

Modulation of the Th1/Th2 bias by an immunoglobulin histamine complex in the ovalbumin allergy mouse model

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Abstract

Vaccination with the antiallergic drug Histaglobin is used to treat a broad range of human allergic diseases including bronchial asthma, allergic rhinitis, and atopic dermatitis. In order to further elucidate its functional activity, Histaglobin was investigated in an in vivo mouse allergy model. Mice were sensitized with ovalbumin either prior to or after Histaglobin treatment, and its antiallergic potential was evaluated. Ovalbumin-sensitized mice exhibited increased serum levels of IL-4, tumor necrosis factor alpha (TNF- α), and an increase of total and ovalbumin-specific IgE; total and ovalbumin-specific IgG levels were also elevated. Subsequent administration (therapeutic treatment) of Histaglobin resulted in a decrease of total and specific serum IgE levels; total and specific IgG1 serum levels were reduced by more than 50% and 45%, respectively; the mice displayed a down-regulation of IL-4 and TNF- α serum levels and showed increased levels of IFN- γ and IgG2a. Mice pretreated with Histaglobin, prior to ovalbumin sensitization (prophylactic treatment), were found to be widely unresponsive to ovalbumin. They exhibited higher serum levels of IFN- γ and IgG2a (total and specific) compared to saline-treated control mice. The inhibitory effects were still observed 1 month post-immunization.

Our data, indicating a Histaglobin-induced modulation of the Th1/Th2 balance in favour of Th1, correspond with the well-known antiallergic activity of Histaglobin observed in patients.

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Keywords: Histaglobin; IL-4; IFN- γ ; TNF- α ; Ovalbumin; Th1/Th2 bias; Lipopeptide

Abbreviations: BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; IFN- γ , interferon-gamma; IL, interleukin; PBS, phosphate-buffered saline; PBST, PBS containing 0.05% Tween 20; P₃CSK₄, N-palmitoyl-S-[2,3-bis(palmitoyloxy)-(2*R*,*S*)-propyl]-(*R*)-cysteiny-l-seryl-(lysyl)₃-lysine; OVA, ovalbumin; TNF- α , tumor necrosis factor alpha.

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1. Introduction

Type 1 allergies are characterized by an elevated production of IgE, and mast cell degranulation involving the release of histamine and other mediators of allergy [1]. The production of IgE by B cells is initiated and regulated by antigen-specific CD4⁺ T cells [2–4]. Cytokines such as IL-4, IL-5, IL-6, and tumor necrosis factor alpha (TNF- α) enhance IgE

synthesis, while IFN- γ , IFN- α , and transforming growth factor- β (TGF- β) inhibit IgE production [5–8]. Production of IgE is critically dependent on the ratio in which the cytokines IL-4 and IFN- γ are elicited after exposure to allergen [5,9,10]. In vivo administration of rIFN- γ or mAb to IL-4 or its receptor strongly inhibits murine IgE production [11,12], suggesting potentially valuable strategies for control of human allergic disease. Several animal models have been utilized testing various approaches including anti-IL-4 antibody and IFN- γ [13,14]. The antiallergic drug Histaglobin is used to treat allergic diseases such as bronchial asthma, allergic rhinitis, and atopic dermatitis [15–17]. Histaglobin has been reported to inhibit NF- κ B nuclear translocation, to down-regulate proinflammatory cytokines [18], and to reduce antigen-induced histamine release from human peripheral blood basophiles and from rat peritoneal mast cells [19]. In addition, Histaglobin has been shown to inhibit the allergen-induced accumulation of eosinophils at inflammation sites [20]. The mechanism underlying the antiallergic functions of Histaglobin has yet to be investigated. In the present study we used the BALB/c mouse strain and the well-characterized OVA model [21–23] to study allergic immune responses and their modulation by Histaglobin.

2. Materials and methods

2.1. Reagents

Mouse Histaglobin, a mixture of 0.15 μ g of histamine dihydrochloride/12 mg of mouse IgG, was provided by Biobasal (Basel, Switzerland). The synthetic lipopeptide *N*-palmitoyl-S-[2,3-bis(palmitoyloxy)-(R,S)-propyl]-(R)-cysteinyl-seryl-(lysyl)₃-lysine (P₃CSK₄) was obtained from ECHAZ microcollections (Tübingen, Germany). Ovalbumin (OVA) grade VII was purchased from Sigma-Aldrich (Steinheim, Germany). Serum levels of IL-4 and IFN- γ were quantified by sandwich ELISA; ELISAs were performed according to the PharMingen Cytokine ELISA protocol (OptEIA™ Mouse IL-4 2651KI, Mouse IFN- γ 2612KI, PharMingen, Heidelberg, Germany). TNF- α was measured using the dual monoclonal sandwich ELISA technology (PharMingen reagents capture antibody G281-2626; 20031D, recombinant mouse TNF-

α (Cat. #19321T) and biotinylated MP6-XT3 antibody (Cat. #18122D).

2.2. Mice

BALB/c mice (female, 6–8 weeks of age), maintained under institutional approved guidelines, were used in all experiments. Animals were purchased from the Max-Planck-Institute for Immunobiology (Freiburg, Germany).

2.3. Sensitization procedure and Histaglobin administration

Animals (BALB/c mice) were divided into four groups of five mice each (groups I–IV). Two groups (I and II) were immunized intraperitoneally on days 0, 14, and 21 with the allergen OVA in the presence of lipopeptide adjuvant P₃CSK₄ (10 μ g OVA/50 μ g P₃CSK₄; total volume 100 μ l) (primary sensitization cluster). Prior to injection, OVA was dissolved in sterile saline (final concentration 1 mg/ml) and emulsified with an equal volume of P₃CSK₄ adjuvant. Subsequently, mice (group II) received Histaglobin (subcutaneously, s.c.) on days 25, 29, 33, 37, 65, 69, 73, and 77 (500 μ g/mouse/injection); the control group (I) received saline instead of Histaglobin. On days 81, 95, and 102, both groups (I and II) were then further treated with OVA/P₃CSK₄ (secondary sensitization cluster). In contrast to groups I and II—which were sensitized prior to Histaglobin/saline treatment—groups III and IV were pretreated—prior to OVA sensitization—with Histaglobin/saline (control); the corresponding saline (group III)/Histaglobin (group IV) injections were performed four times at 4-day intervals followed by further four injections after 1 month. Subsequently (4 days later), mice were sensitized by three injections with OVA/P₃CSK₄ (7-day intervals). Sera were collected after every set of injections. Mice were bled from the plexus retroorbitalis or the lateral tail veins, and sera were stored at –20 °C until determination of indicated parameters.

