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RESEARCH PAPER

Suppression of antibody-mediated arthritis in mice by Fab fragments of the mediating antibodies

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BACKGROUND AND PURPOSE
Fab fragments (Fabs) of antibodies maintain the ability to bind specific antigens, but lack the binding site for complement as well as the site for binding to receptors on effector cells, such as macrophages that play an important role in inflammation. In the present study, we investigated whether Fabs specific for ovalbumin (OVA) were specifically able to suppress anti-OVA antibody-mediated arthritis (AOA-MA) in mice.

EXPERIMENTAL APPROACH
AOA-MA was induced by i.v. injection of purified anti-OVA antibodies into naïve mice followed by intra-articular (left ankle) challenge with the antigen. Anti-OVA Fabs prepared by digestion of anti-OVA antibodies with papain were injected i.v. immediately after administration of the intact antibodies. Normal Fabs were used as a control. Arthritis was assessed by thickness of the joints (caliper) and by histology of paw sections, stained with haematoxylin and eosin.

KEY RESULTS
AOA-MA was markedly suppressed by anti-OVA Fabs, but not by control Fabs. Histologically, mice treated with control Fabs showed marked oedema of synovial tissues with a large number of inflammatory cells including neutrophils, whereas animals given anti-OVA Fabs had mild oedema of the synovium and sparse infiltration of such cells. The antigen-specific suppression of joint inflammation by anti-OVA Fabs was associated with reduced consumption of complement. *In vitro* studies showed that anti-OVA Fabs significantly blocked the binding of intact anti-OVA antibodies to OVA.

CONCLUSIONS AND IMPLICATIONS
Antibody-mediated arthritis appears to be specifically down-regulated by Fabs that competitively inhibit the binding of antibodies to antigens.

Abbreviations
AOA-MA, anti-OVA antibody-mediated arthritis; Fabs, Fab fragments; OVA, ovalbumin; RA, rheumatoid arthritis

Introduction

Antibody-mediated diseases include rheumatoid arthritis (RA), which is a chronic, destructive, inflammatory joint disease (Weissmann, 2004; Panayi, 2005; Bugatti *et al.*, 2007; Okroj *et al.*, 2007). There is strong evidence that serum levels of complement in patients with RA are lower than those in healthy subjects due to its activation by antigen–antibody immune complexes formed (Neumann *et al.*, 2002). Complement cleavage products such as C3a and C5a produced upon its activation have been shown to enhance vascular permeability to be chemotactic for inflammatory

cells and to produce pro-inflammatory cytokines including tumour necrosis factor (TNF)-α and interleukin (IL)-1 via their receptors on varying types of cells including macrophages (el-Lati *et al.*, 1994; Gutzmer *et al.*, 2006; Mullazehi *et al.*, 2006), suggesting an important role of these complement products in inflammation.

Fab fragments (Fabs) produced by the digestion of antibodies with papain maintain the ability to bind specific antigens as effectively as intact antibodies, but unlike intact antibodies, the Fabs lack the binding site for complement and the site(s) that bind to receptors on effector cells such as macrophages. Therefore, treatment with Fabs is expected to result in down-regulation of antibody-mediated diseases, as less complement is activated and hence there is less production of pro-inflammatory cytokines including TNF-α and IL-1 from macrophages. Fabs specific for digoxin have been clinically used for the treatment of patients with toxic levels of digoxin (Woolf *et al.*, 1992; Boss and Pope, 1994; Flanagan and Jones, 2004). However, it was not shown previously *in vivo* that antibody-mediated diseases were specifically regulated by Fabs of the mediating antibodies. Drug therapies for RA include steroidal and non-steroidal anti-inflammatory drugs, immunosuppressive drugs and biological agents such as anti-TNF-α antibodies (O'Dell *et al.*, 1996; Simon, 1996; Bijlsma *et al.*, 2003; Puppo *et al.*, 2005). However, these drugs do not act antigen- or disease-specifically, and therefore serious adverse effects are often observed (Lipsky *et al.*, 2000; Khanna *et al.*, 2004; Layton *et al.*, 2008). The ideal therapy for immune diseases would be to down-regulate only the pathogenic antigen-specific immune responses and not affect those essential for self-defence against bacteria and viruses. Unfortunately, no antigen-specific therapies for immune diseases have been clinically available.

In the present study, we show that antigen-specific suppression of immune diseases is simply and readily achieved by employing Fabs of the mediating antibodies as ovalbumin (OVA)-specific Fabs, but not unrelated, control Fabs were able to down-regulate anti-OVA antibody-mediated arthritis (AOA-MA) in mice.

