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Anti-complement activity of the *Ixodes scapularis* salivary protein Salp20

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Abstract

Complement, a major component of innate immunity, presents a rapid and robust defense of the intravascular space. While regulatory proteins protect host cells from complement attack, when these measures fail, unrestrained complement activation may trigger self-tissue injury, leading to pathologic conditions. Of the three complement activation pathways, the alternative pathway (AP) in particular has been implicated in numerous disease and injury states. Consequently, the AP components represent attractive targets for therapeutic intervention. The common hard-bodied ticks from the family Ixodidae derive nourishment from the blood of their mammalian hosts. During its blood meal the tick is exposed to host immune effectors, including the complement system. In defense, the tick produces salivary proteins that can inhibit host immune functions. The Salp20 salivary protein of *Ixodes scapularis* inhibits the host AP pathway by binding properdin and dissociating C3bBbP, the active C3 convertase. In these studies we examined Salp20 activity in various complement-mediated pathologies. Our results indicate that Salp20 can inhibit AP-dependent pathogenesis in the mouse. Its efficacy may be part in due to synergic effects it provides with the endogenous AP regulator, factor H. While Salp20 itself would be expected to be highly immunogenic and therefore inappropriate for therapeutic use, its emergence speaks for the potential development of a non-immunogenic Salp20 mimic that replicates its anti-properdin activity.

Keywords

Complement activation; Complement alternative pathway; Properdin; *Ixodes scapularis*; Salp20; Salivary protein

1. Introduction

Complement (C), a major component of innate immunity, presents a rapid and robust defense of the intravascular space. Complement marks infectious agents for immune

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clearance or lysis, promotes the local inflammatory response, facilitates T cell lineage commitment, and directs B cell activation and antibody production [1–4]. Complement is also a principal cause of tissue damage: C-related disease and injury can be traced to inappropriate complement activation (humoral autoimmunity) [5] or to inadequate complement regulation (atypical hemolytic uremic syndrome, age-related macular degeneration) [6]. Therapeutic agents designed to modulate complement activity have begun to emerge in the clinical setting [7, 8].

There are three complement activation pathways: the classical pathway responds to antibody:antigen complexes, the lectin pathway responds to microbial surfaces, and the alternative pathway activates spontaneously at low level [1]. Each pathway results in the assembly of the C3 convertases, the enzymes that catalyze the cleavage of C3, forming the C3a and C3b fragments. Nascent C3b can bind covalently to a nearby target surface where it presents a new site for convertase assembly as part of a positive feedback loop (the amplification loop) that drives the rapid and robust complement response. Surface-bound C3 convertases can associate with additional C3b, forming a C5 convertase. C5 convertase cleaves C5 to yield C5a and C5b. C5a, as well as C3a, promotes inflammatory reactions. C5b initiates the complement terminal pathway (TP), leading to the assembly of the membrane attack complex (MAC), membrane perturbation, and cell lysis. Host regulatory proteins protect self-tissues from harmful complement activity [6].

Of the three complement activation pathways, the alternative pathway (AP) in particular has been implicated in numerous disease and injury states [6, 9]. The AP C3 convertase is assembled on a target in a multi-step process that begins with the covalent attachment of nascent C3b to the surface followed by association of C3b with factor B (FB), and cleavage of C3bB by factor D (FD) at a single FB site, forming an active but unstable C3 convertase (T1/2 ~90 sec; [10]), C3bBb. An additional protein, properdin (P), binds to C3bBb, rendering the convertase 5–10-fold more stable [11].

The common hard-bodied *Ixodes* ticks derive nourishment from the blood of their mammalian hosts. During its blood meal, which can last more than 5 days, the tick is exposed to host immune effectors, including the fluid phase complement components. In its own defense, the tick has evolved salivary proteins that can inhibit host immune functions [12–15]. The *Ixodes scapularis* proteins Salp20 and Isac, and the closely related *Ixodes ricinus* proteins IRAC I and II and IXAC-B1–5, inhibit the complement AP [16–19] by binding to properdin, blocking its activity, and displacing it from the C3bB and C3bBb complexes [20, 21].

