Complement C5a Receptor Is Essential for the Optimal Generation of Antiviral CD8$^+$ T Cell Responses[1]

Alfred H. J. Kim,* Ioannis D. Dimitriou,* M. Claire H. Holland,† Dimitrios Mastellos,† Yvonne M. Mueller,* John D. Altman,‡ John D. Lambris,[2†] and Peter D. Katsikis[2*]

The complement system has been long regarded as an important effector of the innate immune response. Furthermore, complement contributes to various aspects of B and T cell immunity. Nevertheless, the role of complement in CD8$^+$ T cell antiviral responses has yet to be fully delineated. We examined the CD8$^+$ T cell response in influenza type A virus-infected mice treated with a peptide antagonist to C5aR to test the potential role of complement components in CD8$^+$ T cell responses. We show that both the frequency and absolute numbers of flu-specific CD8$^+$ T cells are greatly reduced in C5aR antagonist-treated mice compared with untreated mice. This reduction in flu-specific CD8$^+$ T cells is accompanied by attenuated antiviral cytolytic activity in the lungs. These results demonstrate that the binding of the C5a component of complement to the C5a receptor plays an important role in CD8$^+$ T cell responses. ***The Journal of Immunology*****, 2004, 173: 2524–2529.**

The complement cascade plays a role in both innate and adaptive immune responses. Complement functions within the innate immune system through several mechanisms, such as activating and releasing inflammatory mediators, coating pathogens for the purpose of opsonization by phagocytic cells, activating neutrophils and mast cells, and lysis of infected cells and pathogens through membrane-attack complex (1). However, complement has also been shown to directly regulate adaptive immune responses (2). APC activation can occur through the cross-linking of the complement receptor CR1 (CD35) by its ligation to C3b and C4b (3). C3 enhances the response of B cells to both T cell-dependent (4) and -independent (5) Ags. The threshold of B cell activation lowers when C3d binds to CR2 (CD21) (6–8), which cross-links CR2 and CD19 to the BCR and augments the recruitment of BCRs to lipid rafts (9). Mice deficient in CR1 and CR2 (*Cr2*$^{-/-}$ mice) demonstrate an inability to produce Abs that are a product of class switching (10). Strikingly, the functions of complement extend far beyond the immunological realm. Evidence is mounting for a role for complement in the development of various tissues (11), such as bone and cartilage (12–14), spermoocyte fusion (15, 16), limb regeneration in urodeles (17), and liver regeneration (18, 19).

Recently, several observations have been published linking components of complement with the generation of T cell responses. In a murine model of autoimmune myocarditis, Kaya et al. (20) showed that depleting C3 during disease induction prevented the development of disease. The authors observed that the C3 end products act through CR1 and CR2 on memory CD44$^+$CD62L$^-$ CD3$^+$ T cells, and that mice deficient in C3 or CR1 and CR2 fail to secrete TNF-α, IFN-γ, and IL-1 during disease (20). Pratt et al. (21) showed that C3 plays a role in the rejection of a renal allograft transplant by demonstrating that a donor kidney from a C3$^{-/-}$ mouse transplanted into a fully immunocompetent wild-type recipient with a different MHC class I haplotype prolonged survival 8-fold compared with C3$^{+/+}$ kidney transplantation into the same recipients. Furthermore, they demonstrated that the renal tubular epithelium is the major source of C3 in the C3$^{+/+}$ kidneys, which acts as a chemoattractant on recruiting activated CD4$^+$ T cells, expressing both CR1 and CR2 (21). C3 also provides an immunostimulatory signal to responding T cells, thereby reducing the activation threshold (21). Recently, Kopf et al. (22) demonstrated in a primary murine influenza infection model that mice deficient in C3 exhibited delayed viral clearance and increased viral titers in the lung. Additionally, these mice suffered from a drastic loss of effector CD8$^+$ T cells collected by bronchoalveolar lavage. C3$^{-/-}$ mice infected with lymphocytic choriomeningitis virus also possessed reduced CD8$^+$ T cell responses in an epitope-dependent manner that was mouse strain specific (23). Experiments using *Cr2*$^{-/-}$ mice, however, excluded the possibility of complement acting through those receptors for the generation of CD8$^+$ T cell responses (22, 23). This raises the question of whether downstream complement components that require C3 are involved in the generation of CD8$^+$ T cell responses. In this study we show that mice treated with a C5aR antagonist and infected intranasally with influenza type A virus fail to optimally generate a flu-specific CD8$^+$ T cell response, demonstrating that C5a plays a role in the generation of CD8$^+$ T cell responses.

