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Complement and complement receptors (CR) play a central role in immune defense by initiating the rapid destruction of invading microorganisms, amplifying the innate and adaptive immune responses, and mediating solubilization and clearance of immune complexes. Defects in the expression of C or CR have been associated with loss of tolerance to self proteins and the development of immune complex-mediated autoimmune diseases such as systemic lupus erythematosus. In this study, we examined the role of CR on coxsackievirus B3 (CVB3)-induced myocarditis using mice deficient in CR1/2. We found that CR1/2 deficiency significantly increased acute CVB3 myocarditis and pericardial fibrosis resulting in early progression to dilated cardiomyopathy and heart failure. The increase in inflammation was not due to increased viral replication, which was not significantly altered in the hearts of CR1/2-deficient mice, but was associated with increased numbers of macrophages, IL-1β levels, and immune complex deposition in the heart. The complement regulatory protein, CR1-related gene/protein Y (Cry), was increased on cardiac macrophage populations, while immature B220<sup>hi</sup> B cells were increased in the spleen of CR1/2-deficient mice during acute CVB3-induced myocarditis. These results show that expression of CR1/2 is not necessary for effective clearance of CVB3 infection, but prevents immune-mediated damage to the heart.


The complement system consists of 12 soluble plasma proteins that interact with one another in three distinct enzymatic activation cascades, the classical, alternative, and lectin pathways, and in a nonenzymatic cytolytic membrane attack pathway (1). Complement components activate the innate immune response by responding to bound Ab and to molecular patterns found on pathogens. Activation leads to the formation of C split products that have potent proinflammatory properties amplifying both the innate and adaptive immune responses (2, 3). Complement receptor 2 (CR2) (CD21) is the receptor for the cleavage products of C3, including iC3b, C3dg, and C3d. CR2 expression on B cells plays a major role in the induction of Ab responses and has been proposed to be a primary mediator of the immune-enhancing role of C (3–5). CR1 (CD35) has dual roles as a receptor for C3b, C4b, C1q, and mannan-binding lectin, is important in clearing immune complexes (IC), and is a C regulator with decay accelerating and membrane cofactor activities (1, 3). Although CR1 and 2 are encoded on separate genes in humans, in the mouse they are derived from the same gene, Cr2, so that mice deficient in one receptor are also deficient in the other (6, 7). Deficiencies in C can enhance disease by allowing increased susceptibility to infection (8) or by increasing some autoimmune diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), by failing to clear IC and/or by failing to regulate the immune response (4, 5, 9, 10).

Myocarditis is a principal cause of heart disease in young adults and is often a precursor of heart failure due to the progression to dilated cardiomyopathy (DCM) (11, 12). Coxsackievirus B3 (CVB3) infection of susceptible A/J or BALB/c mice results in a disease similar to that observed in humans, with the development of acute myocarditis from days 7 to 14 after infection that progresses to chronic myocarditis and DCM from day 28 to at least day 58 after infection (13, 14). The pathogenesis of myocarditis involves both T cell-mediated and Ab-mediated mechanisms (15–17), with IgG Ab and C deposition in the hearts of patients with DCM or A/J mice inoculated with cardiac myosin and adjuvant to induce experimental autoimmune myocarditis (EAM) (18–21). Proinflammatory cytokines are critical for the development of myocarditis. Increased IL-1β or IL-18 levels in the heart directly
correlate with increased acute CVB3-induced myocarditis in susceptible strains of mice (22–24), while administration of IL-1β during the innate immune response to CVB3 infection induces autoimmune myocarditis in genetically resistant strains of mice (25). However, the role of CR1/2 in the development of CVB3-induced myocarditis has not been previously examined.

To investigate the effect of CR1/2 on the development of CVB3-induced myocarditis, we used A/J mice deficient in CR1/2. We hypothesized that CR1/2 deficiency could 1) increase myocarditis by increasing viral replication, 2) decrease myocarditis due to the “adjuvant effect” of C, or 3) increase myocarditis by preventing clearance of IC. We found that CR1/2 deficiency did not increase viral replication in the heart but significantly increased myocarditis, pericardial fibrosis, and was associated with increased IC deposition in the heart, resulting in early progression to DCM and heart failure during the acute phase of disease. CR1/2 deficiency increased the number of macrophages, CR1-related gene/protein Y (Cryy) expression on macrophages, and proinflammatory IL-1β levels in the heart during acute myocarditis. These results show that CR1/2 expression is not necessary for effective clearance of CVB3 infection, but reduces inflammation and fibrosis in the heart and the progression to severe heart disease.

