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The C5a Anaphylatoxin Receptor (C5aR1) Protects against Listeria monocytogenes Infection by Inhibiting Type 1 IFN Expression

Daniel G. Calame,*† Stacey L. Mueller-Ortiz,* John E. Morales,* and Rick A. Wetsel*‡

Listeria monocytogenes is a major cause of mortality resulting from food poisoning in the United States. In mice, C5 has been genetically linked to host resistance to listeriosis. Despite this genetic association, it remains poorly understood how C5 and its activation products, C5a and C5b, confer host protection to this Gram-positive intracellular bacterium. In this article, we show in a systemic infection model that the major receptor for C5a, C5aR1, is required for a normal robust host immune response against L. monocytogenes. In comparison with wild-type mice, C5aR1−/− mice had reduced survival and increased bacterial burden in their livers and spleens. Infected C5aR1−/− mice exhibited a dramatic reduction in all major subsets of splenocytes, which was associated with elevated caspase-3 activity and increased TUNEL staining. Because type 1 IFN has been reported to impede the host response to L. monocytogenes through the promotion of splenocyte death, we examined the effect of C5aR1 on type 1 IFN expression in vivo. Indeed, serum levels of IFN-α and IFN-β were significantly elevated in L. monocytogenes–infected C5aR1−/− mice. Similarly, the expression of TRAIL, a type 1 IFN target gene and a proapoptotic factor, was elevated in NK cells isolated from infected C5aR1−/− mice. Treatment of C5aR1−/− mice with a type 1 IFNR blocking Ab resulted in near-complete rescue of L. monocytogenes–induced mortality. Thus, these findings reveal a critical role for C5aR1 in host defense against L. monocytogenes through the suppression of type 1 IFN expression. The Journal of Immunology, 2014, 193: 000–000.

Listeria monocytogenes is a major cause of foodborne illness in the United States. A Gram-positive, facultative intracellular pathogen, L. monocytogenes is commonly found in soil, sewage, and ground water, and thus contaminates unpasteurized dairy products, raw meat, and produce. Its threat to the food supply is enhanced by its ability to survive and proliferate at low temperatures and under osmotic stress (1). The elderly and immunocompromised are particularly susceptible to the development of systemic infections that can result in sepsis, meningitis, and/or death. In pregnant women, L. monocytogenes may cross the placenta and infect the fetus, causing miscarriage, stillbirth, or neonatal meningitis. Although cases of listeriosis are relatively uncommon, the mortality rate (20–30%) is much higher than that of more common foodborne illnesses like salmonellosis are relatively uncommon, the mortality rate (20–30%) is much higher than that of more common foodborne illnesses like salmonellosis. As a consequence, L. monocytogenes is the third leading cause of death from food poisoning in the United States (2).

Ultimate clearance of L. monocytogenes is dependent on CD4+ and CD8+ lymphocytes (3, 4). However, a robust innate immune reaction must precede the lymphocyte response to provide early containment and initiate adaptive immunity. For example, studies in knockout mice have revealed that TNF-α, IFN-γ, and IL-6 are required early in the course of infection for mobilization and activation of neutrophils, monocytes, and macrophages (5–8). Although the type 2 IFN, IFN-γ, is critical in provoking early host protection to L. monocytogenes, type 1 IFNs are reported to impair the host response and create a permissive microenvironment to support L. monocytogenes growth. For example, mice deficient in the type 1 IFNR (IFNAR1) are highly protected against L. monocytogenes, containing substantially less bacteria in their spleens 72 h postinfection (9–11). Mechanistically, this difference has been linked to the ability of type 1 IFN to sensitize lymphocytes to apoptosis (10, 12). Substantial amounts of TUNEL+ cells are seen in the spleens of infected wild-type (WT) mice, whereas IFNAR1−/− mice have relatively few. Apoptotic cells reportedly induce the expression of the anti-inflammatory cytokine IL-10 in macrophages, which, in turn, represses innate immunity (12, 13). Macrophages have also been observed to undergo cell death in response to L. monocytogenes in a type 1 IFN–dependent fashion (14). A major downstream target of type 1 IFN is TRAIL. A member of the TNF superfamily, TRAIL is a well-recognized IFN response gene (15). It induces cell death by binding to the death receptors DR4 and DR5. TRAIL expression is induced during L. monocytogenes infection in a type 1 IFN–dependent fashion primarily on the surface of NK cells (9, 16, 17). TRAIL−/− mice resemble IFNAR1−/− mice in their enhanced containment of L. monocytogenes and reduced splenocyte depletion (16, 17).

