The Journal of Immunology

REVIEW ARTICLE | SEPTEMBER 01 2008

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J Immunol (2008) 181 (5): 2953–2959.
https://doi.org/10.4049/jimmunol.181.5.2953

Brief Reviews

THE JOURNAL OF IMMUNOLOGY

Comparative Functional Evolution of Human and Mouse CR1 and CR2[1,2]

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The complement cascade is regulated by a series of proteins that inhibit complement convertase activity. These regulatory proteins, most of which possess binding sites for C3b and/or C4b, can be roughly divided into two groups, one that controls inappropriate complement convertase activity on the surface of cells and another that controls convertase activity on immune complexes in serum. In this review we focus upon the structural and functional comparisons of the CR1 and CR2 proteins of man and mouse. A single gene encodes these proteins in the mouse whereas the human requires two. The analysis of mice lacking the CR1/CR2 proteins demonstrates the requirement of these proteins for the regulation of complement convertase activity within lymphatic tissue immune complexes that is not efficiently controlled by other membrane-bound or serum regulatory proteins.

Complement regulatory proteins

The complement pathway is a series of serum proteins that functions to tag nonself proteins and microbes for phagocytic uptake and destruction. This activation pathway has, by necessity, coevolved a series of serum and membrane bound proteins whose primary goal is to help regulate the pathway, allowing for the targeting of foreign Ags but protecting normal self tissue and cells from deleterious attack. The primary amplification step of the complement cascade is the generation of the activated C3 protein (through the classical, alternative, or lectin activation pathways). The initial cleavage of C3 by these various convertases generates C3a (which is a potent anaphylatoxin recognized by the C3a receptor) (1) and C3b (which may form a covalent thiol-ester bond to substrate) (2). C3b can also join with the C3 convertases to function as a C5 convertase, releasing the anaphylatoxin C5a and the C5b protein (3). C3b can be further degraded into smaller, inactive forms known as iC3b and C3dg by the serine protease factor I (fI),[4] which requires cofactor help (4). Each of these C3b cleavage products can maintain its bond to substrate (and is recognized by a series of receptors) but cannot participate within C3 or C5 convertases.

The C3 complement convertases are the targets of many of the complement regulatory proteins. For example, decay acceleration factor (DAF) enhances the decay of complement convertases by binding to C3b (5, 6). Another set of proteins, typified by membrane cofactor protein (MCP), serves to facilitate fI cleavage of C3b into the smaller, inactive forms (7, 8). An additional protein, known in sub primates as Crry, possesses both MCP and DAF functions (9–11). These complement regulatory proteins are all membrane bound and are relatively small (45,000–70,000 Da), suggesting that their primary function is to protect the membrane of that cell from complement-mediated damage (Table I).

Convertase regulation is also accomplished through a number of serum proteins. C4 binding protein (C4BP) regulates the classical and lectin complement pathways by serving as a cofactor for fI-mediated degradation of C4b proteins within those C3 and C5 convertases as well as by accelerating the decay of these convertases (12, 13). The serum protein factor H (fH) helps regulate the alternative pathway by aiding in fI-mediated cleavage of C3b as well as by destabilizing the convertase (14). fH is also implicated, in association with the acute phase protein C-reactive protein (CRP), of solubilizing immune complexes (15); alleles of fH that do not bind CRP are linked to predisposition to macular degeneration (16–18). The site of action of C4BP and fH are within the blood stream as well as connective tissue, especially during events in which vascular leakage is promoted by responses to infectious agents.

Another set of cellular complement receptor regulatory proteins are typified by much larger membrane-bound proteins. Primate CR1 ranges in size from 190,000 Da to nearly 300,000 Da and serves as a cofactor for the cleavage of activated C3b and C4b into their inactive forms (19, 20). It is also a phagocytic receptor of macrophages and neutrophils for complement-bound immune complexes (21). Primate CR1 is also a key player in the immune adherence phenomenon in which complement-bound immune complexes are first bound to erythrocytes, transported to the spleen and liver, and then removed

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Received for publication May 23, 2008. Accepted for publication July 10, 2008.

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[1] This work was supported by the National Institute of Allergy and Infectious Diseases (AI-24158). A.C.J. was supported by the Training Program in Microbial Pathogenesis, Grant 5T32-AI-055434.

[2] The content is solely the responsibility of the authors and does not necessarily represent the official views of the Institute of Allergy and Infectious Diseases or the National Institutes of Health.

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[4] Abbreviations used in this paper: fI, serine protease factor I; C4BP, C4 binding protein; CVF, cobra venom factor; DAF, decay acceleration factor; FDC, follicular dendritic cell; fH, serum protein factor H; LHR, long homologous repeat; MCP, membrane cofactor protein; SCR, short consensus repeat; WT, wild type.

Table I. *Comparative analysis of C3b/C4b mouse and human regulatory proteins*

Gene	Protein	Size (Da)	Function	Cell Type Expression
Mouse				
Crry	Crry	65,000	Receptor: decay C convertase and cofactor for C3 cleavage	All cells including erythrocytes
Cr2	CR2	145,000	Receptor: C3d, C3dg, C3d-bound complexes	B cells and FDC
Cr2	CR1	200,000	Receptor: C3b, C4b-bound complexes and cofactor for C3 cleavage	B cells and FDC
Daf 1,2	Daf 1,2	70,000	Receptor: decay C convertase	All cells including erythrocytes
CD46	Mcp	60,000	Receptor: sperm acrosome reaction	Testis
Cfh	Factor H	150,000	Serum: cofactor for C3 cleavage and decay C3 convertase	Hepatocytes (serum)
C4BP	C4bp	570,000	Serum: cofactor C4 cleavage	Hepatocytes (serum)
Human				
CR1	CR1	180–280,000	Receptor: C3b, C4b-bound complexes, cofactor for C3 cleavage and decay C convertase	T,B cells, phagocytes FDC, erythrocytes, glomerular podocytes
CR2	CR2	145,000	Receptor: C3d, C3dg, C3d-bound complexes	B cells and FDC
DAF	DAF	70,000	Receptor: decay C convertase	All cells including erythrocytes
MCP	MCP	60,000	Receptor: cofactor, C3 cleavage	Nucleated cells
CFH	Factor H	150,000	Serum: cofactor C3 cleavage, C3 convertase decay	Hepatocytes (serum)
C4BP	C4BP	570,000	Serum: cofactor C4 cleavage	Hepatocytes (serum)

from the erythrocytes for phagocytosis in the liver and spleen (22–24). Both human and mouse CR2 are ~150,000 Da and bind the degraded C3d and C3d,g forms of C3 (25–27). Because CR2 only binds the inactive forms of the C3 protein, it has minimal complement regulatory functions but functions primarily as a member of the B cell coreceptor complex. CR3 and CR4 are integrin phagocytic complement receptors that bind the inactive but partially degraded form of C3, iC3b, bound to immune complexes, leading to internalization of the complexes (28).