Methods

Animals

All animal care and experimental procedures were conducted according to the guidelines of the Ethics Committee of Kobe Pharmaceutical University, Kobe, Japan. Male DBA/1j mice, 7–9 weeks of age, were used in all experiments. The animals were maintained in a temperature-controlled environment with free access to standard rodent chow and water.

Production of anti-OVA antibodies

The animals were immunized by injecting s.c., at the base of the tail, 100 μg OVA (Sigma-Aldrich Fine Chemicals, St Louis, MO, USA) dissolved in 50 μL of phosphate-buffered saline (PBS) and emulsified with an equal volume of complete Freund's adjuvant (CFA; Difco Laboratories, Detroit, MI, USA) (day 0). At day 21, the animals had a booster injection of the OVA and CFA emulsion, and 7 days later sera were collected. To purify anti-OVA antibodies from the sera, 1 mL of saturated ammonium sulphate was added to 1 mL of the sera followed by centrifugation at 2000× *g* at 4°C for 20 min. The pellet was dissolved in 5 mL of PBS and dialysed against 2000 mL of the same buffer for 3 h at 4°C, and this was repeated three times. For further purification of the anti-OVA antibodies, affinity chromatography was used. In brief, OVA (20 mg·mL^{-1}) was coupled to HiTrap NHS-activated HP columns (GE Healthcare UK Ltd, Buckinghamshire, UK), followed by equilibration with binding buffer (20 mM Tris, 0.5 M NaCl, pH 8.0). Then, the proteins recovered from the ammonium sulphate precipitate were applied to the OVA-coupled columns. The columns were washed with binding buffer before the addition of elution buffer (0.1 M glycine, pH 3). The anti-OVA antibody-containing elution buffer was dialysed against PBS. Aliquots of purified protein solution were mixed with Laemmli sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) sample buffer, and the purity of anti-OVA antibodies was assessed according to the methods of Laemmli (1970).

Induction of AOA-MA

To induce AOA-MA, the mice were given i.v. 1 mg of purified anti-OVA antibodies, and 30 min later the animals were intra-articularly injected with 20 μL of PBS containing 10 μg of OVA into the left ankle joints. The right ankle joints were injected with 20 μL of PBS alone as a control. To evaluate the severity of arthritis, the thickness of both ankle joints was measured using a dial gauge caliper (Ozaki Mfg Co., Tokyo, Japan) calibrated with 0.01 mm graduations according to the method described previously (Yoshino, 1998). The net increase in joint thickness attributable to the antigenic challenge was calculated by subtracting the increase in thickness of the right ankle from that in the left ankle. There was no net joint swelling after injection of OVA in untreated naïve mice.

Preparation and administration of anti-OVA Fabs

To prepare anti-OVA Fabs, anti-OVA antibodies were digested by agarose-linked papain (Sigma Aldrich Inc.) at 37°C for 1, 4, 18 and 24 h according to the methods described previously (Katpally *et al.*, 2008). Anti-OVA Fabs were separated by Sephacryl S-200 high-resolution column (GE Healthcare UK Ltd) chromatography. Absorbance at 280 nm was measured using a Gene Quant Pro (GE Healthcare UK Ltd) spectrophotometer to determine protein concentrations. Anti-OVA Fabs were identified by Western blotting using alkaline phosphatase-conjugated anti-mouse kappa/lambda light chain (Sigma Aldrich Inc.) (Towbin *et al.*, 1979). Separated anti-OVA Fabs (0.2, 1 and 5 mg) dissolved in PBS were given i.v. immediately after injection of intact anti-OVA antibodies. PBS and 5 mg of normal IgG Fabs (Sigma-Aldrich Fine Chemicals) were used as controls.

Histology

The mice were killed (exsanguination during ether anaesthesia) at 1 or 5 h after antigenic challenge injection. Hind paws were amputated, fixed in 4% formalin and decalcified in 5% formic acid using the method described previously (Yoshino and Cleland, 1992). The tissues were embedded in paraffin, sectioned at 4 μm and stained with haematoxylin and eosin (H & E).

Measurement of complement fragment C3 and anti-OVA antibodies in sera

The sera of mice with AOA-MA were collected at 5 h after intra-articular challenge with OVA. C3 levels in the sera were measured by using a mouse complement C3 ELISA kit (Kamiya Biomedical Company, Seattle, WA, USA) according to the manufacturer's instructions. Anti-OVA IgG antibodies were measured by an ELISA (Yoshino *et al.*, 1999).

