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C5a Regulates NKT and NK Cell Functions in Sepsis

Michael E. Fusakio,∗1 Javid P. Mohammed,∗1 Yves Laumonnier,† Kasper Hoebe,‡ Jörg Köhl,†,* and Jochen Mattner*‡

Complement, NKT, and NK cells play critical roles in the first line defense against pathogens. Functional roles for both C5a receptors, that is, complement receptor C5a (C5aR) and C5a receptor-like 2 (C5L2), in sepsis have been demonstrated. However, the role of C5a in innate lymphocyte activation during sepsis remains elusive. In this article, we show that naive NKT and NK cells already express high levels of C5aR and minor levels of C5L2 mRNA, but no protein. Upon Escherichia coli-induced sepsis, we found C5aR surface expression on subpopulations of NKT and NK cells, suggesting rapid translation into C5aR protein on bacterial encounter. Importantly, significantly increased survival in the absence of C5aR, NKT, and NK cells, but not of C5L2, was associated with reduced IFN-γ and TNF-α serum levels. Sepsis induction in C5aR−/−C5aR−− mixed bone marrow chimeras identified cognate engagement of C5aR on NKT cells as an important factor for the recruitment of NKT cells. Furthermore, we found synergistic interaction between C5aR and TLRs enhancing the production of TNF-α and IFN-γ from NKT and NK cells in cocultures with dendritic cells. Our results identify C5aR activation as a novel pathway driving detrimental effects of NKT and NK cells during early experimental sepsis. The Journal of Immunology, 2011, 187: 5805–5812.

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tivation of NKT and NK cells is observed upon infection with a broad range of pathogens including bacteria, viruses, parasites, and fungi. Cytokines, particularly IL-12 and IL-18, released from TLR-activated APCs during infection contribute to the activation of both cell populations (1, 2). However, although NKT cells recognize endogenous mammalian or exogenous bacterial glycosphingolipids (GSLs) presented by CD1d on APCs (3) through their conserved semi-invariant mouse CD1d homolog molecules, NK cells preferentially recognize a broad spectrum of antigens presented by MHC class I molecules (4). Unlike NK cells, however, NKT cells also produce TNF-α, a hallmark of their poised effector stage (5). Although NKT cells produce TNF-α at low levels, production increases upon stimulation with IL-12 and IL-18, including IL-18, released from TLR-activated APCs during infection (6).

NKT cells are a unique population of innate lymphocytes that is stress-reactive and activated by engagement of their invariant TCR and CD1d. NKT cells are the first cells of innate lymphocytes to be activated upon infection with pathogens and exhibit rapid cross-activation of NK cells upon NKT cell activation (12). The rapid cross-activation of NK cells upon NKT cell activation (12) implies that innate lymphocyte responses can augment each other dramatically under certain circumstances and in response to defined stimuli. However, the engagement of different receptors may contribute to the opposing effects of NKT cells in the respective models and may interfere with NK cell activation in various ways. Thus, these receptors and their respective effects on NKT and NK cells need to be identified and their mode of action on both cell populations defined.

Sepsis is associated with a strong activation of the complement system and the generation of the anaphylatoxins C3a and C5a in mice and humans (13). Both anaphylatoxins exert their biologic functions through binding and activation of their cognate G-protein-coupled receptors, that is, the C3a receptor and the complement receptor C5a (C5aR/C5L2). C5a and its primary degradation product, C5adesArg, can further bind to another seven-transmembrane receptor, C5a receptor-like 2 (C5L2), which is uncoupled from G proteins (13). In cecal ligation and puncture (CLP)-induced septic peritonitis, functional roles for both C5aR receptors have been demonstrated (14). However, because this is a model involving intestinal flora, the effects of defined bacterial species on the expression of C5aR and C5L2 and the subsequent effects of C5aR and C5L2 expression on the control of bacterial infection and the release of cytokines remain unknown. Up to now, the detrimental effects of C5a in sepsis have been mainly attributed to