One intriguing aspect of the membrane-bound complement regulatory proteins MCP, DAF, Crry, CR1, and CR2 and the soluble complement regulatory proteins fH and C4BP is that they have evolved from a common structural and functional domain, the short consensus repeat (SCR), an ~60-aa sequence with internal disulfide bonds (also known as a Sushi domain or a complement control protein domain) (29). The shorter regulatory proteins possess four to five SCR while the CR1 and CR2 proteins possess 14 or more. These domains make up the entirety of the extracellular sequences of these regulatory proteins and yet have evolved differential specificities for binding to the various ligands, i.e., C4b, C3b, C3d, etc. The numbers, types, and expression patterns of genes that encode this group of complement regulatory proteins are also variable yet redundant. For example, the human has a single DAF gene, yet the mouse appears to possess two functional copies of DAF genes, albeit with restricted tissue expression and structure (30). Additionally, the human MCP protein (CD46; also known as the measles receptor) (31) is expressed by a wide variety of cells while the rodent gene is preferentially expressed in testis (32), regulating sperm acrosome reaction (33). But perhaps the most intriguing difference between the human and mouse complement regulatory proteins is in the structure and expression of the genes encoding the CR1 and CR2 proteins.

Comparative evolution of murine and human CR1

The canonical subprimate *CD21/Cr2* gene produces two proteins, CR1 (~200,000 Da) and CR2 (~145,000 Da), via alternative splicing for an additional six protein N-terminal domains for the CR1 protein (Fig. 1) (34, 35). This CR1 protein can bind both C4b and C3b and possesses cofactor activity for fI-mediated cleavage (27). In the mouse, the *Cr2* gene (encoding CR1 and CR2) is expressed by B cells and follicular dendritic cells (FDCs). Both of these cell types produce both the CR1 and CR2 proteins via alternative splicing; no stimulation has been observed to preferentially splice to either the CR1 or the CR2 form (our unpublished data). It is likely that the duplication of *Crry* sequences within the *Cr2* gene allowed for the creation of the subprimate CR1 protein. A survey of CR1 proteins from nonprimate mammals (by genomic analysis of rat, cow, and dog) encoded from the alternatively spliced CD21/

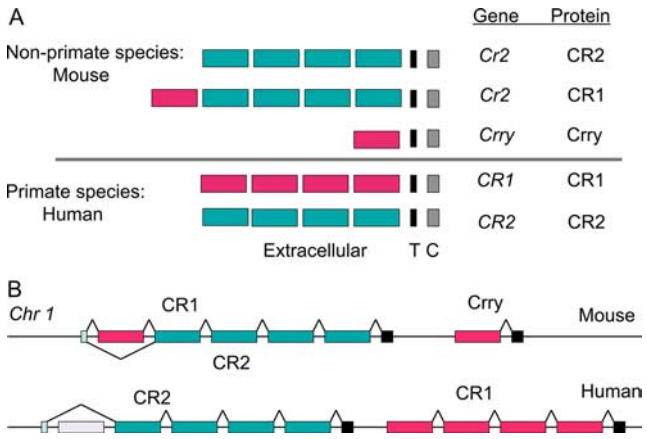

FIGURE 1. Comparative structure of the mouse and human CR1 and CR2 proteins and genes. *A*, Comparison of the functional domains of the CR1, CR2, and Crry proteins. Red blocks denote common sequences used to build the Crry and human CR1 proteins and the N-terminal sequences of the mouse CR1 protein. Green blocks represent common sequences used within the human and mouse CR2 proteins. T, Transmembrane; C, cytoplasmic domains. *B*, Differential genome organization and alternative splicing of the mouse *Cr2* and *Crry* genes and human *CR2* and *CR1* genes present on mouse and human chromosome 1. The light pink box within the human *CR2* gene denotes those *CR1*-like sequences that are not included within mature *CR2* transcripts. The blue box of the mouse and human *Cr2/CR2* genes encodes the signal sequence. The black boxes represent T and C domains. This figure is not drawn to scale nor does it reflect the full exon/intron splicing complexity of these genes.

CR2 gene equivalent suggests these proteins are about the same size as that of murine CR1.

The human (and by genomic analysis, chimp, and rhesus) *CR2* gene only produces the smaller CR2 protein. Exons encoding CR1-like domains are present within the human *CR2* gene between the exons encoding the signal sequence and the first domains of the CR2 protein but are not incorporated into functional transcripts (36). Thus at the step(s) in evolution that separated primates from other species, the *CR2* gene lost the ability to encode the CR1 protein from the *CR2* gene.

Humans do express a protein, CR1, that recapitulates many of the structural domains (and presumed functions) of subprimate *Cr2*-derived CR1. The human CR1 protein is made up of the same SCR domains organized into groups of seven SCR, termed long homologous repeats (LHR), that share a very high degree of homology to one another and hence are indicative of a very recent amplification event in the genome (20, 37). There are a variety of *CR1* alleles in the human population, each consisting of genes possessing three, four, five, or six of these LHR domains, that encode proteins of ~190,000–300,000 Da (38, 39). These LHR possess C3b and C4b binding activity, and the CR1 protein functions as cofactor for fI-mediated cleavage of these substrates (19). Alternative splicing of other primate *CR1* genes may also provide a wider array of protein products from this gene (40).

Sequence homology analysis of the human *CR1* gene indicates that it was derived from the constituents of the *Crry* gene found in subprimates (Fig. 1) (37). The sequences and functions of both gene products are very similar, except that the Crry protein (60,000 Da) is much smaller than the human CR1 isoforms. This common derivation is consistent with the localization of these genes in relation to the *Cr2/CR2* genes in chromosome 1 of mouse and man. In the mouse, the 5′-end of the *Crry* gene is <10,000 bp from the 3′-end of the *Cr2* gene. In the human, the *CR1* gene is also immediately adjacent to the 3′-end of the *CR2* gene although, due to the duplication of sequences, the genomic footprint of the primate *CR1* gene is much larger than that of the subprimate *Crry* gene. The same overall structure of the human *CR1* gene is also found in the chimp, rhesus, and baboon genomes. Other than the mouse, the dog genome is the most complete of the subprimate genomes for the delineation of genes encoding the complement regulatory proteins. The dog genome possesses a single *Cr2*-like gene immediately adjacent to two Crry-like genes flanked by two MCP-like genes.

