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Review

The murine complement regulator Crry: new insights into the immunobiology of complement regulation

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Abstract. Complement has an important role in inflammation and in the normal function of the immune system. Activated complement fragments have the capacity to bind and damage self-tissues. Cells from vertebrates express on their surface regulators of complement activation that protect them from the deleterious effects of cell-bound complement fragments. Abnormalities in these regulators of complement activation may participate in the pathogenesis of autoimmune diseases and inflammatory disorders. Murine Crry is one of these regulators that inhibits the activation of the third component of complement and protects self-tissues from complement-mediated damage. Experimental work on Crry has increased our understanding of the immunobiology of complement regulation and the potential role of complement and complement inhibitors in the development and treatment of human diseases.

Key words. Complement; complement regulators; C3; inflammation; transgenic mice; gene-targeted mice.

Introduction

The complement system consists of a series of distinct but related serum proteins that, once activated, facilitate the immune response and the inflammatory response [1, 2]. Complement activation promotes chemotaxis of inflammatory cells, generates fragments that enhance phagocytosis by neutrophils and monocytes, and expedites the clearance of immune complexes. Abnormalities in cell membrane function are mediated by the formation of the membrane attack complex.

Complement activation occurs by three main mechanisms (fig. 1) [2]. The classical pathway is initiated by antigen-antibody immune complexes. Once formed, these immune complexes activate the first component of complement, C1. C1 cleaves C4, and the major cleavage fragment C4b binds covalently to the target surface in close proximity to C1. C2 then attaches to C4b and is cleaved by C1, generating a fragment known as C2a which is part of the C4b2a bimolecular enzyme known as the classical pathway C3 convertase. C4b2a is one of the enzymes responsible for the activation of the third component of complement C3. Activation of complement through the alternative pathway occurs when a fragment derived from C3, C3b, spontaneously forms in the fluid phase and attaches to a target surface that permits the subsequent binding of factor B. Factor B is then activated by a serum serine esterase known as factor D. This forms a fragment, known as Bb, that is part of the C3bBb bimolecular enzyme known as the alternative pathway C3 convertase. This enzyme is only active when the Bb fragment is attached to the C3b fragment. Properdin, another serum protein of the alternative pathway, can attach and stabilize the C3bBb complex. The interaction of properdin with the alternative pathway C3 convertase preferentially occurs on specific targets such as microbial surfaces, as opposed to host cells. In addition, the alternative pathway serves as an amplification loop for the classical

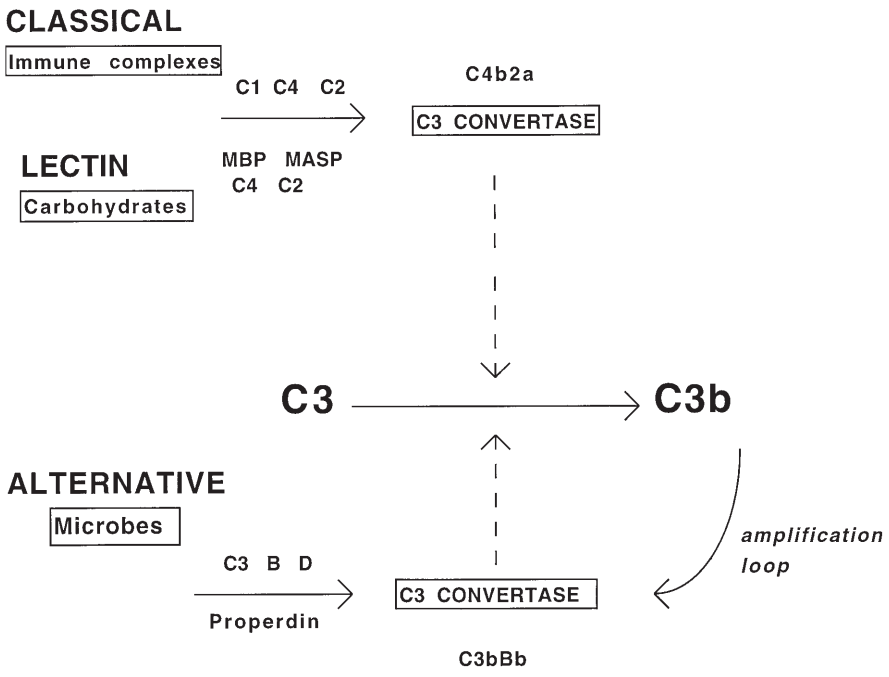

Figure 1. Activation of the complement system. These pathways share a common essential purpose that consists in activation of the third component of complement, C3. See text for more details. MBP, mannose binding protein; MASP, mannose-binding protein-associated serine esterase; B, factor B; D, factor D.