*Division of Immunobiology, Cincinnati Children’s Hospital, Cincinnati, OH 45229; †Institute for Systemic Inflammation Research, University of Lübeck, 23538 Lübeck, Germany; ‡Division of Molecular Immunology, Cincinnati Children’s Hospital, Cincinnati, OH 45229; and ††Mikrobiologisches Institut-Klinische Mikrobiologie, Immunologie und Hygiene, Universitätshospital Erlangen and Friedrich-Alexander Universität Erlangen-Nürnberg, D-91054 Erlangen, Germany

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Address correspondence and reprint requests to Dr. Jörg Köhl or Dr. Jochen Mattner, Institute for Systemic Inflammation Research, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany (J.K.) or Mikrobiologisches Institut-Klinische Mikrobiologie, Immunologie und Hygiene, Universitätshospital Erlangen and Friedrich-Alexander Universität Erlangen-Nürnberg, Wasserturmstrasse 3/5, D-91054 Erlangen, Germany (J.M.). E-mail addresses: joerg.koehl@uk-sh.de (J.K.) and jochen.mattner@uk-erlangen.de (J.M.)

Abbreviations used in this article: BM, bone marrow; C5aR, complement receptor C5a; C5L2, C5a receptor-like 2; CLP, cecal ligation and puncture; DC, dendritic cell; αGalCer, α-galactosyl-ceramide; GSL, glycosphingolipids; wt, wild type.

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the paralysis of neutrophils (15). However, other cell populations may be involved in C5a-mediated effects. Because innate lymphocytes are not only a major cellular source of various cytokines, but also shape the subsequent adaptive immune response, we have assessed in this study the role of C5a in the activation of NK and NKT cells in an Escherichia coli-induced sepsis model.

We observed that NKT and NK cells from naive mice already express both C5a receptors, C5aR and C5L2, at the mRNA but not at the protein level. In vivo exposure to E. coli resulted in the rapid surface expression of C5aR protein on subpopulations of NKT and NK cells, which was associated with an enhanced expression of the early activation marker CD69.

C5aR deficiency resulted in a decreased expression of Nkp46 on NK cells and in a reduced release of IFN-γ and TNF-α by NKT and NK cells, together with an impaired recruitment of NKT and NK cells to the site of infection. Animal survival in response to E. coli-induced sepsis was significantly higher in the absence of C5aR, NKT, and NK cells. Improved survival correlated with reduced IFN-γ and TNF-α levels, suggesting that signaling through C5aR during sepsis contributes to the detrimental effects of NKT and NK cells. Sepsis induction in C5aR−/−C5aR−− mixed bone marrow (BM) chimeras identified cognate engagement of C5aR on NKT cells as a critical factor for NKT cell recruitment, whereas NK cell recruitment required preferentially C5aR engagement on dendritic cells (DCs).

Materials and Methods

Mice

C57BL/6 mice (B6) were purchased from The Jackson Laboratory. Lymphocytes were maintained in the animal facility of Cincinnati Children’s Hospital Medical Center. C5L2−/− mice were kindly provided by Dr. Craig Gerard (Harvard Medical School). All mice were raised in a specific pathogen-free environment according to the respective Institutional Animal Care and Use Committee guidelines.

Bacterial strains and live infection experiments

E. coli (ATCC 25922) were grown overnight in tryptic soy broth, diluted in PBS. A total of 1 × 10⁹ E. coli CFUs were injected i.p. (100 μl).