Materials and Methods

Animals and reagents

Specific pathogen-free, 6- to 8-wk old female C57BL/6J (wild-type) were purchased from The Jackson Laboratory (Bar Harbor, ME). The animals were maintained in an American Association for the Accreditation of Laboratory Animal Care-certified barrier facility at Drexel University College of Medicine, and experiments were performed after obtaining approval from the institutional animal care and use committee. The X31 influenza

*Department of Microbiology and Immunology and Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA 19129; †Protein Chemistry Laboratory, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104; and ‡Department of Microbiology and Immunology, Emory University, Atlanta, GA 30329

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[2] Address correspondence and reprint requests to Dr. Peter D. Katsikis, Department of Microbiology and Immunology and Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA 19129. E-mail address: katsikis@drexel.edu; or Dr. John D. Lambris, Protein Chemistry Laboratory, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104. E-mail address: lambris@mail.med.upenn.edu

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type A virus recombinant strain of A/Aichi/2/68 and A/Puerto Rico/8/34 (H3N2, gift from Dr. R. G. Webster, St. Jude Children's Research Hospital, Memphis, TN) was used. The cyclic hexapeptide AcF[OPdChaWR] was used in this study as a specific C5aR antagonist (24). Peptide synthesis and cyclization were performed as previously described (24). The peptide was purified using preparative reverse phase HPLC, and eluted fractions were characterized by mass spectrometry (MALDI). A C3 inhibitor that is inactive in mice, compstatin (25), was used as a peptide control. All peptides were endotoxin-free. The influenza virus-specific MHC class I H-2D^b-nucleoprotein 366–374 (NP$_{366–374}$)[3] and Sendai virus-specific MHC class I H-2D^b-NP$_{324–332}$ tetramers were prepared as previously described (26). The H-2D^b-binding NP$_{366–374}$, ASNENMETM, was purchased from Genosys (The Woodlands, TX).

Influenza virus infections

Mice were anesthetized by i.p. injection with 2,2,2-tribromoethanol (240 mg/kg; Acros, Geel, Belgium) and intranasally infected with 128 hemagglutinin units of X31 influenza virus in 20 μl of PBS (Mediatech, Herndon, VA) on day 0. The C5a receptor peptide antagonist and control peptide were administered by i.p. injection (1 μg of peptide/1 g of mouse weight) on days 0, 2, 4, 6, and 8. Mice were harvested on day 10 postinfection. All mice were age- and cage-matched, and all statistical analyses were performed by Mann-Whitney *U* test using the JMP statistical analysis program (SAS Institute, Cary, NC).

Pulmonary lymphocyte isolation

Single-cell suspensions of lung parenchymal cells were prepared as previously described (27). Briefly, lungs from individual mice were digested with collagenase A and DNase I (3.0 and 0.15 mg/ml, respectively; Roche, Indianapolis, IN) in sterile RPMI 1640 (Mediatech) with 10% heat-inactivated FBS (Mediatech) for 2 h at 37°C on a rocker. The lung digests were then passed through a 40-μm pore size sterile nylon cell strainer (BD Biosciences, San Jose, CA). Single-cell suspensions were washed with sterile RPMI 1640 with 5% heat-inactivated FBS, and mononuclear cells were isolated by density gradient centrifugation using Lympholyte-M (Cedarlane Laboratories, Hornby, Canada) at 1300 × *g* at room temperature for 20 min. Lung mononuclear cells were resuspended in RPMI 1640 complete medium (with 10% heat-inactivated FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.2 mM L-glutamine, and 5.5 μM 2-ME). Cells were counted with Acridine Orange (3 μg/ml) and ethidium bromide (5 μg/ml) stains (Sigma-Aldrich, St. Louis, MO) to visualize live and dead cells under UV light.