pathway since C3b formed by the latter pathway can interact with factor B and produce C3bBb (see fig. 1). The lectin pathway is initiated by plasma proteins known as mannan-binding lectins, which bind to carbohydrate moieties on the surface of pathogens. Once bound, these lectins interact with serum enzymes known as mannan-binding lectin-associated serine esterase, which participates in the activation of C4 and C2.

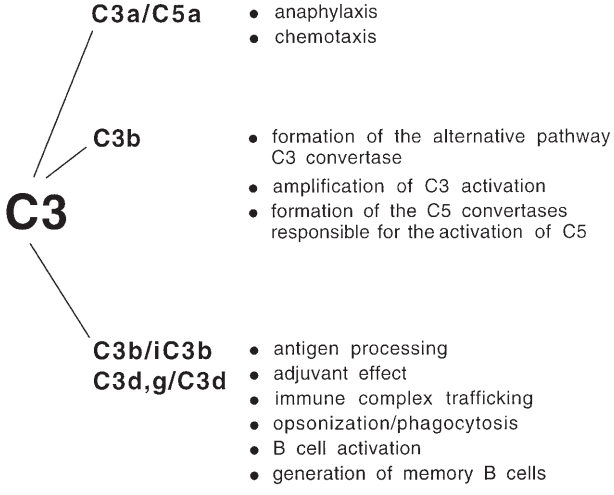

Figure 2. Activation of C3 generates different cleavage products (denoted by the lowercase letters) with specific biological functions.

C3 activation plays a critical role in the biology of the system [2]. Cleavage of C3 generates several biologically active fragments that are responsible for most of the complement functions described above (fig. 2). Furthermore, C3b is responsible for the activation of the alternative pathway. C3b is also an essential part of three enzyme complexes known as the alternative pathway C3 convertase, and the classical pathway and alternative pathway C5 convertase. These enzymes are responsible for activation of C3 and C5, respectively. Blocking the activity of these enzymes by specific regulatory proteins effectively hinders the formation of activated C3 and C5 fragments, and the formation of the membrane attack complex.

Regulation of complement activation

Activated complement fragments have the capacity to bind to tissues, trigger inflammation and cause injury. Autologous bystander cells are protected from the deleterious effects of complement by plasma and membrane-bound proteins that regulate complement activation [3]. Some of this regulation occurs at the level of C3 [4]. Inhibition of C3 activation avoids the formation of most of the complement mediators involved in humoral immunity, inflammation and tissue destruction.

Two mechanisms are involved in the regulation of C3 activation [4]. The first mechanism prevents formation or

Table 1. Regulators of complement activation.

Name	Primary ligand	Decay acceleration	Cofactor activity	Distribution
Complement receptor 1	C3b/C4b	positive	positive	membrane (limited): peripheral blood cells, follicular dendritic cells, glomerular podocytes
Membrane cofactor protein	C3b/C4b	negative	positive	membrane: peripheral blood cells (except erythrocytes), epithelial, endothelial, fibroblast cell lineages
Decay-accelerating factor	C3b/C4b	positive	negative	membrane: ubiquitous
Crry	C3b/C4b	positive	positive	membrane: ubiquitous
C4-binding	C4b	positive	positive	serum
Factor H	C3b	positive	positive	serum

accelerates dissociation of the C3 convertase by a process known as decay-accelerating activity. The second mechanism consists of the cleavage of activated C3 mediated by a serine esterase, factor I, in the presence of protein cofactors.