An ancient and powerful arm of innate immunity is the complement system. L. monocytogenes triggers the alternative pathway of complement activation, resulting in its opsonization by C3b and release of the complement anaphylatoxins C3a and C5a (18–20). Several studies have shown an important role for C3 and its cleavage polypeptides in the host response to L. monocytogenes (19–24). In contrast, little is known about the contribution of C5 reaction must precede the lymphocyte response to provide early containment and initiate adaptive immunity. For example, studies in knockout mice have revealed that TNF-α, IFN-γ, and IL-6 are required early in the course of infection for mobilization and activation of neutrophils, monocytes, and macrophages (5–8). Although the type 2 IFN, IFN-γ, is critical in provoking early host protection to L. monocytogenes, type 1 IFNs are reported to impair the host response and create a permissive microenvironment to support L. monocytogenes growth. For example, mice deficient in the type 1 IFNR (IFNAR1) are highly protected against L. monocytogenes, containing substantially less bacteria in their spleens 72 h postinfection (9–11). Mechanistically, this difference has been linked to the ability of type 1 IFN to sensitize lymphocytes to apoptosis (10, 12). Substantial amounts of TUNEL+ cells are seen in the spleens of infected wild-type (WT) mice, whereas IFNAR1−/− mice have relatively few. Apoptotic cells reportedly induce the expression of the anti-inflammatory cytokine IL-10 in macrophages, which, in turn, represses innate immunity (12, 13). Macrophages have also been observed to undergo cell death in response to L. monocytogenes in a type 1 IFN–dependent fashion (14). A major downstream target of type 1 IFN is TRAIL. A member of the TNF superfamily, TRAIL is a well-recognized IFN response gene (15). It induces cell death by binding to the death receptors DR4 and DR5. TRAIL expression is induced during L. monocytogenes infection in a type 1 IFN–dependent fashion primarily on the surface of NK cells (9, 16, 17). TRAIL−/− mice resemble IFNAR1−/− mice in their enhanced containment of L. monocytogenes and reduced splenocyte depletion (16, 17).

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and its major activation fragments C5a and C5b. The A/J mouse is one of the most susceptible strains to infection with *L. monocytogenes* (25). This susceptibility is largely due to the absence of C5 protein caused by a 2-bp gene deletion in the 5’-exon of the structural gene encoding murine C5 (Hc locus) (26). The C5b fragment that initiates the formation of the C5b-9 complex is unlikely to be a factor in this susceptibility as Gram-positive bacteria are protected against membrane attack complex–mediated lysis by their thick layer of peptidoglycan (27). C5a is a 74-aa peptide that exerts its biological effects through a G protein–coupled receptor, C5aR1 (28). Classically described as an anaphylatoxin because of its ability to cause vasodilatation, histamine release, and smooth muscle contraction, C5a is widely considered to be a proinflammatory molecule. This stems from its anaphylactic and chemotactic properties, as well as its ability to enhance the expression of inflammatory cytokines like TNF-α, IL-6, and IL-1β (29–31). Accordingly, it seemed plausible that C5a might provide protection against *L. monocytogenes* by promoting the expression of cytokines needed for the early cellular immune response. To test this hypothesis, we used a model of systemic *L. monocytogenes* infection in WT and C5aR1−/− mice. Surprisingly, we found that although C5a/cytogenes, C5aR1 was not required for the early production of protective cytokines, including IFN-γ and TNF-α. Instead, C5a/C5aR1 protects the host from *L. monocytogenes* systemic infection through a previously unknown function of C5aR1, the suppression of type 1 IFN expression.

**Materials and Methods**

**Mice**

The C5aR1−/− mice used for these studies have been previously described (32). They were backcrossed for over 10 generations onto the C57BL/6 background. Age-matched C57BL/6 mice from our colony served as WT controls. All mice were housed in HEPA-filtered Techniplast cages in a pathogen-free barrier facility. Male mice between 11 and 14 wk of age were used in these studies. All mouse protocols followed institutional guidelines for animal care and welfare.