Lymph node lymphocyte isolation

Single-cell suspensions of lymph node cells were prepared as previously described (28). Briefly, lymph nodes from individual mice were digested with collagenase D (1.67 mg/ml; Roche) in sterile RPMI 1640 with 2% heat-inactivated FBS and 10 mM HEPES for 30 min at 37°C on a rocker. The lymph node digests were mechanically disrupted by gentle pipetting, and a 1/10 dilution of sterile 0.1 M EDTA in PBS (Mediatech), pH 7.2, was added for 1 min. The digests were diluted with HBSS (Mediatech), 2% FBS, and 5 mM EDTA, pH 7.2, and the single-cell suspension was passed through a 40-μm pore size sterile nylon cell strainer (BD Biosciences). Single-cell suspensions were resuspended in RPMI 1640 complete medium and counted using a solution of Acridine Orange and ethidium bromide under UV light.

Flow cytometry

CD8$^+$ T cells specific for NP$_{366–374}$ were quantified and phenotyped with MHC class I H-2D^b-NP$_{366–374}$ tetramers. The Sendai virus-specific H-2D^b-NP$_{324–332}$ tetramer was used as a control. The tetramers were formed by complexing the peptide-loaded MHC monomers to allophycocyanin-streptavidin (Molecular Probes, Eugene, OR). Cy5PE-anti-CD8α (clone 53-6.7; eBioscience, San Diego, CA) and allophycocyanin-tetramer were used for immunofluorescent staining. Cells were stained and washed in HBSS (Mediatech) with 2% heat-inactivated horse serum (Mediatech) and 0.02% sodium azide. Cell staining was performed at 4°C for 30 min. Cells were washed twice, fixed with 1% paraformaldehyde, and analyzed using a FACSCalibur flow cytometer (BD Biosciences) and FlowJo software (TreeStar, Mountain View, CA).

[3] Abbreviations used in this paper: NP, nucleoprotein; DC, dendritic cell; DLN, draining lymph node.

Cytotoxicity assay

Pulmonary antiviral cytotoxicity was analyzed directly ex vivo against EL-4 target cells that were loaded with 1 μg/ml MHC class I NP$_{366–374}$ peptide for 6 h. Five million peptide-loaded EL-4 target cells were then labeled with 100 μCi of ^{51}Cr for 75 min at 37°C, washed twice, and resuspended in RPMI 1640 complete medium. Target cells (10^4 targets/well) were incubated with varying numbers of effector cells in 96-well, V-bottom Costar cell culture plates (Corning Glass, Corning, NY), centrifuged for 30 s at 200 × *g*, then incubated for 6 h at 37°C in a 5% CO_2 incubator. After incubation, cells were centrifuged for 5 min at 500 × *g*. Culture supernatants (30 μl) were transferred to 96-well Lumaplates (Packard Instruments, Downers Grove, IL), dried overnight, and counted with a Top Count-NXT luminescence scintillations counter (Packard Instruments). The percent lysis was calculated using the formula: 100 × (cpm experimental − cpm spontaneous)/(cpm maximum − cpm spontaneous). Maximum ^{51}Cr release was determined by lysing targets with 5% Triton X-100 solution. Spontaneous ^{51}Cr was typically 10–15% of maximum release.

Intracellular IFN-γ staining

Lung mononuclear cells (10^6) were cultured in 100 μl of RPMI 1640 complete medium with 10 μg/ml brefeldin A in 5-ml, sterile, polystyrene, round-bottom tubes in the presence or the absence of 10 μg/ml NP$_{366–374}$ peptide. Cells were incubated for 6 h at 37°C in a 5% CO_2 incubator. After culture, the cells were first surface-stained with mAbs as detailed above, then fixed and permeabilized with Cytofix-Cytoperm (BD Pharmingen, La Jolla, CA) for 20 min at 4°C. Cells were stained for the intracellular accumulation of IFN-γ with an allophycocyanin-conjugated IFN-γ mAb (eBioscience) for 30 min at 4°C. Cells were washed with Perm/Wash buffer (BD Pharmingen), fixed with 1% paraformaldehyde, and analyzed using a FACSCalibur flow cytometer and FlowJo software.