Several proteins are involved in the regulation of C3 activation [5] (table 1). They belong to a family of proteins called regulators of complement activity (RCA). These molecules are characterized by their capacity to interact with C3 and/or C4 fragments, and by the presence of a common structural motif consisting of repeats of approximately 60 amino acids designated complement control protein (CCP) repeats or short consensus repeats (SCRs). Factor H is a serum protein whose primary ligand is C3b. It is involved in dissociation of alternative pathway C3 convertase and also serves as a cofactor for factor I-mediated cleavage of C3b. C4-binding protein is a serum protein that binds C4b. It is involved in dissociation of the classical pathway C3 convertase and also serves as a cofactor for factor I-mediated cleavage of C4b. In addition, three membrane-bound proteins regulate the activation of C3 on the surface of most human cells [5]. Decay-accelerating factor (DAF) is a ubiquitous 70-kDa protein that is bound to the cell membrane by a phosphatidylinositol linkage. This molecule is responsible for the dissociation of the C3 convertase. Membrane cofactor protein (MCP) is a ~45–70-kDa membrane-bound glycoprotein present on most blood cells (except erythrocytes), and cells of fibroblast, endothelial and epithelial lineages. MCP serves as a cofactor for factor I cleavage of C4b and C3b. Complement receptor 1 (CR1) is a membrane-bound protein that interacts with C3b and C4b. It is present on erythrocytes, monocytes, most B cells and some T cells, polymorphonuclar cells, follicular dendritic cells and glomerular podocytes. It can dissociate the alternative and classical pathway C3 convertases. It also serves as cofactor for factor I cleavage of C3b and C4b.

The importance of these membrane regulators of C3 activation in protecting host cells from complement-mediated damage is highlighted by several experimental observations. In vitro, antibody-induced functional blockade of human DAF and/or MCP increases C3b deposition on the surface of autologous cells and sensitizes them to complement lysis [6, 7]. In addition, expression of human DAF and MCP on the surface of mouse cells protects them from damage mediated by human complement [8]. Conversely, soluble CR1 administration suppresses tissue damage in rat models of post-ischemic myocardial injury and immune complex-mediated vasculitis [9].

There is also considerable evidence that abnormalities in these regulators of complement activation may participate in the pathogenesis of human diseases. Deficiency of DAF and CD59, a membrane-bound protein that controls the formation of the membrane attack complex, is associated with increased C3b deposition and subsequent hemolysis of human erythrocytes in the disease known as paroxysmal nocturnal hemoglobinuria [10–12]. Abnormalities in DAF expression have also been found in diseases such as rheumatoid arthritis and systemic lupus erythematosus, autoimmune disorders characterized by the presence of autoantibodies and damage due to complement activation mediated by immune-complex deposition in organs [13, 14]. Deficiency in factor H is associated with development of membranoproliferative glomerulonephritis in humans and pigs [15, 16]. Inherited forms of the hemolytic uremic syndrome are also strongly associated with functional factor H deficiency [17–19]. Furthermore, decreased expression of CR1 on B cells and neutrophils has been found in patients with systemic lupus erythematosus and other autoimmune diseases [20–22].

Identification of Crry

To perform a more extensive analysis of the role of human C3 regulators in vivo, several investigators characterized their murine homologues. Mouse DAF and MCP have been described elsewhere [23–26] (see below). Interestingly, mouse cells express an additional molecule