The *Crry* gene was sacrificed during primate evolution for the creation of *CR1*; primates lack this gene. The impact of the loss of the Crry gene product during this expansion event would have been critical if not for the continued expression of the new CR1 protein on the surface of erythrocytes and the combined functions of DAF and MCP to help keep cellular surfaces clear of potentially damaging complement convertases. This latter point is especially critical during fetal development in that mice lacking Crry are embryonic lethal unless the mother lacks C3 (41). The evolutionary bottleneck of sacrificing the *Crry* gene to create the larger primate CR1 protein must have had an impact upon the reproductive success of the animal.

Function for human CR1

The study of the function of the human CR1 protein is intriguing in that it has been implicated in a number of biological roles. The evolution of the human *CR1* gene from a *Crry*-like precursor to recreate the lost *Cr2*-derived CR1 protein resulted in the expansion of the coding sequences and the limitation of cell surface expression. Virtually all cells of the mouse express Crry (42), yet mouse CR1 (and CR2) is only expressed on B cells and FDCs (although some reports suggest limited T cell expression) (43–45). The human *CR1* gene has a wider cell expression profile than that of mouse *Cr2-CR1* (including B cells, FDC, macrophages, neutrophils and RBCs) but less so than *Crry*, indicating a contraction of expression in many cell types.

Human CR1 expression on circulating erythrocytes has been demonstrated to be critical for binding complement-bound immune complexes (46) and facilitating the transfer of such complexes to, for example, the Kupfer cells of the liver. Kupfer cells possess the CRIg protein, an Ig domain-containing complement receptor for C3b- and iC3b-bound complexes that has been identified as the critical receptor on mouse and human Kupfer cells for the internalization of complement-bound immune complexes (47). The role of mouse Crry on erythrocytes is analogous to that of human CR1, suggesting that the presence of a receptor for complement-bound immune complexes on erythrocytes is critical. Additionally, human CR1 has demonstrated phagocytic capacity on the surface of neutrophils and macrophages for complement-bound immune complexes, although its functions are highly redundant to those of the integrin CR3 and CR4 receptors that bind and internalize C3b- and iC3b-bound complexes (48). CR1 has also been implicated as a receptor for another two opsonins, C1q and the mannan-binding lectin (49, 50).

The human CR1 protein is a potent regulator of complement activation. Soluble recombinant CR1 proteins have been created and used to control complement activation during heightened complement activation (51–53). The ability of the CR1 proteins to bind to both C3b and C4b allows for the control of classical, alternative, and lectin pathway C3/C5 convertases. Soluble murine Crry proteins have also been used in analogous models to control complement activation (54, 55).

The human *CR1* gene in the human population exists in a variety of alleles derived by the amplification of groups of exons. Although these numerous alleles could be due to the genetic instability of the recent evolutionary expansion of highly homologous sequences, it may also be that there are benefits for having CR1 proteins of varying sizes on the surface of B cells and FDCs in the spleen and on erythrocytes for the binding of C3-bound complexes. Various alleles of human *CR1* have been linked to resistance to malaria and susceptibility to lupus, suggesting differential environmental or genetic stresses could perpetuate *CR1* polymorphisms (56, 57).

Requirement for a CR1 protein

Our primary model is that the primate *CR2* gene, in losing the ability to create both the CR1 and CR2 proteins, generated an immunological stress that could only be alleviated by recreating a new CR1 protein via the use of the *Crry*-like sequences. An alternative scenario can be proposed where the amplification of the *Crry* gene in primate lineages was actively selected to create much larger proteins. The generation of a large CR1 protein with multiple C3b/C4b binding sites (as seen in the human) might have created a more efficient protein for complement control than the subprimate *Cr2*-derived CR1 protein. If the

alternative scenario is correct, then the *Cr2*-derived CR1 protein would have been left as an appendix of the regulatory family, easily lost. Both models, however, predict that a CR1-like protein is critical for the animal. What role could the CR1 protein play in host defense that would make its presence so indispensable and not be covered by the redundant roles of the other membrane-bound and serum complement regulatory proteins? To address this question, the mouse model system is informative.

Analyses of the mouse CR1/CR2 proteins have implicated them in a variety of functions. These proteins expressed by FDCs are not phagocytic but instead, like FDC Fc receptors, serve to hold Ag on the surface of such cells for generating a strong Ab response and for affinity maturation of B cells (58–60). Both human and mouse CR2 have been implicated in the transport of immune complexes. In human cell lines, immune complexes bound to B cell CR2 are transferred to THP-1 monocytic cells (61). In vivo, PE-labeled immune complexes are first captured by macrophages in the subcapsular sinus, transferred to follicular B cells, and subsequently deposited on the surface of FDCs, a function that is dependent upon complement receptor expression on B cells (62). A similar transfer between marginal zone B cells and FDCs in the spleen is observed upon in vivo tracking of CR1/2 mAb (63). These studies imply a role for complement receptors (at least CR2) on both B cells and FDCs in the recognition, processing, and retention of Ags.

The mouse CR1 protein can bind both C4b and C3b complexes whereas mouse (and human) CR2 binds C3dg-bound complexes. CR1 and CR2 on murine B cells form complexes with a coaccessory activation complex including CD19, CD81, and the fragilis/Ifitm proteins (the mouse LEU13 equivalents) (64–67). As seen for human CR2, the coligation of the mouse BCR and complement-bound Ag via CR2/CD19 complexes reduces the threshold of activation for B cell responses, allowing for the activation of Ag-specific, naive B cells with limiting amounts of foreign Ag (68). The human and mouse CR2 proteins have been linked to a variety of intracellular signaling pathways, including a membrane phosphoprotein p53, nucleolin-mediated regulation of PI3K (69), and Ag internalization and processing responses (70). The mouse CR1 protein possesses the same C-terminal protein sequence as the mouse CR2 protein, such that association with and activation through CD19 should be equivalent for both proteins. Human CR1, however, does not associate with CD19 on the surface of B cells and cannot participate in this process (65). Thus, it is unlikely that B cell intracellular signaling is a required function of the human and mouse CR1 proteins.

