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Complement mediated hepatocytes injury in a model of autoantibody induced hepatitis

Zhidan Tu^{1,3}, Qing Li¹, Hong-Shiue Chou², Ching-Chuang Hsieh², Howard Meyerson¹, Marion G. Peters⁵, Hong Bu⁴, John J Fung², Shiguang Qian^{2,3}, Lina Lu^{2,3}, and Feng Lin¹

¹ Department of Pathology, Case Western Reserve University, Cleveland, USA

² Department of Immunology, Lerner Research Institute, Cleveland Clinic, Cleveland, USA

³ Department of General Surgery, Cleveland Clinic, Cleveland, USA

⁴ Department of Pathology, Sichuan University, Chengdu, China

⁵ Division of Gastroenterology, University of California, San Francisco, USA

Abstract

Despite multiple reports on autoantibody-initiated complement activation in autoimmune hepatitis (AIH), how does the humoral immunity contribute to the pathogenesis of AIH remained unclear. In this report, by adoptively transferring a polyclonal rabbit anti-OVA antibody into Hep-OVA Tg mice in which OVA is selectively expressed on the surface of hepatocytes, we found that excessive complement activation initiated by the autoantibody overwhelmed the protection of intrinsic cell surface complement regulators, and induced hepatocytes injury both *in vitro* and *in vivo*. The anti-OVA antibody induced hepatic injury in Hep-OVA Tg but not WT C57BL/6 mice as assessed by serum ALT levels and liver histopathology. Immunohistochemical analyses showed that after the antibody administration, there was massive complement activation on anti-OVA IgG coated hepatocytes in Hep-OVA Tg mice, but not in WT mice. Consistent with these results, depleting complement by cobra venom factor (CVF) prior to antibody injections protected Hep-OVA Tg mice from anti-OVA IgG induced hepatic injury. In addition, treating Hep-OVA Tg mice with recombinant mouse decay accelerating factor, a native complement inhibitor, protected them from autoantibody induced hepatitis. These results suggest that complement could play a pivotal role in liver specific autoantibody mediated hepatocyte injury in AIH, and that complement inhibitors could be, in principle, developed as novel therapeutics against AIH.

Keywords

autoimmune hepatitis; complement; autoantibody; complement regulators

Address correspondence to: Feng Lin, Ph.D., Assistant Professor, Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106. Phone: 216-368-2118, feng.lin@case.edu or Lina Lu, M.D., Associate Professor, Department of Immunology, Cleveland Clinic, Cleveland, OH 44106, Phone: 216-444-2574, Lul2@ccf.org.

Disclosure of potential conflicts of interest

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Introduction

Autoimmune hepatitis (AIH) causes continuing inflammation and necrosis, which progress to cirrhosis and eventually, liver failure (1, 2). Although circulating autoantibodies are hallmarks of AIH (2) and several antibodies against hepatocyte surface antigens have been identified in AIH patients (3–5), whether autoantibodies are integrally involved in the pathogenesis of AIH, and if so, by which mechanism, have not been completely elucidated.

Complement is primarily produced by the liver and participates in many liver diseases [reviewed in (6)]. It can be activated through the classical pathway after the autoantibodies bind to their target antigens (7). Although self tissues are generally protected from autologous complement mediated injury by intrinsic cell surface complement regulators, *i.e.*, decay accelerating factor (DAF) (8), CD46 (9) and CD59 (10), excessive complement activation overwhelming the protection of these complement regulators can cause tissue damage. In fact, the antibody-initiated, complement-mediated cytotoxicity has long been recognized to play a pivotal role in the pathogenesis of many autoimmune diseases in which autoantibodies are present (11). However, despite several reported connections between complement activation and AIH (12–14), the precise role of complement in autoantibody-induced liver injury in AIH remains elusive.