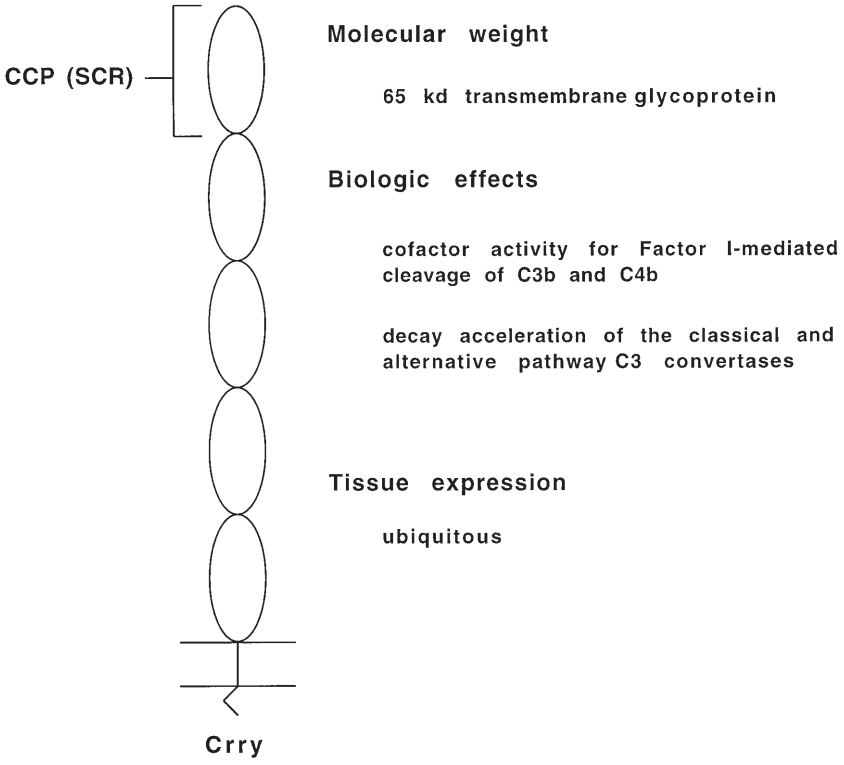

Figure 3. Characterization of mouse Crry. See text for more details. CCP, complement control protein; SCR, short consensus repeat.

that regulates C3 activation [27] (fig. 3). This protein was first identified as a 65-kDa molecule, designated p65, immunoprecipitated from mouse spleen cell membranes using a rabbit polyclonal antibody against human CR1 [28]. Parallel to these experiments, a complementary DNA (cDNA), named *Crry* was cloned from murine spleen libraries probed with a human CR1 cDNA [29, 30]. That the p65 protein was encoded by *Crry* was demonstrated by transfecting this cDNA into human K562 cells lacking CR1 expression [31]. Only cells transfected with the recombinant *Crry* cDNA reacted with the rabbit polyclonal anti-human CR1/p65 antibody described above. Furthermore, Crry-expressing cells were exquisitely protected from deposition of mouse C3b and potential complement-mediated damage as compared with nonexpressing cells [31]. This was the first evidence suggesting that Crry function was related to the regulation of C3 activation. Further work in vitro revealed that the biochemical C3 regulatory properties of Crry were identical to human DAF and human MCP [32]. With MCP it shares the ability to serve as a cofactor for factor I-mediated cleavage of mouse C3b and C4b, and also exhibits decay-accelerating activity for the classical and alternative pathway C3 convertases.

Generation of antibodies specific for mouse Crry helped to further characterize the protein [33]. Crry is a transmembrane glycoprotein expressed in most mouse tissues. The degree of expression varies depending on the tissue, and is most notable in thymus, kidney, uterus, skin, fat and intestine, and to a lesser degree in brain and muscle [33]. All peripheral blood cells express the protein. Substantial differences also exist in molecular weight when comparing different tissues. The reason for tissue-specific isoforms is unknown. Crry has also been identified in rats in tissue specific isoforms. [34, 35]. Again, the role of multiple isoforms of rat Crry remains to be defined.

Crry Complement Regulatory Activities

The importance of Crry in protecting host cells from C3-mediated damage is highlighted by several experimental observations. As pointed out earlier, human cell lines expressing mouse Crry are protected from mouse C3b deposition [31]. Similarly, antibody-induced functional blockade of rat Crry increases C3b deposition on the surface of cultured glomerular endothelial cells and mesangial cells [36]. In vivo, antibody-mediated functional neutralization of Crry in the kidney of experimental rats induces activation of C3 in the area of the glomeruli, tubules, and perivascular capillaries with a concomitant inflammatory reaction and cell injury [37].

A pronounced effect is achieved by systemic administration of neutralizing anti-Crry antibodies. Intravenous injection of these antibodies causes activation of C3 and substantial deposition of C3b around the capillaries of

different organs [38]. Concomitant elevation in the hematocrit level and a rapid fall in blood pressure are seen in these animals due to the extravasation of intravascular fluid. Circulating leucocytes and platelets decrease secondary to complement-mediated activation that results in the margination of these cells in the vessel wall and subsequent migration into the organs affected. Interestingly, these changes are transient and usually subside after 24 h. These acute effects are mostly secondary to the anaphylactic and chemotatic properties of C3a and C5a. It is completely avoided by pretreating the animals with cobra venom factor, a C3b-like protein found in cobra venom that forms a stable C3 convertase with factor B that is not affected by complement regulatory proteins, and causes severe acute systemic complement depletion [39]. Conversely, inhibiting the inactivation of C3a and C5a induces not only an increase in the abnormalities presented above but an increase in associated mortality.