Mice lacking CR1/CR2 expression on FDC and B cells via introduced mutations in the *Cr2* gene show depressed T cell-dependent and -independent Ab responses to low-dose immunizations, specific depression of Ag-specific IgG3 following immunization, a heightened sensitivity to *Streptococcus pneumoniae* infections and diffuse unorganized germinal centers (71–73). IgG3-dependent feedback enhancement of Ab responses was also impaired in CR1/2-deficient mice (74). Analysis of animals expressing lower levels of shorter CR1/CR2 proteins (hypomorph expression) (71, 75) shows that Ag-specific B cells in these mice are eliminated from the germinal centers, presumably through impaired B cell coreceptor signaling (76, 77). Finally, deficiencies in the CR1/2 proteins are implicated in the production of autoreactive Abs in certain autoimmunity models (59, 78, 79). The preceding observations have assumed that the loss (or depressed response) of B cell functions was due to the lack of intracellular signaling via the CR2/CD19 complex or signaling alone.

An alternative explanation for some, if not all, of the phenotypes associated with the *Cr2*-deficient and hypomorphic mice could be due to the loss of the extracellular complement regulatory component of the mouse CR1 protein. The size of this cell surface protein indicates that its function could be to regulate C3/C5 convertase activity on extracellular splenic immune complexes held away from the cell surface and out of the controlling grasp of the Crry and MCP proteins. This regulatory function of the CR1 protein might be particularly critical on the surface of FDCs, in that they do not phagocytize immune complexes that could possess active complement convertases. The absence of the complement convertase regulatory properties of the CR1 protein in the spleen could result in heightened local C3 and C5 convertase activity, leading to increased cellular death and enhanced inflammatory responses due to the release of the anaphylatoxins C3a and C5a.

Recent analyses have shown that the spleen from a naive *Cr2*-deficient animal is in a heightened state of inflammatory activation compared with a naive wild-type (WT) spleen (80). The expressions of a number of inflammatory marker genes are elevated in the *Cr2*-deficient spleen, and these elevated expression profiles can be brought back to WT levels by depleting serum C3 via treatment of the animals with cobra venom factor (CVF) (which consumes serum C3) or by blocking C3a receptor function. The spleens of the *Cr2*-deficient animals also possess elevated numbers of immature myeloid regulatory cells compared with WT spleens, which is consistent with a chronic state of inflammation in the organ (81, 82). The elevated expression of these inflammatory genes can be returned to WT levels by treating *Cr2*-deficient animals with anti-Gr-1 mAb, which transiently eliminates virtually all neutrophils from the spleen.

One of the consistent hallmarks of the *Cr2*-deficient animals has been the relative loss of Ag-specific IgG3 isotypes. When *Cr2*-deficient animals were treated with CVF to deplete C3 at the same time as immunization with a T cell-independent Ag, the levels of Ag-specific IgG3 were returned to the same levels as WT animals treated with CVF. Thus, the presence of C3 in the *Cr2*-deficient animal was partly responsible for the depressed IgG3 production, implicating the byproducts of complement consumption (presumably C3a and/or C5a) as inhibiting the production of this isotype (83, 84).

The preceding data indicated that the removal of C3 relieved the inflammatory stress of the *Cr2*-deficient animal. Thus, the loss of the *Cr2*-derived proteins in the animal can potentially provide two sets of deficiencies, those due to the loss of CR1/CR2 membrane signaling (either with or without CD19 involvement) and those due to the loss of the complement regulatory component of the CR1 protein. It will be of interest to examine a number of the other known phenotypes associated with the *Cr2*-deficient animals to determine whether they are due to the heightened inflammatory state of the animal or the lack of B cell signaling. At least two animal models can be envisioned that could tease these two effects apart. The first would be to create an animal that only possesses the CR2 protein and does not express the alternatively spliced CR1 protein. Such animals would thus lose the complement regulatory contributions

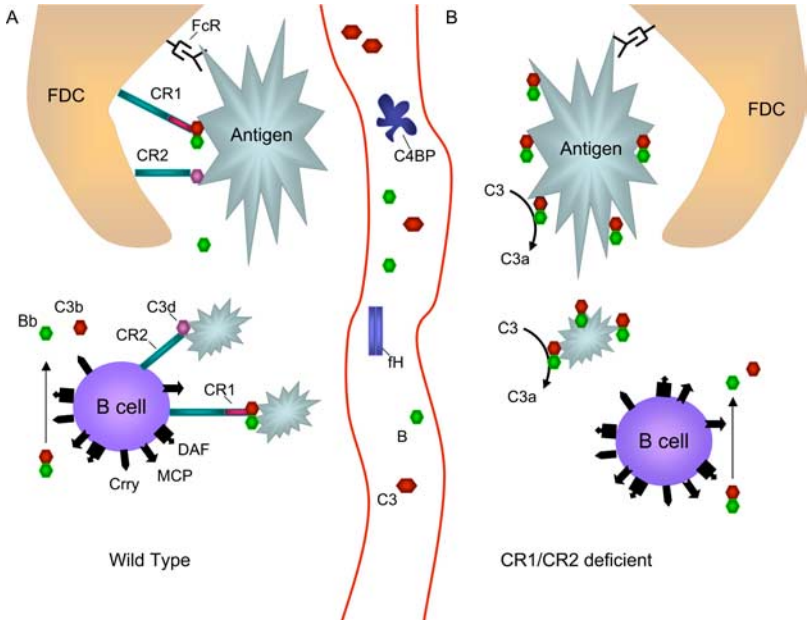

FIGURE 2. Schematic for the regulation of complement convertase activity within lymphatic tissue. The comparison of WT (*left side*, *A*) and *Cr2*-deficient (*right side*, *B*) lymphatic tissue depicting three zones of complement regulation: the zone on the cell surface regulated by Crry, DAF, and MCP; the zone in the blood stream regulated by the soluble factors fH and C4BP; and the zone found with immune complexes associated with FDC and B cells. For clarity, the alternative C3 complement pathway is shown, although classical and lectin pathways could be similarly depicted, as could the regulation of C5 cleavage.

of the CR1 protein but would leave intact the complement-bound Ag B cell signaling pathway (thus creating for the mouse the equivalent of the human *CR2* gene). Alternatively, replacing the sequences encoding the transmembrane and cytoplasmic domains of the *Cr2* gene with those from a nonsignaling protein (such as the MHC class I protein that abrogates the formation of the CR2/CD19 complex) (65) would leave the complement regulatory and receptor functions of the CR1 and CR2 proteins intact but would eliminate the ability of these proteins to effectively provide intracellular signaling. Both of these animal models are currently under analysis (our unpublished data).

