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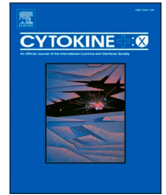

Interferon-gamma impairs phagocytosis of *Escherichia coli* by primary murine peritoneal macrophages stimulated with LPS and differentially modulates proinflammatory cytokine release

Sandra Schütze [a,b,*], Annika Kaufmann [a], Stephanie Bunkowski [a], Sandra Ribes [a], Roland Nau [a,c]

[a] *Institute of Neuropathology, University Medical Center Göttingen, Robert-Koch-Str. 40, D-37075 Göttingen, Germany*
[b] *Department of Geriatrics, Neurogeriatric Section, AGAPLESION Frankfurter Diakonie Kliniken, Wilhelm-Epstein-Str. 4, 60431 Frankfurt am Main, Germany*
[c] *Department of Geriatrics, Evangelisches Krankenhaus Göttingen-Weende, An der Lutter 24, D-37075 Göttingen, Germany*

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ABSTRACT

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 (Pam_3CSK_4), 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 phagozytosed 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 Pam_3CSK_4 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 (E. 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.

* Corresponding author at: Department of Geriatrics, Neurogeriatric Section, AGAPLESION Frankfurter Diakonie Kliniken, Wilhelm-Epstein-Str. 4, 60431 Frankfurt am Main, Germany.
E-mail address: sandra.schuetze@medizin.uni-goettingen.de (S. Schütze).

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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 (1000*g*, 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-stimulated macrophages) or medium (resting macrophages) for 24 h in absence or presence of IFN-γ (100 U/ml; Sigma, Taufkirchen, Germany). Tripalmitoyl-S-glyceryl-cysteine (Pam_3CSK_4; 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 *E. coli* serotype 026:B6 (Sigma, Taufkirchen, Germany). CpG oligodesoxynucleotide (ODN) 1668 (TCC ATG A**CG** TTC CTG ATG CT) from TIB Molbiol (Berlin, Germany) was used as specific ligand of TLR9. After 24 h of stimulation, supernatants were stored at −80 °C until measurement of cytokine levels, and a phagocytosis assay was performed using an *E. coli* strain K1 (serotype O18:K1:H7; originally isolated from a child with meningitis) as previously described [10,11]. Briefly, macrophages (50000 cells/well) were exposed to 5×10^6 colony forming units (CFU) *E. coli* K1/well (100 bacteria per macrophage) for 90 min. Then, extracellular bacteria were killed by treatment with 100 μg/ml gentamicin (Sigma, St. Louis, MO, USA) for 60 min. Cells were lysed with distilled water, and the number of intracellular bacteria was determined by quantitative plating of serial 1:10-dilutions on blood agar plates. For each experiment, the median number of *E. coli* phagocytosed by macrophages treated with IFN-γ without TLR-stimulation was taken as 100%.

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

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 Pam_3CSK_4, 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 [Pam_3CSK_4: 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

