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Targeted mouse complement inhibitor CR2-Crry protects against the development of atherosclerosis in mice

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Abstract

Objective—Atherosclerosis is a chronic inflammatory and immune vascular disease, and clinical and experimental evidence has indicated an important role of complement activation products, including the terminal membrane attack complex (MAC), in atherogenesis. Here, we investigated whether complement inhibition represents a potential therapeutic strategy to treat/prevent atherogenesis using CR2-Crry, a recently described complement inhibitor that specifically targets to sites of C3 activation.

Methods and Results—Previous studies demonstrated that loss of CD59 (a membrane inhibitor of MAC formation) accelerated atherogenesis in Apoe deficient (*Apoe*$^{-/-}$) mice. Here, both CD59 sufficient and CD59 deficient mice in an Apoe deficient background (namely,

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Author Contributions

F.L., L.W., G. W., and X.Q. contributed to the generation of experimental models and characterization of atherosclerosis. S.T. provided insight into complement blockade in the experimental models and supplied the CR2-Crry. F.L., L.W. G.W., C.W. Z.L., S.T., and X.Q. conducted the data analyses; all authors contributed to the project's planning and writing of the manuscript; and X.Q and S.T. supervised the project.

mCd59ab$^{+/+}$/*Apoe*$^{-/-}$ and *mCd59ab*$^{-/-}$/*Apoe*$^{-/-}$) were treated with CR2-Crry for 4 and 2 months respectively, while maintained on a high fat diet. Compared to control treatment, CR2-Crry treatment resulted in significantly fewer atherosclerotic lesions in the aorta and aortic root, and inhibited the accelerated atherogenesis seen in *mCd59ab*$^{+/+}$/*Apoe*$^{-/-}$ and *mCd59ab*$^{-/-}$/*Apoe*$^{-/-}$ mice. CR2-Crry treatment also resulted in significantly reduced C3 and MAC deposition in the vasculature of both mice, as well as a significant reduction in the number of infiltrating macrophages and T cells.

Conclusion—The data demonstrate the therapeutic potential of targeted complement inhibition.

Keywords

Complement; complement regulation; atherosclerosis; Crry; CD59 and therapeutics

Introduction

Atherosclerosis is a chronic inflammatory and immune vascular disease. Extensive human studies indicate that complement, a key mediator of inflammation and immune responses, may play a critical role in atherogenesis[1, 2]. The complement system is activated by three different cascades. All pathways eventually lead to the formation of membrane attack complex (MAC). The results published by us[3] and others[4–6] convincingly demonstrated the anti-atherogenic role of CD59, a membrane-bound inhibitor of MAC formation, which in turn is indicative of the atherogenic role of the MAC. Specifically, we reported that in mice deficient in apolipoprotein E (Apoe), the additional loss of CD59 (*mCd59ab*$^{-/-}$/*Apoe*$^{-/-}$), accelerated atherosclerosis[3]. We also reported that over-expression of human CD59 (hCD59) (*ThCD59*$^{ICAM\text{-}2}$/*Apoe*$^{-/-}$), or inhibition of MAC formation, using a neutralizing anti-mC5 antibody (Ab), attenuated atherogenesis in *Apoe*$^{-/-}$ or *mCd59ab*$^{-/-}$/*Apoe*$^{-/-}$ mice, respectively[3]. Lewis et al demonstrated that deficiency of C6, a necessary component for MAC formation, attenuates atherogenesis in *Apoe*$^{-/-}$ mice[5]. Recently, Manthey et al reported that C5a inhibition reduces atherosclerosis in *Apoe*$^{-/-}$ mice[7]. Although these results highlight an atherogenic role of the terminal complement pathway and the MAC, whether restriction of complement activation has any beneficial effect in the treatment/prevention of atherogenesis remains unclear[3, 5].

The complement system consists of approximately 30 soluble and membrane-bound proteins, and is activated by three distinct pathways (classical, mannose-binding lectin (MBL) and alternative pathways)[8]. All three activation pathways converge at C3 cleavage, leading to the subsequent formation of C5 convertase. The C5 convertase then cleaves C5 to form C5b and C5a. The terminal complement activation pathway is induced initially by C5b, and results finally in the formation of the MAC[8]. The MAC is a macromolecular pore capable of inserting itself into cell membranes and lysing heterologous cells and pathogens[8]. To protect self cells from MAC damage, more than ten plasma- and membrane-bound inhibitory proteins have evolved to restrict complement activation at different stages of the activation pathways[8]. The principle membrane-bound inhibitors of complement in humans that are expressed on the surface of almost all cell types are decay-accelerating factor (DAF or CD55), membrane cofactor protein (MCP or CD46), and CD59[9]. DAF inactivates the C3 (C4b2a and C3bBb) and C5 (C4b2a3b and C3bBb3b) convertases by accelerating the decay