**Bacterial infection**

*Listeria monocytogenes* ATCC strain 13932 (MicroBioLogics), a clinical isolate, was used for all studies. Bacteria were cultured in Bacto brain heart infusion (BHI) broth at 37°C to midlogarithmic phase, pelleted by centrifugation, washed with PBS, and resuspended in PBS. Mice were infected i.v. with 1 × 10^5 bacteria in 100 μl PBS. Control mice received 100 μl PBS. The number of bacteria present in the inoculum was verified by culturing serial dilutions of the inoculum on Bacto BHI agar plates.

**Survival study**

Mice were infected i.v. with 5 × 10^4 *L. monocytogenes* and were observed every 6 h. Mice that showed signs of severe morbidity were euthanized. For rescue experiments, mice were infected i.p. with 1 mg of either the IFNAR blocking Ab, MARI-5A3 (BioXCell), or an isotype control Ab, MOPC-21 (BioXCell), 4 h before infection. Survival curves were generated using GraphPad Prism software, and statistical significance was assessed using the log-rank test.

**Bacterial burden in the liver and spleen**

After exsanguination from the inferior vena cava, the liver and spleen were dissected from mice at either 24 or 72 h, rinsed in PBS, and then placed in 2 ml HBSS. Organs were homogenized using a PRO200 homogenizer (ProScientific) on medium speed and were then placed on ice. Serial dilutions were plated on BHI agar plates to determine bacterial numbers per organ. Data are expressed as mean CFU per organ ± SEM.

**Spleen histology**

The whole spleen was dissected at 72 h, rinsed in PBS, and fixed in 10% neutral-buffered formalin for at least 24 h at 4°C. Organs were dehydrated, embedded in paraffin, cut into 5-μm sections, and stained with H&E. Brightfield images were taken using Spot Advanced software and a Zeiss Axioskop microscope (Carl Zeiss) equipped with a SPOT-RT digital camera (Diagnostic Instruments). For spleen histology, a 20× objective was used for a final magnification ×200.

**Measurement of caspase-3 activity**

Caspase-3 activity was measured in spleen homogenates using the CaspACE assay kit (Promega). In brief, dissected spleens were cut in half. One half was used to enumerate the number of cells in the spleen, whereas the other half was homogenized as described earlier. After clearing the homogenate by centrifugation, caspase-3 activity was measured as per manufacturer’s instructions. The measured activity was normalized by the number of cells per spleen (per 10^7 cells) and is reported as mean absorbance (A405) ± SEM.

**TUNEL staining**

TUNEL staining was quantified from single-cell suspensions of splenocytes using the HT TiterTACS Colorimetric Assay Kit (R&D Systems). In brief, spleens were removed from mice at 72 h and were dissociated into single-cell suspensions using a GentleMACS dissociator (Miltenyi Biotec). Suspensions were filtered successively through 70- and 40-μm filters. Erythrocytes were then lysed with ACK lysis buffer (Lonza). Total live cell numbers were determined by cell counts with a hemocytometer using trypan blue exclusion. An equal number of cells were then used for the TUNEL assay according to the manufacturer’s instructions, with the exception that the assay was performed in 1.7-ml tubes instead of a 96-well plate. The samples were transferred to a 96-well plate at the detection step. The data are reported as mean absorbance (A450) ± SEM.

**Flow cytometry**

Spleens were dissociated into single-cell suspensions using a GentleMACS dissociator. Suspensions were filtered successively through 100- and 40-μm filters. Erythrocytes were then lysed with ACK lysis buffer. Total live cell numbers were determined by cell counts with a hemocytometer using trypan blue exclusion. FeRs were blocked by incubation with an anti-CD16/32 Ab (BD Pharmingen). Cells were subsequently stained with Abs for CD4 (GK1.5), CD8 (53-6.7), CD19 (6D5), NK1.1 (PK136), CD11b (M1/70), CD11c (N418), Ly6G (1A8), Ly6C (HK1.4), and/or TRAIL (N2B2) (Biolegend). During the final wash step, DAPI (Invitrogen) was added as a viability dye. A minimum of 50,000 events was collected on a FACS Aria (BD Biosciences) flow cytometer. For TRAIL expression studies, 1,000,000 events were collected. Data analysis was done using the Kaluza program (Beckman Coulter). Dead cells were excluded from the analysis by gating on DAPI− cells. Data are expressed as mean cell number per organ ± SEM.