Materials and Methods

Mice

Male A/J mice (6–8 wk of age) were obtained from The Jackson Laboratory. CR1/2-deficient mice were backcrossed from the C57BL/6 mouse strain to the A/J strain for five generations, as described previously (26). The murine C2 gene encodes for both CR1 and CR2 transcripts by alternative splicing (6). Furthermore, A/J mice have a 2-bp deletion in the gene encoding C5 that renders mice deficient in functional C5 and unable to form the membrane attack complex C5b-C9 (26, 27). This deficiency allowed us to separate complement effects on the inductive phase of myocarditis from the effector mechanisms related to the membrane attack complex. Mice were maintained under pathogen-free conditions in the animal facility at Johns Hopkins School of Medicine, and approval was obtained from the Animal Care and Use Committee of the Johns Hopkins University for all procedures.

Myocarditis

Individual experiments were conducted at least three times with 7–14 mice/group. Mice, 6–8 wk of age, were inoculated i.p. with a tissue culture-derived stock of CVB3 (Nancy strain) originally obtained from American Type Culture Collection (ATCC) and grown in Vero cells (ATCC). CVB3 was diluted in sterile saline and 10⁵ PFU were injected i.p. on day 0 and tissues were collected on day 2 (innate response) or on day 10 or 12 (acute myocarditis) postinfection (p.i.). Mice inoculated i.p. with PBS or uninfected tissue homogenate did not develop myocarditis (data not shown).

Hearts were cut longitudinally and fixed in 10% phosphate-buffered formalin for 2 min, as described previously (29, 30). Briefly, immune cells were released from the myocardium by digestion with collagenase II (1 mg/ml; Sigma-Aldrich) and protease XIV (0.5 mg/ml; Sigma-Aldrich) in PBS for 7 min at 37°C, and single-cell separation was completed using razor blades to dislodge immune cells from the tissue. Individual cell suspensions from seven mice were pooled by group and immune cells were separated from heart cells using a magnetic column and anti-CD45 para-magnetic beads (30F11.1; Miltenyi Biotec). Splenocytes were isolated, RBCs were lysed with ACK lysing buffer, and samples were pooled by group. Immune cells from the heart and spleen were stained with the following mAbs (BD Pharmingen) diluted in 1% PBS in PBS (Invitrogen Life Technologies): anti-CD3 (total T cells, clone 17A2), anti-CD4 (Th cells, GK1.5), anti-CD8 (CTLs, clone 53-67.6), anti-B220 (B cells, clone RA3-6B2), and anti-GR-1 (granulocytes, clone RB6-8C5). Anti-F4/80 to measure macrophages was purchased from eBioscience (clone BM8). Cell fluorescence was measured using a FACSCalibur flow cytometer (BD Biosciences) and data were analyzed using CellQuest software (BD Biosciences). The same results were obtained in three separate experiments using seven mice per group.

Immunofluorescence

For analysis of IC deposition on the heart during acute CVB3-induced myocarditis, 5-μm cryosections of OCT preserved A/J- or CR1/2-deficient hearts were fixed in acetone and stained at room temperature for 30 min with fluorescence-labeled Abs against FITC-labeled C3 (ICN Biomedicals), Texas Red-labeled IgM or FITC-labeled IgG (Vector Laboratories). Sections were coverslipped with Vectashield mounting medium (Vector Laboratories), viewed with an Olympus fluorescence microscope, and analyzed using Image-Pro Plus version 3.1 software.

Statistical analysis

Normally distributed data were analyzed by the Student t test. The Mann-Whitney U test was used to evaluate nonparametric data. Significant differences were obtained by comparing CVB3-infected knockout mice with infected A/J controls. Test values with a p < 0.05 were considered significantly different from control values; *, p ≤ 0.05; **, p ≤ 0.01; ***, p < 0.001.