of these enzymes[10, 11]. MCP inactivates C3 and C5 convertases by serving as a cofactor for the cleavage of cell-bound C4b and C3b by the serum protease factor I[8]. CD59 restricts MAC formation by preventing C9 incorporation and polymerization in the assembling complex[8]. Humans have only one CD59 gene, while mice have two CD59 genes called *mCd59a* and *mCd59b*[3, 12, 13]. Further, in mice, complement receptor-1 related gene/protein Y (Crry) is a functional and structural analogue of human DAF and MCP[14]. Crry plays a critical role in the regulation of complement activation in mice since the deficiency of mouse Crry results in complement dependent fetal lethality[15]. Crry inhibits all three complement pathways at the central C3 activation stage.

The pharmaceutical modulation of complement activity for therapeutic purposes has been extensively investigated. Until recently, nearly all complement inhibitors investigated both in experimental models and in clinic, systemically restricted complement activity, including eculizumab, an anti-C5 mAb recently approved for the treatment of paroxysmal nocturnal hemoglobinuria[16]. However, since complement activation products have important roles in immunity to infection[17], homeostasis and tissue repair[18], systemic inhibition is likely to have undesirable side effects. This may be particularly true for chronic therapeutic approaches, as may be necessary for the treatment of atherosclerosis. In this context, it has recently been shown that site-specific targeting of a complement inhibitor can obviate the need for systemic complement inhibition, and increase bioavailability and efficacy without compromising host immunity to infection[19, 20]. The strategy was to use a complement inhibitor linked to a complement receptor 2 (CR2) targeting moiety. Natural ligands for CR2 are iC3b, C3dg and C3d, cell-bound breakdown fragments of C3 that mark sites of complement activation. Crry targeted by means of CR2 (CR2-Crry) has been shown to be 10–20 fold more effective than Crry-Ig, a systemic counterpart, in a murine model of intestinal ischemia reperfusion injury (IRI)[21]. Significantly, Crry-Ig but not CR2-Crry enhanced susceptibility to infection in a mouse model of acute septic peritonitis when given at the minimum dose providing protection from intestine IRI[21]. Thus, targeted complement inhibition is much less immunosuppressive than systemic inhibition[22] and may provide a significant potential advantage for patients suffering from complement-related chronic immune and inflammatory diseases such as atherosclerosis. However, whether these targeted complement inhibitors have any beneficial effect in the prevention of atherosclerosis has not been investigated.

Here, we report that CR2-targeted mouse complement regulator Crry protected both *mCd59ab*$^{+/+}$*/Apoe*$^{-/-}$ and *mCd59ab*$^{-/-}$*/Apoe*$^{-/-}$ mice against the development of atherosclerosis. Mice treated with CR2-Crry had significantly less C3b/iC3b/C3c and MAC deposition in the atherosclerotic lesion than mice treated with vehicle. These results indicate that targeted complement inhibitors may provide a novel, effective and safe approach for the treatment of atherosclerosis through the restriction of complement activation and MAC formation.

METHODS

Production and purification of CR2-Crry and measurement of its biological activity

CR2-Crry was produced and characterized as previously described[19, 27]. Briefly, CR2-Crry was expressed in Chinese hamster ovary (CHO) cells transfected with plasmids encoding mouse CR2-Crry and further purified from the cultural supernatants by anti-mouse CR2 affinity chromatography[19, 27]. Biotin-conjugated CR2-Crry was generated by EZ-Link Sulfo-NHS-Biotinylation Kit (21425, Thermo Scientific) and the level of biotin conjugation with CR2-Crry was measured by biotin binding assay according to the manufacturer's instructions. Equivalent amount of biotin was prepared in 1 X phosphate buffered saline (PBS) buffer as vehicle control.