2.4. Quantification of total IgE in serum samples

Total IgE levels were determined by sandwich ELISA using purified anti-mouse IgE monoclonal anti-

body (mAb) (PharMingen, 02111D, clone R35-72) as capture antibody and biotinylated anti-mouse IgE mAb (PharMingen, 2122D, clone R35-92) as a detecting antibody. All steps were performed according to the instructions of the manufacturer. Briefly, purified anti-mouse IgE mAb was diluted in coating buffer (phosphate-buffered saline (PBS), pH 7.2) at a concentration of 2 µg/ml and adsorbed to the wells of a 96-well microtitre plate (Nunc Maxisorp, 446469 Roskilde, Denmark) at 4 °C overnight. The plates were washed three times with PBS containing 0.05% Tween 20 (PBST) using an ELISA washer (SLT-Washer, SLT Lab-instruments, Crailsheim, Germany). Uncoated sites were blocked with blocking buffer (1% bovine serum albumin (BSA) in PBS) for 1 h at 37 °C; subsequently, the wells were washed three times with PBST. Serum samples and IgE standards were diluted in blocking buffer and incubated with the bound anti-IgE mAb overnight at 4 °C. Wells were washed three times with PBST. Biotinylated anti-mouse IgE mAb was added to each well (diluted with blocking buffer, final concentration 2 µg/ml), and the plates were incubated at 37 °C for 1 h. Afterwards, wells were washed six times with PBST, and streptavidin—conjugated to horseradish peroxidase (PharMingen, 13047E, Sav-HRP)—was added (diluted 1:1000 and incubated at 37 °C for 30 min). Wells were washed six times with PBST and the substrate solution was added (0.4 mg/ml *ortho*-phenylenediamine (OPD), 0.02% H₂O₂ (30%) in 0.1 M citrate buffer, pH 5.2). Plates were incubated for 30 min at room temperature for color development, and the reactions were stopped by the addition of 2 N H₂SO₄. Absorbance was monitored by an ELISA plate reader (Dynatech MRX, Denkerdorf, Germany) at 490 nm (reference wavelength: 690 nm). IgE levels were calculated by reference to standard curves of purified mouse IgE (PharMingen, 03121D).

2.5. Quantification of total IgG1 and IgG2a in serum samples

Assessment of total IgG subclasses were performed according to the above-described standard ELISA protocol. Reagents and substances were purchased from PharMingen and Biozol (Eching, Germany). Briefly, total IgG1 and IgG2a levels were determined using purified anti-mouse IgG1 or anti-mouse IgG2a monoclonal antibody (mAb) (Biozol, LO-MG1-13, LO-

MG2a-7) as capture antibodies. Biotinylated anti-mouse IgG1/IgG2a mAb (Biozol, LO-MG1-2, LO-MG2a-9) and purified mouse IgG1 κ isotype, IgG2a κ isotype (PharMingen, 03001D, 03021D) were used as detecting antibodies and standards, respectively.

2.6. Quantification of OVA-specific serum IgE

OVA-specific serum IgE was quantified using a two-step sandwich ELISA. Purified anti-mouse IgE monoclonal antibody (mAb) (PharMingen, 02111D, clone R35-72) was used as a capture antibody (final concentration 2 µg/ml). Plates (96-well microtitre plates, Nunc Maxisorp) were coated at 4 °C overnight. Subsequently, plates were washed, blocked 2 h at 37 °C with 200-µl 3% BSA in PBS, and the wells were washed three times with PBST. Serum samples were diluted in blocking buffer and applied overnight at 4 °C. After washing (three times with PBST), OVA (final concentration 10 µg/ml in blocking buffer) was added and plates were incubated for 1 h at 37 °C. The detecting antibody (horseradish peroxidase (HRP)-conjugated IgG fraction of anti-OVA; Biotrend, Köln, Germany) was then added in a final dilution of 1:20,000. Again plates were incubated for 1 h at 37 °C, then washed six times with PBST. Substrate addition and photometrical determination were performed as described above. IgE levels were calculated by reference to mouse IgE standard curves (PharMingen, 03121D).

2.7. Determination of OVA-specific serum IgG1 and IgG2a

For the detection of OVA-specific IgG1 and IgG2a levels in serum, ELISA plates were coated with OVA (10 µg/ml) overnight at 4 °C, washed with PBST, and blocked with PBS containing 3% BSA for 2 h at room temperature. Serum samples and standards were diluted in PBS containing 1% BSA, added to the plates, and incubated for 4 h at room temperature. After washing, biotinylated anti-mouse IgG1/IgG2a mAb (Biozol, LO-MG1-2, LO-MG2a-9) was added, and the plates were incubated for an additional 1 h at room temperature. Thereafter, the plates were washed six times with PBST, and streptavidin—conjugated to horseradish peroxidase (PharMingen, 13047E, Sav-HRP)—was added (final dilution 1:1000). After a

30-min incubation period at 37 °C, the substrate solution was added (0.4 mg/ml *ortho*-phenylenediamine (OPD), 0.02% H₂O₂ (30%) in 0.1 M citrate buffer, pH 5.2). Plates were incubated again for 30 min at room temperature for colour development, and the reaction was stopped by the addition of 2 N H₂SO₄. Absorbance values were determined by an ELISA plate reader (Dynatech MRX, Denkendorf, Germany) at 490 nm (reference wavelength: 690 nm). OVA-specific IgG1 or IgG2a levels were calculated by reference to mouse IgG1 or IgG2a standard curves (PharMingen, 03001D, 03021D).

3. Results

3.1. Histaglobin inhibits total and allergen-specific IgE production in OVA-sensitized BALB/c mice

Multiple i.p. injections of OVA into BALB/c mice result in high serum levels of both total and OVA-specific IgE antibodies. Using this model, we investigated the role of Histaglobin in the regulation of IgE production. Primary OVA cluster sensitization (days 0, 14, and 21) of mice resulted in a pronounced response indicated by a marked increase in serum IgE levels (5-fold; Fig. 1A, compare days – 7 and 23) and OVA-specific IgE antibodies (~ 100-fold; Fig. 1B, compare days – 7 and 23). Subsequent treatment with Histaglobin resulted in a reduction of total and OVA-specific serum IgE of about 50% and 80%, respectively. An additional OVA cluster immunization (days 81, 95, and 102) resulted in an even higher IgE response in the control group (Fig. 1A,B; group I, compare days 79 and 104/132). In contrast, Histaglobin-treated mice maintained low serum IgE levels (Fig. 1A,B; group II).