Results

Effect of C5a receptor antagonist on CD8$^+$ T cell responses

To determine the role of C5aR stimulation in the generation of CD8$^+$ T cell responses, we examined the virus-specific CD8$^+$ T cell response in the lungs of influenza type A virus-infected mice treated with a C5aR peptide antagonist. We used a total lung digestion procedure to isolate pulmonary lymphocytes (29, 30) that allows for the analysis of individual mice. Virus-specific CD8$^+$ T cells were quantitated by MHC class I tetramers. The C5aR antagonist is a small cyclic hexapeptide (AcF-[OPdChaWR]; M_r = 894 Da) that exhibits specific C5a inhibitory effects in the low nanomolar range (24). As a control peptide, compstatin (25) was selected because it possesses no complement-inhibiting activity in mice. We found that mice treated with C5aR antagonist generated a reduced frequency of NP$_{366–374}$-specific CD8$^+$ T cell response compared with untreated mice and peptide control-treated mice as measured by tetramer staining (7.70 ± 1.58% C5aR antagonist-treated mice vs 13.47 ± 1.60% untreated mice vs 13.73 ± 1.69% peptide control-treated mice; n = 6; Fig. 1, *A* and *C*). The absolute numbers of NP$_{366–374}$-specific CD8$^+$ T cells in the lungs were also greatly diminished in the C5aR antagonist-treated mice (0.62 × 10^5± 0.48 × 10^5 C5aR antagonist-treated mice vs 2.53 × 10^5± 0.61 × 10^5 untreated mice vs 2.33 × 10^5± 0.49 × 10^5 peptide control-treated mice; Fig. 1*D*). Within the draining mediastinal lymph nodes (DLNs) in these mice, NP$_{366–374}$-specific CD8$^+$ T cells were detected at very low frequencies in the untreated, peptide control, or C5aR antagonist-treated mice, with no significant differences seen between these groups (Fig. 1*B*).

The NP$_{366–374}$ CD8$^+$ T cell response was also examined by analyzing IFN-γ-producing CD8$^+$ T cells after in vitro stimulation of lung mononuclear cells with the NP$_{366–374}$ peptide. We observed similar results to the tetramer stains, where C5aR antagonist-treated mice exhibited reduced IFN-γ production in terms of both frequency (8.51 ± 1.59% C5aR antagonist-treated mice vs 15.93 ± 1.96% untreated mice vs 14.29 ± 1.60% peptide control-treated mice; n = 6) and absolute numbers of cells per lung