Administration of the C3a receptor antagonist SB290157 and the H_1 receptor antagonist chlorpheniramine (CPA)

To investigate the role of complement activation in AOA-MA, 30 mg·kg^{-1} of the C3a receptor antagonist SB290157 (Ames *et al.*, 2001; Mathieu *et al.*, 2005; from Calbiochem, San Diego, CA, USA) was given i.p. at the same time as, and then 2 h after, the challenge injection of OVA. PBS was administered as a control. To define the role of histamine in AOA-MA, 30 and 100 mg·kg^{-1} CPA (Rahman *et al.*, 2007; from Sigma Aldrich) were orally administered to mice at 1 h before intra-articular challenge with OVA. PBS was given as a control.

Inhibition analysis

To determine the ability of anti-OVA Fabs to competitively inhibit the binding of whole anti-OVA antibodies to OVA, OVA-coupled CNBr-activated Sepharose 4FF/4B (GE Healthcare Biosciences, Tokyo, Japan) (OVA-beads) was prepared according to the methods described previously (Nakanishi *et al.*, 2008). Similar beads containing the control antigen, keyhole limpet haemocyanin (KLH; Sigma Aldrich) were also prepared. OVA or KLH beads were incubated with 0.5 mg of whole anti-OVA antibodies in the presence or absence of 1 or 5 mg of anti-OVA Fabs followed by centrifugation. Unbound whole anti-OVA antibodies in the supernatants were detected by SDS–PAGE. The amounts of unbound whole anti-OVA IgG antibodies in the supernatants were also determined using antibodies specific for the Fc portion of mouse IgG by ELISA (Inada *et al.*, 1997).

Data analysis

Data are shown as means ± SEM (n = 5). Non-parametric analysis for all experimental data was performed by using the Mann–Whitney U-test. P values < 0.05 were considered statistically significant.

Results

Preparation of anti-OVA Fabs

To prepare anti-OVA Fabs, purified anti-OVA antibodies were incubated with immobilized papain for 1, 4, 18 and 24 h. As shown in Figure 1A, SDS–PAGE analysis revealed that the incubation of the whole antibodies with papain resulted in increased levels of approximately 50 kDa proteins that closely matched the size of standard Fabs. The increase in the levels of these proteins was dependent on the incubation time. In contrast, whole anti-OVA antibody levels including IgG appeared to decrease with time because of their digestion by papain. Then, we attempted to separate Fabs from papain-digested anti-OVA antibodies using gel chromatography, and found that there was a single peak consisting of fractions 40–100 (Figure 1B). To determine whether this single peak contained Fabs, Western blotting analysis was carried out following SDS–PAGE that also showed a protein band of approximately 50 kDa as seen in Figure 1A (data not shown). As shown in Figure 1C, each fraction consisting of the single peak reacted with anti-kappa/lambda chain antibodies, specific for light chains. Thus, anti-OVA Fabs appeared to be successfully separated; therefore, fractions 63–80 were pooled and used for experiments as antigen-specific Fabs.