In a further set of experiments, we investigated the effect of Histaglobin administered prior to OVA sensitization. As shown in Fig. 2A and B (groups III), a marked up-regulation of both total and OVA specific IgE serum levels was induced in response to OVA immunization in control mice. In contrast, mice pretreated with Histaglobin (8 × injections) and subsequently immunized with allergen showed only a marginal increase of both parameters (~ 1.7-/5-fold) (Fig. 2A and B; group IV). Our data thus indicate that Histaglobin is effective in inhibiting the IgE response

when administered prior to allergen sensitization, and reduces an established IgE response.

3.2. Histaglobin inhibits IgG1 and up-regulates IgG2a production

In further investigations, we tested whether Histaglobin modulates additional immunoglobulins involved in allergic responses. Total and OVA-specific serum IgG1 and IgG2a levels were monitored. Primary OVA cluster immunization (three injections on days 0, 14, and 21) induced a marked up-regulation of total and OVA-specific IgG1 (~ 4-fold, >30-fold) (Fig. 3A and B); these levels were still maintained in control mice (saline-treated) up to day 79 (Fig. 3A and B, group I). In contrast, mice treated with Histaglobin (four and eight times) exhibited a strong reduction of total and specific IgG1 serum levels (Fig. 3A and B, group II); more than a 50% reduction of total and an about 45% decrease of OVA-specific IgG1 antibodies was detected. This effect was even more pronounced after a second OVA cluster immunization (three injections on days 81, 95, and 102). Here, serum levels of IgG1 (total and specific) raised about 3-fold in control mice (Fig. 3A and B, group I, days 104 and 132); in contrast, almost no increase was monitored in Histaglobin-treated mice (Fig. 3A and B, group II, days 104 and 132).

When mice were pretreated with Histaglobin prior to OVA sensitization (Fig. 4A and B), we detected a strong reduction of total and specific IgG1 levels (Fig. 4A and B, group IV, days 104 and 132). In contrast, the control group showed markedly enhanced IgG1 serum levels (3-fold increase of total and an about 70-fold increase of OVA-specific IgG1) (Fig. 4A and B, group III, compare days – 7 and 104/132).

We also monitored serum IgG2a levels in mice treated with Histaglobin before or after OVA sensitization. As shown in Fig. 5A, a reduction of total IgG2a was detected in response to OVA immunization (groups I and II, compare days – 7 and 23). Subsequently, a moderate induction of total and OVA-specific IgG2a was observed in serum of control mice (Fig. 5A and B, group I, days 39 and 79). In contrast, Histaglobin-treated mice exhibited a more than 4-fold increase of total and an about 10-fold increase of OVA-specific serum IgG2a levels (Fig. 5A and B, group II, day 79). A second cluster immunization with OVA was accompanied by a reduction of total and

