Interferon-gamma impairs phagocytosis of Escherichia coli by primary murine peritoneal macrophages stimulated with LPS and differentially modulates proinflammatory cytokine release

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A R T I C L E   I N F O

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A B S T R A C T

Introduction: Interferon-γ levels are increased upon viral infections and during inflamm-aging. Resistance to infections due to Escherichia coli (E. coli), a major cause of bacteriaemia and sepsis, is impaired in aged individuals, partly due to altered phagocytic capacity and cytokine release of immune cells. Here, we analyzed the effect of IFN-γ on phagocytosis of E. coli K1 and release of proinflammatory cytokines by macrophages in resting condition and upon stimulation with different bacterial Toll-like receptor (TLR) agonists.

Methods: Primary peritoneal macrophages from C57BL/6 mice were exposed to medium or stimulated with agonists of TLR4 (LPS), 1/2 (Pam3CSK4), and 9 (CpG-DNA) in the presence and absence of IFN-γ (100 U/ml) for 24 h. TNF-α, IL-6, and KC were measured in the cell culture supernatant by ELISA. Macrophages were exposed to viable E. coli K1. After 90 min, intracellular phagocytozed bacteria were quantified by quantitative plating.

Results: Macrophages treated with LPS 1 µg/ml in the presence of IFN-γ ingested more than 10-fold lower numbers of E. coli than macrophages treated with LPS alone. Phagocytosis of E. coli by macrophages in resting condition or upon stimulation with Pam3CSK4 or CpG was not significantly affected by IFN-γ. Cytokine release was differentially modulated by IFN-γ, with reduced KC release by TLR-stimulated macrophages in the presence of IFN-γ being the most striking effect.

Conclusions: In vitro, IFN-γ reduces the phagocytosis of E. coli by LPS-stimulated macrophages and differentially modulates cytokine release of macrophages activated by different bacterial TLR agonists. Elevated levels of IFN-γ might lead to reduced bacterial clearance and worse outcome of bacterial infections, e.g., in aged individuals and after viral infections and other inflammatory events.

1. Introduction

The type II interferon interferon-gamma (IFN-γ) is an important modulator of the innate immune system and involved in the host response to bacterial infections. Levels of IFN-γ and IP-10 (IFN-γ induced protein, CXCL10) are increasing during the aging process in serum and different tissues as part of a process named inflamm-aging [1]. Furthermore, elevated serum levels of IFN-γ and/or IP-10 have been detected in patients with frailty and sarcopenia [2], and after inflammatory events, e.g., viral infections, myocardial infarction and stroke [1,3,4]. IFN-γ modulates cytokine release by monocytes and macrophages [5] and has been shown to reduce phagocytosis of different pathogens including Streptococcus pneumoniae (S. pneumoniae), Staphylococcus aureus (S. aureus), and Escherichia coli [6,7,8].

E. coli is a major cause of bacteriaemia and sepsis, which is still associated with high mortality. Incidence of E. coli infections increases with age, and outcome is worse in elderly patients [9]. Altered phagocytic capacity and cytokine release of macrophages is supposed to be one reason for the impaired resistance to infections in aged individuals [10].

Here, we analyzed the effect of IFN-γ on phagocytosis of E. coli K1 and the release of proinflammatory cytokines by primary murine peritoneal macrophages in resting condition and upon stimulation with different bacterial Toll-like receptor (TLR) agonists.