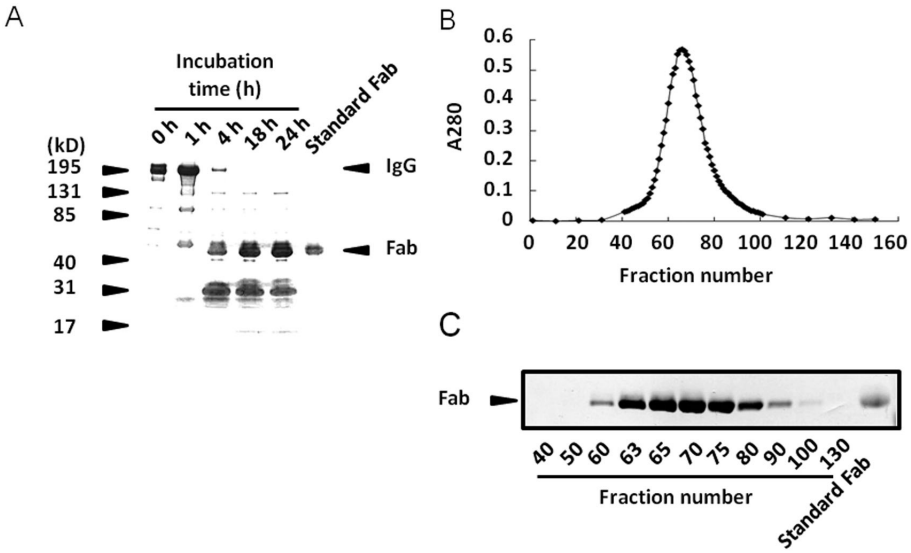

Figure 1

Preparation of anti-OVA Fabs. (A) Production of Fabs from anti-OVA antibodies by treatment with papain. Anti-OVA antibodies were incubated with immobilized papain for 1, 4, 18 and 24 h, and SDS–PAGE analysis was performed to show the production of anti-OVA Fabs. (B) Separation of Fabs from anti-OVA antibodies treated with papain. Anti-OVA Fabs were separated by Sephacryl-S-200 high-resolution columns. (C) Western blotting analysis. Proteins in fractions 40–130 collected following gel chromatography were stained with anti-mouse kappa/lambda antibodies to identify anti-OVA Fabs. Data are representative of three independent experiments.

Effect of anti-OVA Fabs on AOA-MA

To induce AOA-MA, the mice were injected i.v. with 1 mg of purified anti-OVA antibodies followed by intra-articular challenge with 10 μg of OVA. As shown in Figure 2A, the animals developed joint inflammation, reaching a peak at 30 min after antigenic challenge followed by subsidence to some extent by 1 h. Thereafter, however, arthritis began to flare-up again, reaching a peak at 3 h. To test the effect of anti-OVA Fabs on AOA-MA, 0.2, 1 and 5 mg of the Fabs were given by i.v. injection immediately after the administration of 1 mg of whole anti-OVA antibodies. Fabs (5 mg) from naïve normal animals were used as a control. The results showed that anti-OVA Fabs suppressed AOA-MA in a dose-related manner throughout the period examined, whereas control Fabs failed to affect joint inflammation. The suppression of arthritis by 5 mg of anti-OVA Fabs at 0.5 and 3 h was 68 and 74% respectively. Figure 2B shows the difference in joint swelling of hind paws between control Fabs- and anti-OVA Fabs-treated mice. Histologically, mice injected with control Fabs had marked oedema of synovial tissues and infiltration of a large number of inflammatory cells including neutrophils, while those given anti-OVA Fabs had mild swelling of the synovium and a minimum of inflammatory cell infiltration (Figure 2C).

Levels of C3 in the sera of mice treated with anti-OVA Fabs

Because complement appeared to be consumed due to its activation during the development of AOA-MA, serum levels of the complement fragment C3 were determined. As shown in Figure 3, mice treated with PBS or control Fabs-treated mice had lower levels of C3 compared to normal naïve mice. In contrast, there was no difference in C3 levels between normal naïve mice and anti-OVA Fabs-treated animals.

Effects of the C3a receptor antagonist SB290157 and the histamine H_1 receptor antagonist CPA on AOA-MA

Because the activation of C3 appeared to be prevented in anti-OVA Fabs-treated mice, we examined whether C3 activation was indeed involved in AOA-MA. For this examination, the mice were treated with the C3a receptor antagonist SB290157. As shown in Figure 4A, significant suppression of joint inflammation by SB290157 was observed at 3–5 h after antigen challenge. However, the C3a antagonist failed to affect joint oedema induced before 3 h. Because chemical mediators including histamine released from mast cells via their receptors for anti-OVA IgE might have played a role in the earlier phase of joint inflammation, the effect of the H_1