Results from these experiments underscore several interesting points. First, the investigators used the $F(ab')_2$ fragment of the neutralizing anti-Crry antibody. These bivalent fragments lack the Fc portion of the antbody, but still recognize the target. Consequently, the classical pathway may not be involved in the induction of C3 activation in these rats since the Fc portion of an antibody is required for activation of this pathway. That the alternative or lectin pathway is involved has not been formally addressed. Second, this effect is directly related to Crry blockade since injecting an isotype-matched $F(ab')_2$ fragment of a blocking antibody against CD59, the complement regulatory molecule that inhibits the formation of the membrane attack complex, causes no such adverse effects. This is particularly informative because the tissue expression of both molecules is similar.

Additional evidence pointing to the importance of Crry in protecting host cells from C3-mediated damage is found in Heymann nephritis [40]. Induction of neutralizing auto-antibodies against Crry is the causative factor in this animal model of membranous glomerulonephropathy. In this model, a rat renal tubular preparation (fraction 1A or Fx1A) is injected into rats and induces generation of anti-Fx1A antibodies that bind to the glomeruli. Subsequently, animals develop proteinuria associated with deposition of C3b and other complement components 8 weeks after Fx1A injection. Only Fx1A that contains Crry generates the disease. Injection of Crry-deficient Fx1A induces heavy deposition of anti-Fx1A antibodies within the rat glomeruli but fails to elicit C3b deposition and renal disease. Interestingly, this Crry-deficient Fx1A preparation can be made nephritogenic by adding Crry to it. In addition, the Crry-deficient Fx1A can also cause disease if the $F(ab')_2$ portion of a neutralizing anti-Crry antibody is given 10 weeks after Crry-deficient Fx1A immunization. Administration of control antibodies in the latter experiments does not cause disease. Based on these observations, the authors of this study conclude that Crry is the key nephritogenic auto-immunogen in Heymann nephritis. Abnormalities in Crry function induced by these blocking antibodies cause complement activation and subsequent renal damage.

If blocking the function of Crry causes increased complement activation and subsequent complement-mediated organ damage, it is fair to assume that increasing the activity of this molecule may be protective against inflammatory disorders, especially those induced by abnormal complement activation. In fact, this is the case. A recombinant soluble protein has been generated containing the extracytoplasmic domain of Crry followed by the Fc portion of mouse immunoglobulin G1 (IgG1), an antibody isotype that does not activate complement [41]. This soluble form of Crry (Crry-Ig) retains the complement regulatory functions of the membrane-bound form.

Mice injected with Crry-Ig are resistant to complement-mediated organ damage in a murine model of antibody-induced glomerulonephritis [41]. The amount of proteinuria is dramatically inhibited with Crry-Ig, and is comparable to animals with complement depletion induced by cobra venom factor, or to C3- and C4-deficient mice. IgG derived from the nephrotoxic serum binds to Crry-Ig-treated and control glomeruli, but C3b deposition is present only in control animals and not in the Crry-Ig-treated mice. Interestingly, Crry-Ig is not protective when using a high dose of the nephrotoxic serum, suggesting that as the dose of nephrotoxic antibody is raised, other complement-independent mechanisms of immune-mediated tissue damage are recruited.

Transgenic animal models

The use of transgenic mouse models has expanded our understanding of the role of Crry in vivo. Transgenic mice expressing a soluble functional form of Crry have been generated [42]. The transgenic construct consists of the Crry signal peptide and the extracellular domains, without the transmembrane or intracellular domains, under the transcriptional regulation of the heavy-metal-inducible metallothionein (MT)-1 promoter. These mice have high levels of functional recombinant soluble Crry in their serum. These recombinant soluble Crry transgenic mice are resistant to complement-mediated organ damage in the same murine model of antibody-induced glomerulonephritis described above [42].