**Cytokine measurements**

Most cytokines and chemokines were measured in sera or clarified liver homogenates taken at 24 and 72 h by the Milliplex Mouse Cytokine/Chemokine 22-plex kit (#MPXMCYTO70KPMX22; Millipore) on the Luminex 200 system. Serum IFN-α and IFN-β levels at 24 h were measured using the VeriKine Mouse IFN-α ELISA kit and VeriKine Mouse IFN-β ELISA kit (R&D Systems), respectively, as per manufacturer’s instructions.

**FIGURE 1.** Impaired survival in C5aR1−/− mice during *L. monocytogenes* infection. For survival studies, WT and C5aR1−/− mice were infected i.v. with 5 × 10^6 CFU *L. monocytogenes* and followed for 2 wk. Data are pooled from two independent experiments. n = 9 for WT and n = 7 for C5aR1−/− mice. p = 0.0103 by log-rank test.
Statistical analysis
Statistical analysis was done with GraphPad Prism 5. All values are expressed as mean values with the SEM as error bars. For experiments involving two groups, data were analyzed via unpaired two-tailed t test. In experiments involving multiple groups, one-way ANOVA with the Tukey posttest was used to determine significance. Survival curves were analyzed by the log-rank (Mantel–Cox) test. The p values <0.05 were considered significant.

Results
C5aR1 deficiency results in increased susceptibility to L. monocytogenes
We began our assessment of the role of C5aR1 in host defense against L. monocytogenes with a survival experiment. WT and C5aR1−/− mice were injected i.v. with 5 × 10^4 CFU L. monocytogenes and then followed for 2 wk. At this dose, no mortality was observed in WT mice (9/9 survived). In contrast, ∼60% of C5aR1−/− mice succumbed to the infection within the first week (3/7 survived, p = 0.0103; Fig. 1). To determine whether C5aR1 contributes to the control of L. monocytogenes, we infected WT and C5aR1−/− mice, and then harvested livers and spleens at 24 and 72 h. At 24 h, a modest 2-fold elevation of L. monocytogenes was observed in the spleens of C5aR1−/− mice compared with WT mice (p = 0.0276; Fig. 2A). No difference in bacterial burden was observed in the liver between the two genotypes at this time (Fig. 2A). By 72 h, a marked difference in CFUs was observed between WT and C5aR1−/− mice (Fig. 2B). This elevation of bacterial burden roughly coincides with the onset of mortality in C5aR1−/− mice (Fig. 2A). By 72 h, there was a 43% reduction in splenocyte numbers compared with infected WT controls (p = 0.0097). In accord with the histological data, at 72 h, the infected C5aR1−/− mice had ∼80% fewer splenocytes than infected WT mice (p = 0.0007; Fig. 2B).

The depletion of splenocytes during listeriosis results primarily from caspase-3–dependent apoptotic cell death (9, 10, 17, 35). Caspase-3 is the key executioner caspase that initiates apoptosis and is often used to quantify apoptosis. Therefore, to test whether C5aR1 protects splenocytes from L. monocytogenes–induced apoptosis, we examined caspase-3 activity in spleen homogenates from PBS-treated and infected WT and C5aR1−/− mice. As expected, PBS-treated animals had little caspase-3 activity (Fig. 3C). In addition, splenocytes from infected C5aR1−/− mice exhibited significant increases in caspase-3 activity. Consistent with the data shown in Fig. 3A and 3B, infected C5aR1−/− mice had significantly more caspase-3 activity on a per-cell basis than infected WT mice (p < 0.0001; Fig. 3C). In addition, splenocytes