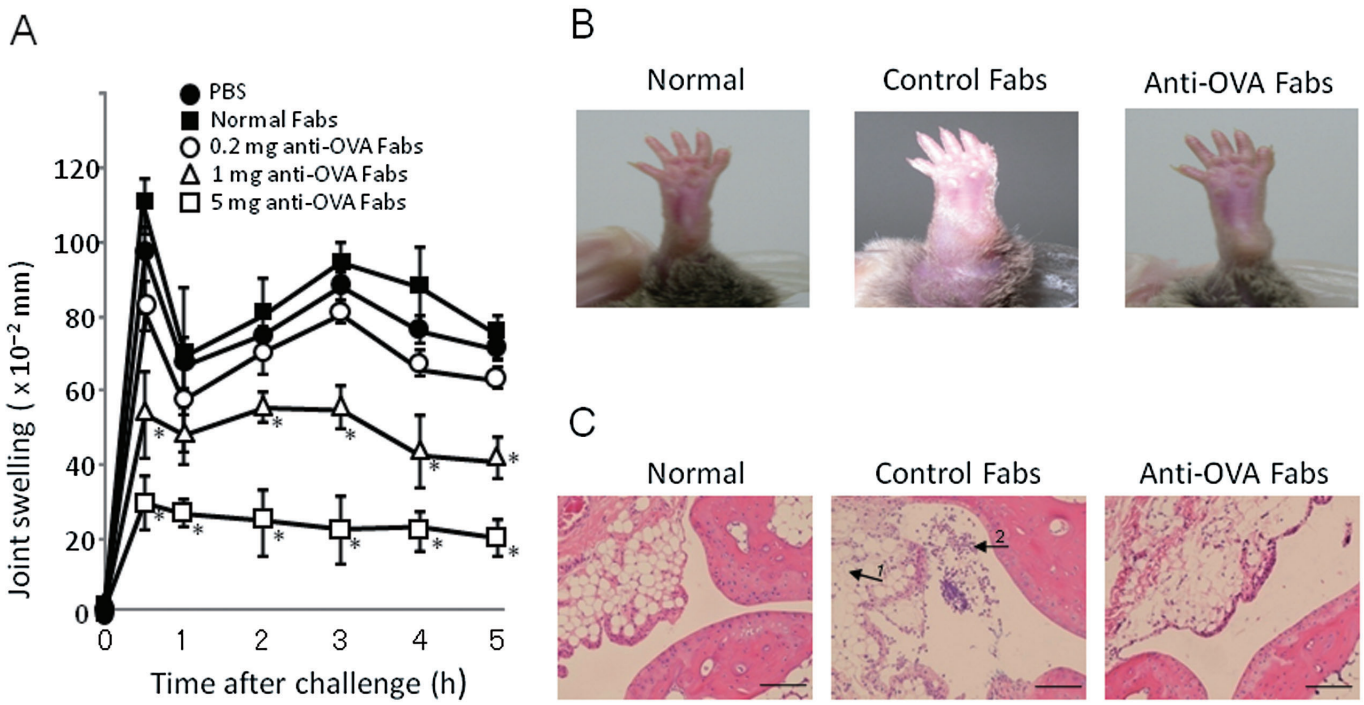

Figure 2

Effect of anti-OVA Fabs on AOA-MA in mice. (A) Anti-OVA Fabs suppress AOA-MA in a dose-related manner. AOA-MA was induced by i.v. injection of 1 mg of whole anti-OVA antibodies followed by intra-articular injection of 10 μg of OVA. A range of doses of anti-OVA Fabs (0.2, 1 and 5 mg) were given i.v. immediately after injection of whole anti-OVA antibodies. PBS and 5 mg of normal Fabs were used as controls. Data are means ± SEM ($n = 5$). *$P < 0.05$ versus controls. (B) Joints of mice treated with anti-OVA Fabs. Photographs of the hind paws of mice treated with control Fabs and anti-OVA Fabs were taken at 3 h after antigenic challenge and compared to those of normal mice. (C) Histological changes in joints of mice treated with anti-OVA Fabs. Ankle joints of mice treated with control Fabs and anti-OVA Fabs were amputated 5 days after challenge injection of OVA, decalcified, embedded in paraffin, sectioned at 4 μm and stained with H & E. Scale bar: 100 μm. Arrow 1 shows oedema of synovial tissues, and arrow 2 indicates infiltration of many inflammatory cells. Data are representative of three independent experiments.

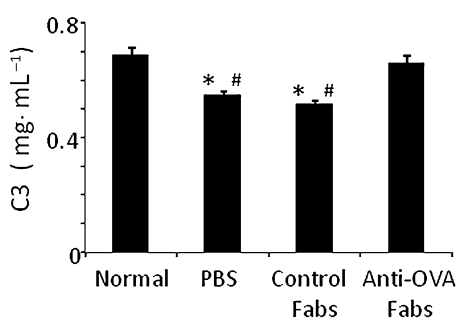

Figure 3

Treatment with anti-OVA Fabs prevents consumption of the complement fragment C3. Sera of mice with AOA-MA treated with PBS, control Fabs and anti-OVA Fabs were collected 5 h after antigenic challenge, and serum levels of C3 in these treatment groups determined by ELISA were compared to those of normal naïve mice. Data are means ± SEM ($n = 5$). *$P < 0.05$ and #$P < 0.05$ versus normal naïve and anti-OVA Fab-treated mice respectively. Data are representative of two independent experiments.

receptor antagonist CPA on AOA-MA was also investigated. The results are shown in Figure 4B. CPA significantly down-regulated joint inflammation seen at 0.5–1 h, but not at 2–5 h after challenge with OVA. The down-regulation of arthritis by CPA was histologically confirmed (Figure 4C,D), indicating an important role for histamine in the earlier phase of joint reaction.