In another recombinant soluble Crry transgenic mouse model, the recombinant cDNA encoding for the soluble Crry protein was placed under the transcriptional control of a glial fibrillary acidic promoter that directs the production of the protein to brain astrocytes [43]. These animals have recombinant soluble Crry present in their cerebrospinal fluid. Moreover, these transgenic mice are pro-

tected from the development of neurological deficits in a model of murine experimental allergic encephalomyelitis, a mouse model of multiple sclerosis. Interestingly, this protection is dependent on the mouse strain. Recombinant soluble Crry transgenic mice derived form the SJL/J strain are protected from experimental allergic encephalomyelitis as compared with nontransgenic SJL/J mice, with a decreased number of inflammatory lesions and no complement deposition detected in the few lesions present. In contrast, recombinant soluble Crry transgenic mice derived from the C57BL/6 strain, or the (SJL/J × C57BL/6) F1 strain, have delayed onset of disease but otherwise no clinical protection. These mice have an increased number of inflammatory lesions similar to nontransgenic C57Bl/6 controls.

The authors of this study indicate that this strain-dependent difference in clinical and histological outcome is probably related to strain-dependent differences in experimental allergic encephalomyelitis disease expression. SJL/J wild-type mice have an acute monophasic demyelinating disease, whereas C57BL/6 mice have a severe chronic demyelinating form of experimental allergic encephalomyelitis. The initial phase of the disease may be dependent on complement in both strains of mice. This may explain the beneficial outcome in the SJL/J mice expressing the recombinant soluble Crry and the delayed onset of disease in the C57BL/6 mice expressing the recombinant soluble Crry. The chronic phase of the disease, present mainly in C57BL/6 mice, may be secondary to the recruitment of other inflammatory pathways that render the complement system dispensable. It is not known what other genetic factors affect the degree of tissue inflammation in the C57BL/6 strain responsible for the differences noted above. Notwithstanding the strain differences in disease manifestation, the authors demonstrate a therapeutic potential for complement inhibition in the treatment of demyelinating diseases, and possibly other inflammatory diseases of the central nervous system in which complement has been implicated.

The use of gene-targeted mice provides further insight into the role of Crry in complement-dependent tissue inflammation [44]. We have generated mice deficient in this protein using gene-targeting techniques. Surprisingly, the Crry deficiency leads to embryonic lethality that occurs at approximately 10.5 days post coitus (dpc) (table 2). The developing embryos died from their inability to suppress complement activation and subsequent complement-induced tissue inflammation. First, immunohistochemical detection of Crry in cryosectioned wild-type embryos indicated that Crry is highly expressed in wild-type trophoblasts as early as 7.5 dpc, and at very low levels in the embryo proper. Trophoblast cells are involved in generation of extraembryonic tissues, including the placenta. Second, 7.5 dpc *Crry*$^{-/-}$ embryos exhibit distinct positive staining for activated surface-deposited C3 fragments in their trophoblasts and in the early placenta (trophoectoderm and ectoplacental cone) as compared with minimal or no staining in wild-type embryos. This observation indicates that the lack of Crry promotes abnormal activation and deposition of complement. In addition, *Crry*$^{-/-}$ embryos exhibit extensive invasion by inflammatory cells as shown by the presence of polymorphonuclear granulocytes in areas around the ectoplacental cone and the surrounding trophoectoderm. Finally, the embryonic lethality present in the Crry-deficient animals is completely rescued if these mice are bred to C3-deficient animals. Thus, failure to control C3 activation leads to an inflammatory response in the *Crry*$^{-/-}$ fetuses and eventually to embryonic demise.

Table 2. Genotype analysis of littermates from *Crry*$^{-/-}$ matings*.

Age	*Crry*$^{+/+}$	*Crry*$^{+/-}$	*Crry*$^{-/-}$	Total
Live births	113 (46%)	133 (54%)	0 (0%)	246
16d embryo	5 (56%)	4 (44%)	0 (0%)	9
13d embryo	19 (31%)	40 (64%)	3 (5%)	62
11d embryo	22 (33%)	40 (61%)	4 (6%)	74
10d embryo	21 (40%)	26 (50%)	5 (10%)	52
9d embryo	25 (20%)	44 (49%)	21 (23%)	90

* From [44].