Properdin (P) is an attractive target for therapeutic inhibition since it is AP-specific, and known properdin-deficient individuals, nearly all males as properdin is encoded by the X chromosome [22], are well except for increased susceptibility to meningococcal disease [23, 24], a condition that can be addressed by vaccination [25]. Here we examine Salp20 activity in various AP-mediated animal models. Our results indicate that Salp20 can inhibit AP-dependent pathogenic processes in the mouse. Its efficacy may be part in due to synergic effects it shares with the endogenous AP regulator, factor H.

2. Materials and methods

2.1 Proteins and sera

Purified complement proteins and pooled normal human serum were purchased from CompTech. The Salp20 protein used in this investigation was a recombinant protein that carries a V5 epitope and a 6× Histidine tag fused to the carboxy terminus of the Salp20 sequence (S20NS). The protein was produced in High Five cells and purified as previously described [19]. Activity was confirmed by C3b deposition assay [26] and/or by hemolytic assay (below). Recombinant Factor B D254G [27] was prepared and quantified as previously described [28]. Briefly, human 293T kidney cells were transfected in serum-free medium with factor B D254G cDNA cloned in pSG5 (Stratagene) expression vector. Supernatants containing FB protein were treated with benzamidine-Sepharose (Amersham Biosciences cat#17-5123-10) to remove nonspecific proteases, dialyzed and stored in phosphate buffer supplemented with 25 mM NaCl. Recombinant factor B was quantified by ELISA and examined by Western blotting. A negative control supernatant was prepared with the expression vector incorporating the FB coding sequence in reverse orientation.

2.2 Sheep erythrocyte AP convertase assay

Antibody-sensitized sheep erythrocytes (CompTech cat#B200) were opsonized with C3b as previously described [29] and stored at a final concentration of 10^8 cells per mL at 4°C for up to a week. For each reaction, 100 μ L C3b-opsonized cells were treated with the stabilizing D254G FB variant (50–400 pg, as indicated), FD (5 ng), and properdin (50 ng) in 10 mM MgEGTA buffer (total volume 250 μ L) for 30 min at 30 °C. The convertases formed were detected by treating the cells with 2.5% guinea pig serum in 40 mM EDTA buffer for 60 min at 37°C on an orbital shaker at 190 RPM to permit the assembly of the membrane attack complex and subsequent cell lysis. The resulting reaction mixtures were centrifuged at 2000 RPM for 5 min and the OD₄₁₄ of the supernatants were determined. Negative control (blank) reactions were performed in the absence of FB and 100% lysis was determined with reactions performed with distilled water. The fraction of cells lysed (y) = (OD reaction - OD blank)/(OD 100% lysis - OD blank). Average lytic sites per cell (Z value) were calculated assuming “one-hit” kinetics: $Z = [1 - \ln(y)]$ [30, 31]. In some cases, cells were treated with Salp20 and/or FH along with FB, FD and properdin to determine the effects of the regulators on net convertase formation. The inhibitory effects of Salp20 were expressed as mean $\pm$ SD derived from the Z values obtained with 300 pg FB in three separate experiments. The values used to quantify the additive effects of Salp20 and FH were derived from the Z values obtained with 200 ng FB in three separate experiments.

2.3 LPS-dependent AP activation assay

C57BL/6J mice (The Jackson Laboartory) were administered i.p. with various concentrations of Salp20. Plasma samples were collected at various time points (2 to 24 h) for an LPS-dependent AP activation assay [32–34]. Briefly, plasma was diluted (1:10 in Mg²⁺-EGTA GVB²⁺ buffer), applied to LPS-coated ELISA plates (2 μ g /well), and incubated at 37°C for 1 h. After washing, goat anti-mouse C3 Ab (1:4000 dilution, MP Cappel, cat#55463) was added followed by HRP-conjugated anti-goat IgG (1:2000 dilution; Jackson ImmunoResearch), after which substrate reagent (cat#DY999, R&D Systems) was

added for 10 min. The reaction was stopped with 1M H₂SO₄ and the OD of samples measured at 450 nm.