The regulation of complement activation has focused upon blocking convertase formation on cell surfaces via the proteins DAF, MCP and Crry and disrupting active convertases in the serum using the soluble regulators C4BP and fH. Together, these sets of proteins should be able to control complement activation pathways in the body. However, as shown in Fig. 2, the analysis of the *Cr2*-deficient mouse suggests that lymphoid tissues require an additional level of convertase control. The deposition of immune complexes in lymphoid tissues places convertases too far from the cell surface for DAF, MCP, or Crry to regulate, but out of the reach of the serum regulators. The large size of CR1 would allow it to modulate the activity of the complement convertases bound to such extracellular immune complexes. As depicted in Fig. 2, misregulation of complement convertases due to lack of the CR1 protein would allow for the excessive release of C3a and C5a. Although only misregulation of the alternative pathway is shown in Fig. 2, any of the pathways could be similarly affected. fH is ~150,000 Da while C4BP is much larger at ~570,000 Da. The larger size of the C4BP protein may pose a greater restriction for its exit from the circulatory system and entry into the lymphoid tissue matrix of the spleen than fH. Thus CR1 proteins (both mouse and human) may be more critical for the neutralization of C3/C5 convertases generated from the classical or lectin pathway than for the alternative pathway C3/C5 convertase.

Concluding remarks

The analysis of animals possessing engineered genetic defects has always been limited by the preconception of phenotypes and the penetrance of the defect. Apart from the vagaries associated with the techniques (85), immunological concerns of such genetically engineered animals can include the lack of the optimal test for their analysis, the potential of other proteins providing complementary and overlapping functions, and the difficulties in discriminating a specific phenotype by proteins with multiple functions. The *Cr2*-deficient mice are illustrative of such concerns in that separating the extracellular complement regulatory effects of the deficiency from those implicit with the diminished capacity of the CD19-associated B cell signaling pathway has complicated their analysis. No doubt similar concerns exist for many such gene deficiency models, lending credence to the application of nonbiased assays (such as gene expression analyses) as a complement to their initial characterization.

Acknowledgments

We thank Janis Weis and members of the laboratory for critical reading of this work.

Disclosures

The authors have no financial conflict of interest.

References

1. Haas, P. J., and J. van Strijp. 2007. Anaphylatoxins: their role in bacterial infection and inflammation. *Immunol. Res.* 37: 161–175.
2. Law, S. K., and A. W. Dodds. 1997. The internal thioester and the covalent binding properties of the complement proteins C3 and C4. *Protein Sci.* 6: 263–274.
3. Gerard, N. P., and C. Gerard. 1991. The chemotactic receptor for human C5a anaphylatoxin. *Nature* 349: 614–617.
4. Kohl, J. 2006. Self, non-self, and danger: a complementary view. *Adv. Exp. Med. Biol.* 586: 71–94.
5. Medof, M. E., T. Kinoshita, and V. Nussenzweig. 1984. Inhibition of complement activation on the surface of cells after incorporation of decay-accelerating factor (DAF) into their membranes. *J. Exp. Med.* 160: 1558–1578.
6. Nicholson-Weller, A., J. Burge, D. T. Fearon, P. F. Weller, and K. F. Austen. 1982. Isolation of a human erythrocyte membrane glycoprotein with decay-accelerating activity for C3 convertases of the complement system. *J. Immunol.* 129: 184–189.
7. Cole, J. L., G. A. Housley, Jr., T. R. Dykman, R. P. MacDermott, and J. P. Atkinson. 1985. Identification of an additional class of C3-binding membrane proteins of human peripheral blood leukocytes and cell lines. *Proc. Natl. Acad. Sci. USA* 82: 859–863.
8. Seya, T., J. R. Turner, and J. P. Atkinson. 1986. Purification and characterization of a membrane protein (gp45–70) that is a cofactor for cleavage of C3b and C4b. *J. Exp. Med.* 163: 837–855.
9. Kim, Y. U., T. Kinoshita, H. Molina, D. Hourcade, T. Seya, L. M. Wagner, and V. M. Holers. 1995. Mouse complement regulatory protein Crry/p65 uses the specific