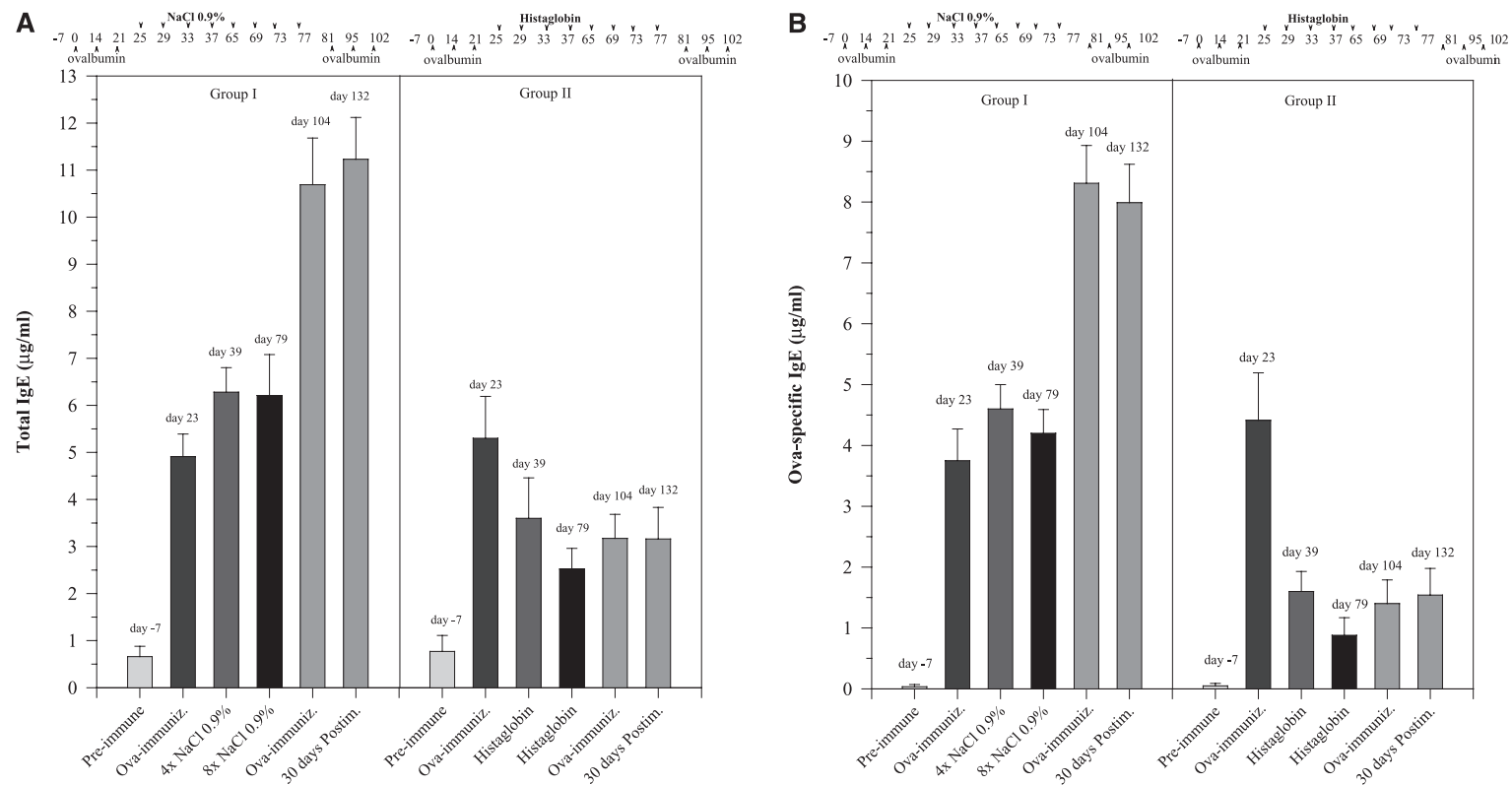


Fig. 1. Determination of total (A) and OVA-specific (B) serum IgE after OVA sensitization and Histaglobin treatment. BALB/c mice were sensitized with OVA as described in Materials and methods. Time schedules for sensitization and further treatment are indicated at the top of each graph. Control mice (A, B/group I) received saline instead of Histaglobin (A, B/group II). Sera were taken on days indicated and IgE was quantified by ELISA. Results are expressed as mean $\pm$ S.E.M. for $n=5$ mice/group.

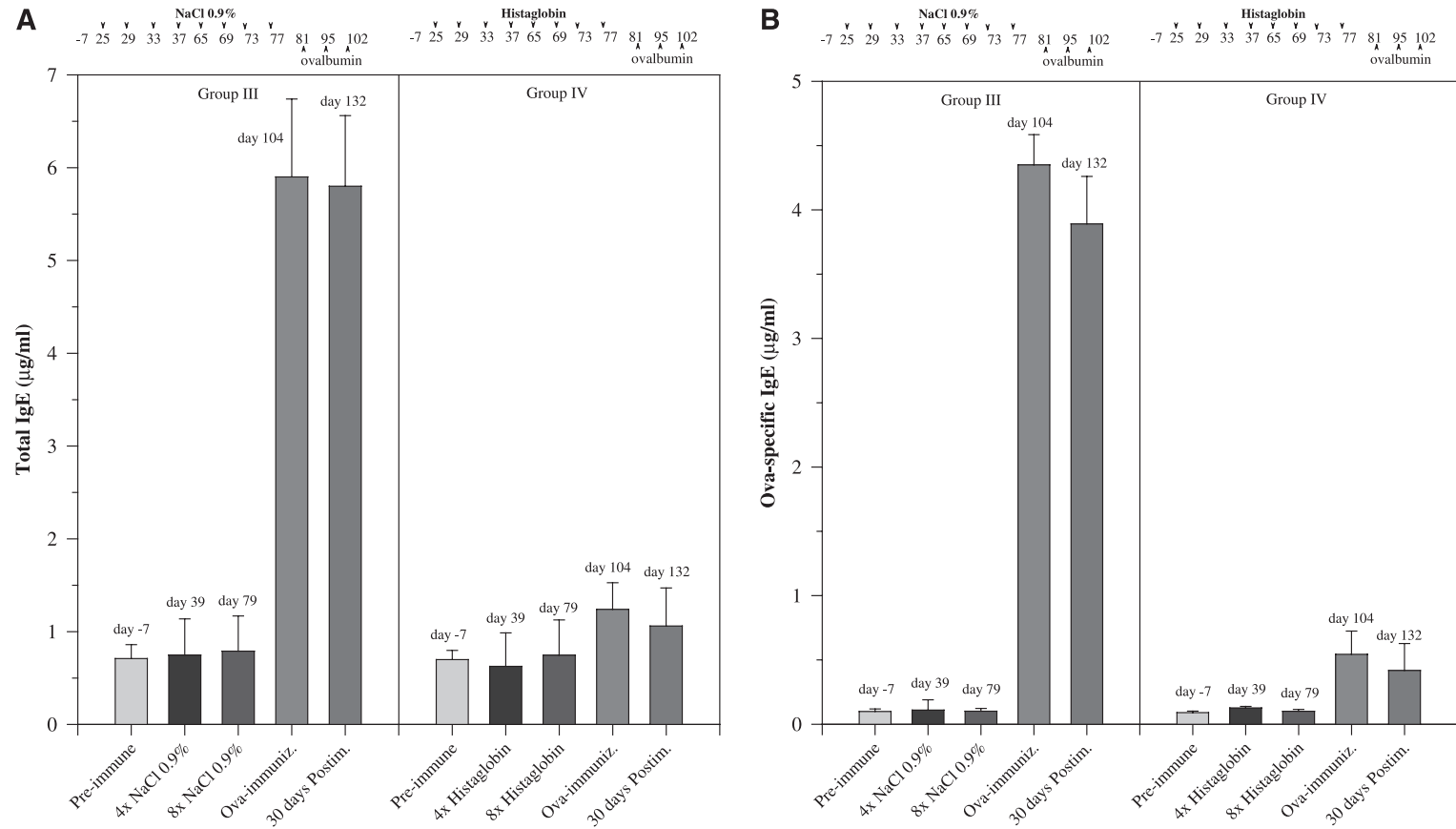


Fig. 2. Determination of total (A) and OVA-specific (B) serum IgE after Histaglobin treatment and subsequent OVA sensitization. BALB/c mice were pretreated with Histaglobin and subsequently sensitized with OVA as described in Materials and methods. Time schedules for Histaglobin administration, OVA sensitization, and further treatment are indicated at the top of each graph. Control mice (A, B/group III) received saline instead of Histaglobin (A, B/group IV). Sera were taken on days indicated and IgE was quantified by ELISA. Results are expressed as mean $\pm$ S.E.M. for $n = 5$ mice/group.