To assess the AP activity in the OVA-induced asthma model, mice were sacrificed on day 17 (see below) and their lungs lavaged 3 times with 1 mL of sterile PBS. Samples were cleared by centrifugation at 14,000 rpm for 20 min at 4°C. Cleared samples were diluted at 1:2 in Mg²⁺-EGTA GVB²⁺ buffer prior to being applied to LPS-coated plates. Post lavage, lungs were harvested and homogenized in 2 mL of cold PBS. Homogenates were cleared twice with centrifugation at 14,000 rpm × 20 min at 4°C. Samples were normalized by protein concentration based on absorbance at 280 nm and diluted 1:2 in Mg²⁺-EGTA GVB²⁺ buffer prior to being applied to LPS-coated plates. AP activity was measured as above.

2.4 OVA-induced asthma model

Adult male C57BL/6 mice (8–12 weeks) were immunized i.p. on day 0 and day 7 with 20 µg OVA suspended in 2.25 mg of aluminum hydroxide (total volume 100 µL). On days 14, 15, 16 mice were challenged intranasally (i.n.) with 30 µg OVA in PBS (total volume 30 µL) following anesthesia with isoflurane. Mice were sacrificed on day 17 and their bronchoalveolar lavage fluid (BALF) and lungs were harvested for analysis and histology. In some experiments mice were also administered heat inactivated or active Salp20 (9 µg i.n.) concomitantly with OVA (total volume 45 µL).

IL-13 level in the BALF was measured with an ELISA kit (cat#DY413, R&D Systems). For C3a ELISA, BALF (100 µL) was applied to plates coated with anti-mouse C3a monoclonal antibody (4 µg/mL, cat#558250, BD Pharmingen) and incubated for 2 h at RT, followed by biotinylated anti-mouse C3a monoclonal antibody (250 ng/mL, cat#558251, BD Pharmingen). After washing and incubation with streptavidinperoxidase (400 ng/mL, cat#890803, R&D Systems), 100 µL of peroxide-chromogen solution (cat#DY999, R&D Systems) was added to each well and color development was read at 450 nm with a SpectraMax Plus Reader (Molecular Devices). Mouse recombinant C3a (Cat# 558618 BD Pharmingen) was used to establish the standard curve.

2.5 Elastase-induced abdominal aortic aneurysm (AAA) model

AAA was induced in 8 to 12-week old male C57BL/6 mice as previously described [35, 36]. Briefly, mice were anesthetized with KXA cocktail (ketamine 87 mg/kg; xylazine 13 mg/kg; and acepromazine 2 mg/kg). A laparotomy was performed under sterile conditions. The abdominal aorta was isolated and the pre-perfused AD was measured with a calibrated ocular grid. Temporary 7-0 silk ligatures were placed around the proximal and distal aorta to interrupt proximal flow. An aortotomy was created at the inferior ligature using the tip of a 30-gauge needle and the aortic lumen was perfused for 5 min at 100 mm Hg with a solution containing 0.145 U/mL type 1 porcine pancreatic elastase (Sigma). After removal of the catheter, the aortotomy was repaired without constriction of the lumen to restore the flow. At day 14, a second laparotomy was performed and the perfused segment of the abdominal aorta was re-exposed and quantitative digital readout of AD was obtained in situ by two individual observers prior to euthanasia and tissue procurement. AAA is defined as an

increase in AD of greater than 100% over pre-perfused diameter. In some experiments, mice were randomly assigned to receive an i.p. injection of Salp20 (20 µg i.p.) 3 h prior to and again at 1 and 24 h after elastase perfusion (3 doses total).