In this report, we systematically studied the distribution of intrinsic cell surface complement inhibitors on primary mouse hepatocytes, and the role of complement in a hepatitis model in which the liver injury was induced by administering a polyclonal rabbit anti-chicken ovalbumin (OVA) IgG into the Hep-OVA Tg mice, which selectively express membrane bound chicken OVA protein on their hepatocytes and have been employed to study the cellular autoimmunity in AIH (15). Our results indicate that complement activation is the primary mechanism underlying autoantibody induced liver injury in this model, and complement inhibitors could be developed as new therapeutics for AIH management.

Methods and reagents

Mice and antibodies

Hep-OVA transgenic (Tg) mice on C57BL/6 (B6) background which selectively express membrane-bound OVA on hepatocytes were developed as previously described (15). 8–12 weeks male Hep-OVA Tg mice and age matched B6 mice (Jackson laboratory, ME) were used in all studies. Polyclonal rabbit anti-OVA antibodyI was purchased from Millipore (Billerica, MA), rat anti-mouse CD59 mAb (clone ER-MP20) was ordered from AbCam Inc. (Cambridge, MA) and rat anti-mouse Crry mAb (clone 1F2) was purchased from BD Biosciences (San Jose, CA). Rat anti-mouse C3 mAb (Clone RmC11H9) was ordered from Cedarlane Laboratories (Burlington, NC) and the rabbit anti-human C5b-9 antibody which cross-reacts with mouse C5b-9 was from Abcam Inc (Cambridge, MA). Rat anti-mouse DAF mAb (clone 2C6) (16) was kindly provided by Dr. BP Morgan (Cardiff University, U.K.). All studies were conducted using an approved Institutional Animal Care Protocol.

Primary hepatocyte isolation

The mouse liver was *in situ* perfused with 0.05% collagenase H (Roche Molecular Biochemicals, IN) via portal vein, and the primary hepatocytes were isolated as described before (17). Cells were cultured in William's medium E with 10% fetal bovine serum (FBS) for 2 hours and collected for analyses.

Complement mediated cytotoxicity assay

A BCECF-AM based complement mediated cytotoxicity assay was employed as described before (18). Briefly, 2×10^5 freshly isolated primary hepatocytes were first loaded with 3 μ M BCECF-AM (Invitrogen, CA) in MEM medium at 37°C for 1 hr. After washing, labeled hepatocytes were incubated at 37°C with 50 μ g/ml rabbit anti-OVA IgG and 30% mouse serum in 100 μ l GVB/ Ca^{2+} Mg^{2+} buffer (veronal-buffered saline supplemented with 0.1% gelatin, 5 mM CaCl_2 and 3 mM MgCl_2) for another 30 min. 1 mM EDTA was included to inhibit complement activation in the controls. Following incubation, complement mediated cell injury was assessed by measuring levels of converted BCECF released into the supernatants using a fluorescence microtiter plate reader (Molecular Devices, CA) with excitation and emission wavelengths of 485 and 538 nm. To calculate the percentage of BCECF release after complement mediated cellular injury, the following equation was used: percentage of BCECF release = $[(A-B)/(C-B)] \times 100\%$; where A represents the mean experimental BCECF release, B represents the mean spontaneous BCECF release and C represents the mean maximum BCECF released which was induced by incubating cells with 0.1% SDS. The cells were also collected and assessed for C3b deposition by staining with an anti-mouse C3 mAb followed by flow cytometry analysis as described before (19).

Induction of autoimmune hepatitis

0.5 mg of the rabbit anti OVA IgG was injected into Hep OVA Tg mouse through the tail vein. Livers and sera were collected 4 hrs later. Serum ALT levels were measured by an automatic biochemical analyzer in the Clinical Core Laboratory of University Hospital Case Medical Center, and livers were sectioned and analyzed by H&E staining and immunohistochemical staining.