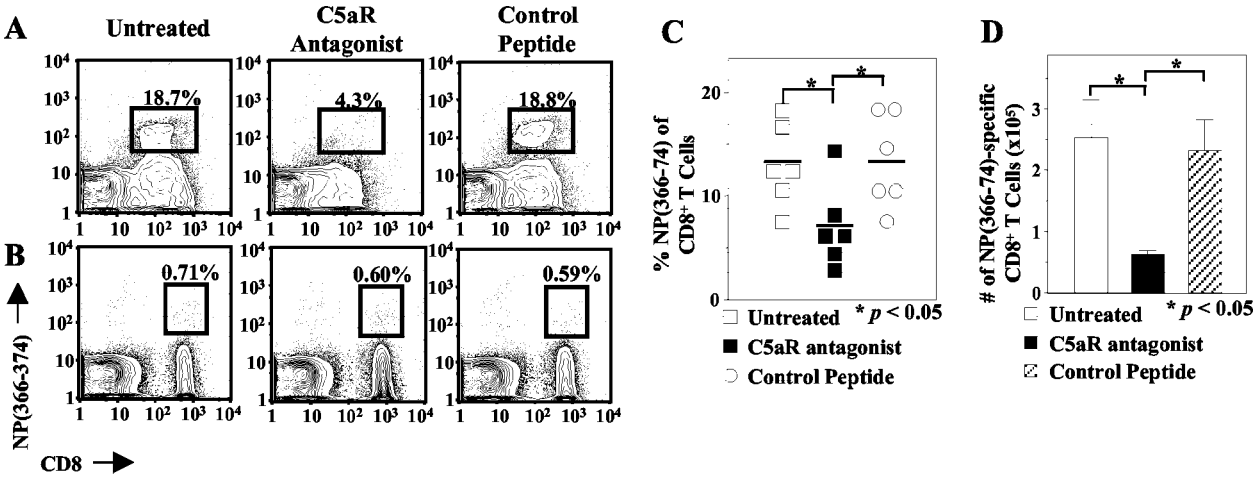

FIGURE 1. C5aR peptide antagonist inhibits the expansion of CD8$^+$ T cells specific for the immunodominant $NP_{366-374}$ influenza epitope in influenza A-infected mice. Lung lymphocytes and mediastinal lymph nodes from wild-type mice differentially treated with C5aR peptide antagonist, control peptide, or no peptide were harvested 10 days postinfection with influenza A virus. Lymphocytes were then stained with anti-CD8 mAb and D^b-$NP_{366-374}$ tetramer. Representative FACS plots show $NP_{366-374}$ tetramer-staining cells in the lungs (*A*) or mediastinal DLNs (*B*) of infected mice. Numbers indicate the percentages of CD8$^+$ T cells staining positively for the $NP_{366-374}$ tetramer. Pooled data showing the frequency (*C*) and absolute numbers (per animal; *D*) of $NP_{366-374}$-specific CD8$^+$ T cells from the lungs of infected animals. Pooled data from two independent experiments are shown ($n = 6$ mice/group). Significant differences, determined by Mann-Whitney *U* test, are indicated by asterisks and *p* values.

($0.61 \times 10^5 \pm 0.33 \times 10^5$ C5aR antagonist-treated mice vs $2.00 \times 10^5 \pm 0.58 \times 10^5$ untreated mice vs $1.52 \times 10^5 \pm 0.33 \times 10^5$ peptide control-treated mice; Fig. 2, *A–C*).

Finally, C5aR antagonist-treated mice demonstrated a reduced ability of lung lymphocytes to kill $NP_{366-374}$-loaded target cells in a ^{51}Cr release assay compared with control peptide-treated or untreated mice (Fig. 2*D*).

Discussion

Given the role complement plays in the adaptive immune response (2) and the recent studies demonstrating that C3$^{-/-}$ mice show greatly reduced CD8$^+$ T cell responses, whereas *Cr2*$^{-/-}$ mice, which lack CR1 and CR2, had normal responses (22, 23), we examined whether complement components downstream of C3, such