Purification of NKT and NK cells and co-stimulation assays

Lymphocyte preparations, cell staining, and sorting with CD1d-galactosyl-ceramide (αGalCer) tetramers were performed as described previously (3, 16). In brief, spleen cells were incubated with CD1d-galCC tetramers for 2 h at room temperature. Other mAbs, specific for either TCRβ, C5aR, or CD69, were then added and cells were incubated for 30 min on ice. Spleen cells, staining double positive for TCRβ and α-GalCer tetramer (NKT cells), or for Nkp46 and NK1.1 (NK cells) were purified using a FACSAria II (BD Bioscience) cell sorter resulting in purities >98 and >95% respectively. APCs were BM-derived GM-CSF (100 ng/ml; R&D Systems) cultured DCs (2.5 × 10⁶/200 μl/well). Spleen cells and cocultures of DCs with purified NKT or NK cells were stimulated with recombinant human C5a (100 nM; Sigma), 100 ng/ml Pam3CSK4, polyinosin-polycytidylic acid, LPS, standard flagellin of Salmonella typhimurium, FSL-1, or ssRNA-40 each, 10⁸ heat-killed E. coli (ATCC 25922) were grown overnight in tryptic soy broth, diluted in PBS. A total of 1 × 10⁹ E. coli CFUs and from uninfected control mice. After an initial peak of C5a within the first minutes postinfection (not shown), high C5a levels were detected 1 h later (Fig. 1A). Genetic deletion of C5aR resulted in improved survival in response to E. coli from 0 to 30% (Fig. 1B), similar to what had been reported in a CLP-induced sepsis model (14). The absence of C5L2 improved survival only marginally (Fig. 1B). Improved survival in C5aR−/− mice correlated with reduced serum levels of cytokines including IFN-γ and TNF-α (Fig. 1C). A detrimental role has been attributed to both cytokines during sepsis (20). Furthermore, NK and NKT cells together with myeloid cells are the major cellular sources for these two cytokines during the initial immune response (21). Genetic deletion of NK and NKT cells improved the survival of mice up to 50% (Fig. 1D), which was associated with decreased serum IFN-γ and TNF-α levels (Fig. 1E).

The absence of NK and NKT cells did not affect C5a plasma levels (Fig. 1F), excluding a role of both cell populations in modulating the generation of C5a. Collectively, these data suggest that 1) NKT and NK cells play an important role in the pathogenesis of E. coli-mediated sepsis; and 2) C5a contributes to the activation of NK and NKT cells and subsequent cytokine production during E. coli-mediated sepsis.

Differential regulation of C5aR and C5L2 expression in NKT and NK cells upon bacterial encounter

C5aR expression has been described on various cell populations including α/β and γ/δ T cells (22, 23). When we assessed C5aR expression by single-cell populations including α/β and γ/δ T cells (22, 23). When we assessed C5aR
and C5L2 expression on splenic NKT and NK cells from uninfected mice, we detected already multiple C5aR mRNA but only few C5L2 copies (Fig. 2A, 2B). Surprisingly, although C5aR preferentially modulated the survival rates (Fig. 1B), C5aR mRNA levels remained unchanged upon exposure to *E. coli*, whereas C5L2 mRNA copy numbers slightly but significantly increased.

As the survival of the mice was improved in the absence of C5aR, NK cells, and NKT cells, and as substantially more C5aR than C5L2 mRNA was detected in NKDtg and NK cells, C5a likely acts on NKT and NK cells preferentially via the engagement of C5aR. Therefore, we tested the regulation of C5aR protein expression upon infection by flow cytometry. Gating on CD1d-αGalCer tetramer+ TCRβ+ (Fig. 2C) and NK1.1+ Nkp46+ (Fig. 2D) cells in the spleen, we observed that only few, if any, NKT or NK cells expressed C5aR protein in naive, uninfected mice (Fig. 2C–E). However, the number of C5aR+ NKT and NK cells increased 24 h postinfection (Fig. 2C–E), indicating that C5aR mRNA is rapidly translated into C5aR protein.