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2. Material and methods

Cultivation and treatment of primary peritoneal macrophages from C57BL/6 mice was performed as previously described [10]. Briefly, peritoneal macrophages were harvested by peritoneal lavage with pre-cooled phosphate-buffered saline (PBS; 5 × 1 ml). Cells were collected by centrifugation (1000g, 10 min, 4 °C), and the pellet was resuspended in cell culture medium (DMEM with Glutamax I (Gibco Invitrogen, Karlsruhe, Germany) supplemented with 10% FCS). Cells were plated in 96-well cell culture plates at a density of 50,000 cells/well. After 2 h, culture medium was changed completely. After incubation overnight, cultured macrophages were treated with different TLR agonists (TLR-agonists) or medium (resting macrophages) for 24 h in absence or presence of IFN-γ (100 U/ml; Sigma, Taufkirchen, Germany). Tripalmitoyl-S-glyceryl-cysteine (Pam3CSK4; EMC Microcollections GmbH, Tübingen, Germany) was used as specific agonist of TLR1/2. For activation of TLR4, microglial cells were exposed to endotoxin (LPS) from TIB Molbiol (Berlin, Germany) was used as specific ligand of TLR9. A godesoxynucleotide (ODN) 1668 (TCC ATG A CG CGA TCT TGC ATG CT) from TIB Molbiol (Berlin, Germany) was used as specific ligand of TLR9.

Fig. 1. Phagocytosis of E. coli by resting peritoneal macrophages was not significantly influenced by treatment with IFN-γ (100(22/171) % with IFN-γ versus 44(22/74) % without IFN-γ, p = 0.06; n = 21/group; Fig. 1 A). Macrophages stimulated with LPS, a cell wall component of Gram-negative bacteria, ingested more than 10-fold lower numbers of E. coli in the presence of IFN-γ than macrophages treated with LPS alone [50 (42/111) % versus 526(156/2368) %, p < 0.0001; n = 23/group; Fig. 1 C]. The amounts of E. coli phagocytosed by peritoneal macrophages stimulated with the TLR1/2 agonist Pam3CSK4, a mimic of bacterial lipopeptide, or the TLR9 agonist CpG, a mimic of bacterial DNA, did not differ in the presence and absence of IFN-γ [Pam3CSK4: 22(16/44) % versus 44(19/519) %, p = 0.46; n = 8/group; Fig. 1 B / CpG: 256(12/472) % versus 188(39/267) %, p = 0.87; n = 8/group; Fig. 1 D).

Release of the three proinflammatory cytokines TNF-α, IL-6 and KC by primary murine peritoneal macrophages was quantified by measuring their concentrations in the cell culture supernatants. Cytokine release was differentially influenced by IFN-γ (Table 1). IFN-γ slightly enhanced TNF-α release only in resting macrophages (p < 0.0001) and did not influence TNF-α release in macrophages stimulated with agonists of TLR1/2, 4, and 9. In the presence of IFN-γ, IL-6 release

Concentrations of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and keratinocytes-derived chemokine (KC; the mouse equivalent of chemokine (C-X-C motif) ligand 1 (CXCL1)) in cell culture supernatants were measured by ELISA [10]. TNF-α levels were determined using antibody pairs from BioLegend (Biozol, Munich, Germany), and DuoSet ELISA Development Kits (R&D Systems, Wiesbaden, Germany) were used for the measurement of IL-6 and KC.

GraphPad-Prism-software 5.0 (GraphPad-Software, San Diego, California, USA) was used for statistical analyses and graphical presentation. All data are expressed as medians and interquartile ranges and were compared using the Mann-Whitney U test. P values < 0.05 were considered statistically significant.

3. Results

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was increased in macrophages stimulated with PamCSK4 (10 µg/ml; n = 8), LPS (1 µg/ml; n > 20), and CpG (10 µg/ml; n = 8) in absence and presence of IFN-γ (100 U/ml). [Medians (25th/75th percentiles); Mann-Whitney U test: ***p < 0.001, **p < 0.01, *p < 0.05].