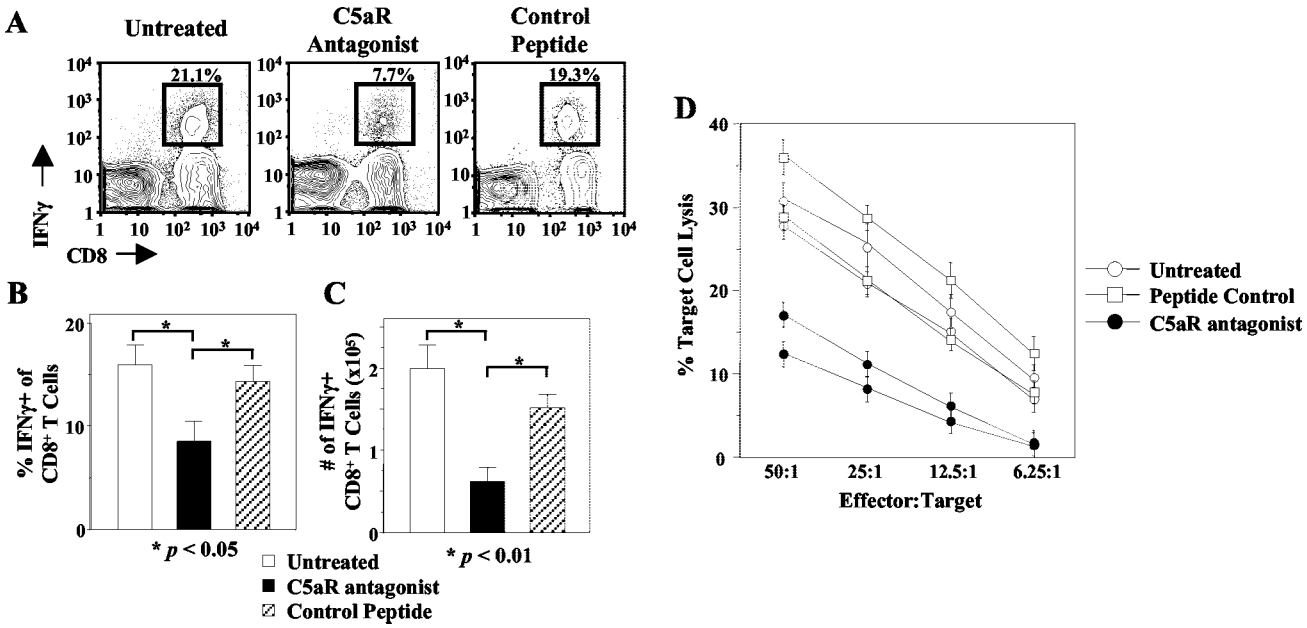

FIGURE 2. Blocking C5aR receptor inhibits flu-specific, IFN-γ-producing CD8$^+$ T cells and cytotoxicity during the primary response in the lungs of influenza A-infected mice. Lung lymphocytes were harvested 10 days postinfection, and intracytoplasmic IFN-γ was determined by flow cytometry after peptide stimulation. *A*, Representative histogram plots showing intracytoplasmic IFN-γ staining after $NP_{366-374}$ influenza peptide stimulation. Numbers indicate the percentages of CD8$^+$ lymphocytes that stained positively for IFN-γ. Pooled data are presented, showing the frequency (*B*) and absolute numbers (*C*) of virus-specific IFN-γ-producing CD8$^+$ T cells. Pooled data from two independent experiments are shown ($n = 6$ mice/group). *D*, Representative experiment of direct ex vivo cytotoxic activity of CD8$^+$ T cells from lungs of infected mice against $NP_{366-374}$. Significant differences, determined by Mann-Whitney *U* test, are indicated by asterisks and *p* values.

as C5a, may be critical to mounting optimal $CD8^+$ T cell responses in viral infections. Our findings show that blocking the C5a receptor with a C5aR peptide antagonist inhibits the generation of flu-specific cytotoxic $CD8^+$ T cell responses. This reduction of flu-specific $CD8^+$ T cell responses in C5aR antagonist-treated mice does not appear to be secondary to a defect in the migratory capacity of activated $CD8^+$ T cells out of the DLNs to the site of infection, because that would create a buildup of flu-specific $CD8^+$ T cells within the DLN, and the numbers that we find within the DLNs do not account for the absence of cells seen in the lungs of infected mice. Rather, it appears that there may be an intrinsic defect in either the activation or the extranodal proliferation of these flu-specific $CD8^+$ T cells. Additionally, previous observations demonstrated that influenza A virus infection significantly increases the levels of C5a in bronchial lavage fluids from human subjects who developed influenza illness (31), further suggesting a role for C5a in the development of immune responses against influenza. Our own preliminary data using a sensitive ELISA (32) show that 1–3% of plasma C3 complement is activated within 24 h of influenza virus lung infection in mice (data not shown), and this suggests that C5a is being generated during infection. That complement activation and C5a production occur in the lungs of mice similar to those in human infection, although likely, remains to be established. These data add to the growing list of roles that complement plays in supporting the adaptive immune response. The requirement for inflammation to occur at the time of Ag delivery for the generation of an effective immune response is well established (33, 34), yet the products of inflammation, such as anaphylatoxin C5a, and their requirement for functional $CD8^+$ T cell responses have not been investigated.

The C5aR peptide antagonist used in this study possesses excellent affinity for the C5aR, with an IC_{50} in the nanomolar range (35, 36). Furthermore, the antagonist is highly selective for C5aR compared with other similarly structured, G protein-coupled, seven-transmembrane proteins, such as C3aR (24). This peptide has been extensively studied and has been shown to antagonize the binding of C5a to C5aR on human PBMCs in vitro (37); inhibit C5a-mediated neutrophil chemotaxis and macrophage cytokine production in vitro (38); protect rat intestine and lung from neutrophil-associated injury in an abdominal aortic aneurysm animal model in vivo (39); attenuate acute limb (40), mesenteric (41, 42), and kidney (43, 44) ischemia-reperfusion injury in separate rat models in vivo; improve survival rates in septic rats in vivo while inhibiting the reverse-passive Arthus reaction (35, 45–48); reduce joint pathology in an in vivo rat model of Ag-induced arthritis (41); and protect against intestinal pathology associated with inflammatory bowel disease in rats in vivo (49).