Flow cytometry analysis and immunohistochemical staining

To examine the distribution of intrinsic cell surface complement regulators, 2×10^5 freshly isolated primary hepatocytes were incubated with 5 μ g/ml of mAbs against mouse DAF, CD59 or Crry, respectively, or the same concentration of non-relevant rat IgG as negative controls. Mouse erythrocytes known to express all the three intrinsic cell surface complement regulators were included as positive controls. For immunohistochemical stainings, liver tissues were snap frozen in liquid nitrogen, then 7 micron cryosections were cut and stained with mAbs against rabbit IgG (rabbit anti-OVA IgG), mouse C3, mouse C5b-9 and mouse CD11b using a Vectastain ABC kit (Vector Labs, CA) following the manufacturer provided protocol. Non-relevant isotope IgGs were used as controls.

Complement depletion by CVF

To deplete complement, 20 μ g of purified cobra venom factor (CVF) (Sigma, MO) was injected *i.p.* in each mouse. Serum samples were collected from the tail vein before and after CVF injection for standard E^{shA} C3b uptake assays (20) to verify the depletion of complement. In brief, 5×10^5 E^{shA} were incubated at 37°C with 10% of the serum samples collected before and after CVF injection in 100 μ l GVB/ Ca^{2+} Mg^{2+} buffer for 30 min, then stained with 5 μ g/ml FITC labeled anti-mouse C3 mAb followed by flow cytometry analysis on a flow cytometer (LSR I, BD Biosciences, CA).

Recombinant soluble mouse DAF preparation and treatment

Yeast *Pichia pastoris* expressing soluble mouse DAF CCP 1–4 with a C-terminus 6 $\times$ his tag was developed in the lab (20). For soluble mouse DAF preparation, recombinant yeast was cultured in YPD media and induced with 1% methanol for 2 days. Secreted soluble mouse DAF protein was purified from the supernatants by Ni^{2+} affinity chromatography (Qiagen, CA) and dialyzed against PBS. The concentrations of the resultant mouse DAF was

measured using a Bio-Rad protein assay kit (Bio-Rad, CA) following the manufacturer provided protocol.

For DAF-based treatment, 200 µg of purified recombinant mouse DAF protein was injected *i.p.* per mouse 40 min before anti- OVA IgG administration. Sera and livers were collected 4 hr after induction of hepatitis. Inhibition of systemic serum complement by administrated DAF protein was assessed by C3b uptake assays using antibody sensitized sheep erythrocytes (E^{shA}) as described above. Serum ALT levels and liver histopathology were assessed as described above.

Statistics

All experiments were repeated at least twice. Results were compared using the ANOVA test. A *p* value <0.05 was considered significant.

Results

Mouse hepatocytes constitutively express DAF, CD59 and Crry

To systematically examine the distribution of intrinsic cell surface complement inhibitors, *i.e.* DAF, CD59 and Crry on mouse hepatocytes, we isolated murine primary hepatocytes by collagenase digestion with high purify (> 95%, data not shown) following an established protocol (17). After the isolation, we stained the hepatocytes with respective mAbs followed by flow cytometry analysis. These assays showed that mouse primary hepatocytes constitutively express all intrinsic cell surface complement regulator DAF, CD59 and Crry (Fig. 1).

The anti-OVA antibody induces complement mediated hepatocytes injury *in vitro*

We next tested whether the rabbit anti-OVA IgG activates complement and induces complement mediated hepatocyte injury *in vitro* using primary Hep-OVA Tg mouse hepatocytes by standard C3b uptake and BCECF-based cytotoxicity assays (21). These experiments showed that Hep-OVA hepatocytes exhibited markedly increased C3b deposition after incubating with anti-OVA IgG and 30% normal mouse serum (Fig. 2A) compared to those with complement inactivation (1 mM EDTA). Consistent with the elevated C3b deposition, antibody sensitized Hep-OVA hepatocytes with complement activation exhibited significantly heightened cell injury as indicated by > 10 fold greater amount of leaked BCECF than those in which complement activation was inhibited by EDTA (Fig. 2B). These results suggest that the anti-OVA IgG activates complement on Hep-OVA hepatocytes, which overwhelms the protection of intrinsic cell surface complement regulators, and induces complement mediated hepatocytes injury *in vitro*.