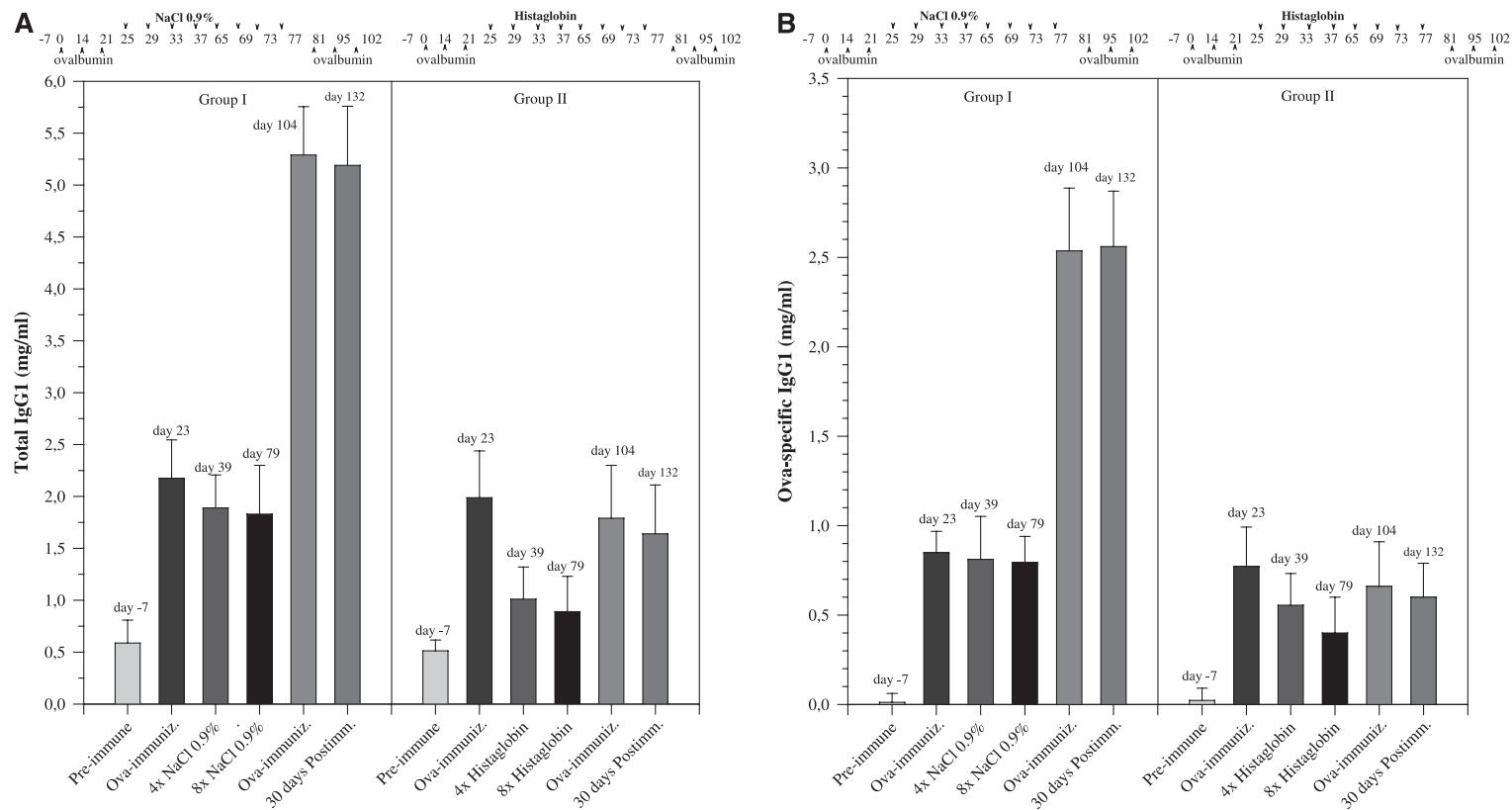


Fig. 3. Determination of total (A) and OVA-specific (B) serum IgG1 after OVA sensitization and Histaglobin treatment. BALB/c mice were sensitized with OVA as described in Materials and methods. Time schedules for sensitization and further treatment are indicated at the top of each graph. Control mice (A, B/group I) received saline instead of Histaglobin (A, B/group II). Sera were taken on days indicated and IgG1 was quantified by ELISA. Results are expressed as mean $\pm$ S.E.M. for $n=5$ mice/group.