As mentioned above, Kopf et al. (22) observed that $C3^{-/-}$ mice showed severely reduced flu-specific $CD8^+$ T cells, yet $Cr2^{-/-}$ mice exhibited normal $CD8^+$ T cell responses and viral clearance. Because C3 can be broken down into the anaphylatoxins C3a and C3b, an important component of the C5 convertase that cleaves C5 into C5a and C5b, the role C3 plays in $CD8^+$ T cells responses could be attributed to the C5 complement products. This is supported by the finding that C3b binding to the complement receptor CR1 or CR2 is not involved in the $CD8^+$ T cell response, as $Cr2^{-/-}$ mice show no defect in the ability to mount $CD8^+$ T cell responses (22). Our findings suggest that the effect seen by Kopf and colleagues (22) in $C3^{-/-}$ mice is probably due to the lack of C3b, preventing the C5 convertase from being assembled and the downstream generation of C5a.

How C5a mediates its effects on $CD8^+$ T cell function is unknown, but several mechanisms could explain how C5a could modulate the priming of $CD8^+$ T cells. One possible explanation involves the role of C5a as a chemotactic molecule for both APCs and $CD8^+$ T cells. The release of C5a during the early inflammatory stage of a pathogen infection could be used as a homing signal for dendritic cells (DCs) to the source of Ag, optimizing the priming conditions for the generation of effector T cells. The C5a receptor (C5aR, CD88) is a 40-kDa, G protein-coupled, seven-transmembrane domain receptor expressed on numerous cell types (50, 51), including mast cells (52, 53), neutrophils, macrophages, basophils (54), human DCs (55, 56), murine memory B cells (57), and human T cells (58). Furthermore, several observations have been published demonstrating the role of C5a as a potent chemoattractant for human T cells (58, 59), human B cells (60), and immature and mature DCs (55, 56, 61, 62). The inability to recruit DCs to Ag depots due to the absence of C5a would, therefore, severely compromise the initiation of functional $CD8^+$ T cell responses.

C5a, however, may also play a role in the direct control of $CD8^+$ T cell activation. Other complement components, such as C3d, are known to modulate the threshold of B cell activation (9), and it is possible that C5a also enhances $CD8^+$ T cell signaling in a similar manner. Both C5aR and chemokine receptors possess seven transmembrane domain receptors coupled to G proteins; the latter are known to activate Ca^{2+} mobilization and lipid phosphorylation, initiating the process of T cell activation. During chemokine receptor signaling, T cell polarization occurs, inducing actin-based protrusions from the lamella podium that contain clusters of highly sensitized TCRs (63). This polarization of T cells prepares them for Ag recognition before encountering an APC. It is possible that C5aR signaling exerts its action on $CD8^+$ T cells in a similar fashion to chemokines, thus enhancing $CD8^+$ TCR sensitivity and lowering the activation threshold.

Similarly, $CD4^+$ T cells appear to respond to C5a, which may alter the function of $CD4^+$ T cells, thereby enhancing $CD8^+$ T cell responses. Indeed, C5aR is expressed on human $CD4^+$ T cells and C5a induces their chemotaxis, either directly or indirectly (58, 59, 64–68). Although direct activation of $CD4^+$ T cells by C5aR signaling has yet to be demonstrated, this is very likely, because chemotaxis itself is known to induce early T cell activation signaling cascades in the absence of Ag (63). However, an indirect effect of C5a on the $CD8^+$ T cell response mediated through $CD4^+$ T cells may not be critical, as primary $CD8^+$ T effector cells can develop independently of $CD4^+$ T cells (69).