mechanisms of both human decay-accelerating factor and membrane cofactor protein. *J. Exp. Med.* 181: 151–159.
10. Paul, M. S., M. Aegerter, K. Cepek, M. D. Miller, and J. H. Weis. 1990. The murine complement receptor gene family. III. The genomic and transcriptional complexity of the Crry and Crry-ps genes. *J. Immunol.* 144: 1988–1996.
11. Quigg, R. J., C. F. Lo, J. J. Alexander, A. E. Sneed, III, and G. Moxley. 1995. Molecular characterization of rat Crry: widespread distribution of two alternative forms of Crry mRNA. *Immunogenetics* 42: 362–367.
12. Scharfstein, J., A. Ferreira, I. Gigli, and V. Nussenzweig. 1978. Human C4-binding protein. I. Isolation and characterization. *J. Exp. Med.* 148: 207–222.
13. Gigli, I., T. Fujita, and V. Nussenzweig. 1979. Modulation of the classical pathway C3 convertase by plasma proteins C4 binding protein and C3b inactivator. *Proc. Natl. Acad. Sci. USA* 76: 6596–6600.
14. Zipfel, P. F., J. Hellwage, M. A. Friese, G. Hegasy, S. T. Jokiranta, and S. Meri. 1999. Factor H and disease: a complement regulator affects vital body functions. *Mol. Immunol.* 36: 241–248.
15. Laine, M., H. Jarva, S. Seitsonen, K. Haapasalo, M. J. Lehtinen, N. Lindeman, D. H. Anderson, P. T. Johnson, I. Jarvela, T. S. Jokiranta, et al. 2007. Y402H polymorphism of complement factor H affects binding affinity to C-reactive protein. *J. Immunol.* 178: 3831–3836.
16. Klein, R. J., C. Zeiss, E. Y. Chew, J. Y. Tsai, R. S. Sackler, C. Haynes, A. K. Henning, J. P. SanGiovanni, S. M. Mane, S. T. Mayne, et al. 2005. Complement factor H polymorphism in age-related macular degeneration. *Science* 308: 385–389.
17. Edwards, A. O., R. Ritter, III, K. J. Abel, A. Manning, C. Panhuysen, and L. A. Farrer. 2005. Complement factor H polymorphism and age-related macular degeneration. *Science* 308: 421–424.
18. Haines, J. L., M. A. Hauser, S. Schmidt, W. K. Scott, L. M. Olson, P. Gallins, K. L. Spencer, S. Y. Kwan, M. Noureddine, J. R. Gilbert, et al. 2005. Complement factor H variant increases the risk of age-related macular degeneration. *Science* 308: 419–421.
19. Krych-Goldberg, M., and J. P. Atkinson. 2001. Structure-function relationships of complement receptor type 1. *Immunol. Rev.* 180: 112–122.
20. Wong, W. W., J. M. Cahill, M. D. Rosen, C. A. Kennedy, E. T. Bonaccio, M. J. Morris, J. G. Wilson, L. B. Klickstein, and D. T. Fearon. 1989. Structure of the human CR1 gene. Molecular basis of the structural and quantitative polymorphisms and identification of a new CR1-like allele. *J. Exp. Med.* 169: 847–863.
21. Changelian, P. S., R. M. Jack, L. A. Collins, and D. T. Fearon. 1985. PMA induces the ligand-independent internalization of CR1 on human neutrophils. *J. Immunol.* 134: 1851–1858.
22. Hess, C., and J. A. Schifferli. 2003. Immune adherence revisited: novel players in an old game. *News Physiol. Sci.* 18: 104–108.
23. Cornacoff, J. B., L. A. Hebert, W. L. Smead, M. E. VanAman, D. J. Birmingham, and F. J. Waxman. 1983. Primate erythrocyte-immune complex-clearing mechanism. *J. Clin. Invest.* 71: 236–247.
24. Reinagel, M. L., and R. P. Taylor. 2000. Transfer of immune complexes from erythrocyte CR1 to mouse macrophages. *J. Immunol.* 164: 1977–1985.
25. Weis, J. J., T. F. Tedder, and D. T. Fearon. 1984. Identification of a 145,000 Mr membrane protein as the C3d receptor (CR2) of human B lymphocytes. *Proc. Natl. Acad. Sci. USA* 81: 881–885.
26. Nemerow, G. R., R. Wolfert, M. E. McNaughton, and N. R. Cooper. 1985. Identification and characterization of the Epstein-Barr virus receptor on human B lymphocytes and its relationship to the C3d complement receptor (CR2). *J. Virol.* 55: 347–351.
27. Molina, H., T. Kinoshita, C. B. Webster, and V. M. Holers. 1994. Analysis of C3b/C3d binding sites and factor I cofactor regions within mouse complement receptors 1 and 2. *J. Immunol.* 153: 789–795.
28. van Lookeren Campagne, M., C. Wiesmann, and E. J. Brown. 2007. Macrophage complement receptors and pathogen clearance. *Cell. Microbiol.* 9: 2095–2102.
29. Liszewski, M. K., T. C. Farries, D. M. Lublin, I. A. Rooney, and J. P. Atkinson. 1996. Control of the complement system. *Adv. Immunol.* 61: 201–283.
30. Spicer, A. P., M. F. Seldin, and S. J. Gendler. 1995. Molecular cloning and chromosomal localization of the mouse decay-accelerating factor genes. Duplicated genes encode glycosylphosphatidylinositol-anchored and transmembrane forms. *J. Immunol.* 155: 3079–3091.
31. Manchester, M., A. Valsamakis, R. Kaufman, M. K. Liszewski, J. Alvarez, J. P. Atkinson, D. M. Lublin, and M. B. Oldstone. 1995. Measles virus and C3 binding sites are distinct on membrane cofactor protein (CD46). *Proc. Natl. Acad. Sci. USA* 92: 2303–2307.
32. Tsujimura, A., K. Shida, M. Kitamura, M. Nomura, J. Takeda, H. Tanaka, M. Matsumoto, K. Matsumiya, A. Okuyama, Y. Nishimune, et al. 1998. Molecular cloning of a murine homologue of membrane cofactor protein (CD46): preferential expression in testicular germ cells. *Biochem. J.* 330: 163–168.
33. Inoue, N., M. Ikawa, T. Nakanishi, M. Matsumoto, M. Nomura, T. Seya, and M. Okabe. 2003. Disruption of mouse CD46 causes an accelerated spontaneous acrosome reaction in sperm. *Mol. Cell. Biol.* 23: 2614–2622.
34. Kurtz, C. B., E. O'Toole, S. M. Christensen, and J. H. Weis. 1990. The murine complement receptor gene family. IV. Alternative splicing of Cr2 gene transcripts predicts two distinct gene products that share homologous domains with both human CR2 and CR1. *J. Immunol.* 144: 3581–3591.
35. Kinoshita, T., J. Takeda, K. Hong, H. Kozono, H. Sakai, and K. Inoue. 1988. Monoclonal antibodies to mouse complement receptor type 1 (CR1). Their use in a distribution study showing that mouse erythrocytes and platelets are CR1-negative. *J. Immunol.* 140: 3066–3072.
36. Holguin, M. H., C. B. Kurtz, C. J. Parker, J. J. Weis, and J. H. Weis. 1990. Loss of human CR1- and murine Crry-like exons in human CR2 transcripts due to CR2 gene mutations. *J. Immunol.* 145: 1776–1781.