![FIGURE 2](http://www.jimmunol.org/) Defective bacterial clearance in C5aR1−/− mice during L. monocytogenes infection. WT and C5aR1−/− mice were infected i.v. with 1 × 10^3 CFU L. monocytogenes, and at 24 (A) and 72 h (B) spleens and livers were dissected, homogenized, and CFU per organ was determined. Data are pooled from two independent experiments and are presented as mean CFU per organ ± SEM. n = 9–12 mice per group per time point. *p = 0.0276, **p = 0.0010, ***p < 0.0001 by t test. n.s., not significant.
taken from infected C5aR1<sup>−/−</sup> mice were also significantly more TUNEL<sup>+</sup> than those from infected WT mice at 72 h (Fig. 3D). Taken together, these results indicate that C5aR1 protects against the destruction of splenocytes during listeriosis by limiting L. monocytogenes–induced apoptosis.

**C5aR1 broadly protects splenocytes in L. monocytogenes–infected mice**

The spleen consists of many types of immune cells that play different roles in the course of an infection. C5aR1 is thought to be expressed by many of them, albeit with considerable variation in expression levels. Recent work indicated a role for C5aR1 in promoting T cell survival both in vitro and in vivo (36, 37). Therefore, it seemed important to determine whether the increased cell loss observed in C5aR1<sup>−/−</sup> mice was specific for particular subsets of splenocytes. To test this, we dissociated spleens, counted the number of viable cells, and then used cell-surface staining along with a viability dye to identify the major subsets of live lymphocytes and myeloid cells. Similar numbers of B cells (CD19<sup>+</sup>), CD4<sup>+</sup> and CD8<sup>+</sup> T cells, NK cells (NK1.1<sup>+</sup>), neutrophils (CD11b<sup>+</sup>, Ly6G<sup>+</sup>/Ly6C<sup>+</sup>), monocytes (CD11b<sup>+</sup>, Ly6G<sup>−</sup>/Ly6C<sup>−</sup>), and DCs (CD11c<sup>+</sup>) were observed in uninfected WT and C5aR1<sup>−/−</sup> mice (Supplemental Fig. 1), and in infected mice at 24 h (Fig. 4A). However, by 72 h, every cell type examined was significantly reduced in C5aR1<sup>−/−</sup> mice relative to their WT counterparts (Fig. 4B). C5aR1 thus broadly protects against splenocyte depletion in listeriosis.

**C5aR1 is not required for the expression of protective inflammatory cytokines and chemokines**

Early resistance to L. monocytogenes infection has been attributed to the production of IFN-γ and TNF-α, as well as other ILs, cytokines, and chemokines important for the recruitment and activation of monocytes/macrophages and neutrophils (5–8). Because C5a potentiates inflammatory cytokine and chemokine expression in many models, we anticipated that the production of IFN-γ, TNF-α, and other cytokines/chemokines protective during listeriosis might be defective in C5aR1<sup>−/−</sup> mice. We therefore examined serum cytokine and chemokine levels in WT and C5aR1<sup>−/−</sup> mice at 24 and 72 h through the Luminex platform. Contrary to our expectations, the expression of protective ILs, cytokines, and chemokines were either similar or elevated in C5aR1<sup>−/−</sup> mice compared with WT mice as early as 24 h (Fig. 5). Cytokine and chemokine levels in the liver showed a similar pattern as those in the serum (Supplemental Fig. 2). Currently, it is not clear why the absence of C5aR1 during L. monocytogenes infection resulted in increased production of proinflammatory ILs, cytokines, and chemokines. Possibly, the increased L. monocytogenes infection that occurred in the C5aR1<sup>−/−</sup> mice caused elevated inflammation that was mediated by other compensatory mechanisms.
proinflammatory receptors. In any event, these data clearly indicate that there is no reduction of critical ILs, cytokines, or chemokines that would cause the increased \( L.\) monocytogenes infection observed in the C5aR1\(^{-/-}\) mice.