Results

CR1/2 deficiency increases acute CVB3-induced myocarditis and fibrosis

CR1/2 deficiency is associated with certain autoimmune diseases such as SLE (4, 5, 9, 10). To investigate the role of CR1/2 in CVB3-induced myocarditis, A/J mice deficient in CR1/2 were examined for the development of acute myocarditis at day 10 or 12 after infection with 10⁵ PFU of CVB3 i.p. We found, by histological examination of H&E sections, that CR1/2 deficiency significantly increased the level of inflammation in the heart at day 10 (data not shown) or 12 p.i. compared with infected wild-type A/J controls (Fig. 1A). Fibrosis usually develops during the progression to chronic myocarditis following CVB3 infection (28). In this study, we found a significant increase in myocardial fibrosis in CR1/2-deficient hearts (Fig. 1, B and D) compared with wild-type A/J hearts (Fig. 1, B and C). Areas of myocardial fibrosis were closely associated with inflammation. Thus, we show here that CR1/2 deficiency increases acute CVB3-induced myocarditis and fibrosis.
myocarditis (\(B\rightarrow D\)) or fibrosis (\(B\rightarrow D\)) compared with wild-type A/J controls (\(A\rightarrow C\) for the development of acute myocarditis (\(A\)) or fibrosis (\(B\rightarrow D\)). Mice received 10^3 PFU of CVB3 i.p. on day 0 and hearts were collected on day 12 p.i. Myocardial sections were stained with H&E to detect inflammation or Masson’s trichrome to detect collagen deposition (stains bright blue, \(C\) and \(D\)) and assessed as the percentage of the heart section with inflammation or fibrosis compared with the overall size of the heart section. Individual experiments were conducted four times with 7–14 mice per group. Data are presented as the mean ± SEM. *, \(p < 0.05\); **, \(p < 0.01\). Magnification, ×2.5.

CR1/2 deficiency reduces survival following CVB3 infection

Because adults rarely die from acute viral myocarditis (11), we developed a model of CVB3-induced myocarditis where 100% of mice survive acute myocarditis (from days 7 to 14 p.i.) and progress to develop chronic autoimmune myocarditis and DCM (days 35 to 56 p.i.) (13, 14). In this study, we found survival was reduced by ~50% on days 7–8 p.i. in CR1/2-deficient mice (Fig. 2A), indicating that CR1/2 deficiency results in premature death during acute myocarditis.

CR1/2 deficiency increases DCM

DCM is characterized by progressive myocardial hypertrophy and dilation, as well as contractile (systolic) dysfunction. It often develops following myocarditis (12) and is a major cause of heart failure in patients (12, 31) and mice (28, 32, 33). DCM in mice can be determined in heart cross-sections as dilation (28) and by assessing cardiac function (32, 33). DCM is not observed during acute CVB3-induced myocarditis (days 7 to 12 p.i.), but develops in susceptible strains of mice (i.e., BALB/c and A/J) by day 35 p.i. during chronic myocarditis (14, 28). We previously reported that DCM is more severe in mice deficient in IFN-\(\gamma\) during chronic CVB3-induced myocarditis or EAM, and is associated with increased heart failure and death (28, 32, 33). In this study, we found that wild-type A/J control mice did not develop DCM (0%) during acute myocarditis (Fig. 2B), whereas ~50% of surviving CR1/2-deficient mice (Fig. 2A) developed DCM, by histological analysis, during the acute phase of CVB3-induced myocarditis (Fig. 2C). Thus, CR1/2-deficient mice rapidly develop DCM following CVB3 infection.

CR1/2 deficiency does not increase viral replication in the heart

Complement is a critical component of the innate immune response that protects the host against invading microorganisms. During viral infections, C3 and C4 directly coat virions, including CVB3, preventing or neutralizing viral infection of target cells and facilitating lysis of virus-infected host tissues (34, 35). Complement deficiencies are often associated with reduced resistance to bacterial and viral infections (8, 36, 37). Hence, increased myocarditis (Fig. 1) and deaths (Fig. 2A) in CR1/2-deficient mice could be due to increased viral replication in the heart. However, when we examined the level of infectious CVB3 in the heart by plaque assay, we did not find a significant difference in viral replication at day 2 (Fig. 3A), day 10 (Fig. 3B), or day 12 (Fig. 3C) p.i. By day 14 p.i., CVB3 could no longer be detected in the heart of wild-type A/J or CR1/2-deficient mice by plaque assay (data not shown). Thus, CVB3 infection is effectively cleared from the heart independent of CR1/2 expression.