Animal treatment and characterization of atherosclerotic lesions

To investigate anti-atherogenic effect of CR2-Crry, we treated six-week-old *mCd59ab*$^{+/+}$/*Apoe*$^{-/-}$ or *mCd59ab*$^{-/-}$/*Apoe*$^{-/-}$ mice with CR2-Crry and maintained on high fat diet (HFD) for 4 or 2 months, respectively. The selection of the treatment periods was based on our previous observation that *mCd59ab*$^{-/-}$/*Apoe*$^{-/-}$ on HFD for 2 months and *mCd59ab*$^{+/+}$/*Apoe*$^{-/-}$ on HFD for 4 months developed extensive atherosclerosis in both aorta and aortic root[3]. Briefly, mice were treated with either CR2-Crry (tail vein injection *or i.v.*[28], 0.25mg/mouse, twice per week for 2 or 4 months) or PBS buffer (vehicle control, which does not affect complement activity[19]), and simultaneously fed with HFD (or atherogenic diet) (C12108; Research Diets Inc.) containing 20.1% saturated fat, 1.37% cholesterol, and 0% sodium cholate[3]. Harvard Medical School Institutional Animal Care and Use Committee approved all experimental procedures and protocols. The selection of the CR2-Crry dosing regimen was based on previously published data showing the protective effect of CR2-Crry in prolonged treatment protocol, together with data showing that CR2-Crry does not induce any anti-CR2-Crry antibodies [27, 29]. To directly investigate the localization of CR2-Crry, we treated 3-month-old *mCd59ab*$^{+/+}$/*Apoe*$^{-/-}$ mice with biotin-conjugated CR2-Crry (*i.v*, 0.25mg/mouse) (experimental group) or biotin (*i.v*, 3ug/mouse, equivalent molar amount of biotin vs. biotin-CR2-Crry) (vehicle control) twice per week for 1 month. Unlabeled CR2-Crry (*i.v*, 1mg/mouse, which is 4 fold higher than the dose of the biotin-conjugated CR2-Crry used) plus biotin-conjugated CR2-Crry (*i.v.* 0.25mg/mouse) (competitive inhibition control) were simultaneously administered twice per week for 1 month. All groups of mice were maintained on HFD for 1 month.

After fasting the mice overnight, we sacrificed the mice by CO_2 asphyxiation and blood collected by heart puncture. Serum was prepared and stored at −80°C. The entire aorta was analyzed from the heart outlet to the iliac bifurcation, with Oil red-O as previously described[3]. Sections of the aortic roots (5 μm) were stained with hematoxylin and eosin (H&E)[3]. The total and Oil red-O stained area in entire aorta, and H&E stained lesion area in aortic root for each section were recorded and two independent investigators performed all measurements in a blinded fashion with respect to the origin of the coded samples.

To investigate deposition of Biotin-conjugated CR2-Crry, frozen sections (5 μm) of the aortic roots were stained with VECTASTAIN Elite avidin-biotin complex (ABC) Kit

(Standard, Vector labs) following the manufacturer's instructions and counterstained with Harris Hematoxylin for 3 min. Sections were dehydrated by passing the slides through a series of increasing alcohol concentrations.

Immunofluorescence and histology

Frozen sections of aortic root (5 μm) were stained with rat anti-mouse C3, IgG2a (clone: 3/26, Hycult Biotechnology), which recognizes mouse complement protein C3 as well as activated C3 fragments C3b, iC3b, and C3c, and rabbit anti-rat C9, which cross-reacts with mouse C9 (Kindly provided by Dr. P. Morgan, University of Wales)[3]. Cellular components in the atherosclerotic plaques (aortic root) were characterized by immunostaining with the following reagents: (1) rat anti-mouse CD68, IgG_{2a} (clone: FA-1, AbD Serotec) for mononuclear phagocytes; and (2) rat Anti-mouse CD4, (Lou/WS1) $IgG_{2a,\,k}$ (clone: H129.19, BD Biosciences). All the primary antibodies were detected using corresponding fluorescein isothiocyanate (FITC)-conjugated secondary antibodies and compared with negative controls which were stained with the secondary antibody alone. We quantified immunofluorescence and histological results from three serial sections from each mouse using Image ProPlus 6.0 software as described[30]. The mean of the quantitative results of three sections obtained from each mouse was used to perform the statistical analysis.

Serum lipid measurement

Serum cholesterol and triglyceride profiles were measured at the Clinical Pathology Laboratory of Children's Hospital, Boston.

Statistical analysis

Experimental results are shown as the Mean ± s.e.m. The difference between the two groups was examined with a nonparametric Mann-Whitney test. All statistical tests with $P< 0.05$ were considered significant.

