) [28], synthetic mycoplasmal macrophage activating lipoprotein (R-MALP)-2 was from Dr. Muehlradt (Braunschweig, Germany) [29], ultra pure LPS of *Salmonella minnesota Re595* was from List Laboratory (Campbell, California, USA), recombinant murine IL-1β was from Peprotech (London, England), and phorbol 12-myristate 13-acetate (PMA) was from Sigma (Deisenhofen, Germany).

2.2. Mice

TLR2$^{-/-}$ [30] mice were kindly provided by Tularik (South San Francisco, California) and nine-fold crossed towards a C57BL/6 genetic background.

2.3. Generation of mouse TLR2ECD specific antibody and enzyme linked immunosorbent assay (ELISA)

We subcloned a cDNA fragment upon amplification from a murine monocytic cDNA library encoding the extracellular domain encompassing amino acid residues 25–587 of premature mouse TLR2 [31] (mTLR2ECD) into a mammalian expression vector enabling overexpression as a human Fc fusion protein (mT2ECD) with a thrombin cleavage site between the TLR2ECD and the C-terminal Fc moiety. For control, the mouse TLR9ECD was subcloned equivalently. The mT2ECD was found to be expressed and released to the cell supernatant upon overexpression in HEK293 cells. The supernatant of stably transfected HEK293 cells was collected and applied to a protein A column (Amersham, Freiburg, Germany). The mTLR2ECD portion was released from the column upon thrombin digest over night (o.n.) and collected by washing. Fifty micrograms of purified mTLR2ECD was intraperitoneally injected into a *TLR2*$^{-/-}$ mouse in the presence of 10 nmol of a thiolated DNA oligonucleotide 1668 (TIB MOLBIOL, Berlin, Germany) as adjuvant [32]. Antibody responses were boosted for two times by injection of the same amounts of recombinant protein as antigen and CpG DNA. Mice were sacrificed eight weeks after the first injection and hybridomas generated [33]. Briefly, spleen cells from immunized mice were fused with hypoxanthine aminopterin thymidine (HAT) sensitive P3X mouse bone marrow tumor cells, cultured in the presence of hybridoma fusion and cloning supplement (HFCS, Roche, Mannheim, Germany) and selected through addition of HAT to the cell culture medium. Hybridomas were subcloned three times by limiting dilution and their supernatants analyzed by sandwich ELISA. Antibodies were purified by applying hybridoma supernatants to protein A bead columns (Amersham). For identification of specific antibodies, ELISA plates were coated with goat anti human IgG Fcγ; antibody (Dianova, Hamburg, Germany), blocked and incubated with cellular lysates of HEK293 cells stably overexpressing the TLR2ECD. As negative controls, lysates of cells stably overexpressing TLR9ECD, as well as parental HEK293 control cells were applied simultaneously to different wells. Next, ELISA plates were incubated with hybridoma supernatants. Subsequently, horse radish peroxidase (HRP) coupled anti mouse IgG antibodies (Dianova) were applied and incubated prior to washing of the plates. HRP activity was measured spectrometrically upon enzymatic assay in a reader (Tecan, Crailsheim, Germany). For isotype analysis of antibodies identified as mouse TLR2 specific, separate wells of ELISA plates were coated with either anti mouse IgG1, IgG2a, IgG2b, or IgG3 antibodies (Dianova). MAb specificities for mTLR2ECD, as well as cyto- and chemokine concentrations in cell supernatants were analyzed by ELISA (R&D systems, Minneapolis, Minnesota, USA).

2.4. Flow cytometry

For light chain isotype determination, phycoerythrin labelled anti mouse IgGκ and fluorescein isothiocyanate labeled anti mouse IgGλ antibodies (BD Pharmingen, Heidelberg, Germany) were applied in flow cytometry analysis. Stably transfected HEK293 cell clones, RAW264.7 cells, as well as peritoneal wash-out macrophages were seeded and

Download English Version:

https://daneshyari.com/en/article/9266228

Download Persian Version:

https://daneshyari.com/article/9266228

Daneshyari.com