CR1/2 deficiency increases pericardial fibrosis

We have recently shown that an adhesive, fibrotic pericarditis is associated with increased DCM and reduced survival in IFN-\(\gamma\)-deficient mice following CVB3 infection (28). The development of fibrosis is an important feature in a number of pathological conditions including myocarditis, and is a key determinant in the clinical outcome of chronic heart disease (28, 31). Fibrosis involves proliferation of fibroblasts and deposition of extracellular matrix proteins like collagen (38). Fibroblasts are a major component of cardiac tissue and so it is not surprising that fibrosis is an important contributor to the development of DCM and congestive heart failure (38, 39). In our model of CVB3-induced myocarditis, fibrosis is not observed during acute myocarditis but develops with the progression to chronic disease (14, 28, 40). In this study, we observed increased fibrotic pericarditis during acute CVB3 myocarditis (day 10 or 12 p.i.) in CR1/2-deficient hearts (Fig. 4, A and C) compared with wild-type A/J controls (Fig. 4, A and B). Pericardial fibrosis was indicated by bright blue staining for collagen deposition (Fig. 4, B and C) and was associated with pericardial inflammation. Pericarditis was observed in A/J mice in some experiments, and was always increased in CR1/2-deficient mice, but was
deficient mice contained fewer CD4+ T cells than CVB3 infected wild-type A/J mice by FACS analysis (Fig. 5A). The percentage of B cells was also decreased in the hearts of CR1/2-deficient mice (A/J 20.7% vs CR1/2−/− 13.6%) (Fig. 5B). The reduced percentage of T and B cells in the hearts of CR1/2-deficient mice following CVB3 infection is consistent with previous reports of reduced B cell and T cell activation in virally infected or adjuvant immunized C or CR-deficient mice (26, 41–43). Thus, CR1/2 deficiency reduces T and B cells in the heart during acute CVB3-induced myocarditis.

**CR1/2 deficiency increases B220low B cell populations in the spleen**

Splenic B cells can be divided into mature B2 cells that express high levels of B220 (B220high) and immature or marginal zone (MZ) B cells that express lower levels of B220 (B220low). MZ B cells function very early in the immune response to infection or immunization by binding IgM-IC via C and CR2 and transporting IC to the follicles for Ag presentation to dendritic cells (3, 4, 44, 45). Increased percentages of MZ B cells have been reported in various immune deficiencies and are believed to account for the poor immune responses in these individuals or mice (44). The MZ B cell compartment has also been shown to contain higher levels of clones with autoreactivity, and is associated with certain autoimmune diseases such as SLE (46, 47), but has not been previously examined during CVB3-induced myocarditis. In this study, we found no differences in T cell or macrophage percentages in the spleen of CR1/2-deficient mice compared with A/J control mice by FACS analysis during acute myocarditis (data not shown). Total B cell (B220) numbers were also unaltered between CR1/2-deficient and A/J control splenocytes (A/J 16.9% vs CR1/2−/− 18.3%) (Fig. 6, R3). However, there was an increase in the percentage of B cells with a B220low phenotype in CR1/2-deficient mice (A/J 5.1% vs...
from heart cells using magnetic beads, and the relative proportions of each
12 p.i. after enzymatic digestion. Individual immune cells were separated
ing) responses in mice (51). When we examined inflammatory
costimulatory molecule on T cells and to favor Th2 (IL-4-producing)
function as a receptor for C or facilitate clearance of IC (50). Crry
cay accelerating and membrane cofactor activities like CR1 (7,
the heart during acute CVB3-induced myocarditis.

cells isolated from the heart at day 12 p.i. for Crry expression by
FACS, we found that Crry levels were unchanged on T and B cells
(data not shown) but were increased on macrophages by ~30%
(A/J 43.2% vs CR1/2−/− 56.8%) over the isotype control (Fig.
7B). Not only were absolute numbers of macrophages increased in
the hearts of CR1/2-deficient mice compared with A/J controls
(7A), but relative Crry levels on CR1/2-deficient macrophages
were increased when equal numbers of macrophages were com-
pared (Fig. 7B). Thus, in the absence of CR1/2, macrophage num-
bbers and Crry expression on macrophages is increased during acute
CVB3-induced myocarditis.