RESULTS

CR2-Crry protects *mCd59ab*$^{+/+}$/*Apoe*$^{-/-}$ and *mCd59ab*$^{-/-}$/*Apoe*$^{-/-}$ mice against the development of atherosclerosis

We first investigated whether CR2-Crry had any beneficial effect in protecting against atherogenesis in a CD59 sufficient background, a more physiologically relevant condition than a CD59 deficient background. We administered CR2-Crry to 6 week old *mCd59ab*$^{+/+}$/*Apoe*$^{-/-}$ mice twice per week for 4 months and maintained the mice on HFD. Mice treated with CR2-Crry developed significantly less atherosclerosis in the aortic surface (as evaluated by en face preparation) and aortic root compared to mice treated with vehicle (Figure 1A, 1B, and supplemental figure 1). In addition, serum cholesterol levels, and serum triglyceride levels, and body weight were not significantly different between the two groups (Supplemental figure 2A). These results show that the targeted complement inhibitor CR2-Crry protects *mCd59ab*$^{+/+}$/*Apoe*$^{-/-}$ mice against the development of atherosclerosis.

Our previous data using *mCd59ab*$^{-/-}$/*Apoe*$^{-/-}$ mice, CD59 over-expressing transgenic mice, together with data obtained using anti-C5 Ab therapy, indicated an atherogenic role of the

MAC[3]. Recent reports have shown that C5 cleavage and activation of the terminal pathway can occur independently of C3 activation via thrombin-mediated cleavage[23]. To further define the role of C3 activation in the development of the atherosclerosis occurring in *mCd59ab*$^{-/-}$*/Apoe*$^{-/-}$ mice, we therefore investigated whether restricting complement activation at the C3 level with CR2-Crry would modulate the development of MAC-associated atherogenesis. We administered CR2-Crry to 6 week old *mCd59ab*$^{-/-}$*/Apoe*$^{-/-}$ mice for 2 months while mice were maintained on a HFD. Compared to control treatment, CR2-Crry treatment resulted in significantly fewer atherosclerotic lesions in the aorta and aortic root, and inhibited the accelerated atherogenesis seen on the CD59 deficient background (Figures 2A–2B). Further, there were no significant differences in serum cholesterol and triglyceride levels, as well as body weight among the groups (Supplemental figure 2B). Together, these results indicate that the inhibition of complement activation at C3 level with CR2-Crry antagonizes the MAC-accelerated atherogenesis in CD59 deficient mice. The different periods of CR2-Crry treatment in CD59-sufficient and CD59-deficient mice precluded us from directly comparing the anti-atherogenic effects of CR2-Crry in both groups.

CR2-Crry-treated mice have significantly less MAC and C3 deposition on the atherosclerotic lesion than vehicle-treated mice

It has been shown previously that CR2-Crry has a short circulatory half life (8.5 hours), has no significant effect on serum complement activity at therapeutic doses, and targets specifically to sites of complement activation and C3 deposition[19]. To investigate whether the anti-atherogenic role of CR2-Crry is associated with the targeted inhibition of complement activation and C3 deposition on the vessel wall where atherosclerosis occurs, we stained the aortic roots with anti-C3b/iC3b/C3c specific antibodies. Immunofluorescence studies using an antibody specific for C3 activation (C3b/iC3b/C3c) revealed significantly less C3 deposition in aortic roots from both CD59 sufficient or deficient mice treated with CR2-Crry compared to vehicle (Fig. 3A and 3B). Further, to experimentally evaluate whether CR2-Crry targets the atherosclerotic plaque, we administered biotin-conjugated CR2-Crry, biotin alone (vehicle control), or unlabeled CR2-Crry plus biotin-conjugated CR2-Crry (competitive inhibition control) to *mCd59ab*$^{+/+}$*/Apoe*$^{-/-}$ mice for one month, followed by analysis of biotin deposition in the plaque of the aortic root using ABC staining. Biotin staining was readily detected on cells in plaques from mice treated with biotin-conjugated CR2-Crry, but there was little to no staining in plaques from mice treated with biotin alone (vehicle control) or unlabeled CR2-Crry plus biotin-conjugated CR2-Crry (competitive inhibition control) (Figure 4). This result suggests that CR2-Crry localizes in the plaque of the mice after administration. Taken together, CR2-Crry inhibited complement activation at the site of complement activation in the vasculature in our experimental models.