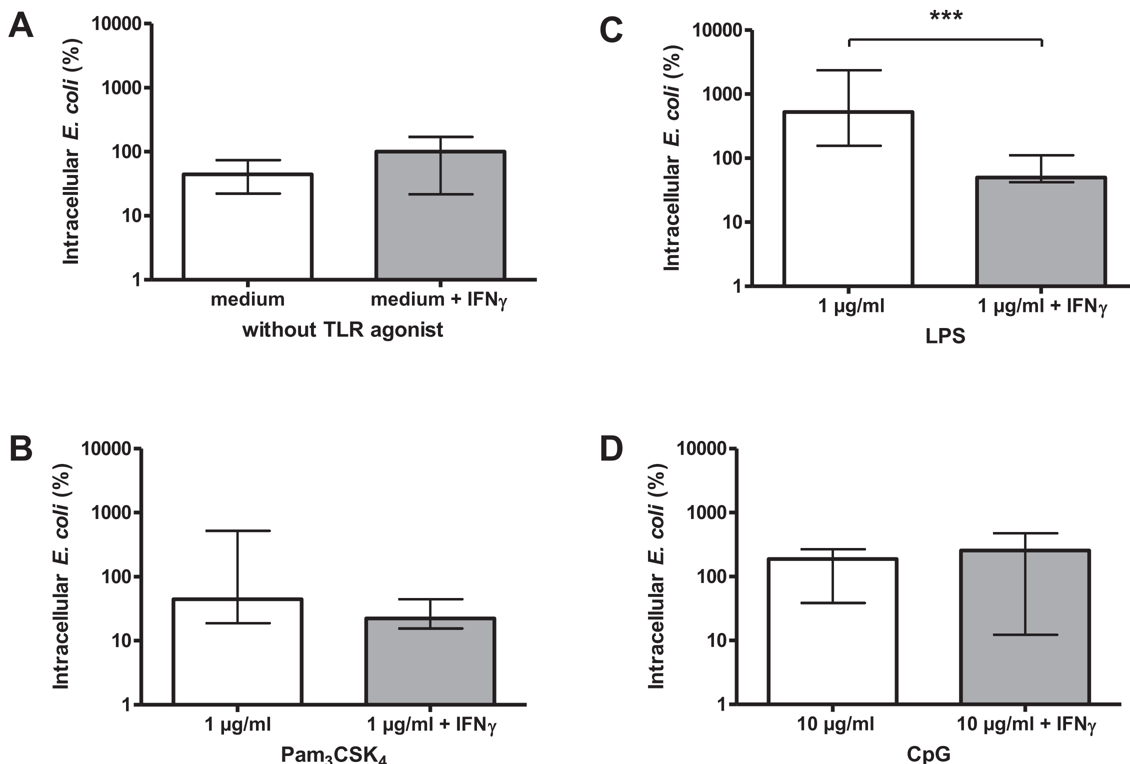

Fig. 1. Phagocytosis of *E. coli* K1 by primary murine peritoneal macrophages without TLR-stimulation (n = 21; **A**), and after stimulation with Pam_3CSK_4 (n = 8; **B**), LPS (n = 23; **C**), and CpG (n = 8; **D**) in the absence (left colums, white) and presence (right colums, grey) of IFN-γ (100 U/ml) in % (median phagocytosis rate of macrophages treated with IFN-γ only = 100%) [Medians (25th/75th percentiles); Mann-Whitney *U* test: ***p < 0.001].

Table 1
Release of TNF-α, IL-6 and KC by primary murine peritoneal macrophages (pg/ml cell culture supernatant) without TLR stimulation (n > 20) and after treatment with Pam_3CSK_4 (1 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	without IFN-γ	+ IFN-γ	p	+ IFN-γ/- IFN-γ
TNF-α (pg/ml) [median (25th/75th percentile)]	none	**29** (17/38)	**64** (43/116)	**<0.0001*** **	↑ (x 2.21)
	Pam_3CSK_4	1374 (1260/1539)	1592 (1383/1653)	0.09	↔
	LPS	5255 (4579/6049)	4974 (4642/5787)	0.73	↔
	CpG	94 (61/120)	109 (96/118)	0.33	↔
IL-6 (pg/ml) [median (25th/75th percentile)]	none	29 (15/31)	17 (15/49)	0.99	↔
	Pam_3CSK_4	**772** (648/962)	**1654** (1638/1684)	**0.0002*** **	↑ (x 2.14)
	LPS	**10,084** (9261/13244)	**7561** (6748/10231)	**0.0004*** **	↓ (x 0.75)
	CpG	**471** (453/514)	**1057** (792/1255)	**0.0002*** **	↑ (x 2.24)
KC (pg/ml) [median (25th/75th percentile)]	none	142 (25/210)	60 (22/150)	0.38	↔
	Pam_3CSK_4	**10,258** (8906/11457)	**4185** (3606/4814)	**0.0002*** **	↓ (x 0.41)
	LPS	**18,107** (16755/25360)	**5833** (3614/7190)	**< 0.0001*** **	↓ (x 0.32)
	CpG	**342** (186/445)	**76** (68/184)	**0.01***	↓ (x 0.22)

was reduced in LPS-stimulated macrophages (p < 0.0004), whereas it was increased in macrophages stimulated with Pam_3CSK_4 (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 phagocytose *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-acetat) 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. Remarkably, 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* particles 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/EBPβMARCO 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 inflammation caused by ischemic events (e.g., myocardial infarction and stroke) are associated with a further increase of IFN-γ serum levels [1,3,4]. 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. **Annika 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.