2.6 Animals

C57BL/6J mice (The Jackson Laboratory) and P^{Null} mice [37] were kept in pathogen-free facility at Washington University School of Medicine. All animal experiments were performed in compliance with federal laws and in strict accordance with the guidelines established by the Division of Comparative Medicine at Washington University. The animal protocol is subjected to annual review and approval by The Animal Studies Committee of Washington University.

3. Results

3.1 Salp20 inhibits murine AP activity

The Salp20-like proteins have been shown by in vitro assay to be active inhibitors of complement AP activity in numerous mammalian species [21]. To examine the capacity of Salp20 to block the murine AP in vivo we first assessed AP activity in plasma derived from Salp20-treated animals. WT mice were injected i.p. with various concentrations of Salp20; at 3 h their plasma was collected and assayed for LPS-dependent AP activity as previously described [32–34]. Plasma obtained from properdin-deficient (P^{Null}) mice [37] served as negative control. Salp20 at concentration as low as 5 µg efficiently inhibited AP activity while heating destroyed its function-blocking activity (Fig. 1). Inhibition of the AP pathway by Salp20 was no longer detectable at 6 h post injection (data not shown).

3.2 Salp20 attenuates AP-dependent OVA-induced asthma phenotype

Asthma is a chronic inflammatory disease characterized by mucous cell metaplasia, airway hyper-responsiveness (AHR) and remodeling. Inflammatory leukocyte recruitment and cytokine/chemokine secretion have all been implicated in the development of these responses in sensitized hosts following allergen challenge of the lung. Using P^{Null} mice [37, 38], we observed a significant reduction in bronchoalveolar lavage (BAL) eosinophilia in the OVA-sensitized and challenged P^{Null} mice as compared to WT animals (Fig. 2A). Moreover, OVA-induced IL-13 and C3a levels in the BAL fluid were significantly decreased in P^{Null} mice (Fig. 2B & C). Histological analysis of lung sections revealed reduced mucous cell metaplasia in P^{Null} mice (Fig. 2D). These results are consistent with a recent report demonstrating that properdin plays a critical role in OVA-induced asthma phenotype [39]. To examine Salp20 activity in this asthma model, WT mice were sensitized with OVA as above. On day 14 mice were challenged i.n. with OVA and concomitantly administered heat inactivated (HI) or active Salp20. Intranasal administration of active Salp20 significantly reduced airway inflammation and eosinophilia (Fig. 3A), BAL- and lung-associated AP activity (Fig. 3B–C) to the levels observed in P^{Null} mice (Fig 2). There was a trend in the reduction of airway IL-13 and C3a generation that did not reach statistical significance (Fig. 3D–E).

3.3 Salp20 ameliorates AP-dependent elastase-induced AAA

AAA is an inflammatory and immune-mediated disease. Using a well-characterized elastase-induced model of experimental AAA [40] we previously established that the AP is required for disease development and absence or blockade of properdin activity abrogated aneurysm formation. To examine the activity of Salp20 in AAA development, mice were given Salp20 (20 µg i.p.) 3 h prior to and again at 1 and 24 h after elastase perfusion (3 doses total). AAA was assessed on day 14 as previously described [40]. Salp20 administration suppressed elastase-induced AAA (Fig. 4).

3.4 Salp20 and Factor H act synergistically to inhibit surface-level AP activation

Factor H (FH) is a plasma protein that inhibits AP activity. FH acts in the fluid phase to prevent excessive C3 turnover, and it is recruited by its polyanion-binding sites to self-tissue where it prevents inappropriate AP-dependent damage [41]. FH bound to C3b inhibits AP convertase assembly by promoting factor I (FI)-mediated C3b cleavage (cofactor activity). In addition, FH accelerates C3bBb dissociation (decay acceleration activity). Properdin suppresses FH activity: Properdin bound to C3b inhibits FH cofactor activity [10, 42] and properdin bound to C3bBb may impede FH-dependent decay acceleration [43]. Consequently, properdin blockade would be expected to enhance the activity of endogenous FH. In principle, Salp20 would be particularly effective since it can both sequester properdin and dissociate properdin from C3bP and C3bBbP. We tested this hypothesis by comparing the effects of Salp20 and FH alone and together on the AP-dependent lysis of sheep erythrocytes. Sheep erythrocytes were chosen because they harbor surface polyanions that facilitate FH activity [44–46].