The data shown above using CD59 deficient mice indicate that CR2-Crry modulates the development of MAC-associated atherogenesis, and to further explore this functional connection between CR2-Crry treatment and MAC inhibition, we determined the effect of CR2-Crry on MAC deposition (using an antibody to C9, a component protein of the MAC). Mice treated with CR2-Crry had significantly less C9 deposition on the atherosclerotic

lesion than the mice treated with vehicle (Fig. 5A and 5B). Furthermore, immunofluorescence studies showed that mice treated with CR2-Crry had a significantly lower content of inflammatory cells on the atherosclerotic lesion (macrophage and T-cells) compared to mice treated with vehicle (Supplemental figure 3). The above results taken together are consistent with a pathogenic role of the MAC in the atherosclerotic phenotype of our experimental mice, and demonstrate the therapeutic potential of targeted complement inhibition.

DISCUSSION

Here, we document that CR2-Crry protects both CD59 sufficient and CD59 deficient mice in an *Apoe* deficient background from the development of atherosclerosis through complement inhibition that is specifically targeted at the site of complement activation. The targeted complement inhibitor CR2-Crry, which was prepared by linking a fragment of CR2 to Crry, was initially reported to ameliorate tissue injury in a mouse model of intestinal ischemia/reperfusion injury[19]. Further studies have demonstrated that CR2-Crry attenuates inflammatory disease development in multiple models, including models of mouse spinal cord injury, collagen-induced arthritis and murine lupus[20, 24, 25]. CR2-Crry specifically targets to the sites of complement activation and has no significant effect on serum complement activity[19, 20, 25]. Here, we define the anti-atherogenic role of CR2-Crry, and demonstrate the therapeutic potential of this targeted approach.

Previously, we reported that the pre-treatment of *mCd59ab* deficient mice in an *Apoe*$^{-/-}$ background with anti-mouse C5 Ab attenuates the development of atherosclerosis[3]. Anti-mC5 Ab systemically inhibits complement activation by restricting MAC formation and C5a generation[3]. Further, either genetic deficiency of C6, a necessary component for MAC formation, or systemic blockage of C5a receptor attenuates atherosclerosis in *Apoe*$^{-/-}$ mice[5, 7]. It is notable that targeted complement inhibition has significant potential advantages over systemic inhibition for the treatment of complement-related diseases such as atherosclerosis. However, specific benefits of targeted vs. systemic complement inhibition for treating atherosclerosis remains to be determined. In this context, the immunosuppressive properties of CR2-targeted complement regulators, as well as potential side-effects will require further investigation clinically even though these types of targeted inhibitor have minimal immunosuppressive and off-target effects in mouse models[27,29].

Crry restricts all three complement activation pathways at the C3 activation step, leading to the inhibition of C3 opsonization, C3a and C5a production, and MAC formation. Previous clinical and experimental results indicate that the MAC plays a critical role in atherogenesis[1, 3–6], and here we show that CR2-Crry protects against the development of atherosclerosis in the mice. This result further confirms the critical atherogenic role of complement including MAC. Nevertheless, a recent study showed that deficiency of mouse DAF attenuates atherogenesis in *Apoe*$^{-/-}$ mice, and data indicated that this was due to a C3a modulating effect on lipid metabolism[6, 26]. In contrast, we found that CR2-Crry inhibits at the same point in the complement pathway as DAF[14] but did not change body weight or the lipid profiles, including serum cholesterol and triglyceride, in both *mCd59ab*$^{+/+}$*/Apoe*$^{-/-}$ and *mCd59ab*$^{-/-}$*/Apoe*$^{-/-}$ mice. Since CR2-Crry is targeted and has higher complement

inhibitory activity than Crry[21], the reported effects of C3a may be due to systemic production in DAF deficient mice. The relative roles of the MAC vs. C3a/C5a in the development of atherosclerosis will require further investigation. Regardless, the current data indicate that targeted complement inhibition at the C3 activation step may provide a novel approach for the treatment of atherosclerosis.

CONCLUSION

The data demonstrate the therapeutic potential of targeted complement inhibitor CR2-Crry in treatment of atherosclerosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlight

- Targeted complement inhibitor CR2-Crry treatment resulted in significantly fewer atherosclerotic lesions in *mCd59ab*$^{+/+}$*/Apoe*$^{-/-}$ mice.
- CR2-Crry inhibited the accelerated atherogenesis seen in *mCd59ab*$^{-/-}$*/Apoe*$^{-/-}$ mice.
- CR2-Crry treatment reduced C3 and MAC deposition, infiltrating macrophages and T cells in the vasculature of both mice
- The data demonstrated the therapeutic potential of targeted complement inhibition in prevention/treatment of atherosclerosis