The anti-OVA antibody induces liver injury *in vivo*

After confirming that the anti-OVA IgG binds to Hep-OVA hepatocytes and induces complement mediated cell injury *in vitro*, we next studied whether administration of the hepatocytes-specific antibodies could induce liver injury *in vivo*. We injected the anti OVA antibody (0.5 mg/mouse) *i.v.* into Hep-OVA Tg mice, and 4 hrs later, assessed serum ALT levels and processed liver sections for immunohistochemical and H&E staining. At the same time, we treated WT C57BL/6 mice (without OVA expression) with the same protocol as the controls. As shown in Fig. 3A, after the same amount of anti-OVA IgG administration, serum ALT levels were 101± 30 U/L in Hep-OVA Tg mice compared to 58± 11 U/L in WT C57BL/6 mice (*p*<0.05). H&E staining showed that livers from Hep-OVA Tg mice with treatment exhibited interrupted hepatocyte clusters with increased numbers of leukocytes infiltration (Fig. 4A: B1), compared to normal liver histology in the control WT mice (Fig. 4A: A1). Consistently, immunohistochemical analysis showed that, compared to negative

staining in liver sections from control WT mice (Fig. 4A: A2), Hep-OVA Tg mouse livers demonstrated intensive rabbit IgG staining (Fig. 4A: B2), indicating the selective binding of the rabbit anti-OVA IgG on Hep-OVA Tg mouse hepatocytes. More importantly, these assays also showed massive complement activation product C3b and C5b-9 accumulations in livers from Hep-OVA mice (Fig. 4A: B3, B4) but not on livers from the control WT B6 mice (Fig. 4A: A3, A4). These data indicate that, as suggested by the above *in vitro* results, administration of hepatocyte specific antibodies selectively activates complement on hepatocytes and induces liver injury *in vivo*.

Anti-OVA IgG induced liver injury in Hep-OVA Tg mice is mediated by complement

To determine the role of complement in the anti-OVA antibody induced liver injury in Hep-OVA mice, we first injected CVF *i.p.* to deplete complement, and then gave the same amount of anti-OVA IgG as done above. In a pilot study, we have defined that depletion of complement was established 18 hrs after *i.p.* administration of 20 μ g of CVF and the deficiency of complement lasted for at least 42 hrs (data not shown), therefore, this protocol was used in this study. As shown in Fig. 3, after complement depletion, liver injury induced by anti-OVA IgG administration in Hep-OVA Tg mice was significantly attenuated with serum ALT levels decreased to 53 ± 29 U/L, compared to 101 ± 30 U/L in complement sufficient Hep-OVA Tg mice ($p < 0.05$). In agreement with these results, H&E staining showed normal liver histology (Fig. 4A: C1) and immunohistochemical staining showed that although large amounts of rabbit IgG bind to hepatocytes in the CVF- treated Hep-OVA Tg mice (Fig. 4A: C2), decreased levels of C3b and C5b-9 were detected (Fig. 4A: C3, C4) after complement depletion. We further stained the infiltrated cells with CD11b (Fig. 4B), which is expressed on most leukocytes, and quantitated numbers of the CD11b⁺ cells in each section. We found that average CD11b⁺ cell numbers in five high power fields were 4.0 ± 1.1 in WT B6 mice, 28.8 ± 2.8 in Hep-OVA mice, and 11.0 ± 0.8 in Hep-OVA mice with CVF complement depletion ($p < 0.05$). Under high power magnification, these infiltrated CD11b⁺ cells appear to be neutrophils (Fig. 4B).