**CR1/2 deficiency increases IL-1β levels in the heart**

For some time proinflammatory cytokines have been known to
contribute to the development of chronic myocarditis in mice (22,
23, 25, 28). We have previously shown that IL-1β and IL-18 levels
in the heart directly correlate with the severity of acute CVB3-
induced myocarditis (22). Prolinflammatory cytokines are likely to
be produced by macrophages and neutrophils during acute myo-
carditis, as these cells constitute over 60% of the inflammatory
infiltrate (29, 30). In this study, we examined the levels of TNF-α,
IL-1β, IL-12, IL-18, IFN-γ, and IL-4 in heart or spleen homoge-
nates from CR1/2-deficient or wild-type A/J control mice that had
been infected with CVB3 10 days earlier. We found that CR1/2
deficient mice had significantly decreased levels of TNF-α
and significantly increased levels of IL-1β in the heart during acute
myocarditis (Fig. 8). There were no significant changes in the
levels of IL-12, IL-18, IFN-γ, or IL-4 in the hearts of these mice (Fig.
8). We did not observe significant differences in any of the cyto-
kines we tested in spleen homogenates at this time point (data not
shown). Our results indicate that CR1/2 deficiency reduces TNF-α

**CR1/2 deficiency reduces T cell and B cell numbers in the
heart.** Flow cytometry dot plots depict the percentage of T cells (A) and B
cells (B) isolated from the hearts of A/J control (left) and CR1/2-deficient
(right) mice during acute myocarditis. Mice received 10^3 PFU of CVB3 i.p.
on day 0 and CD4^+ immune cells were isolated from the heart on day
12 p.i. after enzymatic digestion. Individual immune cells were separated
from heart cells using magnetic beads, and the relative proportions of each
of the following cell types were evaluated by FACS: CD4^+ T cells, CD8^+
T cells, and B cells (B220). Individual experiments were conducted three
times using 7–14 mice per group.

**FIGURE 5.** CR1/2 deficiency reduces T cell and B cell numbers in the
heart. Flow cytometry dot plots depict the percentage of T cells (A) and B
cells (B) isolated from the hearts of A/J control (left) and CR1/2-deficient
(right) mice during acute myocarditis. Mice received 10^3 PFU of CVB3 i.p.
on day 0 and CD4^+ immune cells were isolated from the heart on day
12 p.i. after enzymatic digestion. Individual immune cells were separated
from heart cells using magnetic beads, and the relative proportions of each
of the following cell types were evaluated by FACS: CD4^+ T cells, CD8^+
T cells, and B cells (B220). Individual experiments were conducted three
times using 7–14 mice per group.

**FIGURE 6.** CR1/2 deficiency increases immature B cell numbers in the
spleen. Flow cytometry dot plots depict the percentage of immature B220^−/−
B cells (R2) and total B cells (R3) from the spleen of control A/J
(A) and CR1/2-deficient (B) mice. Mice received 10^3 PFU of CVB3 i.p. on
day 0 and splenocytes were analyzed by FACS at day 12 p.i. for the
percentage of B220^−/− B cells. Individual experiments were conducted three
times using 7–14 mice per group.
and increases IL-1β levels in the heart during acute CVB3-induced myocarditis.