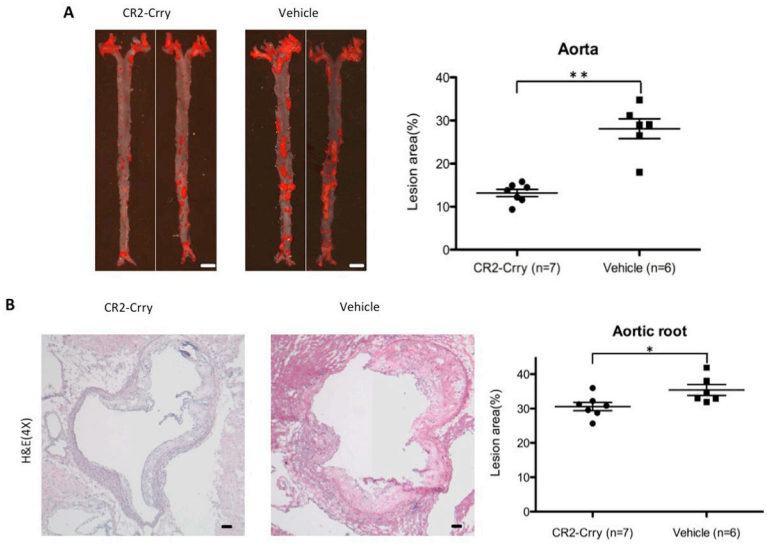

Figure 1. CR2-Crry protects against atherosclerosis in *mCd59ab*$^{+/+}$/*Apoe*$^{-/-}$ mice

A, Atherosclerosis analysis of en face aorta with oil red O staining in the HFD-fed *mCd59ab*$^{+/+}$/*Apoe*$^{-/-}$ mice treated with CR2-Crry (n=7 males) or vehicle (n=6 males) for four months. Lesion area (%) is (oil red O staining area/aortic area) × 100. Scale bar: 2mm. B, H&E staining of aortic root in *mCd59ab*$^{+/+}$/*Apoe*$^{-/-}$ mice shows that CR2-Crry treated mice have a smaller plaque than vehicle group. Lesion area (%) is (Plaque area/ventricle area) ×100. Scale bar: 100μm. *P<0.05;**P<0.005.

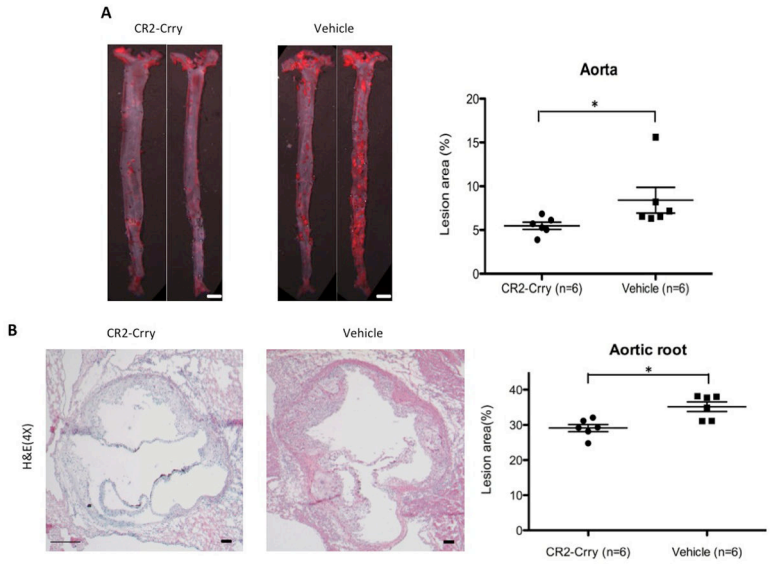

Figure 2. Inhibition of C3 activation with CR2-Crry protects against atherosclerosis in *mCd59ab*$^{-/-}$/*Apoe*$^{-/-}$ mice

A, Atherosclerosis analysis of en face aorta with oil red O staining in the HFD-fed *mCd59ab*$^{-/-}$/*Apoe*$^{-/-}$ mice treated with CR2-Crry (n=6 with 3 males and 3 females) or vehicle (n=6 with 2 males and 4 females) for two months. Lesion area (%) is (oil red O staining area/aortic area) × 100. Scale bar: 2mm. B, H&E staining of aortic root in *mCd59ab*$^{-/-}$/*Apoe*$^{-/-}$ mice shows that CR2-Crry treated mice have a smaller plaque than vehicle group. Lesion area (%) is (H&E staining area/ventricle area)×100. Scale bar: 100μm.*P<0.05;**P<0.005.