References

[1] N.M. Harris, M. Roy-O'Reilly, R.M. Ritzel, A. Holmes, L.H. Sansing, L.M. O'Keefe, L.D. McCullough, A. Chauhan, Depletion of CD4 T cells provides therapeutic benefits in aged mice after ischemic stroke, Exp Neurol. 326 (2020) 113202, https://doi.org/10.1016/j.expneurol.2020.113202.

[2] E. Marzetti, A. Anna Picca, F. Marini, A. Biancolillo, H.J. Coelho-Junior, J. Gervasoni, M. Bossola, M. Cesari, G. Onder, F. Landi, R. Bernabei, R. Calvani, Inflammatory signatures in older persons with physical frailty and sarcopenia: The frailty "cytokinome" at its core, Exp Gerontol. 122 (2019) 129–138, https://doi.org/10.1016/j.exger.2019.04.019.

[3] D.T.T. Hang, E.-J. Choi, J.-Y. Song, S.-E. Kim, J. Kwak, Y.-K. Yeun-Kyung Shin, Differential effect of prior influenza infection on alveolar macrophage phagocytosis of Staphylococcus aureus and Escherichia coli: involvement of interferon-gamma production, Microbiol Immunol. 55 (2011) 751–759, https://doi.org/10.1111/j.1348-0421.2011.00383.x.

[4] F.F. Hoyer, K. Naxerova, M.J. Schloss, M. Hulsmans, A.V. Nair, P. Dutta, D. M. Calcagno, F. Herisson, A. Anzai, Y. Sun, G. Wojtkiewicz, D. Rohde, V. Frodermann, K. Vandoorne, G. Courties, Y. Iwamoto, C.S. Garris, D.L. Williams, S. Breton, D. Brown, M. Whalen, P. Libby, M.J. Pittet, K.R. King, R. Weissleder, F. K. Swirski, M. Nahrendorf, Tissue-specific macrophage responses to remote injury impact the outcome of subsequent local immune challenge, Immunity 51 (5) (2019) 899–914.e7, https://doi.org/10.1016/j.immuni.2019.10.010.

[5] K.G. Häusler, M. Prinz, C. Nolte, J.R. Weber, R.R. Schumann, H. Kettenmann, U.-K. Hanisch, Interferon-gamma differentially modulates the release of cytokines and chemokines in lipopolysaccharide- and pneumococcal cell wall-stimulated mouse microglia and macrophages, Eur. J. Neurosci. 16 (2002) 2113–2122, https://doi.org/10.1046/j.1460-9568.2002.02287.x.

[6] K. Sun, D.W. Metzger, Inhibition of pulmonary antibacterial defense by interferon-gamma during recovery from influenza infection, Nat Med. 14 (2008) 558–564, https://doi.org/10.1038/nm1765.

[7] D. Frausto-Del-Río, I. Soto-Cruz, C. Garay-Canales, X. Ambriz, G. Soldevila, J. Carretero-Ortega, J. Vázquez-Prado, E. Ortega, Interferon gamma induces actin polymerization, Rac1 activation and down regulates phagocytosis in human monocytic cells, Cytokine 57 (1) (2012) 158–168, https://doi.org/10.1016/j.cyto.2011.11.008.

[8] Z. Wang, S. Zhou, C. Sun, T. Lei, J. Peng, W. Li, P. Ding, J. Lu, Y. Zhao, Interferon-gamma inhibits nonopsonized phagocytosis of macrophages via an mTORC1-c/EBPbeta pathway, J Innate Immun. 7 (2015) 165–176, https://doi.org/10.1159/000366421.