37. McLure, C. A., J. F. Williamson, B. J. Stewart, P. J. Keating, and R. L. Dawkins. 2005. Indels and imperfect duplication have driven the evolution of human complement receptor 1 (CR1) and CR1-like from their precursor CR1 α: importance of functional sets. *Hum. Immunol.* 66: 258–273.
38. Fearon, D. T., L. B. Klickstein, W. W. Wong, J. G. Wilson, F. D. Moore, Jr., J. J. Weis, J. H. Weis, R. M. Jack, R. H. Carter, and J. A. Ahearn. 1989. Immunoregulatory functions of complement: structural and functional studies of complement receptor type 1 (CR1; CD35) and type 2 (CR2; CD21). *Prog. Clin. Biol. Res.* 297: 211–220.
39. Hourcade, D., V. M. Holers, and J. P. Atkinson. 1989. The regulators of complement activation (RCA) gene cluster. *Adv. Immunol.* 45: 381–416.
40. Birmingham, D. J., X. P. Shen, D. Hourcade, M. W. Nickells, and J. P. Atkinson. 1994. Primary sequence of an alternatively spliced form of CR1. Candidate for the 75,000 M_r complement receptor expressed on chimpanzee erythrocytes. *J. Immunol.* 153: 691–700.
41. Xu, C., D. Mao, V. M. Holers, B. Palanca, A. M. Cheng, and H. Molina. 2000. A critical role for murine complement regulator crry in fetomaternal tolerance. *Science* 287: 498–501.
42. Paul, M. S., M. Aegerter, S. E. O'Brien, C. B. Kurtz, and J. H. Weis. 1989. The murine complement receptor gene family. Analysis of mCRY gene products and their homology to human CR1. *J. Immunol.* 142: 582–589.
43. Qin, D., J. Wu, M. C. Carroll, G. F. Burton, A. K. Szakal, and J. G. Tew. 1998. Evidence for an important interaction between a complement-derived CD21 ligand on follicular dendritic cells and CD21 on B cells in the initiation of IgG responses. *J. Immunol.* 161: 4549–4554.
44. Kurtz, C. B., M. S. Paul, M. Aegerter, J. J. Weis, and J. H. Weis. 1989. Murine complement receptor gene family. II. Identification and characterization of the murine homolog (Cr2) to human CR2 and its molecular linkage to Crry. *J. Immunol.* 143: 2058–2067.
45. Kaya, Z., M. Afanasyeva, Y. Wang, K. M. Dohmen, J. Schlichting, T. Tretter, D. Fairweather, V. M. Holers, and N. R. Rose. 2001. Contribution of the innate immune system to autoimmune myocarditis: a role for complement. *Nat. Immunol.* 2: 739–745.
46. Emlen, W., V. Carl, and G. Burdick. 1992. Mechanism of transfer of immune complexes from red blood cell CR1 to monocytes. *Clin. Exp. Immunol.* 89: 8–17.
47. Helmy, K. Y., K. J. Katschke, Jr., N. N. Gorgani, N. M. Kljavin, J. M. Elliott, L. Diehl, S. J. Scales, N. Ghilardi, and M. van Lookeren Campagne. 2006. CRIg: a macrophage complement receptor required for phagocytosis of circulating pathogens. *Cell* 124: 915–927.
48. Holers, V. M., T. Kinoshita, and H. Molina. 1992. The evolution of mouse and human complement C3-binding proteins: divergence of form but conservation of function. *Immunol. Today* 13: 231–236.
49. Ghiran, I., S. F. Barbashov, L. B. Klickstein, S. W. Tas, J. C. Jensenius, and A. Nicholson-Weller. 2000. Complement receptor 1/CD35 is a receptor for mannan-binding lectin. *J. Exp. Med.* 192: 1797–1808.
50. Klickstein, L. B., S. F. Barbashov, T. Liu, R. M. Jack, and A. Nicholson-Weller. 1997. Complement receptor type 1 (CR1, CD35) is a receptor for C1q. *Immunity* 7: 345–355.
51. Weisman, H. F., T. Bartow, M. K. Leppo, H. C. Marsh, Jr., G. R. Carson, M. F. Concino, M. P. Boyle, K. H. Roux, M. L. Weisfeldt, and D. T. Fearon. 1990. Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science* 249: 146–151.
52. Ramaglia, V., R. Wolterman, M. de Kok, M. A. Vigar, I. Wagenaar-Bos, R. H. King, B. P. Morgan, and F. Baas. 2008. Soluble complement receptor 1 protects the peripheral nerve from early axon loss after injury. *Am. J. Pathol.* 172: 1043–1052.
53. Mulligan, M. S., C. G. Yeh, A. R. Rudolph, and P. A. Ward. 1992. Protective effects of soluble CR1 in complement- and neutrophil-mediated tissue injury. *J. Immunol.* 148: 1479–1485.
54. Quigg, R. J., Y. Kozono, D. Berthiaume, A. Lim, D. J. Salant, A. Weinfeld, P. Griffin, E. Kremmer, and V. M. Holers. 1998. Blockade of antibody-induced glomerulonephritis with Crry-Ig, a soluble murine complement inhibitor. *J. Immunol.* 160: 4553–4560.
55. Briggs, D. T., C. B. Martin, S. A. Ingersoll, S. R. Barnum, and B. K. Martin. 2007. Astrocyte-specific expression of a soluble form of the murine complement control protein Crry confers demyelination protection in the cuprizone model. *Glia* 55: 1405–1415.
56. Nath, S. K., J. B. Harley, and Y. H. Lee. 2005. Polymorphisms of complement receptor 1 and interleukin-10 genes and systemic lupus erythematosus: a meta-analysis. *Hum. Genet.* 118: 225–234.
57. Cockburn, I. A., M. J. Mackinnon, A. O'Donnell, S. J. Allen, J. M. Moulds, M. Baisor, M. Bockarie, J. C. Reeder, and J. A. Rowe. 2004. A human complement receptor 1 polymorphism that reduces *Plasmodium falciparum* rosetting confers protection against severe malaria. *Proc. Natl. Acad. Sci. USA* 101: 272–277.
58. Fang, Y., C. Xu, Y. X. Fu, V. M. Holers, and H. Molina. 1998. Expression of complement receptors 1 and 2 on follicular dendritic cells is necessary for the generation of a strong antigen-specific IgG response. *J. Immunol.* 160: 5273–5279.
59. Prodeus, A. P., S. Goerg, L. M. Shen, O. O. Pozdnyakova, L. Chu, E. M. Alicot, C. C. Goodnow, and M. C. Carroll. 1998. A critical role for complement in maintenance of self-tolerance. *Immunity* 9: 721–731.
60. Wu, X., N. Jiang, Y. F. Fang, C. Xu, D. Mao, J. Singh, Y. X. Fu, and H. Molina. 2000. Impaired affinity maturation in $Cr2^{-/-}$ mice is rescued by adjuvants without improvement in germinal center development. *J. Immunol.* 165: 3119–3127.
61. Lindorfer, M. A., H. B. Jinivizian, P. L. Foley, A. D. Kennedy, M. D. Solga, and R. P. Taylor. 2003. B cell complement receptor 2 transfer reaction. *J. Immunol.* 170: 3671–3678.