Administration of recombinant mouse DAF protein protects antibody-induced liver injury

If the pathology is primarily mediated by excessive complement activation, then administration of exogenous complement regulator(s) should protect mice from the autoantibody-induced hepatic injury. To test this hypothesis, we next treated Hep-OVA Tg mice with purified recombinant mouse DAF protein before anti-OVA antibody administration. After this, we assessed their complement activities and hepatitis severities by C3b uptake assays, serum ALT level measurements and liver histopathological analyses. These studies showed that compared to sera collected before treatments, sera from Hep-OVA Tg mice received 200 μ g/mouse recombinant DAF treatment had > 5 fold reduced serum complement activity as assessed by E^{shA} C3b uptake assays (Fig. 5A). In accordance with these results, Hep-OVA Tg mice received DAF treatment exhibited significantly reduced serum ALT levels (50 ± 12 u/ml vs. 101 ± 36 U/ml, $p < 0.05$) (Fig. 5B). This reduced ALT levels was associated with markedly improved liver histopathology (Fig. 6B1). In addition, immunohistochemical staining showed that while recombinant DAF administration did not interfere with anti-OVA IgG's binding to hepatocytes in Hep-OVA Tg mice (Fig. 6B2), it markedly reduced complement C3b and C5b-9 deposition on them (Fig. 6B3, B4), indicating that the recombinant DAF protein protects Hep-OVA mice by inhibiting complement activation.

Discussion

In this report, using the same Hep-OVA Tg mice that have been employed in understanding the role of cellular autoimmunity in AIH (15), we studied the role of the humoral

autoimmunity in the pathogenesis of this liver disease. We found that although mouse hepatocytes express all the intrinsic cell surface complement regulators, complement activation initiated by a hepatocyte specific antibody overwhelms the protection of these complement regulators, resulting in liver injury. Furthermore, depleting complement by CVF or inhibiting complement activation by recombinant DAF protein attenuates the liver specific antibody induced hepatitis.

Knowledge of expression patterns of intrinsic cell surface complement regulators on hepatocytes is important for understanding the role of complement in AIH and in transplanted liver/hepatocyte rejection. The current known intrinsic cell surface complement regulators in humans are DAF, CD59 and CD46. DAF inhibits complement activation on cell surfaces by accelerating the decay of C3/C5 convertases (8), while CD46 serves as a cofactor for factor I to inactivate C3b/C4b therefore preventing the assembly of C3/C5 convertases (9). CD59 protects cells from complement mediated injury by inhibiting the formation of the membrane attack complexes (10). Not all cells express all the three intrinsic complement regulators on their surface. For examples, human erythrocytes do not express CD46 (22), and natural killer cells do not express DAF (23). Earlier work on human liver tissues indicated that only CD46 is expressed on normal hepatocytes (24), but later studies using human hepatoma cell lines showed that CD46, DAF and CD59 are all present on these tumorized hepatocytes (25). Recently, a detailed study using isolated primary hepatocytes found that human hepatocytes express all the intrinsic cell surface complement regulators (26). Although mice are commonly used to study human hepatitis and liver transplantation, surprisingly, expression patterns of the intrinsic cell surface complement regulators on mouse hepatocytes have not been systematically studied. Different from that in humans, CD46 expression in rodents is limited only in testis (27). But there is another rodent specific cell surface complement regulator termed complement receptor-related gene y (Crry) (28, 29), which is widely distributed and considered the counterpart of human CD46 in mouse tissues. Using isolated primary murine hepatocytes, we found that similar to that in humans, murine hepatocytes constitutively express all intrinsic cell surface complement regulators DAF, CD59 and Crry, which could help to protect hepatocytes from autologous complement mediated injury.

It is the current understanding that autoreactive T cells induce liver injury in AIH. Despite the long time observations that autoantibodies are developed in most of the AIH patients (30, 31), the precise role of autoantibodies in AIH remains elusive. While many of the autoantibodies are not liver specific and are against soluble antigens (31), several autoantibodies against hepatocyte surface antigens have been identified from patients (3–5). There has been one study showing that an IgM isolated from AIH patients reacts to a ~190 kDa unknown hepatocytes surface antigen, activates complement, and induces acute liver injury in mice (12). However, the precise role of complement in this intriguing model of AIH was not elucidated.