C5a may also be acting on DCs, altering their homing, functionality, and differentiation and thus enhancing their ability to initiate and propagate $CD8^+$ T cell responses. As mentioned above, numerous studies have demonstrated that immature DCs can be actively recruited by C5a (55, 56, 61, 62, 70–72). Furthermore, the presence or the absence of PGE_2 affects the expression of C5aR on human DCs stimulated with LPS or TNF-α (73). The DCs responsive to C5a may include a novel subset of human blood DCs that is M-$DC8^+$ and has been recently shown to uniquely express C5aR and C3aR, an expression pattern not shared by either DC1s or DC2s (74). C5a induces the migration and cytokine production by these DCs (74). Finally, C5a can directly recruit monocytes and program them to differentiate into mature inflammatory DCs by stimulating the release of TNF-α and PGE_2 (70). Thus, C5aR stimulation on DCs may enhance both the number and the immunogenicity of mature DCs at sites of inflammation and thereby indirectly affect $CD8^+$ T cell responses.

It is possible, though, that the effects of C5a on the enhancement of $CD8^+$ T cell function may work indirectly through other inflammatory mediators generated by complement activation. $CD8^+$ T cells and DCs may be indirectly activated by downstream inflammation products induced by C5aR activation on other cell

types. C5a induces inflammatory responses in mast cells, neutrophils, monocytes/macrophages, and basophils, causing those cells to release cytokines such as IL-12 and TNF-α and chemokines such as MIP-1α. IL-12 is a strong activator of CD8$^+$ T cells, and TNF-α can induce T cells to undergo transendothelial migration by up-regulating vascular adhesion molecules and induce the expression of IFN-γ by those T cells (68). As mentioned previously, the endothelium also becomes activated through up-regulation of adhesion molecules upon exposure to C5a-mediated TNF-α and serotonin exposure (59, 64–66, 75, 76), further assisting T cell recruitment to inflamed tissue. C5a activation may also indirectly activate DC migration into the inflamed tissue through the release of chemokines, such as alveolar macrophage secretion of MCP-1 (77).

One cannot ignore the potential contribution C3a may also have to the generation of antiviral CD8$^+$ T cell responses, considering the number of structural and functional similarities between C3a and C5a. The anaphylatoxin C3a is generated by the cleavage of C3 and, like C5a, possesses a wide spectrum of proinflammatory effects on numerous cell types. Furthermore, C3aR, like C5aR, is a G protein-coupled receptor with seven transmembrane domains (54). Thus, C3a may be acting independently of C5a to enhance the antiviral properties of CD8$^+$ T cells. C3a has been shown to induce the migration of human mast cells (52–54, 78), basophils (79, 80), and eosinophils (81) and to inhibit the exodus of hemopoietic stem cells from bone marrow (82). Furthermore, human monocytes and macrophages release PGE_2 and Ca^{2+} ions upon exposure to C3a (81, 83). Human CD4$^+$ T cells, CD8$^+$ T cells, and DCs express functional C3aR, which also induces calcium fluxes in these cell types, suggesting a chemotactic and activating effect of C3a on C3aR-expressing cells (72, 84). Type I IFNs can stimulate the expression of C3a (85, 86) and C3aR in lymphocytes (84), suggesting that C3a may also link adaptive and innate immune responses as does C5a. Alternatively, C3 activation could be acting through C5a in the activation of antiviral CD8$^+$ T cell responses. We suggest that the production of C3b contributes to the construction of the C5 convertase, which would produce C5a and help induce antiviral CD8$^+$ T cell responses; however, we cannot exclude that both C3a and C5a are required for the optimal CD8$^+$ T cell response.

Our data show that the interaction of C5a and C5aR is critical for the generation of functional antiviral CD8$^+$ T cell responses. Mice treated with C5aR antagonist and infected intranasally with influenza type A virus have severely attenuated frequency and absolute numbers of CD8$^+$ T cells specific for the immunodominant $NP_{366-374}$ epitope, IFN-γ production in response to $NP_{366-374}$ influenza peptide stimulation, and cytotoxic activity. These data strongly support a requisite role for C5a and C5aR in CD8$^+$ T cell responses to viruses and suggest that manipulating C5 may prove useful as a strategy to modulate cytotoxic CD8$^+$ T cell responses.

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