Effect of anti-OVA Fabs on the interaction between OVA and whole anti-OVA antibodies

Next, we examined whether anti-OVA Fabs were able to interfere with OVA and whole anti-OVA antibody interactions. For this study, OVA and the control antigen KLH beads were incubated with 0.5 mg of whole anti-OVA antibodies alone or 0.5 mg of whole anti-OVA antibodies plus 1 and 5 mg of anti-OVA Fabs followed by centrifugation. Then, whole anti-OVA antibodies in the

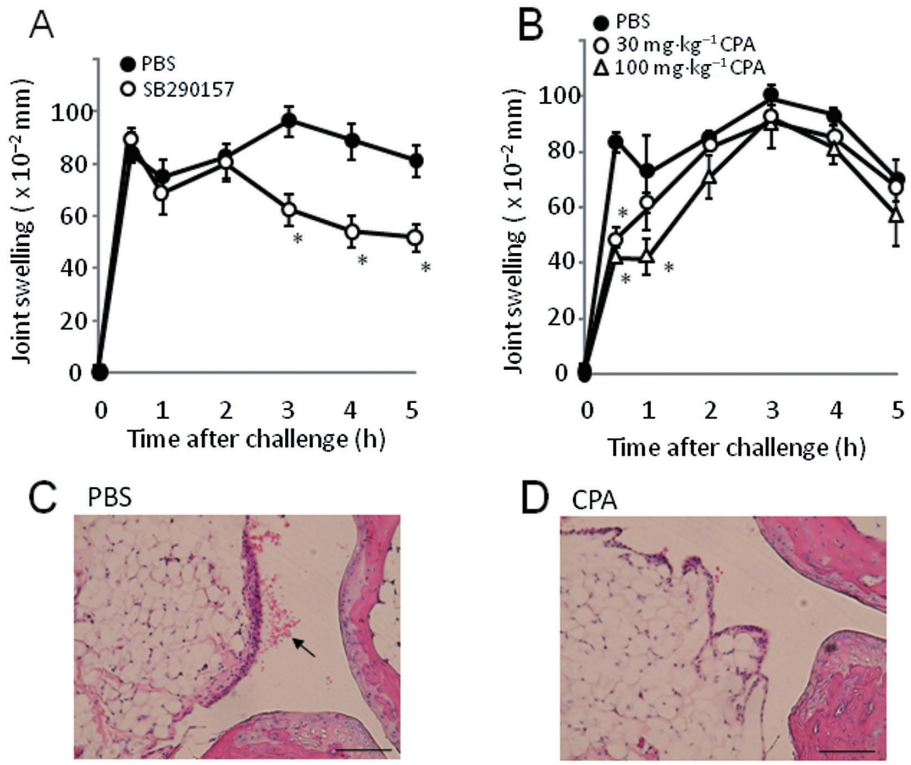

Figure 4

Roles of C3a and histamine in AOA-MA in mice. (A) C3a plays a role in the late phase of AOA-MA. Mice were given 30 mg·kg^{-1} i.p. of the C3a receptor antagonist SB290157 at the same time as, and at 2 h after, antigen challenge with OVA. PBS was given as a control. (B–D) Histamine plays a role in the early phase of AOA-MA. Mice were orally given 30 or 100 mg·kg^{-1} of the H_1 receptor antagonist CPA at 1 h before the challenge injection of OVA. PBS was given as a control. Data are means ± SEM (n = 5). *P < 0.05 versus PBS. Data are representative of two independent experiments. For histological analysis, ankle joints of mice treated with PBS or CPA were amputated 1 h after challenge injection of OVA, decalcified, embedded in paraffin, sectioned at 4 μm and stained with H & E. An arrow shows infiltration of inflammatory cells. Scale bar: 100 μm.

supernatants were measured. Figure 5A shows the results of SDS–PAGE analysis. Incubation of OVA, but not KLH, beads with whole anti-OVA antibodies alone resulted in few free whole anti-OVA antibodies in the supernatants, indicating binding of most of the whole antibodies to OVA beads that were precipitated on centrifugation. However, incubation of OVA beads with whole anti-OVA antibodies plus anti-OVA Fabs increased the amount of free whole antigen-specific antibodies in the supernatants in a dose-related manner, suggesting that Fabs blocked the interaction between anti-OVA antibodies and OVA, because of the ability of the fragments to bind OVA. To quantitatively demonstrate the interference with OVA and anti-OVA antibody interactions by anti-OVA Fabs, unbound free anti-OVA IgG in the supernatants was measured by ELISA. As shown in Figure 5B, unbound free anti-OVA antibodies in the supernatants increased dose-dependently when OVA beads were incubated with anti-OVA plus anti-OVA Fabs.