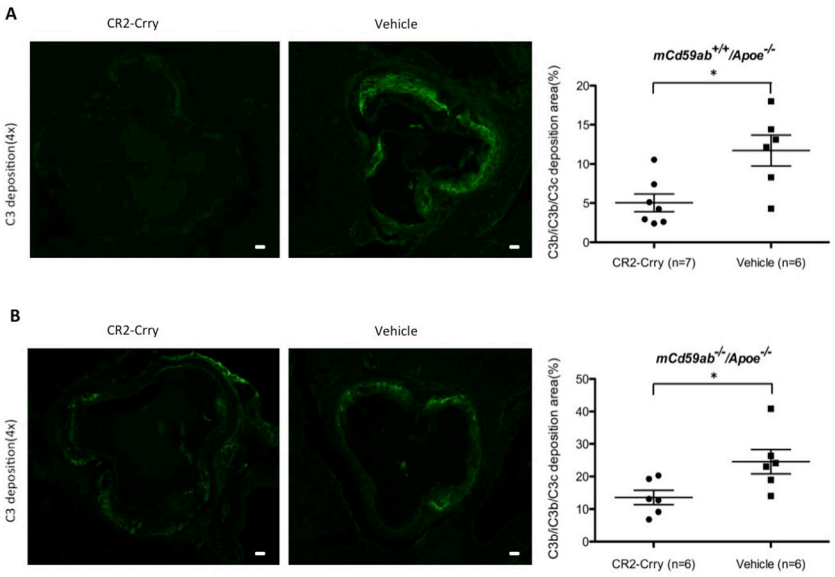

Figure 3. C3b/iC3b/C3c deposition was reduced in atherosclerotic lesions of CR2-Crry-treated mice

A and B, C3b/iC3b/C3c deposition in aortic root of *mCd59a*$^{+/+}$*/Apoe*$^{-/-}$ (A) and *mCd59ab*$^{-/-}$*/Apoe*$^{-/-}$ mice (B) (Left, CR2-Crry; Right, PBS). The mean of the quantitative results of three sections obtained from each mouse was used to perform the statistical analysis. Scatter plot graph shows levels of C3b/iC3b/C3c deposition (percentage of positive area vs. lesion area) detected in atherosclerotic lesions. Scale bar: 100μm *P<0.05.