| Cytokine | TLR agonist | IFN-γ | p | +IFN-γ | p |
|----------|-------------|-------|---|--------|---|
| TNF-α (pg/ml) | none | 29 (17/38) | 64 (43/116) | <0.0001*** | 1 (x) | 2.21 (0.38) |
| PamCSK4 (25th/75th percentile) | 1374 | 1592 | 0.09 | ** |
| LPS | 5255 | 4974 | 0.73 | ** |
| CpG | 94 (61/120) | 109 | 0.33 | ** |
| IL-6 (pg/ml) | none | 29 (15/31) | 17 (15/49) | 0.99 | ** |
| PamCSK4 (25th/75th percentile) | 772 (648/962) | 1634 | 0.0002*** | 1 (x) | 2.14 (2.14) |
| LPS | 10,084 | 7561 | 0.0004*** | 1 (x) | 0.75 (0.75) |
| CpG | 471 (453/514) | 1057 | 0.0002*** | 1 (x) | 2.24 (2.24) |
| KC (pg/ml) | none | 142 (25/210) | 60 (22/150) | 0.38 | ** |
| PamCSK4 (25th/75th percentile) | 10,258 | 4185 | 0.0002*** | 1 (x) | 0.41 (0.41) |
| LPS | 18,107 | 5833 | <0.0001*** | 1 (x) | 0.32 (0.32) |
| CpG | 342 (186/445) | 76 (68/184) | 0.01 * | 1 (x) | 0.22 (0.22) |

was reduced in LPS-stimulated macrophages (p < 0.0004), whereas it was increased in macrophages stimulated with PamCSK4 (p < 0.0002) and CpG (p < 0.0002). IFN-γ did not affect KC release in resting macrophages, but reduced KC release in macrophages stimulated with agonists of TLR1/2 (p = 0.0002), 4 (p < 0.0001), and 9 (p = 0.01).

4. Discussion

We have previously shown that stimulation of primary cultures of murine microglial cells and peritoneal macrophages by agonists of TLR1/2, 4, and 9, the principal TLRs activated during infections with Gram-negative bacteria, increases their ability to phagocyte E. coli and modulates their cytokine release pattern, including the release of TNF-α, IL-6, and KC [10,11,12]. In these in-vitro-experiments, co-treatment with IFN-γ was essential to induce a substantial release of nitric oxide (NO) [10,12] reflecting the activation of inducible NO synthase (iNOS) by IFN-γ. In the present in-vitro-study, we demonstrate a strong inhibitory effect of IFN-γ on E. coli phagocytosis by primary murine peritoneal macrophages stimulated with the TLR4 agonist LPS (Fig. 1). In the presence of IFN-γ, LPS-stimulated macrophages ingested more than 10-fold lower numbers of E. coli than in the absence of IFN-γ.

Our data complement previous studies demonstrating an impact of IFN-γ on bacterial phagocytosis by macrophages which appears to depend on IFN-γ dose, macrophage source, subtype, and activation state, as well as the type of bacteria [3,4,13]. These studies mainly demonstrated an impaired phagocytosis of S. pneumoniae or S. aureus by alveolar macrophages upon high IFN-γ levels [3,6]. In macrophages obtained from human PBMCs (peripheral blood mononuclear cells), pre-treatment with 420 U/ml IFN-γ – a more than 4-fold higher dose compared to the IFN-γ dose used in our study - time-dependently enhanced production of reactive oxygen species (ROS) upon stimulation with PMA (phorbol-12-myristat-13-acetate) and increased intracellular killing of S. aureus [14]. This might contribute the beneficial effects of IFN-γ on infections in patients with chronic granulomatous disease (CGD), an inherited disorder of the NADPH oxidase, rendering phagocytes unable to generate reactive oxygen species. Importantly, internalization of bacteria – the early step of phagocytosis which was addressed in our experimental setting - by human macrophages was not influenced by pre-treatment with IFN-γ [14]. Clearance of S. pneumoniae in mouse lungs was increased after intratracheal administration of low IFN-γ doses (10 ng) but decreased after administration of higher IFN-γ doses (10 µg) [4]. Considering the reported data from experimental models, elevated IFN-γ levels in individuals after influenza infection might be one cause of increased susceptibility to bacterial pneumonia in this patient group [3].