Discussion and conclusions

The present study demonstrates that antibody-mediated inflammatory diseases are able to be specifically regulated by Fabs of the mediating antibodies, as AOA-MA in mice was markedly suppressed by anti-OVA Fabs, but not by control Fabs. The idea that antigen and antibody interactions would be inhibited by the antibody Fabs is well established. However, surprisingly, no previous studies had shown *in vivo* that antibody-mediated joint inflammatory diseases were indeed able to be down-regulated by Fabs from the mediating antibodies, although Fabs specific for digoxin have been clinically used to treat patients who had toxic levels of the drug (Woolf *et al.*, 1992; Boss and Pope, 1994; Flanagan and Jones, 2004). Current therapeutic drugs for RA include steroidal and non-steroidal anti-inflammatory drugs, immunosuppressive agents, disease-modifying anti-rheumatic drugs such as methotrexate and biological agents

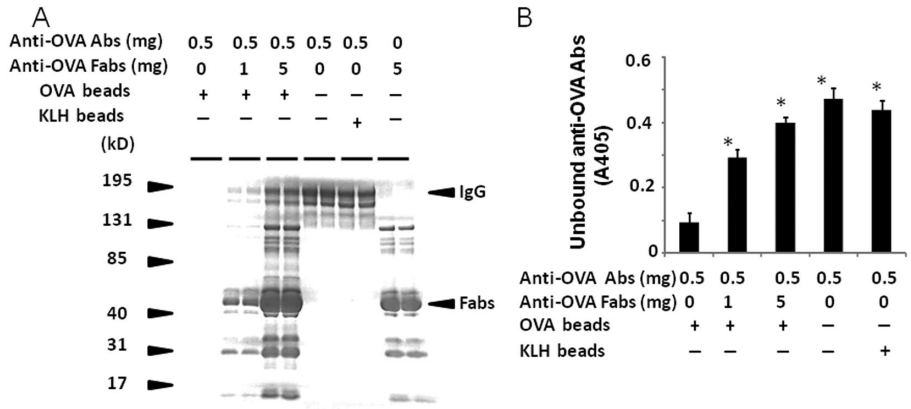

Figure 5

Anti-OVA Fabs interfere with OVA and anti-OVA antibody interactions. OVA or the control antigen KLH beads were incubated with 0.5 mg of whole anti-OVA antibodies alone or 0.5 mg of whole anti-OVA antibodies plus 1 and 5 mg of anti-OVA Fabs. After centrifugation, unbound free whole anti-OVA antibodies in the supernatants were detected by SDS–PAGE (A). The amounts of whole anti-OVA antibodies in the supernatants were also measured by ELISA (B). *$P < 0.05$ versus anti-OVA Abs/OVA beads. Data are representative of three independent experiments.

including infliximab (O'Dell *et al.*, 1996; Simon, 1996; Bijlsma *et al.*, 2003; Puppo *et al.*, 2005). However, these drugs act non-specifically (i.e. they suppress the function of not only inflammatory or immune cells, but also other normal cells), and therefore serious adverse effects are often observed when patients with RA are treated with them (Lipsky *et al.*, 2000; Khanna *et al.*, 2004; Layton *et al.*, 2008). No antigen-specific drug therapy for antibody-mediated diseases is clinically available currently.