The lack of a good animal model for AIH contributes to limited understanding of the disease pathogenesis and inadequate development of effective therapeutics. In addition to liver transplantation, current therapeutic interventions for AIH are mainly immunosuppressive drugs such as Prednisone and Azathioprine, which are not always effective and possess many side effects (31). It is important to understand the pathogenesis underlying AIH to develop better therapeutics for disease management. OVA is a model antigen which has been widely employed to study the pathogenesis of many diseases in their respective models. The reagents and assays required for studies on OVA specific T cell and antibody responses have been well developed. The generation of the unique OVA transgenic mice which selectively express OVA antigen on hepatocytes surface provides a simple model to better understand the role of both the cellular and humoral autoimmunity in the pathogenesis

of AIH, and to develop novel therapies against this disease. Previous studies (15) using the same mouse have shown that hepatitis can be induced by adoptive transfer of OVA specific CD8⁺ T cells from OT-I mice, providing a powerful tool to study the role of hepatocyte specific autoreactive T cell responses in AIH. In current report, we have demonstrated that by adoptive transfer of an anti-OVA antibody into the same Hep-OVA Tg mouse, the liver specific antibody-initiated, complement mediated hepatocytes injury can be reliably induced. And this autoantibody induced hepatitis depends on the selective expression of OVA antigen on hepatocytes surface because WT C57BL/6 mice which do not express OVA protein exhibited little, if any, liver injury after the same antibody treatment. We propose that this model could be used to study the pathogenesis of AIH from the humoral side of the autoimmunity, which should be helpful in understanding the role of autoantibodies in AIH.

In many autoantibody induced autoimmune diseases and their animal models, excessive complement activation initiated by autoantibodies that overwhelms self complement inhibitors' protection play a pivotal role in the pathology, indicating that exogenous complement inhibitors can be used to treat these diseases. One of the examples is myasthenia gravis, in which anti-acetylcholine receptor (AChR) autoantibodies bind to AChR at neuromuscular junctions, initiates complement activation leading to endplates damage and eventually muscle paralysis (32, 33). In addition to conventional immunosuppressive drugs and thymectomy, a complement inhibitor (anti-C5 mAb) has shown promise in attenuating muscle weakness in an animal model of MG (34) and the humanized anti C5 mAb (Eculizumab) is being evaluated in Phase II clinical trials as a new therapy for MG patients. Using the Hep-OVA Tg mice, we found that autoantibodies activate complement on surface of hepatocytes which overwhelms the protection of intrinsic cell surface complement regulators, resulting in C5b-9 deposition and neutrophils infiltration which lead to liver injury. Depletion of complement by CVF or administration of recombinant soluble DAF protein, a potent native complement inhibitor, effectively suppresses complement activation and thereby protecting Hep-OVA Tg mice from anti-OVA IgG induced liver injury. These results suggest that like MG, complement activation could be the primary mechanism underlying autoantibody mediated liver injury in AIH, and complement inhibitors, in principle, could be developed for AIH management.

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Figure 1.

Mouse primary hepatocytes express DAF, Crry and CD59. 2×10^5 freshly isolated B6 primary hepatocytes were stained with mAbs against DAF (A), Crry (B) or CD59 (C), followed by flow cytometry analysis. Representative results of three separate experiments.

Figure 2.