C3b-coated sheep erythrocytes were incubated with FB, FD, and P in the presence of Mg^{2+} , the divalent cation required for convertase assembly. In place of WT FB, we used FB D254G, a gain-of-function FB mutant that partially stabilizes both C3bBbP and C3bBb [27], making possible the detection of the otherwise highly labile C3bBb. In some reactions, Salp20 and/or FH were included to examine their impact on convertase formation. Cells were then treated with 1/40 guinea pig serum in EDTA buffer, permitting convertase-dependent formation of the membrane attack complex (MAC) and cell lysis [31]. Hemolytic activity was quantified in terms of Z value, the average productive lytic sites per cell [30, 31]. As seen in Fig. 5A, hemolysis was dependent on the amount of FB present in the assembly step, and the additional presence of properdin resulted in ~3-fold increase in Z value. The addition of 200 ng Salp20 in the presence of P (50 ng) reduced lytic activity to $36\% \pm 4\%$ (n=3), the same level observed in the absence of P, and consistent with the established Salp20 activity. Addition of Salp20 in the absence of P had no detectable effect on cell lysis.

We next used the hemolysis assay to compare the effects of Salp20 and FH separately and together on net convertase assembly and function. Keeping Salp20 constant at 200 ng, we supplemented the reaction mixture with FH (20 or 50 ng per reaction). As seen in Fig. 5B–C, the two inhibitors together had a much greater effect than either alone: 200 ng Salp20 reduced hemolysis to $35\% \pm 4\%$ while 20 ng FH reduced hemolytic activity to $48\% \pm 6\%$; when combined, Salp20 and FH reduced hemolytic activity to less than 1 % (n=3). Similar

results were obtained with 50 ng FH (Fig. 5C). These findings lend strong support to our hypothesis that Salp20 and FH work synergistically to inhibit AP activity on a cell surface.

4. Discussion

Complement presents a formidable first line of defense against pathogenic microorganisms. Many pathogens, however, circumvent complement activity with surface receptors that recruit protective host regulatory proteins. The *Ixodes* species have evolved a unique approach to evade host immune response. During its blood meal, the tick salivary glands secrete several structurally similar glycoproteins, among them Salp20, that block the host AP by inhibiting properdin, the sole positive regulator of the complement alternative pathway. In principle, properdin inhibition would facilitate the feeding process by protecting the tick from complement-mediated tissue damage and by reducing swelling and irritation that could be detected by the host and result in tick removal.

The complement alternative pathway (AP) has been implicated in numerous disease and injury states [9] including age-related macular degeneration [47–51], paroxysmal nocturnal hemoglobinuria (PNH) [52], atypical hemolytic uremic anemia (aHUS) [53–56], rheumatoid arthritis [57], osteoarthritis [58], abdominal aortic aneurysms [38, 59], and ischemic reperfusion injury [60]. Due to these considerations, the AP components, factor B, factor D and properdin, have become attractive targets for therapeutic intervention. Indeed, a humanized anti-Factor D Fab fragment (AFD, lampalizumab) is being used in ongoing trials in the intravitreal treatment of dry age-dependent macular degeneration [61]. AFD would not, however, be suitable for systemic treatment wherein it is rapidly neutralized through high steady state factor D production [61]. Because the properdin gene is located on the X chromosome, a sizable number of properdin-deficient males have been identified and they are well except for increased susceptibility to fulminant meningococcal disease [23], a condition that can be addressed by vaccination [25]. For the above reasons properdin-directed therapeutics merit further consideration.