C5aR deficiency reduces the activation and the recruitment of NKT and NK cells in response to *E. coli*

As shown earlier, the number of NKT and NK cells responding to C5a increases during infection. To investigate whether C5aR plays a role in the activation of both cell populations, we tested the expression of CD69, an early activation marker, on NKT and NK cells from wild type (wt), C5aR−/−, and C5L2−/− mice 4 h postinfection (Fig. 3A). As expected, CD69 was upregulated on NK (Fig. 3A, upper panel) and NKT cells (Fig. 3A, lower panel) from infected wt mice. In contrast, CD69 upregulation was impaired on NKT and NK cells from infected C5aR−/− mice, whereas C5L2 deletion had no impact on NKT and NK cell activation (Fig. 3A), as evidenced by CD69 expression levels similar to the ones observed in wt mice. The observation that C5aR is critical for early NKT and NK cell activation, and the fact that C5aR expression was comparable between NKT and NK cells from wt and C5L2−/− mice (6.51 ± 5.17 versus 8.84 ± 6.83% for NK and 4.86 ± 3.71 versus 4.22 ± 3.31% for NK cells in infected mice) suggested that NKT and NK cell activation by C5a is predominantly regulated through the engagement of C5aR and not C5L2, at least in the initial response phase to *E. coli*.

Because C5a is a well-known chemoattractant for leukocytes (13), we next evaluated the numbers of NKT and NK cells in the spleens, livers, peritoneal cavities, and the blood of uninfected and infected wt, C5aR−/−, and C5L2−/− mice. Although C5aR deficiency did not affect NKT and NK cell numbers in naive mice, we found markedly reduced numbers of NKT and NK cells in the spleen (Fig. 3B) and the peritoneal cavity (Fig. 3C) of C5aR−/−, but not of C5L2−/− mice 20 and 48 h postinfection when compared with wt mice. Although there was a tendency that C5L2−/− mice had a slight defect in recruiting NK cells (Fig. 3B, 3C), we concluded that C5aR preferentially regulates the recruitment of NKT and NK cells to the site of infection in response to C5a.

Cognate engagement of C5aR activates NKT cells during *E. coli sepsis*

To test whether cognate C5a–C5aR interactions are required for NKT and NK cell activation, we reconstituted irradiated B6 CD45.1 mice with a mixture of B6 CD45.1+ C5aR+/+ and B6 CD45.2+ C5aR−/− BMs. These mixed BM chimeras expressed similar numbers of CD45.2+ (47.2 ± 4.9) and CD45.1+ (52.1 ± 6.8) cells as determined by FACS analysis in the peripheral blood. Using CD45.1 and CD45.2 to distinguish cells of C5aR+/+ and C5aR−/− origin and knowing that NKT and NK cell numbers accumulate in the spleen postinfection, we observed significantly

![FIGURE 1](http://www.jimmunol.org/DownloadedFrom)
increased percentages of C5aR+ CD45.1+ NKT cells when the BM chimeras were exposed to E. coli 12 h before (Fig. 4A), suggesting that direct cognate engagement of C5aR on NKT cells affects NKT cell recruitment. Although there was a tendency that more C5aR+ NK cells were recruited, the effect was not statistically significant. Bystander effects through NK–DC interactions may play an important role here (see later). No significant differences in the percentages of NKT cells were detected in C5L2+/+ mixed BM chimeras (data not shown).

To test whether additional receptors are regulated on NKT and NK cells upon C5aR engagement, we evaluated a range of activating and inhibitory NK cell receptors including NKG2D, Ly49H, Nkp46, and Ly49D that are expressed on both cell populations. Nkp46 has been described as a killer receptor (24) critical for the elimination of influenza virus (25) and the production of IFN-γ.

Nkp46 expression was significantly reduced on NK cells from C5aR−/−, but not from wt mice (Fig. 4B) postinfection, correlating with a reduced systemic IFN-γ production in C5aR−/− mice (Fig. 1C, 1E). Thus, next to various bystander effects of sepsis-mediated immune activation, direct cognate C5a/C5aR interaction contributes to the activation of NKT and NK cells and, thus, to the detrimental effects of NKT and NK cells during sepsis.