62. Phan, T. G., I. Grigorova, T. Okada, and J. G. Cyster. 2007. Subcapsular encounter and complement-dependent transport of immune complexes by lymph node B cells. *Nat. Immunol.* 8: 992–1000.
63. Whipple, E. C., R. S. Shanahan, A. H. Ditto, R. P. Taylor, and M. A. Lindorfer. 2004. Analyses of the in vivo trafficking of stoichiometric doses of an anti-complement receptor 1/2 monoclonal antibody infused intravenously in mice. *J. Immunol.* 173: 2297–2306.
64. Matsumoto, A. K., J. Kopicky-Burd, R. H. Carter, D. A. Tuveson, T. F. Tedder, and D. T. Fearon. 1991. Intersection of the complement and immune systems: a signal transduction complex of the B lymphocyte-containing complement receptor type 2 and CD19. *J. Exp. Med.* 173: 55–64.
65. Matsumoto, A. K., D. R. Martin, R. H. Carter, L. B. Klickstein, J. M. Ahearn, and D. T. Fearon. 1993. Functional dissection of the CD21/CD19/TAPA-1/Leu-13 complex of B lymphocytes. *J. Exp. Med.* 178: 1407–1417.
66. Bradbury, L. E., G. S. Kansas, S. Levy, R. L. Evans, and T. F. Tedder. 1992. The CD19/CD21 signal transducing complex of human B lymphocytes includes the target of antiproliferative antibody-1 and Leu-13 molecules. *J. Immunol.* 149: 2841–2850.
67. Smith, R. A., J. Young, J. J. Weis, and J. H. Weis. 2006. Expression of the mouse fragilis gene products in immune cells and association with receptor signaling complexes. *Genes and immunity* 7: 113–121.
68. Lee, Y., K. M. Haas, D. O. Gor, X. Ding, D. R. Karp, N. S. Greenspan, J. C. Poe, and T. F. Tedder. 2005. Complement component C3d-antigen complexes can either augment or inhibit B lymphocyte activation and humoral immunity in mice depending on the degree of CD21/CD19 complex engagement. *J. Immunol.* 175: 8011–8023.
69. Barel, M., M. Balbo, M. Le Romancer, and R. Frade. 2003. Activation of Epstein-Barr virus/C3d receptor (gp140, CR2, CD21) on human cell surface triggers pp60src and Akt-GSK3 activities upstream and downstream to PI 3-kinase, respectively. *Eur. J. Immunol.* 33: 2557–2566.
70. Barrault, D. V., and A. M. Knight. 2004. Distinct sequences in the cytoplasmic domain of complement receptor 2 are involved in antigen internalization and presentation. *J. Immunol.* 172: 3509–3517.
71. Ahearn, J. M., M. B. Fischer, D. Croix, S. Goerg, M. Ma, J. Xia, X. Zhou, R. G. Howard, T. L. Rothstein, and M. C. Carroll. 1996. Disruption of the Cr2 locus results in a reduction in B-1a cells and in an impaired B cell response to T-dependent antigen. *Immunity* 4: 251–262.
72. Molina, H., V. M. Holers, B. Li, Y. Fung, S. Mariathasan, J. Goellner, J. Strauss-Schoenberger, R. W. Karr, and D. D. Chaplin. 1996. Markedly impaired humoral immune response in mice deficient in complement receptors 1 and 2. *Proc. Natl. Acad. Sci. USA* 93: 3357–3361.
73. Haas, K. M., M. Hasegawa, D. A. Steeber, J. C. Poe, M. D. Zabel, C. B. Bock, D. R. Karp, D. E. Briles, J. H. Weis, and T. F. Tedder. 2002. Complement receptors CD21/35 link innate and protective immunity during *Streptococcus pneumoniae* infection by regulating IgG3 antibody responses. *Immunity* 17: 713–723.
74. Diaz de Stahl, T., J. Dahlstrom, M. C. Carroll, and B. Heyman. 2003. A role for complement in feedback enhancement of antibody responses by IgG3. *J. Exp. Med.* 197: 1183–1190.
75. Hasegawa, M., M. Fujimoto, J. C. Poe, D. A. Steeber, and T. F. Tedder. 2001. CD19 can regulate B lymphocyte signal transduction independent of complement activation. *J. Immunol.* 167: 3190–3200.
76. Barrington, R. A., M. Zhang, X. Zhong, H. Jonsson, N. Holodick, A. Cherukuri, S. K. Pierce, T. L. Rothstein, and M. C. Carroll. 2005. CD21/CD19 coreceptor signaling promotes B cell survival during primary immune responses. *J. Immunol.* 175: 2859–2867.
77. Fischer, M. B., S. Goerg, L. Shen, A. P. Prodeus, C. C. Goodnow, G. Kelsoe, and M. C. Carroll. 1998. Dependence of germinal center B cells on expression of CD21/CD35 for survival. *Science* 280: 582–585.
78. Chen, Z., S. B. Koralov, and G. Kelsoe. 2000. Complement C4 inhibits systemic autoimmunity through a mechanism independent of complement receptors CR1 and CR2. *J. Exp. Med.* 192: 1339–1352.
79. Wu, X., N. Jiang, C. Deppong, J. Singh, G. Dolecki, D. Mao, L. Morel, and H. D. Molina. 2002. A role for the Cr2 gene in modifying autoantibody production in systemic lupus erythematosus. *J. Immunol.* 169: 1587–1592.
80. Jacobson, A. C., J. J. Weis, and J. H. Weis. 2008. Complement receptors 1 and 2 influence the immune environment in a B cell receptor-independent manner. *J. Immunol.* 180: 5057–5066.
81. Delano, M. J., P. O. Scumpia, J. S. Weinstein, D. Coco, S. Nagaraj, K. M. Kelly-Scumpia, K. A. O'Malley, J. L. Wynn, S. Antonenko, S. Z. Al-Quran, et al. 2007. MyD88-dependent expansion of an immature GR-1^{+}CD11b^{+} population induces T cell suppression and Th2 polarization in sepsis. *J. Exp. Med.* 204: 1463–1474.
82. Ezernitchi, A. V., I. Vaknin, L. Cohen-Daniel, O. Levy, E. Manaster, A. Halabi, E. Pikarsky, L. Shapira, and M. Baniyash. 2006. TCR ζ down-regulation under chronic inflammation is mediated by myeloid suppressor cells differentially distributed between various lymphatic organs. *J. Immunol.* 177: 4763–4772.
83. Morgan, E. L., W. O. Weigle, and T. E. Hugli. 1984. Anaphylatoxin-mediated regulation of human and murine immune responses. *Fed. Proc.* 43: 2543–2547.
84. Fischer, W. H., and T. E. Hugli. 1997. Regulation of B cell functions by C3a and C3^{desArg}: suppression of TNF-α, IL-6, and the polyclonal immune response. *J. Immunol.* 159: 4279–4286.
85. Schmidt-Supprian, M., and K. Rajewsky. 2007. Vagaries of conditional gene targeting. *Nat. Immunol.* 8: 665–668.