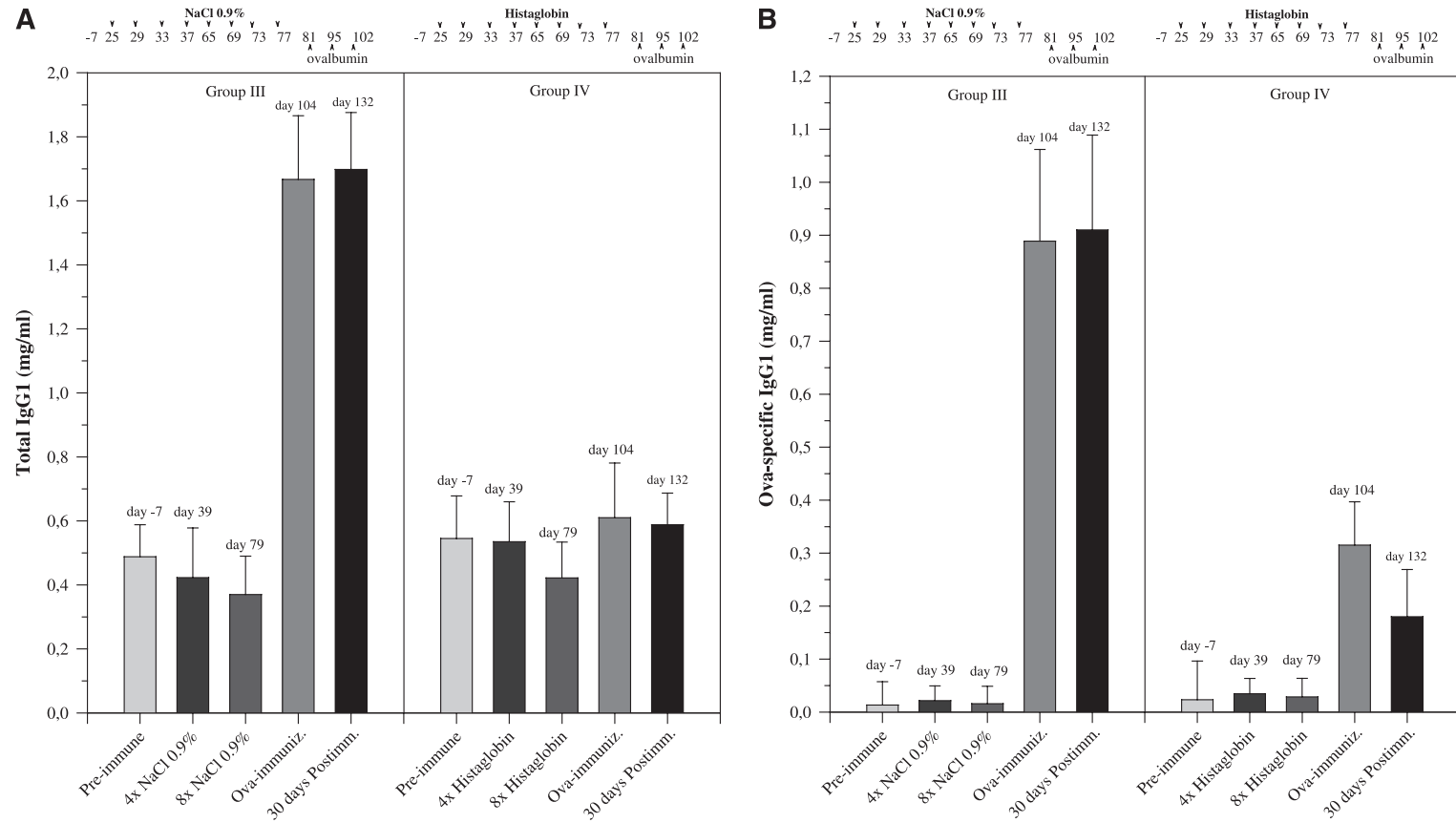


Fig. 4. Determination of total (A) and OVA-specific (B) serum IgG1 after Histaglobin treatment and subsequent OVA sensitization. BALB/c mice were pretreated with Histaglobin and subsequently sensitized with OVA as described in Materials and methods. Time schedules for Histaglobin administration, OVA sensitization, and further treatment are indicated at the top of each graph. Control mice (A, B/group III) received saline instead of Histaglobin (A, B/group IV). Sera were taken on days indicated and IgG1 was quantified by ELISA. Results are expressed as mean $\pm$ S.E.M. for $n = 5$ mice/group.

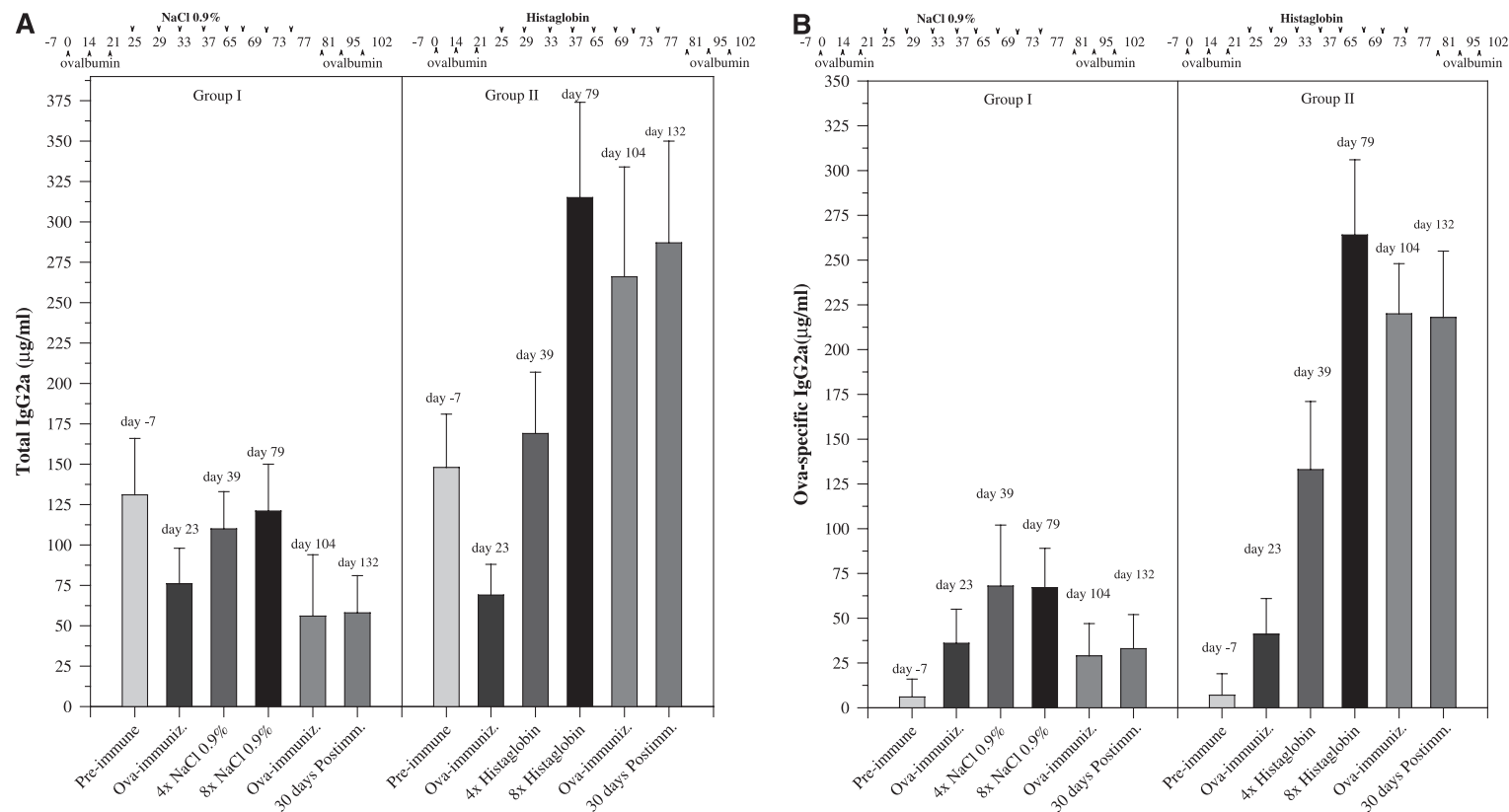


Fig. 5. Determination of total (A) and OVA-specific (B) serum IgG2a after OVA sensitization and Histaglobin treatment. BALB/c mice were sensitized with OVA as described in Materials and methods. Time schedules for sensitization and further treatment are indicated at the top of each graph. Control mice (A, B/group I) received saline instead of Histaglobin (A, B/group II). Sera were taken on days indicated and IgG2a was quantified by ELISA. Results are expressed as mean $\pm$ S.E.M. for $n = 5$ mice/group.

specific serum IgG2a in control mice (Fig. 5A and B, group I, days 104 and 132) whereas high levels of both total and OVA-specific IgG2a were still maintained in sera of Histaglobin-treated mice (Fig. 5A and B, group II, days 104 and 132). Pretreatment of mice with Histaglobin and subsequent OVA sensitization resulted in enhanced levels of total and specific serum IgG2a (Fig. 6A and B, group IV, days 104 and 132) when compared to control mice receiving saline instead of Histaglobin (Fig. 6A and B, group III, days 104 and 132). In both groups—saline- and Histaglobin-treated mice—we detected a moderate induction of total IgG2a serum levels prior OVA sensitization (Fig. 6A, groups III and IV, days 39 and 79).