It has been suggested that complement may be important in the reproductive system and in pregnancy [45–48]. The *Crry* gene-targeted model indicates that complement regulation is indeed important in fetoplacental survival, maintenance of normal pregnancy and adequate reproductive function by maintaining a form of fetomaternal tolerance against immunological mechanisms of tissue damage related to natural immunity. These studies are also relevant to the possible involvement of these molecules in pathologic pregnancies, both in animals and in humans, in which complement is believed to be involved in the disease pathogenesis [49]. CR1 and mouse DAF are not expressed in early embryos and trophoblasts [44], and mouse MCP are preferentially expressed in testis [26], thus leaving Crry as the critical regulator of complement activation during early murine embryonic development. These observations explain the difference in phenotype of the Crry-deficient embryos and mutant mice lacking DAF, in which embryonic development is not affected [25]. In contrast, both DAF and MCP are heavily expressed in human placentas [45, 47], and there is no direct human counterpart to Crry. Thus, human DAF or MCP should play a similar role to mouse Crry during early embryonic development by controlling effector components of natural immunity in the form of complement regulation and polymorphonuclear granulocyte recruitment and activation, to protect fetomaternal tissues from tissue inflammation and destruction.

Crry effect on T cells

Recent experimental work has revealed a unique role for Crry that is unrelated to complement regulation. A mouse monoclonal antibody (3F10) raised against a rat cortical thymic epithelial cell line (R-TNC.1) recognized rat Crry [50]. Using this antibody, the authors show that thymic epithelial cells and primary thymocytes express this complement regulator. Interestingly, incubation of thymocytes with this antibody induces homotypic aggregation of these cells in vitro, and promotes heterotypic adhesive interactions between thymocytes and thymic epithelial cell lines. These interactions are mediated by an adhesion molecule known as leukocyte function-associated antigen-1, also known as LFA-1. In addition, 3F10 stimulates proliferation of primary thymocytes induced with suboptimal concentrations of concavalin A. It does not affect spontaneous proliferation or proliferation induced by optimal doses of concavalin A. The data suggests that Crry expressed in the thymus may regulate processes involved in early T cell development by mediating proadhesive and activation signals on thymocytes.

In related experiments, Fernández-Centeno et al. demonstrated that antibodies against Crry induced in vitro proliferation of a particular primary spleen T cell expressing a molecule known as CD4 on their surface [51]. This proliferation is dependent on coincubation of antibodies against Crry and against CD3, a molecule on the surface of T cells involved in antigen recognition and subsequent cell activation. This only happens when both antibodies are bound to tissue culture plastic plates. Anti-Crry antibodies in solution have no effect. In addition, independent crosslinking of these molecules has minimal effect on T cell proliferation suggesting that Crry-dependent enhancement of CD3-mediated T cell activation needs the proximity of both proteins on the cell membrane. Anti-Crry antibodies also potentiate CD3-mediated T cell activation induced by costimulatory signals derived from CD28 engagement or the use of phorbol esters. In addition, CD3 and Crry coligation on T cells enhances the production of cytokines such as interleukin 4 (IL-4), with minimal effect on the production of other cytokines such as interferon γ (IFN-γ). Since IL-4 is mainly secreted by, and is involved in the differentiation of, a subpopulation of T cells known as Th2 cells, whereas IFN-γ is involved in the differentiation of another subpopulation of T cells known as Th1, these findings suggest a potential role for Crry in the selective development of Th1 and Th2 cells [51].

These effects on T cell activation induced by Crry are related to alterations in early T cell receptor and CD3 signaling. The authors show that after Crry and CD3 stimulation of T cells, tyrosine phosphorylation of different intracellular protein substrates involved in T cell activation is increased and appears earlier as compared with T cells stimulated by the combination of anti-CD3 and a control antibody. For example, the amount of active phosphorylated ERK2 is increased. Furthermore, anti-Crry antibodies coprecipitate proteins with kinase activity, and experimental observations demonstrate that Crry is associated to $p56^{lck}$. The in vivo significance of the above findings is unclear.