[9] Bonten M, Johnson JR, van den Biggelaar AHJ, Georgalis L, Geurtsen J, de Palacios PI, Gravenstein S, Verstraeten T, Hermans P, Poolman JR. Epidemiology of Escherichia coli bacteremia: a systematic literature review. Clin Infect Dis. 2020; doi: 10.1093/cid/ciaa210.

[10] S. Schütze, S. Ribes, A. Kaufmann, A. Manig, J. Scheffel, S. Redlich, S. Bunkowski, U.-K. Hanisch, W. Brück, R. Nau, Higher mortality and impaired elimination of bacteria in aged mice after intracerebral infection with *E. coli* are associated with an age-related decline of microglia and macrophage functions, Oncotarget 5 (24) (2014) 12573–12592, https://doi.org/10.18632/oncotarget.v5i2410.18632/oncotarget.2709.

[11] S. Ribes, S. Ebert, D. Czesnik, T. Regen, A. Zeug, S. Bukowski, A. Mildner, H. Eiffert, U.-K. Hanisch, S. Hammerschmidt, R. Nau, Toll-like receptor prestimulation increases phagocytosis of Escherichia coli DH5alpha and Escherichia coli K1 strains by murine microglial cells, Infect Immun. 77 (2009) 557–564, https://doi.org/10.1128/IAI.00903-08.

[12] S. Ebert, J. Gerber, S. Bader, F. Mühlhauser, K. Brechtel, T.J. Mitchell, R. Nau, Dose-dependent activation of microglial cells by Toll-like receptor agonists alone and in combination, J Neuroimmunol. 159 (1-2) (2005) 87–96, https://doi.org/10.1016/j.jneuroim.2004.10.005.

[13] J.T. Barbieri, T.S. Hamrick, E.A. Havell, J.R. Horton, P.E. Orndorff, Host and bacterial factors involved in the innate ability of mouse macrophages to eliminate internalized unopsonized *Escherichia coli*, Infect Immun. 68 (1) (2000) 125–132, https://doi.org/10.1128/IAI.68.1.125-132.2000.

[14] M.C. Greenlee-Wacker, E.M. Nauseef, IFN-γ targets macrophage-mediated immune responses toward Staphylococcus aures, J Leukoc Biol. 101 (2017) 751–758, https://doi.org/10.1189/jlb.4A1215-565RR: 10.1189/jlb.4A1215-565RR.

[15] K. Schroder, P.J. Hertzog, T. Ravasi, D.A. Hume, Interferon-γ: an overview of signals, mechanisms and functions, J. Leukoc. Biol. 75 (2) (2004) 163–189.

[16] H. Qin, A.T. Holdbrooks, Y. Liu, S.L. Reynolds, L.L. Yanagisawa, E.N. Benveniste, SOCS3 deficiency promotes M1 macrophage polarization and inflammation, J. Immunol. 189 (7) (2012) 3439–3448.

[17] S. Paudel, P. Baral, L. Ghimire, S. Bergeron, L. Jin, J.A. DeCorte, J.T. Le, S. Cai, S. Jeyaseelan, CXCL1 regulates neutrophil homeostasis in pneumonia-derived sepsis caused by *Streptococcus pneumoniae* serotype 3, Blood 133 (2019) 1335–1345, https://doi.org/10.1182/blood-2018-10-878082.

[18] Jones-Carson J, Balish E, Uehling DT. Susceptibility of immunodeficient gene-knockout mice to urinary tract infection. J Urol. 1999; 161:338-341.

[19] X. Hu, C. Herrero, W.P. Li, T.T. Antoniv, E. Falck-Pedersen, A.E. Koch, J.M. Woods, G.K. Haines, L.B. Ivashkiv, Sensitization of IFN-gamma Jak-STAT signaling during macrophage activation, Nat Immunol. 3 (2002) 859–866, https://doi.org/10.1038/ni828.