**Engagement of TLRs and C5aR induces synergistic innate lymphocyte responses**

Cross talk between C5aR and different TLRs on APCs has been described (26). However, the interaction between α-GalCer, the prototypical NKT cell TCR ligand, and C5a has not been evaluated. To assess the direct functional impact of C5aR expression on NKT and NK cells, we tested the cytokine production from spleen cells of E. coli-infected B6 and C5aR−/− mice in response to different TLR ligands and the α-GalCer homolog, PBS-57 (27). Stimulation of spleen cells with PBS-57 or with different TLR ligands resulted in IFN-γ and TNF-α production (Fig. 5A, 5C). Importantly, IFN-γ production was significantly reduced in the absence of C5aR in response to ligands for TLR5, TLR9, and the
FIGURE 3. C5aR, but not C5L2, drives the early activation and recruitment of NKT and NK cells. A. Analysis of CD69 expression on NK (upper panel) and NKT cells (lower panel) in the spleen performed 4 h postinfection. n = 9/group. The experiment was repeated three times with similar results. B. Analysis of NK (upper panel) and NKT (lower panel) cell recruitment to the spleen performed 20 h postinfection. n = 4/group. The experiment was repeated once with similar results. C. Two days after i.p. E. coli infection, absolute NK (upper panel) and NKT (lower panel) cell numbers within the peritoneal cavity were determined. n = 7/group. The experiment was repeated once with similar results. Statistical significance was calculated using ANOVA. *p < 0.05.

combination of TLR1/2/6 or TLR1/2/6, TLR4, TLR5, and TLR9 (Fig. 5A). However, C5aR deficiency did not affect LPS-induced IFN-γ (Fig. 5A). TNF-α production was also significantly reduced, preferentially after stimulation with ligands for TLR1/2/6 or TLR1/2/6, TLR4, TLR5, and TLR9 (Fig. 5C). In contrast, we observed enhanced TNF-α production in C5aR−/− spleen cells in response to PBS-57, suggesting that C5a suppresses TCR-mediated induction of TNF-α from NKT cells. Thus, our data indicate that C5a may exert a dual role in NKT cell activation: it suppresses cognate Ag-mediated NKT cell activation, but synergizes with TLRs to drive the production of the proinflammatory cytokines TNF-α and IFN-γ.

FIGURE 4. C5aR engagement during E. coli sepsis directly activates NKT cells. A and B, Irradiated B6 CD45.1 mice were reconstituted with a 1:1 mixture of C5aR-deficient CD45.2 and C5aR-sufficient CD45.1 BM cells or reconstituted with a 1:1 mixture of C5L2-deficient CD45.2 and C5L2-sufficient CD45.1 BM cells (not shown). Representative FACS dot plots of NK and NKT cells 12 h postinfection are displayed. The expression of CD69 (A) and NKp46 (B) was measured on NKT (gated on TCRβ+ CD1d-tetramer+) and NK (NKp46+ NK1.1+) cells using CD45.1-specific Abs to assign their origin to wt or C5aR-deficient cells. A. Right graphs depict the frequencies of CD45.1+ C5aR+ (black bars) and CD45.2+ C5aR− (white bars) NK cells (n = 4). *p < 0.05. **p < 0.01.

FIGURE 3. C5aR, but not C5L2, drives the early activation and recruitment of NKT and NK cells. A. Analysis of CD69 expression on NK (upper panel) and NKT cells (lower panel) in the spleen performed 4 h postinfection. n = 9/group. The experiment was repeated three times with similar results. B. Analysis of NK (upper panel) and NKT (lower panel) cell recruitment to the spleen performed 20 h postinfection. n = 4/group. The experiment was repeated once with similar results. C. Two days after i.p. E. coli infection, absolute NK (upper panel) and NKT (lower panel) cell numbers within the peritoneal cavity were determined. n = 7/group. The experiment was repeated once with similar results. Statistical significance was calculated using ANOVA. *p < 0.05.
To directly test the contribution of NKT and NK cells to the TLR and C5aR-induced cytokine production, we stimulated spleen cells from C5aR−/−, NK/NK cell double-deficient and wt mice with different TLR ligands in the presence or absence of C5a (Fig. 5B, SD). The ligation of TLR1/2/6, TLR4, TLR5, and TLR9 resulted in IFN-γ and TNF-α production from wt mice. C5a stimulation enhanced the TLR1/2/6 and TLR9-driven production of IFN-γ (Fig. 5D), as well as the TLR1/2/6 and TLR5-driven production of TNF-α from wt mice (Fig. 5D). Surprisingly, the engagement of C5aR was essential for the LPS-mediated production of TNF-α in spleen cells similar to what we observed with NK/NK double-deficient cells. Because LPS can trigger the release of TNF-α preferentially by myeloid cells that constitute only a minor population in the spleen, C5aR engagement affects other cellular sources of TNF-α including NKT cells that fail to promote TNF-α production by LPS-stimulated myeloid cells. Altogether, NK/NK double-deficient spleen cells produced only minor amounts of IFN-γ and TNF-α on in vitro stimulation with TLR ligands in the presence or absence of C5a, suggesting that both innate lymphocyte populations are the major sources for IFN-γ and TNF-α in the early stages of sepsis.