**C5a represses type 1 IFN expression in vivo**

Bacterial counts and spleen pathologies of \( L.\) monocytogenes–infected C5aR1\(^{-/-}\) mice were in many ways completely opposite to those observed in \( L.\) monocytogenes–infected IFNAR\(^{-/-}\) mice (9–12). For example, IFNAR\(^{-/-}\) mice have significantly enhanced bacterial clearance (less CFUs) at 72 h. In contrast, at 72 h, C5aR1\(^{-/-}\) mice exhibited a dramatic increase in bacterial counts compared with WT mice. In addition, IFNAR\(^{-/-}\) mice are protected against splenocyte death, whereas C5aR1\(^{-/-}\) mice have greatly enhanced splenocyte depletion. These opposing parallels led us to suspect that C5a/C5aR1 might inhibit the type 1 IFN pathway in listeriosis. We therefore examined type 1 IFN expression in \( L.\) monocytogenes–infected WT and C5aR1\(^{-/-}\) mice. There are two major types of type 1 IFN, IFN-\(\alpha\) and IFN-\(\beta\). In \( L.\) monocytogenes models, IFN-\(\alpha\) expression is partially dependent on IFN-\(\beta\), and type 1 IFN expression peaks at 24 h (38–40). At this time point, both IFN-\(\alpha\) and IFN-\(\beta\) were significantly elevated in the serum of C5aR1\(^{-/-}\) mice \((p \leq 0.0004; \text{Fig. 6A})\). As in prior reports, serum IFN-\(\beta\) levels were quite low (40). However, a consistent difference was seen between WT and C5aR1\(^{-/-}\) mice.

TRAIL is a type 1 IFN response gene and an important mediator of splenocyte depletion during \( L.\) monocytogenes infection (15–17). A member of the TNF superfamily recognized for its ability to trigger apoptosis in immune cells, TRAIL expression is restricted to the surface of NK cells at 72 h (16). To determine whether the elevation of type 1 IFN observed in C5aR1\(^{-/-}\) mice was biologically significant, we examined TRAIL expression on NK1.1\(^{+}\) NK cells by flow cytometry. In PBS-treated animals, little to no TRAIL\(^{+}\) NK cells were observed (Fig. 6B, 6C). By 72 h, a small fraction of NK cells was TRAIL\(^{+}\) in WT animals. The percentage of TRAIL\(^{+}\) NK cells was significantly higher in C5aR1\(^{-/-}\) mice \((p = 0.0001; \text{Fig. 6B, 6C})\). These data therefore show that C5aR1 inhibits type 1 IFN expression and its downstream target TRAIL during listeriosis.

**Blockade of the IFNAR1 rescues C5aR1\(^{-/-}\) mice**

IFN-\(\alpha\) and IFN-\(\beta\) bind and signal through a common receptor composed of the subunits IFNAR1 and IFNAR2 (41). If dysregulation of type 1 IFN expression is indeed responsible for the mortality observed in C5aR1\(^{-/-}\) mice, then inhibition of the type 1 IFN axis should rescue them. To test this hypothesis, we administered either an isotype Ab or an IFNAR1 blocking Ab (MAR1-5A3) i.p. 4 h before i.v. infection (42). Similar to our earlier survival study, at a dose of \( 5 \times 10^{7} \) CFU, WT mice showed no mortality (12/12 survived), whereas C5aR1\(^{-/-}\) mice were highly susceptible to \( L.\) monocytogenes infection (Fig. 7). C5aR1\(^{-/-}\) mice given MAR1-5A3 were almost completely rescued (13/14 survived), whereas an isotype Ab failed to rescue them (3/14 survived; \( p < 0.0001 \)). Taken together, our data suggest that C5a protects mice during listeriosis through the repression of type 1 IFN expression.

**Discussion**

In this study, we have demonstrated that the complement anaphylatoxin receptor C5aR1 protects mice against \( L.\) monocytogenes through the inhibition of type 1 IFN expression. The absence of C5aR1 in mice resulted in increased mortality and bacterial burden in the liver and spleen. Splenocyte depletion, a major feature of listeriosis that impedes the host response to.
L. monocytogenes, was sharply elevated in L. monocytogenes–infected C5aR1<sup>−/−</sup> mice. This splenocyte depletion was associated with increased caspase-3 activity in the spleens of C5aR1<sup>−/−</sup> mice, suggesting that it resulted from elevated L. monocytogenes–induced apoptosis. Because L. monocytogenes causes lymphocyte apoptosis in a type 1 IFN–dependent fashion, we examined serum IFN-α and IFN-β levels at 24 h and found that both were significantly elevated in C5aR1<sup>−/−</sup> mice in comparison with WT mice. This elevation was associated with increased NK expression of TRAIL, a type 1 IFN-response gene and a major driver of L. monocytogenes–induced splenocyte depletion. Finally, we showed that blockade of IFNAR rescued C5aR1<sup>−/−</sup> mice from L. monocytogenes–induced mortality, thereby demonstrating that the elevation of type 1 IFN seen in C5aR1<sup>−/−</sup> mice is responsible for their increased susceptibility to L. monocytogenes.