Here we show that the Salp20 protein can inhibit AP-dependent pathogenesis in the mouse. In the naïve WT mouse, as little as 5 µg of Salp20 administered systemically suppressed AP-dependent C activation to the levels observed in P-deficient animals. The suppression, however, is short-lived, lasting less than 6 h. Although transient, i.n. delivery of Salp20 consistently suppressed local AP activation and reduced airway inflammation in a mouse model of asthma, as evidenced by a reduction in total cell count and eosinophilia in the BAL fluid of OVA-sensitized/challenged mice. Systemic administration of Salp20 also suppressed aneurysm development in an AP-dependent mouse model of AAA. These experiments provide a rationale for pursuing Salp20-based therapeutic AP inhibition in human disease processes. While Salp20 itself would be expected to be highly immunogenic and therefore inappropriate for therapeutic use, its emergence speaks for the potential development of a non-immunogenic Salp20 mimic that replicates its anti-properdin activity. Future directions will seek to find short peptides/small molecules that recapitulate Salp20 activity and various stealth mechanisms and/or chemical modifications will be explored to suppress potential immunogenicity if the need arises.

Factor H is a plasma protein that inhibits alternative pathway activity [62]. It serves as a cofactor in the cleavage of C3b by factor I and it dissociates the AP convertases. Several factor H polyanionic binding sites direct its regulatory activities to surfaces that bear glycosaminoglycans (GAGs) and/or sialic acid. Properdin and factor H compete directly on these surfaces and the outcome affects the balance between complement regulation and complement activation: Properdin bound to C3b inhibits factor H-mediated C3b cleavage [10, 42] and properdin bound to C3bBb may impede factor H-dependent C3bBb dissociation [43]. In principle, a properdin antagonist such as Salp20 would be particularly effective in conjunction with factor H in protecting cell surfaces from AP-dependent damage: Salp20 can displace properdin from C3bP, leaving C3 vulnerable to FH-mediated cleavage, and Salp20 can displace properdin from C3bBbP, leaving C3bBb vulnerable to FH-mediated decay [20, 21]. Alternatively, Salp20 can simply bind to properdin and effectively remove it from the system. Our findings strongly support a synergistic relationship between the two proteins in the inhibition of AP convertase assembly and function (Fig. 5).

Human pathogens have evolved a repertoire of mechanisms to evade complement [63]. Many cases involve the recruitment of factor H from the fluid phase [41]. The properdin/FH dynamic observed with Salp20 may account in part for the vulnerability of properdin-deficient individuals to fulminant meningococcal disease since the responsible pathogen, *Neisseria meningitidis*, is protected from host AP activity by FH recruited to the pathogen surface by sialic acid-bearing glycoproteins [64]. Consequently, properdin deficient individuals would lack a major countermeasure against the FH-protected pathogen. Of further note, Lyme disease is transmitted by the Ixodes tick, *Borrelia burgdorferi*, the spirochete that causes Lyme disease, also recruits FH to its surface [65]. It is possible that during the tick blood meal the family of anti-P salivary proteins function in concert with host FH to protect this pathogen [19].

Properdin is composed of 53 kD linear monomers of about 25 nm in length that associate head-to-tail in dimers, trimers, tetramers and pentamers that form linear, triangular, square and pentagonal structures, respectively [43, 66]. Higher order forms are more active than the lower order forms [67]. While Salp20 binds properdin with high affinity and does not appear to interact directly with either C3b or C3bBb [20, 21], its mechanism of action is not completely understood. Recent EM-based studies have shown that the properdin vertices bind C3bBb via both C3b and Bb subunits, accounting for its stabilizing effect [43]. Salp20 could act by successfully competing with C3b for its properdin-binding site. Alternatively, Salp20 may act indirectly by altering the properdin conformation to one with lower C3b affinity. Deeper insights into the Salp20 mechanism of action could facilitate the production of small molecules that possess Salp-20 like regulatory activity.

Conclusions

The *Ixodes scapularis* salivary protein Salp20 can inhibit complement AP-dependent pathogenesis in the mouse. Its efficacy may be in part due to synergistic effects it shares with the endogenous AP regulator, factor H.