**CR1/2 deficiency increases IC deposition in the heart**

Deposition of IC in the heart contributes to the pathogenesis of chronic myocarditis in patients with DCM or A/J mice with EAM (18–21). CR1 in humans and CR1/2 in mice is known to be critical for disposing of IC as well as the products of inflammatory injury such as apoptotic cells (3). However, the role of CR1/2 deficiency in IC deposition in the heart during CVB3-induced myocarditis has not been investigated. We report here that IC deposition occurs in both A/J- and CR1/2-deficient hearts during acute CVB3 myocarditis (Fig. 9). Specific fluorescence staining for IC was observed on the myocardium and pericardium of all mice examined. Areas of the heart that showed increased pericarditis and/or fibrosis also had increased deposition of IgM, IgG, and C3, with the highest intensity of staining along the pericardium (Fig. 9). Although staining was fairly similar between A/J- and CR1/2-deficient hearts for IgM and C3 deposition, IgG deposition was greater in CR1/2-deficient hearts (Fig. 9). Thus, CR1/2 deficiency is associated with increased IC deposition in the heart during acute CVB3-induced myocarditis.

**Discussion**

We report here, for the first time, that CR1/2 deficiency increases CVB3-induced inflammatory heart disease. CR1/2-deficient mice rapidly succumb to heart failure during the acute phase of CVB3 myocarditis due to increased inflammation, pericardial fibrosis, and DCM. Increased inflammation is associated with increased numbers of macrophages, IL-1β levels, and IC deposition in the heart, whereas viral replication is unchanged. Overall, these findings provide evidence that CR1/2 expression reduces immune-mediated damage in the heart during acute CVB3-induced myocarditis by reducing IC deposition and fibrosis.

Complement deficiencies are often associated with reduced resistance to bacterial or viral infections (36, 37). However, a previous study showed that depletion of C3 with cobra venom factor did not alter viral replication or myocarditis in CVB3-infected BALB/c mice (17). In the present study, CVB3 replicated in the heart at similar levels in wild-type A/J and CR1/2-deficient mice (Fig. 3). These results indicate that CR1/2 expression is not required for effective clearance of CVB3 infection, and show that increased myocarditis, fibrosis, and deaths in CR1/2-deficient mice are not due to increased viral replication. Because CVB3 infection is effectively cleared without expression of CR1/2, other factors must be necessary for viral clearance. We recently demonstrated that IL-12-induced IFN-γ reduces CVB3 replication during myocarditis (22, 28, 30). In this study, IL-12 and IFN-γ levels were unaltered in the heart (Fig. 8) or spleen (data not shown) of CR1/2-deficient mice, indicating that IFN-γ could protect CR1/2-deficient mice.
against viral infection. So, what mechanisms are responsible for increasing heart disease in CR1/2-deficient mice?

Increased levels of proinflammatory cytokines have been associated with increased heart disease and heart failure following CVB3 infection of mice (22, 23, 25, 28, 40). Proinflammatory cytokines are released from a number of inflammatory cells in the heart, including T cells and macrophages, as well as by resident myocytes and mast cells (22, 23). Reduced levels of TNF-α in the heart of CR1/2-deficient mice in this study may be due to reduced numbers of T cells (Figs. 5 and 8), and suggest a protective role for TNF-α during acute CVB3 myocarditis. CD4+ T cells can be an important source of local TNF-α production during inflammation (52). Increased levels of IL-1β in the heart may reflect greater numbers of macrophages in the hearts of CR1/2-deficient mice. Increased IL-1β levels in the heart directly correlate with increased inflammation during acute CVB3-induced myocarditis (22), and are known to alter myocyte function (53). Furthermore, we have found that increased IL-1β levels, a strongly profibrotic cytokine, are associated with increased pericardial fibrosis and DCM in chronic CVB3-induced myocarditis (28). Thus, increased IL-1β levels in the heart of CR1/2-deficient mice may contribute to increased myocarditis, fibrosis, and DCM.

CR1/2-deficient mice have reduced numbers of T and B cells in the heart following CVB3 infection (Fig. 5). Because T and B cells are important in the pathogenesis of myocarditis (13–19), the reduced numbers of T and B cells in CR1/2-deficient mice would be expected to reduce disease. However, other pathogenic factors may outweigh the effects of reduced T and B cell inflammation in the heart. For example, macrophage infiltration is increased (Fig. 7). Another possibility is that a protective population of T cells, such as CD25+ T regulatory (Treg) cells, may be reduced in the heart resulting in increased myocarditis. In support of this idea, we found that LPS-stimulated T cells from the spleen of CR1/2-deficient mice have reduced CD25+ expression (43), a molecule expressed on Treg cell populations that is important in reducing autoimmune disease.