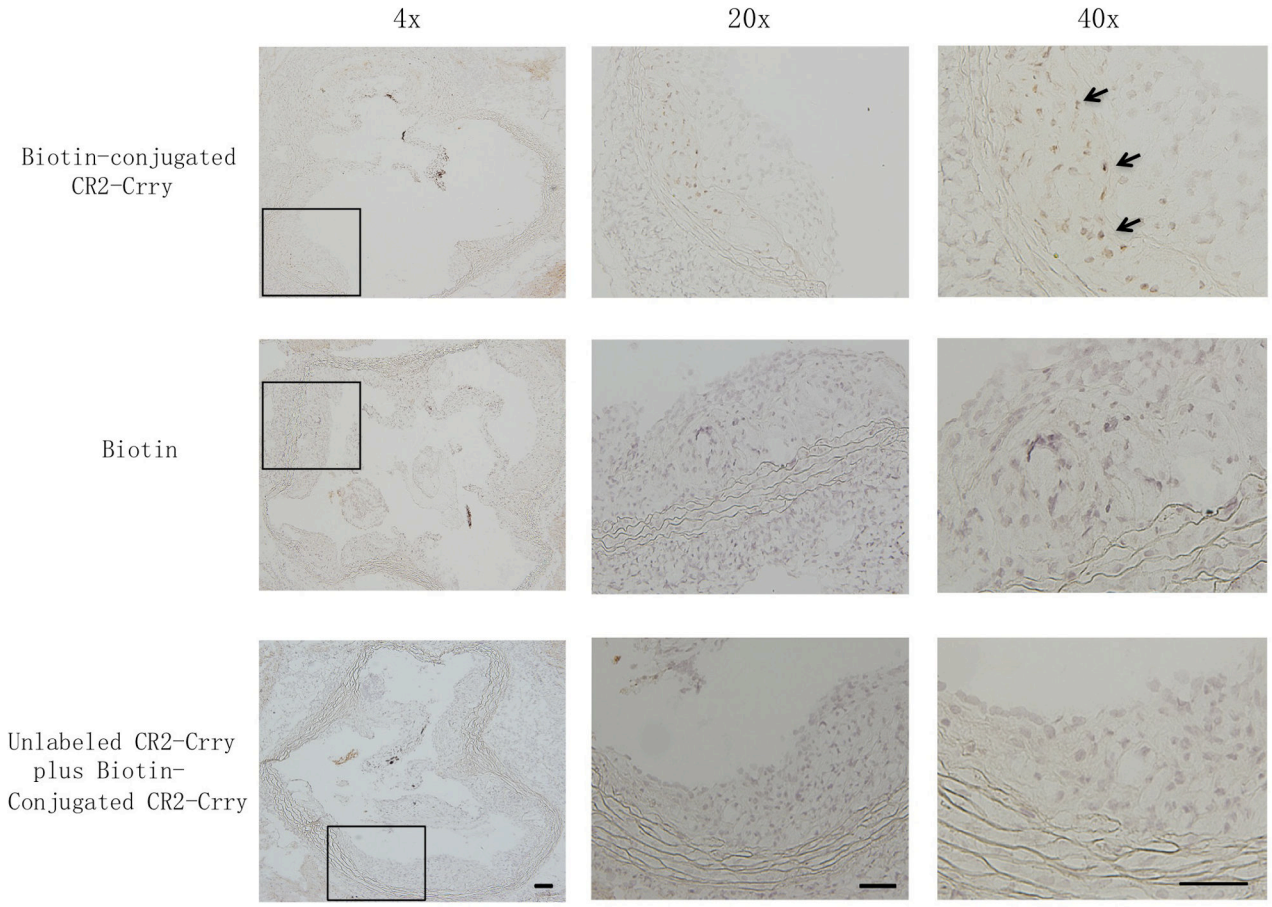

Figure 4. Localization of CR2-Crry on the atherosclerotic plaque of *mCd59ab*$^{+/+}$/*Apoe*$^{-/-}$ mice

4-month-old *mCd59ab*$^{+/+}$/*Apoe*$^{-/-}$ mice were treated with biotin-conjugated CR2-Crry (upper), biotin alone (middle), or with unlablled CR2-Crry plus biotin-conjugated CR2-Crry (lower). Deposition of biotin in the aortic root was detected using ABC staining. Arrows indicate biotin positive (brown stained) cells. Left, middle and Right columns respectively show 4X, 20X and 40 X magnifications of the area outlined by the black rectangle in the image at left. Scale bar, 100μm.

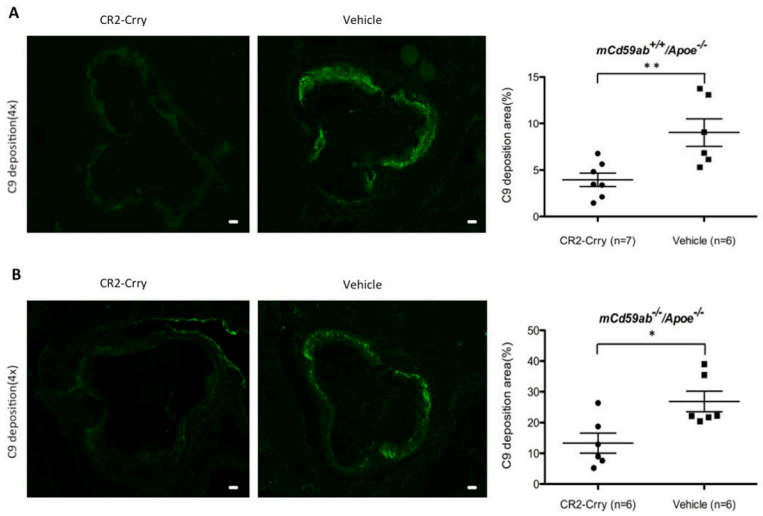

Figure 5. C9 deposition was reduced in atherosclerotic lesions in CR2-Crry-treated mice

A and B, C9 deposition in aortic root of *mCd59a*$^{+/+}$*/Apoe*$^{-/-}$ (A) and *mCd59ab*$^{-/-}$*/Apoe*$^{-/-}$ mice (B) (Left, CR2-Crry; Right, PBS). Of note, the aortic roots of *mCd59ab*$^{-/-}$*/Apoe*$^{-/-}$ mice were used to stain with the antibody. The mean of the quantitative results of three sections obtained from each mouse was used to perform the statistical analysis. Scatter plot graph shows levels of C9 deposition (percentage of positive area vs. lesion area) detected in atherosclerotic lesions. Scale bar: 100μm. *P<0.05;**P<0.005.