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Original article

Recognition via the class A scavenger receptor modulates cytokine secretion by human dendritic cells after contact with *Neisseria meningitidis*

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Abstract

Proinflammatory cytokines play a major role in the pathogenesis of meningococcal disease and their serum levels in patients are correlated with the outcome of infection. Dendritic cells initiate immunity against *Neisseria meningitidis* and are a major source of proinflammatory cytokines. Here we show that physical interaction of human DC with *N. meningitidis* via the class A scavenger receptor (SRA) modulates cytokine release by DC. Phagocytosis and uptake via SRA were shown to increase release of TNF-alpha, IL-1 beta and IL-6. In contrast, secretion of IL-8 is enhanced after recognition of *N. meningitidis* via SRA and not dependent on phagocytosis. Binding of *N. meningitidis* results in dephosphorylation of SRA but not in upregulation of SRA transcription. Unlike previously thought, not all meningococcal strains are recognized via SRA. A constitutively unencapsulated carriage isolate could be shown not to bind to SRA and induce proinflammatory cytokines independent of this receptor. In conclusion, recognition via SRA by dendritic cells is likely to play a central role in the immune response to *N. meningitidis*.
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Keywords: *Neisseria meningitidis*; Scavenger receptor; Cytokines

1. Introduction

Neisseria meningitidis, the meningococcus, is an important cause of bacterial meningitis and septicaemia in children and infants. Meningococci colonize the nasopharyngeal mucosa of approximately 10% of the healthy population. In rare cases they cross the epithelial barrier of their habitat, enter the bloodstream and may penetrate the blood–cerebrospinal fluid barrier to cause systemic disease [1]. The major virulence factor of *N. meningitidis* is a polysaccharide capsule, which shields meningococci from recognition and phagocytosis by host cells [2,3]. Capsule expression is – like many virulence associated traits in *N. meningitidis* – subject to phase variation [4]. Loss of capsule expression, which frequently occurs during colonization, is thought to favour intimate attachment of *N. meningitidis* to the nasopharyngeal epithelium [4]. Immature dendritic cells (DC) reside within this epithelium and represent a first line of defence against invading *N. meningitidis* [5]. DC are highly phagocytic cells and able to initiate and regulate primary immune responses [6]. After contact with invading pathogens, they undergo a process of phenotypic maturation and become potent antigen presenting cells migrating to secondary lymphoid organs. DC have been suggested to be a major source of proinflammatory and regulatory cytokines in meningococcal disease [7]. Cytokine production in meningococcal infection is directly correlated with severity of disease and high levels of cytokines are associated with lethality [8]. Toll-like receptors (TLRs) play a central role in recognizing *N. meningitidis* and mediating activation of DC and cytokine release. Meningococcal lipopolysaccharide (LPS) is a potent agonist of TLR4 [9]. Neisserial porin PorB is able to activate TLR2 [10]. Both TLR4 and TLR2 induce MyD88 dependent signalling and expression of inflammatory cytokines [11]. Despite their major role in sensing pathogens, TLRs do not play a role in phagocytosis. For *N. meningitidis*, class A scavenger receptor (SRA) has been identified as the main receptor for recognition and uptake by DC.

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However, *N. meningitidis* is protected from SRA mediated recognition by its capsule and full length LPS [12–14]. It has been shown that SRA mediated phagocytosis is essential for the induction of the regulatory cytokine IL-10 [13]. The aim of this study was to determine the influence of SRA mediated recognition on proinflammatory cytokine release by human DC.

2. Material and methods

2.1. Bacteria and mutant strains

Meningococcal strains used in this study were 2120 (serogroup C, ST-11c) [15], its isogenic Δ*siaD* mutant [13], MC58 (serogroup B, ST-32c) [16], its isogenic Δ*siaD* mutant [13] and the unencapsulated carriage isolate alpha14 (capsule null locus, ST-53) [17]. An Δ*lgtA* mutant of strain alpha14 was constructed as described previously [13]. Strains were stored at −80 °C, plated on GC agar (BD Difco, Heidelberg, Germany) supplemented with PolyVitex (BioMerieux, Marcy l'Etoile, France) and grown at 37 °C in 5% CO_2 for 24 h.