During the past few years, there has been growing evidence that C5a is critical in protecting cells from damage during tissue regeneration by providing prosurvival/antiapoptotic signals via C5aR1 (42). For example, when C5aR1-deficient mice are subjected to liver injury, they exhibit severe hepatic apoptosis, preventing normal liver regeneration (43). In this model, C5a protects regenerating hepatocytes from apoptotic death indirectly by acting as an upstream mediator that increases STAT-3–dependent IL-6 and TNF-α gene expression via activation of C5aR1-bearing Kupffer cells (44). TNF-α and IL-6 are crucial regulators of the priming phase of liver regeneration, and IL-6 in particular is a major prosurvival factor for regenerating hepatocytes via the PI3K/AKT/mTOR pathway (45). C5a has also received considerable attention recently as an important mediator of T cell survival during the immune response. In vitro, constitutive signaling through C5aR1 was reported as necessary for optimal T cell survival (36). Similarly, T cell activation and expansion in vivo have been reported to require C5aR1 signaling, in part because C5aR1 signaling inhibits activation-induced T cell apoptosis (36, 37). Other in vivo studies that support a role for C5aR1 signaling in T cell survival include a mouse model of influenza where the absence or antagonism of C5aR1 caused a reduction in the numbers of CD8<sup>+</sup> T cells specific for influenza type A virus (46) and a mouse model of GVHD where the absence or antagonism of C5aR1 impaired T cell expansion (47). These in vitro and in vivo investigations collectively make a strong, compelling case for the importance of C5aR1 in providing prosurvival signals to activated T cells. Our investigations also support the importance of C5a/C5aR1 in providing antiapoptotic signals to activated T cells; but in contrast with the findings of Strainic et al. (36), we did not observe any reduction in the number of T cells in the spleens of naive uninfected C5aR1<sup>−/−</sup> mice (Figs. 3B, and Supplemental Fig. 1). The C5aR1<sup>−/−</sup> mice used in our studies and those of Strainic et al. (36) originated from distinct colonies of founder C5aR1 knockout mice (32, 48); therefore, possible genetic variability affecting survival of naive T cells (distinct or in concert with C5aR1 deficiency) may account for the different results obtained in these two investigations.

Similar to regenerating hepatocytes, the prosurvival/antiapoptotic effect of C5a on T cells is thought to occur indirectly through C5aR1-mediated production of cytokines that affect the PI3K/AKT/mTOR pathway.
AKT/mTOR pathway (36, 37). In addition to indirect protection, it has been reported that C5aR1 may also provide prosurvival signals directly to T cells expressing C5aR1 (36, 37). However, the possibility of direct protection by C5a has been challenged by recent studies indicating that T cells (naive or activated) do not express C5aR1 (49). In this study, we have discovered a novel means by which C5aR1 can provide prosurvival activity through another indirect mechanism. Instead of bolstering the expression of protective cytokines such as TNF-α and IL-6 as in liver injury, C5aR1 protects against *L. monocytogenes*–induced splenocyte loss through the inhibition of type 1 IFN expression.

Although generally thought of as a proinflammatory molecule, C5a can also adopt a regulatory role in certain contexts. The first demonstration of this came over a decade ago in two articles showing that C5a inhibits the expression of IL-12 in human macrophages in response to LPS and *Staphylococcus aureus* (50, 51). Since then this C5a inhibitory activity has been extended to include most members of the IL-12 cytokine family and additional stimuli such as CD40 activation and the intracellular bacteria *Porphyromonas gingivalis* (52, 53). Beyond the IL-12 family, C5a also represses the production of IL-17A in LPS-activated macrophages in vitro and in a mouse model of endotoxemia in vivo (54). Furthermore, the inhibitory effects of C5a are not limited to cytokines, because C5aR1 also suppresses the expression of the chemokines CCL17 and CCL22 in DCs during allergic asthma.