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Highlights

1. Salp20, a salivary protein of the common deer tick *Ixodes scapularis*, inhibits complement alternative pathway dependent pathogenesis in mouse disease models.
2. Salp20 efficacy may be in part due to synergistic effects it provides with the host complement regulatory protein factor H.
3. A non-immunogenic Salp20 mimic that replicates its anti-properdin activity could be a valuable therapeutic agent.

Fig. 1. Salp20 inhibits AP activity. Salp20 was injected i.p. into mice at the indicated concentrations. Plasma was collected at 3 h and assayed for AP activity as indicated by the LPS-dependent surface deposition of C3 fragments. P^{Null} plasma and heat-inactivated (HI) Salp20 served as controls.

Figure 2.

Properdin deficiency attenuates the inflammatory response in OVA-induced asthma model.

Mice were sensitized i.p. with OVA (20 µg mixed with alum) on days 0 and 7 and challenged with OVA (30 µg i.n.) on days 14, 15, 16. (A) On day 17 mice were sacrificed and their bronchoalveolar lavage fluid (BALF) analyzed for total cell count (TCC) and differentials (PMN, polymorphonuclear leukocytes; Eo, eosinophils; lymph, lymphocytes; Mφ, macrophages). BALF was also assayed for IL-13 (B) and C3a (C) levels by ELISA. Data represent mean ± SD (n = 4–5 mice per genotype). ND = not detected. (D) Day 17 lungs were harvested, fixed in formalin, embedded in paraffin, sectioned and stained with Periodic Acid-Schiff (PAS) for mucous cells. Insets represent higher magnification of boxed areas. Representative photomicrograph of each genotype is shown. *p < 0.05, **p < 0.01

Figure 3.

Salp20 attenuates OVA-induced asthma phenotype. Mice were sensitized and challenged with OVA as detailed in Materials and Methods. Heat-inactivated (HI) or active Salp20 (9 μ g/mouse) was administered i.n. concomitantly with OVA. On day 17, mice were sacrificed and BALF enumerated for leukocyte subpopulations (A). AP activity was assessed in the BALF (B) and the lung (C). IL-13 (D) and C3a generation (E) were assessed in the BALF.

* $P < 0.05$

Fig. 4.

Salp20 suppresses aneurysm formation. Mice were perfused with elastase on day 0. Aortic diameter (AD) was assessed on day 14 and presented as percent (% **A**) or mm (**B**) increase. AAA is defined as an increase in AD of >100% over the pre-perfused diameter. Salp20 (20 µg given 3 h prior to and again 1 h and 24 h after elastase perfusion) suppressed aneurysm formation. N= at least 3 mice per treatment group. ***p < 0.001 compared to WT.

Fig. 5.

Salp20 and FH work synergistically to inhibit AP activity. (A) A hemolysis assay was employed to assess Salp20 inhibition of the assembly and function of the AP convertases. C3bBb or C3bBbP were assembled with the stabilizing FB D254G mutant on a sheep erythrocyte surface in the presence or absence of 200 ng of Salp20. Functional convertases were quantified by their capacity to direct cell lysis, as described in the Materials and Methods. Hemolysis was dependent on the amount of FB present in the assembly step, and the additional presence of properdin resulted in ~3-fold increase in hemolytic activity. The

addition of 200 ng Salp20 in the presence of P (50 ng) reduced lytic activity to the same level observed in the absence of P. Addition of Salp20 in the absence of P had no detectable effect on cell lysis. **(B–C)** Comparison of the inhibitory effects of Salp20 and factor H alone and together on AP convertase assembly and function. AP convertase assembly and function were assayed as in Panel A. Salp20 (200 ng/reaction) and/or FH (level indicated) were included in some of the assembly steps. The two inhibitors together had clear synergistic effect. Results in panels A and B are representative of three independent experiments. Results in panel C are representative of two independent experiments.