The specific regulation of AOA-MA by anti-OVA Fabs, but not by control Fabs, was associated with a reduced consumption of complement in the sera of mice treated with the antigen-specific Fabs that lacked the Fc region containing the binding site for complement, thus preventing its activation. This suggests that the prevention of the activation of complement by anti-OVA Fabs appears to be the mechanism by which AOA-MA was suppressed by the fragments. Complement activation has been shown to be involved in antibody-mediated arthritis (Makinde *et al.*, 1989; Wouters *et al.*, 2006). For instance, serum levels of C3 in patients with RA are lower compared to those of healthy subjects (Neumann *et al.*, 2002), suggesting that complement appears to be consumed because of its activation by antigen and antibody immune complexes. C3 cleavage products including C3a and C5a are potent mediators involved in inflammation, as they enhance vascular permeability, are chemotactic for inflammatory cells and induce pro-inflammatory cytokines including TNF-α and IL-1 through their receptors on various types of cells such as macrophages (el-Lati *et al.*, 1994; Gutzmer *et al.*, 2006; Mullazehi *et al.*, 2006). However, the prevention of complement activation by anti-OVA Fabs appears to play a role in the late, but not early, phase of AOA-MA as the C3a receptor antagonist SB290157 (Ames *et al.*, 2001; Mathieu *et al.*, 2005) significantly inhibited only the late phase of AOA-MA (i.e. at 3–5 h after antigenic challenge). The early phase of AOA-MA observed before 3 h was not affected by the C3a receptor antagonist. It is of note that the suppression of AOA-MA by anti-OVA Fabs was seen at all time-points examined.

Fabs lack not only the binding site for complement, but also the site for Fc receptors including FcεRI for IgE on mast cells and basophils, resulting in the failure of activation of such effector cells (Kalesnikoff and Galli, 2008). Therefore, the suppression of the early phase of AOA-MA by anti-OVA Fabs might be explained by the failure of degranulation of such effector cells in mice treated with the antigen-specific Fabs. Anti-OVA antibodies used in our experiments were polyclonal, and therefore they should have contained not only IgG and IgM that have the ability to activate complement, but also IgE that plays an important role in the activation of such mast cells and basophils. Therefore, varying kinds of chemical mediators such as histamine released following the activation of mast cells and basophils via the interaction between receptor-bound IgE and OVA might have contributed to the induction of the early phase of AOA-MA. This appears to be supported by the result that the H_1 antagonist CPA (Rahman *et al.*, 2007) markedly suppressed joint oedema seen at 0.5 and 1 h after antigenic challenge, but not thereafter. Recent studies demonstrated that mast cells were abundant in synovial tissues of patients with RA (Eklund, 2007; Nigrovic and Lee, 2007; Sandler *et al.*, 2007). There

is also evidence that IgE might be in part involved in RA because IgE rheumatoid factors and IgE-containing immune complexes are present in the arthritic joints (Gruber *et al*., 1988; De Clerck *et al*., 1989). These findings appear to show that chemical mediators including histamine and various types of cytokines released by the activation of mast cells in addition to complement cleavage products such as C3a might also play a role in RA, especially in its initiation. Furthermore, the suppression of the early phase of joint inflammation by anti-OVA Fabs also suggests that allergic diseases such as allergic rhinitis and asthma in which pathogenic allergen-specific IgEs are critically involved (Galli *et al*., 2008; Gould and Sutton, 2008) might be specifically regulated by the pathogenic IgE Fabs.

Our studies also demonstrate that the specific regulation of AOA-MA by anti-OVA Fabs appears to be due to the competitive inhibition of the binding of whole anti-OVA antibodies to OVA by anti-OVA Fabs, because much greater amounts of free unbound whole anti-OVA antibodies were observed in the supernatant when OVA beads were incubated *in vitro* with the antigen-specific antibodies plus anti-OVA Fabs than when incubated with anti-OVA antibodies alone.

Pathogenic antibody Fabs appear to be effective only in diseases in which the intact antibodies play a critical role, because arthritis induced by active immunization with OVA followed by intra-articular injection of the antigen in which both antibodies and T cells specific for OVA were involved was unaffected by anti-OVA Fabs (unpublished data).

Kagari *et al*. (2003) previously demonstrated that the systemic injection of anti-type II collagen antibody $F(ab')_2$ fragments failed to induce arthritis in mice, although the intact antibodies caused joint inflammation. However, in their experiments, they did not investigate the effect of the $F(ab')_2$ on the arthritogenic antibody-induced arthritis.

In summary, the ideal therapy for immune diseases is to down-regulate pathogenic antigen-specific immune responses, but not those essential for self-defence against bacteria and viruses. However, unfortunately, such immunotherapy is unavailable clinically. On the other hand, the present study clearly demonstrates that antibody-mediated arthritis was simply and readily down-regulated by the mediating antibody Fabs via immunologically fundamental mechanisms (i.e. the interference with antigen–antibody interactions by the fragments). Thus, antibody-mediated diseases such as RA might be specifically treated with antigen-specific Fabs of the mediating antibodies, although the antigen specificity of antibodies in the rheumatic disease is still to be elucidated, and therefore at present the results shown in the present study would not directly lead to a human therapy.

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Conflict of interest

The authors declare no conflict of interest.

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