The pleitropic cytokine IFN-γ exerts its functions through transcriptional control of a variety of immunologically relevant genes [for review: [15]]. IFN-γ is a potent stimulator of suppressor of cytokine signaling (SOCS) 1 and 3, which both can inhibit inflammatory functions of macrophages by feedback inhibition of cytokines that use the Jak/STAT pathway [16]. Deletion of SOCS3 enhanced phagocytosis of E. coli by murine macrophages [16]. Thus, the inhibitory effect of IFN-γ on E. coli phagocytosis observed in our study might be partly due to the modulation of SOCS3 expression by IFN-γ. E. coli phagocytosis by monocyte-derived-macrophages was reduced upon treatment with IFN-γ by modulation of actin polymerization and cytoskeleton rearrangement [7]. IFN-γ inhibited phagocytosis of nonopsonized E. coli in unstimulated peritoneal macrophages, partly through the mTOR-c/EBP/mIRACO pathway [8,13]. In contrast, E. coli phagocytosis by resting peritoneal macrophages was not significantly influenced in our experiments, in which a 10-fold higher IFN-γ dose was administered. In addition to the previous studies, we investigated the influence of IFN-γ on the phagocytic ability of TLR-stimulated macrophages mimicking the situation during bacterial infections. Here, IFN-γ abandoned the LPS-induced increase of E. coli phagocytosis.

Releases of TNF-α, IL-6, and KC, representative for key cytokines in the host immune response to bacterial infections, were differentially modulated by IFN-γ in resting and TLR-stimulated peritoneal macrophages. In line with previously reported results in primary murine microglia [5], we found the reduction of KC release by TLR-stimulated peritoneal macrophages to be the strongest and most striking effect of IFN-γ. The chemotactic cytokine (chemokine) KC, the mouse equivalent of CXCL1, has neutrophil-attracting properties and improves bacterial control and survival of bacterial infections by regulating neutrophil homeostasis [17]. Thus, reduction of KC levels is supposed to impair the resistance to bacterial infections.

Low levels of IFN-γ appear to be important for the resistance to infections, e.g., urinary tract infections by E. coli [18] or S. pneumoniae pneumonia [4]. Low IFN-γ levels sensitize myeloid cells to subsequent IFN-γ stimulation, and higher IFN-γ concentrations provoke ligand-induced feedback inhibition and desensitization [19]. Therefore, IFN-γ levels exceeding a certain threshold presumably impair resistance to bacterial infections. Higher basal serum concentrations of IFN-γ have been detected in aged individuals, especially in persons with physical frailty and sarcopenia [1.2]. Viral or bacterial infections as well as inflammatory events caused by pathogenic agents (e.g., myocardial infarction and stroke) are associated with a further increase of IFN-γ serum levels [13,4]. IFN-γ-induced reduction of bacterial phagocytosis and KC release, which we observed in activated macrophages in vitro, might contribute to the increased susceptibility and the impaired outcome after bacterial infections in these patient groups. Analyzing the effect of IFN-γ inhibition, e.g., by using a neutralizing anti-IFN-γ antibody, on the clinical course of bacterial infections in aged mice seems promising.

As a side note it shall be mentioned that in murine cell culture
experiments, IFN-γ is used regularly as a co-stimulant [12]. Our present experiments emphasize that the direct IFN-γ effects on functions of immune cells must be considered for appropriate interpretation of results.

5. Conclusions

In vitro, IFN-γ reduces phagocytic capacity of LPS-stimulated macrophages and differentially modulates cytokine release of macrophages activated by bacterial TLR agonists. Elevated levels of IFN-γ might lead to reduced bacterial clearance and worse outcome of bacterial infections, e.g., in aged individuals and after viral infections and other inflammatory events. Thus, IFN-γ inhibition appears an interesting target for the prevention and therapy of bacterial infections, especially in these vulnerable patients.

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CRediT authorship contribution statement

Sandra Schütze: Conceptualization, Project administration, Supervision, Validation, Writing – original draft. Anniaka Kaufmann: Methodology, Writing – review & editing. Stephanie Bunkowski: Writing – review & editing. Sandra Ribes: Methodology, Supervision, Writing – review & editing. Roland Nau: Resources, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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