**FIGURE 6.** Type 1 IFN and its target TRAIL are elevated in C5aR1−/− mice during *L. monocytogenes* infection. WT and C5aR1−/− mice were injected i.v. with $1 \times 10^5$ CFU *L. monocytogenes* or PBS, and serum was isolated from the mice at 24 h. (A) IFN-α and IFN-β were measured by ELISA. Sera from PBS-injected animals had no detectable type 1 IFN (data not shown). Data from two independent experiments were combined and are presented as mean pg/ml ± SEM. $n = 9–11$ per genotype. **p ≤ 0.0004 by t test. (B and C) WT and C5aR1−/− mice were treated with PBS or infected with *L. monocytogenes* and their spleens removed at 72 h. Splenocytes were stained with the viability dye DAPI, TRAIL-PE, and NK1.1-allophycocyanin to determine the percentage of live TRAIL−/− NK cells. Representative plots of TRAIL expression in DAPI−/− NK1.1+ cells are shown (B). The percentage of live TRAIL−/− NK cells in mice from two independent experiments are depicted (C). $n = 6$ per group. **p = 0.0001 by ANOVA with the Tukey posttest.

**FIGURE 7.** Blocking IFNAR1 rescues C5aR1−/− mice from *L. monocytogenes*–induced mortality. Four hours before infection, C5aR1−/− mice were administered 1 mg of either the IFNAR1 blocking Ab MAR1-5A3 or an isotype Ab control MOPC-21 i.p. in PBS. WT and C5aR1−/− mice were then infected i.v. with $5 \times 10^6$ CFU *L. monocytogenes* and followed for 2 wk. Data are pooled from two independent experiments. $n = 12–14$ mice per condition. $p < 0.0001$ by log-rank test.
models (55). Pathogens even exploit the regulatory activity of C5a for their own gain. The gingival pathogen Porphyromonas gingivalis actively cleaves C5 to trigger cross talk between C5aR1 and TLR2 that, in turn, inhibits the release of NO (56). Although the effects of C5a/C5aR1 on a variety of cytokines have been examined, to date no one has examined how they regulate the type 1 IFNs. This report therefore adds type 1 IFNs to the scope of the regulatory functions of C5a/C5aR1 for the first time, to our knowledge.

It is increasingly appreciated that type 1 IFN can impair the host response to bacteria (57, 58). Studies with Salmonella typhimurium, Chlamydia muridarum, Brucella abortus, and L. monocytogenes have illustrated that type 1 IFN broadly promotes macrophage and lymphocyte death during intracellular bacterial infections (9, 10, 59–61). Beyond this, type 1 IFN can also repress antibacterial activity in other ways. For example, type 1 IFN appears to dampen the responsiveness of macrophages to IFN-γ during L. monocytogenes infection (62). Although type 1 IFN is believed to induce IL-10 expression during listeriosis through lymphocyte apoptosis, in other models, type 1 IFN directly triggers IL-10 expression in macrophages and lymphocytes (57). Compared with WT infected mice, IL-10 serum levels in infected C5aR1−/− mice were increased by ~200-fold at 72 h postinfection (Fig. 5), providing further evidence that increased levels of type 1 IFNs result in increased IL-10 production. Furthermore, type 1 IFN suppresses the expression of IL-17, a key antibacterial cytokine, in both innate γδ T cells and Th17 cells (63–65). C5aR1 may therefore have developed a regulatory role for type 1 IFN expression to limit detrimental effects during intracellular bacterial infections.

In summary, this study reveals in a mouse model the previously unknown, yet important function of C5aR1 in providing host defense against L. monocytogenes systemic infection through the impairment of L. monocytogenes–induced apoptosis of both myeloid and lymphoid cells required for ultimate clearance of this intracellular bacterium. Moreover, C5aR1 impairs cellular apoptosis during listeriosis not by increasing the production of prosurvival/antiapoptotic cytokines such as TNF-α and IL-6, which are important in C5aR1-mediated liver regeneration and T cell activation, but rather by suppressing the expression of type 1 IFNs and their downstream target TRAIL.

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Disclosures

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