2.2. Cell culture

Generation of human DC from peripheral blood monocytes of healthy volunteers has previously been described [3]. DC precursors were cultured for 6–8 days in RPMI 1640 medium supplemented with glutamine, 100 ng recombinant human IL-4 (Strathmann Biotec, Hamburg, Germany), 800 U recombinant human GM-CSF (Immunex, Seattle, USA) and 10% heat inactivated fetal calf serum. Medium and cytokines were replenished twice during the incubation period. THP-1 cells (DSMZ: ACC16) were cultured in RPMI 1640 medium containing 10% FCS. For differentiation of THP-1 cells to macrophages, cells were seeded in a 6-well plate (1×10^6 cells/well in 2 ml RPMI 1640), treated with 20 ng/ml phorbol 12-myristate-13-acetate (PMA) for 24 h and further incubated for 48 h for complete differentiation.

2.3. Infection experiments

On day 7 non-adherent DC were collected and infection experiments were performed in 96-well microtitre plates. Immature dendritic cells (1×10^5) were seeded in 100 µl of RPMI per well. All experiments were performed in serum-free media as serum does not influence *N. meningitidis*–DC interaction [7]. Infection was performed with 10 bacteria/cell (MOI = 10). The number of non-adherent bacteria was assessed by plating three serial dilutions of the supernatants (10^{-3}, 10^{-4}, 10^{-5}) to GC agar. The number of adherent and viable intracellular bacteria was determined after removal of supernatants and subsequent washing (2× with PBS) by adding 200 µl of RPMI 1640 + 1% saponin and incubation at 37 °C for 15 min until complete lysis of the cells. Serial dilutions (10^{-3}, 10^{-4}, 10^{-5}) of the lysed-cell suspension, containing adherent and viable intracellular bacteria were plated on GC agar. The following inhibitors/additives were used when indicated: poly G (500 µg/ml, Sigma), poly C (500 µg/ml, Sigma), cytochalasin D (4 µg/ml, Merck), swinholide A (500 nM, Merck), colominic acid (up to 100 µg/ml, Sigma).

2.4. Cytokine assays

Supernatants from infection experiments were collected and immediately stored at −80 °C. Cytokine concentrations in the supernatants were measured with the Luminex technology (obtained from BioSource, Nivelles, Belgium) as described previously [13].

2.5. Immunoprecipitation

Cells (1×10^6) were infected with meningococcal strains (MOI = 10). At the indicated time points reaction tubes were put on ice, followed by a centrifugation step for 3 min, $500 \times g$ at 4 °C. Cell pellets were resuspended in 200 µl of ice cold TBS and then incubated for 10 min at 100 °C. Afterwards 200 µl of 2% Triton-X 100 in 2× TBS (2 mM EDTA, 2 mM PMSF, 10 mM NaF, 2× Complete from Roche) was added. Lysates were incubated for 30 min on ice and vortexed every 10 min for 5 s. Afterwards lysates were centrifuged for 15 min at 14,000 rpm, 4 °C and supernatants were transferred into a new tube. Equilibrated SRA–Antibody–Protein G-Sepharose complex was added and incubated overnight, rotating at 4 °C. Next day the precipitate was washed twice with 1% Triton-X 100 in 1× TBS for 10 min on ice. Finally beads with precipitates were resuspended with 20 µl 2× sample solution and boiled for 10 min. Ten microlitres of the samples were loaded on a 10% SDS–polyacrylamide gel. The following antibodies were used for Western blot analysis: rabbit α-human SRA A2403 (1:500, Santa Cruz Biotechnology), goat α-rabbit conjugated with HRP B2607 (1:5000, Santa Cruz Biotechnology), mouse α-phospho-Ser/Thr/Tyr 04230705 (1:500, Biomol), goat α-mouse conjugated with HRP 67374 (1:5000, Jackson Immuno Research).

2.6. Reverse transcriptase real time PCR

Total cellular RNA was extracted with Qiagen shredder tubes and RNeasy Mini (Qiagen) and reversely transcribed with QuantiTect reverse transcription kit from Qiagen. The resulting cDNA was analysed with SYBR Green (ABgene) reverse transcriptase real time PCR for scavenger receptor Class A (SRA) and GAPDH (housekeeping control). SRA primers were designed to cover all three isotype forms of SRA: forward 5′-TGC ACT GAT TGC CCT TTA C-3′ and reverse 5′-TCC TCT TCG CTG TCA TTT C-3′ (product size: 168 bp). GAPDH (glycerinaldehyde-3-phosphate-dehydrogenase) was amplified using the primers: forward 5′-GAT GAC ATC AAG AAG GTG GTG-3′ and reverse 5′-TCA TAC CAG GAA ATG AGC TTG-3′ (product size: 179 bp). The following conditions were used for amplification: denaturation for 15 s at 95 °C, annealing for 20 s at 56 °C and elongation for 20 s at 72 °C for 55 cycles in Roche LightCycler system. Quantification of mRNA was determined by relative comparison and normalization to the housekeeping control.

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