3.3. Histaglobin inhibits allergen-induced serum IL-4 production

In a further set of experiments, we assayed the effect of Histaglobin treatment on IL-4 production either prior to or after OVA sensitization. We detected elevated levels of serum IL-4 in mice sensitized with OVA (Fig. 7A, groups I and II, day 23) corresponding to the observed elevation in IgE production (Fig. 1A, groups I and II, day 23). Subsequent treatment with Histaglobin suppressed the induction IL-4 (>60%) (Fig. 7A, group II, days 39 and 79) compared to control mice (Fig. 7A, group I, days 39 and 79). An about 2-fold increase of IL-4 was detected in response to a secondary allergen cluster immunization in the control group (Fig. 7A, group I, days 104 and 132) whereas mice treated with Histaglobin showed only a marginal increase in IL-4 levels (Fig. 7A, group II, days 104 and 132). Pretreatment with Histaglobin resulted in an effective down-regulation of allergen-induced IL-4 production. As can be seen in Fig. 7B, control mice, receiving saline (8 ×) and subsequently sensitized with OVA, showed a significant increase in serum IL-4 levels (Fig. 7B, group III, days 104 and 132). In contrast, Histaglobin-treated mice showed only a marginal increase in IL-4 levels after allergen immunization (Fig. 7B, group IV, days 104 and 132).

3.4. Histaglobin augments serum IFN- γ production in OVA-treated mice

As shown in Fig. 8A, no reduction of serum IFN- γ was observed in response to OVA immunization (day

23). Successive application of saline (8 ×) did not influence the IFN- γ profile. However, mice treated with Histaglobin exhibited an about 2-fold increase of IFN- γ levels (Fig. 8A, group II, days 39 and 79). This effect was amplified in Histaglobin-treated mice in response to a second OVA cluster immunization (4-fold) (Fig. 8A, group II, days 104 and 132). In control animals, we observed only a slight increase in IFN- γ levels after the second sensitization (Fig. 8A, group I, days 104 and 132). As shown in Fig. 8B, administration of saline or of Histaglobin alone did not induce a change in IFN- γ levels (Fig. 8B, groups III and IV, days 39 and 79). However, pretreatment of mice with Histaglobin (8 ×) led to a markedly increased production of IFN- γ (~ 3-fold) in response to OVA sensitization (Fig. 8B, group IV, days 104 and 132). In contrast, only a minor increase of IFN- γ production was detected in serum of saline-treated mice following OVA immunization (Fig. 8B, group III, days 104 and 132). Interestingly, high IFN- γ serum levels were still maintained in Histaglobin-treated mice 1 month after sensitization with allergen.

3.5. Histaglobin effect on the proinflammatory cytokine TNF- α

To further characterize the immunomodulatory effects of Histaglobin on other cytokines playing a role in inflammatory diseases, we monitored TNF- α serum levels in Histaglobin treated mice. Primary OVA immunization triggered an increase in TNF- α (~ 3-fold) as shown in Fig. 9A (day 23, groups I and II); subsequent Histaglobin application (8 ×) induced a marked decrease of TNF- α (>70%) (Fig. 9A, group II, day 79). An even higher level of TNF- α was observed in response to the secondary OVA cluster sensitization in the control group (Fig. 9A, group I, days 104, 132). In contrast, Histaglobin-treated mice exhibited levels of serum TNF- α similar to those found in pre-immune serum (Fig. 9A, group II, days 104 and 132).

Histaglobin pretreatment of mice effectively inhibited the induction of TNF- α production in response to OVA (Fig. 9B, group IV). In contrast, allergen immunization without Histaglobin pretreatment resulted in high levels of TNF- α (Fig. 9B, group III).