C5a receptor signaling is critical for TLR-driven TNF-α production from NKT cells and for IFN-γ production resulting from NK and DC cell cross talk

Importantly, several other innate immune cells including DCs can produce TNF-α in response to TLR and C5aR stimulation. Furthermore, cross talk between NKT cells and DCs can affect the production of this cytokine. The comparison of monocultures of BM-derived DCs with cocultures of DCs and purified NKT cells from infected but not from uninfected mice suggests a synergism between NKT cells and BM-DCs for TNF-α production through combined C5aR engagement and TLR1/2/6, TLR5, or TLR9 ligation (Fig. 6A). Albeit significantly lower than in the cocultures with BM-DCs, NKT cells alone produced TNF-α in response to selected combinations of TLR ligands (Fig. 6A, right panel) correlating with the expression of TLR5, TLR6, and TLR7, together with TLR3, as determined by RT-PCR (data not shown). Importantly, this TLR-driven TNF-α production was almost completely absent in cells from C5aR-deficient mice, suggesting that cross talk between TLRs and C5aR is critical for genuine TNF-α production from NKT cells. Furthermore, IFN-γ responses could be induced in NKT cells in response to all TLR ligand combinations tested that were reduced in the absence of C5aR (data not shown). In contrast with PBS-57–mediated IFN-γ production, which was a direct effect of the NKT cell TCR engagement, TLR-induced IFN-γ was due to the release of IL-12 and IL-18 in response to TLR engagement on DCs (data not shown).

It has been reported that NK cells from naive mice do not respond to TLR9 ligation (28). Similarly, we found no IFN-γ production in monocultures of BM-DCs or NK cells in response to TLR activation (data not shown). In contrast, IFN-γ responses could be induced in cocultures of BM-DCs with purified NK cells from infected mice in response to TLR4 alone or in response to a combination of all tested TLR ligands (Fig. 6B). This TLR-induced IFN-γ production by NK cells was almost completely abolished in the absence of C5aR, suggesting that DCs drive NK cell activation upon C5aR engagement.

Discussion

Our data suggest a synergism for cytokine production by NKT and NK cells when synergistically activated by defined TLR ligands in combination with C5aR. The NKT and NK cell-mediated “cytokine storm” in this context likely contributes to the impaired survival during sepsis. Although C5aR engagement induces various bystander effects on NK cells through activation of DCs, cognate engagement of C5aR has a more prominent role on NKT than on NK cell recruitment. Thus, NKT cells may guide NK cells to follow them to the site of infection. This novel mechanism of complement-driven NKT cell activation with unique cytokine profiles may provide an explanation why NKT cells can exert suppressive and stimulatory properties (6, 7). Depending on whether TLRs are ligated in the context of complement activation,
NKT cells may exert distinct immunomodulatory effects on NK cells because of an altered magnitude of cytokine production.