Prospective panorama

A major question when studying the biology of Crry is its relevance in controlling complement activation in the context of other RCAs molecules, especially since DAF and MCP are also expressed in mouse tissues [23, 24]. In addition, human cells have no direct Crry homologue and regulate complement activation on their surface by expressing DAF and MCP [27]. Concerning mouse MCP, it is preferentially expressed in the testis [26]. The reason for this limited expression, as compared with the widespread presence of MCP in human tissues, is unknown. Nevertheless, this situation leaves Crry as the only ubiquitously expressed transmembrane molecule that serves as a cofactor for factor I-mediated cleavage of membrane-bound mouse C3b and C4b**.** Although DAF is widely expressed, DAF-deficient mice have no apparent problem with abnormal complement activation in vivo [25]. Moreover, some mouse cells, such as rat T cells and early mouse trophoblast cells, have weak or absent DAF expression but seem resistant to complement activation [44, 52]. In addition, although DAF controls the activation of C3, DAF cannot control the biological consequences of membrane-bound C4b. This is not a trivial observation since membrane-bound C4b can interact with complement receptors and can act as an opsonin. In the case of CR1, this molecule also has limited tissue expression in humans, and a more restricted tissue expression in mice, being present mainly on murine B cells and follicular dendritic cells [4, 27] (table 1). CR1 discrete tissue distribution limits its effectiveness as a regulator of complement activation in most of human and mouse tissues. These observations suggest that Crry is a critical membrane-bound molecule that controls complement activation in murine tissues by means of its C3 and C4 regulatory properties. This idea is further supported by the fact that isolated Crry deficiency results in C3-mediated embryonic lethality [44].

Another major question is how the study of a rodent molecule not present in human tissues will aid in understanding human clinical problems. Given that mouse Crry and human DAF and MCP control C3 activation by the same biochemical mechanisms in vitro, analysis of Crry function is relevant to the possible involvement of these regulatory molecules in human pathologic conditions in which complement is believed to be involved in the dis-

ease pathogenesis [32]. Studies on Crry are providing significant insight into the roles of the corresponding functionally related human molecules in vivo that was not appreciated by in vitro analysis or by examination of structural orthologues (see above). Both DAF and MCP are heavily expressed in human cells and play a similar role to mouse Crry by controlling effector components of natural immunity, in the form of complement regulation, protecting tissues from inflammation and destruction [3–5].

As noted above, characterization of the functional roles of Crry in vivo has brought new insights into the role of complement and complement regulators in the development of pathological conditions. Conditions such as autoimmune glomerulopathies, abnormalities during pregnancy, degenerative central nervous system conditions, ischemic events, septic shock, and abnormalities in T cell development and T cell activation may be affected by complement and complement regulatory molecules (table 3). In addition, Crry transgenic and gene-targeted mice are excellent tools to analyze the role of these molecules in human autoimmune diseases. Experimental techniques are available that obviate the embryonic lethality found in Crry-deficient animals. Adoptive transfer of particular $Crry^{+/+}$ or $Crry^{-/-}$ cell populations and/or tissues into syngeneic $Crry^{+/+}$ or $Crry^{-/-}$ recipients, and the use of conditional gene-targeting technology, will enable us to study the effect of Crry deficiency in adult mice. The availability of other transgenic and gene-targeted mouse models that affect the expression of complement components and complement-related molecules will aid in a more profound analysis of the role of Crry in the conditions detailed above.

Another area in which the study of Crry is informative relates to the use of these regulators of complement activation in the treatment of inflammatory, ischemic and autoimmune diseases [53]. Studies using rat models have shown that soluble forms of human CR1 have beneficial therapeutic effects in these conditions [9, 54–58]. Other disease processes, such as Alzheimer's disease [59, 60], systemic lupus erythematosus [61] and rheumatoid arthritis [62], may be potential targets for treatment with complement regulatory molecules. Nevertheless, the majority of these analyses have examined relative acute events. There is no long-term investigation exploring the effectiveness of these C3 regulators in chronic diseased states and the potential side effects resulting from long-term inhibition of complement activation. First, extensive analysis of experimental mouse models is precluded by the failure of human CR1 to control the mouse classical pathway [63]. This is specially discouraging since excellent naturally occurring and transgenic mouse models exist with chronic pathological conditions resembling those found in humans. Second, the generation of mouse anti-human CR1-neutralizing antibodies in vivo limits the chronic use of the soluble form of human CR1 as a therapeutic agent in murine models of disease [41]. Here is where the use of soluble Crry will greatly enhance our understanding of the role of these regulators in chronic inflammatory diseases.

Table 3. Conditions that may be affected by Crry-mediated regulation of complement activation.

Thymocyte development [50]
T cell activation [51]
Th1/Th2 development [51]
Fetomaternal tolerance [44, 64, 65]
Immune complex-mediated glomerulonephritis [36, 37, 40–42]
CNS degenerative conditions (experimental allergic encephalomyelitis) [43]
Ischemia-reperfusion injury [66]
Acute septic schock [38, 67]

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