The hepatocyte-specific antibody activates complement and induces complement mediated hepatocyte injury *in vitro*. **A:** 1×10^5 hepatocytes freshly isolated from Hep-OVA mice were incubated with 50 $\mu\text{g/ml}$ anti OVA IgG in MEM media for 20 min. After washing, cells were resuspended in 50 μl GVB²⁺ buffer with 20% normal mouse serum with (dotted line) or without 1 mM EDTA (solid line), and incubated at 37°C for another 30 min. Deposition of complement activation product C3b was detected by staining with 5 $\mu\text{g/ml}$ FITC-anti C3 mAb followed by flow cytometric analysis. Representative results of two separate experiments. **B:** 1×10^5 freshly isolated Hep-OVA hepatocytes were labeled with 1 μM BCECF-AM, then incubated with 50 $\mu\text{g/ml}$ anti-OVA IgG and 20% normal mouse serum in GVB²⁺ buffer with (control) or without 1 mM EDTA (experimental) for 30 min. Cell injury was assessed by measuring released BCECF in the supernatants then calculated as described in Methods. The experimental results were normalized against the controls (with EDTA). Combined results from 3 experiments were shown. (* $p < 0.05$)

Figure 3.

Administration of the anti-OVA IgG selectively induces complement mediated liver injury in Hep-OVA Tg mice. WT or Hep-OVA Tg mice were *i.v.* injected with 0.5 mg the anti-OVA IgG and serum ALT levels were measured 4 hrs later. To determine the role of complement in the hepatocyte specific antibody-induced liver injury, complement was depleted in Hep-OVA mice by *i.p.* injection of 20 μ g/mouse CVF, followed by the anti-OVA IgG administration. Each dot represents one mouse and the bars represent the averaged serum ALT values. * $p < 0.05$ between the (Hep-OVA Tg + anti-OVA IgG) group and other groups

Figure 4A

Figure. 4B

Figure 4.

Panel A, Representative results of liver histopathological and immunohistochemical analyses of WT (A), Hep-OVA Tg (B) and Hep-OVA Tg mice with CVF complement depletion (C) followed by rabbit anti-OVA IgG administration. Liver samples were collected 4 hr after anti-OVA IgG injections. For H&E stainings, livers were fixed in 4% formaldehyde and 7 μ M paraffin-embedded sections were cut and stained (A1, B1 and C1). Arrows indicate infiltrated inflammatory cells. For bound rabbit IgG (A2, B2 and C2), deposited C3b (A3, B3 and C3) and C5b-9 (A4, B4 and C4) staining, livers were snap-frozen in liquid nitrogen and 7 μ M cryosections were made. After this, sections were treated with 4% H₂O₂, followed by the anti-rabbit IgG (A2, B2 and C2), anti-C3 mAbs (A3, B3 and C3) and anti C5b-9 Ab (A4, B4 and C4) with an ABC kit. The staining with non-relevant IgGs were negative (not shown). Panel B. Staining of CD11b⁺ infiltrated cells, showing neutrophil-like CD11b⁺ cells in the liver after anti-OVA IgG administration.

Figure 5.

Administration of recombinant DAF protein protects anti-OVA-mediated liver injury in Hep-OVA mice. A. E^{shA} - based C3b uptake assays showing that recombinant DAF protein administration inhibited > 80% of the serum complement activity (mean fluorescence intensity 5 vs 25). B. Administration of recombinant DAF protein significantly reduced serum ALT levels. Each dot represents one mouse and the bars represent the averaged serum ALT values. * $p < 0.05$

Figure 6.

Representative H&E and immunohistochemical staining pictures of livers from Hep-OVA Tg mice without (A) and with (B) recombinant DAF protein treatment. A1, H&E stainings of Hep-OVA mouse liver sections 4 hr after anti-OVA IgG administration, showing infiltrated inflammatory cells (arrows); B1, H&E staining of Hep-OVA mouse liver sections with recombinant DAF treatment 4 hr after anti-OVA IgG administration, showing little, if any infiltrated cells; A2, B2, Rabbit IgG staining, showing that rabbit anti-OVA IgG binds to Hep-OVA mouse hepatocytes without (A2) and with (B2) DAF treatments; A3, B3, and A4, B4, complement activation products C3b and C5b-9 staining, showing that C3b accumulation and C5b-9 deposition on hepatocytes are markedly reduced in Hep-OVA Tg mice with DAF treatment (B3, B4) compared to that without DAF treatment (A3, B4). Staining with non-relevant IgGs was negative (not shown).