Although TLR- and C5aR-mediated signals act synergistically on NKT cells, administration of α-GalCer appears to have the opposite effect for NKT cell-mediated TNF-α production. Thus, C5a-mediated signals may contribute to the activation and recruitment of NKT cells to the site of infection in an environment where multiple TLRs are ligated, whereas the engagement of the NKT cell TCR by GSL Ags may promote the attachment of NKT cells to the site of pathogen persistence. In this context, it has been reported that NKT cells crawl along liver sinusoids under physiologic conditions and rapidly arrest once they detect GSL Ags like α-GalCer (29). Similar mechanisms may apply for NK cells; NKG2D, for example, may detect infected NKG2D ligand-expressing cells and consequently lead to the arrest of NK cells at the site of infection. This may also explain why most of the NK cell receptors on NKT and NK cells are not regulated upon C5aR ligation. However, during sepsis, various receptors on NKT and NK cells will be engaged including the ones for C5a, and the sequence and hierarchy of these interactions need to be delineated in future studies. Although our data indicate that the absence of C5aR increased the amount of TNF-α released in spleen cell cultures stimulated with α-GalCer, it remains to be determined whether endogenous GSLs that are available at the site of infection in higher numbers than in uninfected tissues (30) can arrest NKT cells at a septic focus when various other stimuli are around. In fact, endogenous GSL Ags exhibit much lower stimulatory capacity than α-GalCer (3, 7).

Our data suggest that only C5aR activation directly triggers NKT and NK cell activation; however, the influence of the second C5a receptor, C5L2, needs to be evaluated in more detail during sepsis. The increase of TNF-α in spleens cells in C5aR-deficient mice in response to combined C5a and TLR5 or TLR1/2/6 engagement suggests synergistic effects between C5L2 and TLRs in our model (Fig. 5D). Similarly, recent studies have uncovered critical roles of C5L2 in inflammatory diseases such as CLP-mediated septic peritonitis (14) and allergic asthma (18). In fact, C5L2-deficient mice showed increased survival in a model of CLP-induced midgrade septic peritonitis. The minor survival of C5L2-deficient mice that we found in E. coli-mediated sepsis may result from the distinct experimental model. C5L2 is likely not to serve solely as a decoy receptor, as initially thought. C5L2 has been shown to affect C5aR signaling via the β-arrestin pathway in neutrophils, demonstrating that both receptors can be linked independent of G-protein–coupled pathways (31). Further elucidation of the connection between the two receptors for C5a is important to better understand the role of C5a at different phases of sepsis and to therapeutically target them.

In summary, this is, to our knowledge, the first report showing the expression of the two C5a receptors on NK and NKT cells and the requirement of C5aR engagement on NKT and NK cells for host survival under septic conditions. We show that C5aR signaling not only regulates TLR but also TCR-mediated cytokine production from NKT cells and the cross talk with DCs in sepsis highlighting the crucial role of C5a in disease pathogenesis. Thus, targeting of C5a will not only affect neutrophil dysfunction, cell apoptosis,

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**FIGURE 6.** Cross talk of C5aR and defined TLRs on DCs and NKT cells, as well as DCs and NK cells, drives synergistic TNF-α and IFN-γ production. A and B, C5aR and TLR-mediated cross talk between DCs and NKT (A, left panel) cells promotes early TNF-α production, whereas cross talk between DCs and NK cells (B) drives IFN-γ production in early E. coli-mediated sepsis. Sorted NKT cells (purity > 98%), NK cells (purity > 95%), as well as BM-derived DCs from infected B6 or C5aR−/− were exposed to the indicated TLR ligands. Cytokine release was determined 48 h later by ELISA. Monocultures of DCs (A, middle panel) and NKT cells (A, right panel) produced TNF-α. In contrast, neither monocultures of BM-derived DCs nor of purified NK cells produced IFN-γ (not shown). Statistically significant differences were determined using ANOVA. The experiment was repeated once with similar results. *p < 0.05, **p < 0.01.
tissue factor expression, and cardiomyopathy (32), but also the function of innate lymphocytes in sepsis.

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Disclosures

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