Increased numbers of immature or MZ B (B220low) cells are associated with increased autoantibodies and increased IC-mediated autoimmune diseases like SLE (44, 46, 47). In this study, we observed increased numbers of B220low B cells in the spleen of CR1/2-deficient mice (Fig. 6). Bone marrow-derived B2 cells characteristically have high levels of B220, whereas B1 cells display low to intermediate levels of B220 but reside predominantly in the pleural or peritoneal cavities of the adult mouse (42, 44, 45). Immature B or MZ B cells also have low levels of B220 but reside in the spleen. Hence, the population of B220low B cells increased in CR1/2-deficient splenocytes in this study may be immature or MZ B cells (Fig. 6). This population of immature B cells may contribute to the development of CVB3-induced heart disease by increasing autoantibody levels and IC-mediated pathology. In support of this, we found increased deposition of IgG associated with areas of increased inflammation and fibrosis in the heart of CR1/2-deficient mice (Fig. 9).

A body of circumstantial evidence links the development of many autoimmune diseases with preceding infections, such as SLE with EBV and myocarditis with CVB3 (24). Environmental factors like infections are thought to trigger autoimmune disease in susceptible individuals, although the precise mechanisms remain unclear (24). CR1 levels are significantly lower in SLE patients, and genetically susceptible CR1/2-deficient mice develop increased lupus-like disease (5, 9, 10, 54). Molecular genetics studies and analysis of twin pairs discordant for the presence of SLE suggest that reduced levels of CR1 are an acquired phenomenon (55, 56). Thus, it is possible that viral or bacterial infections could reduce CR1 expression resulting in increased autoimmune disease in susceptible individuals. It has been shown that LPS or bacterial infection in mice results in reduced CR2 transcript levels (57). It is important to keep in mind that not all results obtained from mice may be directly applicable to humans due to the fact that humans encode two different genes for CR1 and CR2 while mice use alternatively spliced variants of the same gene, which differ in their extracellular and intracellular signaling domains (6, 58). Thus, it is difficult to separate the individual roles of CR1 and CR2 in the mouse.

A major function of CR1 is to remove IC from the circulation (1, 4, 5). Pathology in systemic autoimmune diseases like SLE is believed to involve increased autoantibody-IC deposition and vasculitis. IC-mediated cardiac involvement in SLE occurs in >50% of cases, with myocarditis and pericarditis occurring most frequently (40, 59). IgG autoantibodies specific for cardiac myosin are found in myocarditis and DCM patients (60), and IgG1 is deposited in the myocardium and pericardium of A/J mice during EAM (20). An explanation for the increased pericardial fibrosis and DCM observed in CR1/2-deficient mice in this study (Figs. 2C and 4) is damage to the heart due to IC deposition and reduced clearance of IC by CR1 (Fig. 9). The increased levels of Crry on macrophages in CR1/2-deficient mice may be an attempt by the immune system to compensate for the loss of CR1. However, Crry does not bind C3b and C4b and does not facilitate clearance of IC (50). Thus, CR1/2 is important in reducing IC-mediated damage to the heart following CVB3 infection in A/J mice.

In this study, we show that CR1/2 deficiency hastens the development of chronic, fibrotic perimyocarditis and DCM following CVB3 infection. CR1/2 deficiency results in increased macrophages, IL-1β, and IC deposition in the heart—factors that all contribute to fibrosis, pericarditis, and DCM. Dysfunctional or absent CR1 expression promotes the development of IC-mediated diseases like SLE and RA in humans, while IC-mediated damage is considered to be an important pathological mechanism in the development of DCM and heart failure in patients.

**FIGURE 9.** CR1/2 deficiency increases IgG deposition in the heart during acute CVB3 myocarditis. Deposition of IgM, C3, and IgG was observed in the myocardium and pericardium of wild-type A/J and CR1/2-deficient (CR1/2−/−) mice compared with isotype controls (Control) by immunofluorescent staining. Frozen sections of A/J or CR1/2-deficient hearts were collected on day 12 p.i. with 103 PFU of CVB3 i.p. Analysis of IC deposition was conducted in two separate experiments with seven mice per group, with one representative heart shown for each group. Magnification, ×20.
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