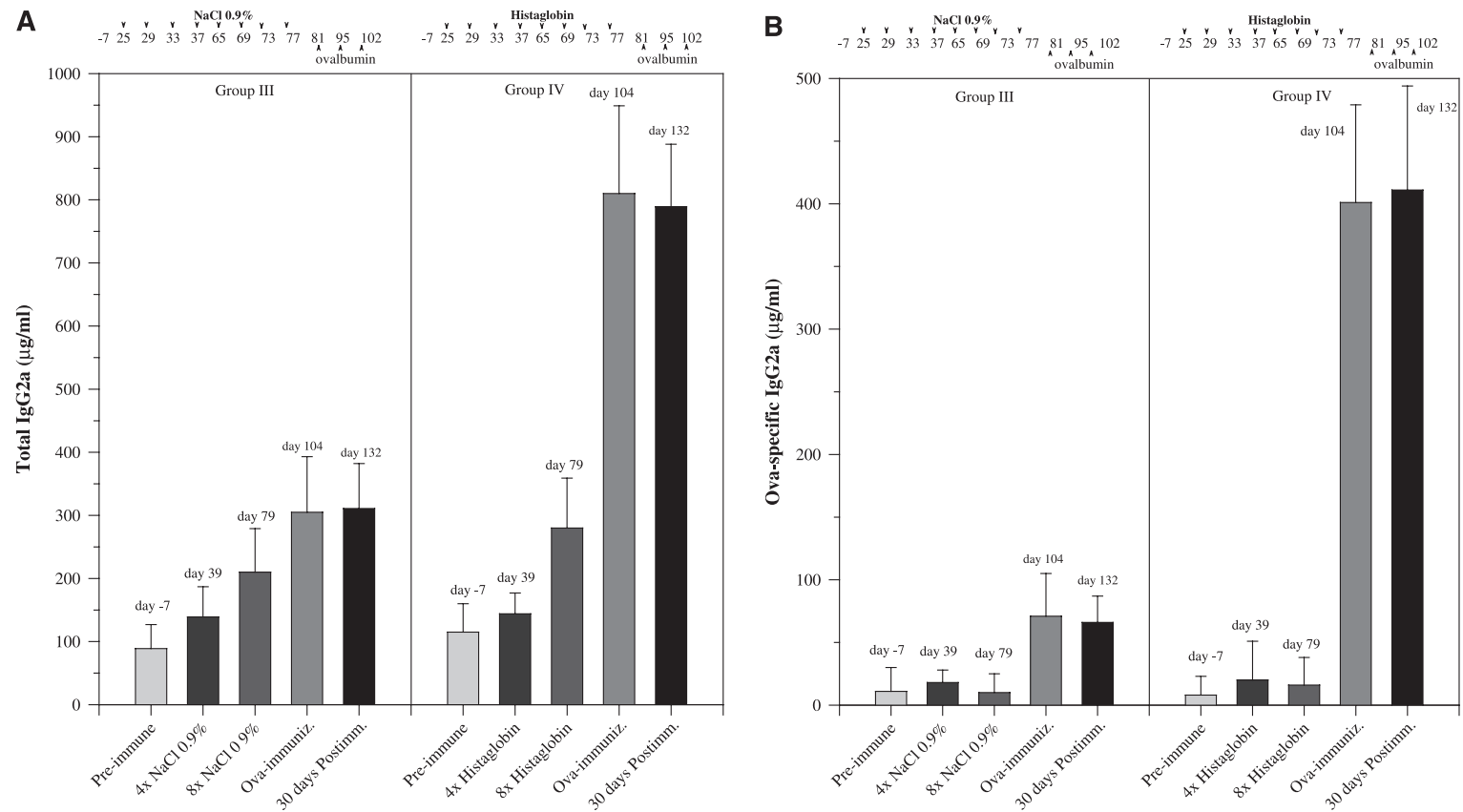


Fig. 6. Determination of total (A) and OVA-specific (B) serum IgG2a after Histaglobin treatment and subsequent OVA sensitization. BALB/c mice were pretreated with Histaglobin and subsequently sensitized with OVA as described in Materials and methods. Time schedules for Histaglobin administration, OVA sensitization, and further treatment are indicated at the top of each graph. Control mice (A, B/group III) received saline instead of Histaglobin (A, B/group IV). Sera were taken on days indicated and IgG2a was quantified by ELISA. Results are expressed as mean $\pm$ S.E.M. for $n = 5$ mice/group.

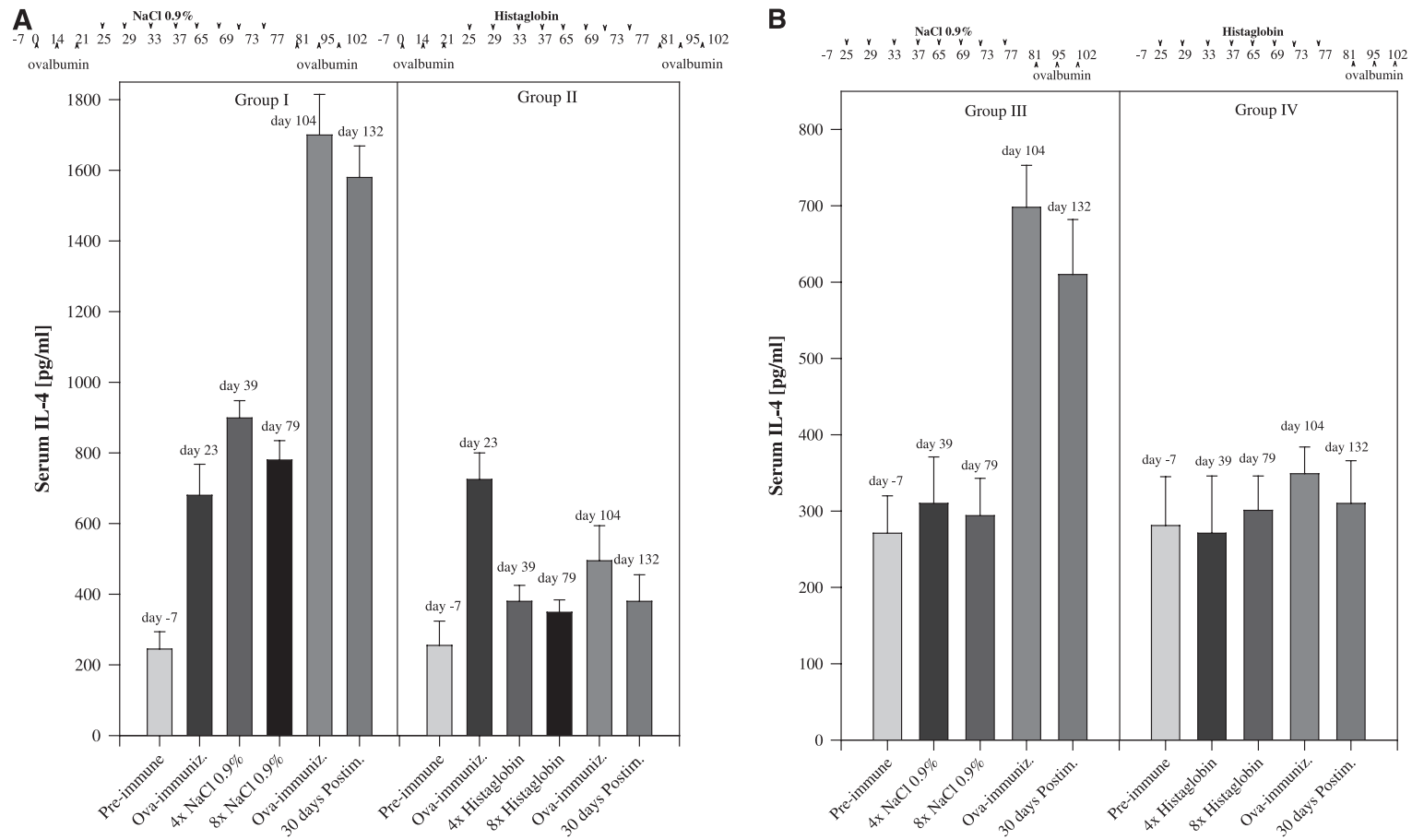


Fig. 7. (A) Determination of serum IL-4 after OVA sensitization and Histaglobin treatment. (B) Determination of serum IL-4 after Histaglobin treatment and subsequent OVA administration. BALB/c mice were either sensitized with OVA and subsequently treated with Histaglobin (A) or pretreated with Histaglobin and afterwards immunized with OVA (B). Time schedules for Histaglobin application, sensitization, and further treatment are indicated at the top of each graph. Control mice (A, group I; B, group III) received saline instead of Histaglobin (A, group II; B, group IV). Sera were taken on days indicated and IL-4 was quantified by ELISA. Results are expressed as mean $\pm$ S.E.M. for $n = 5$ mice/group.

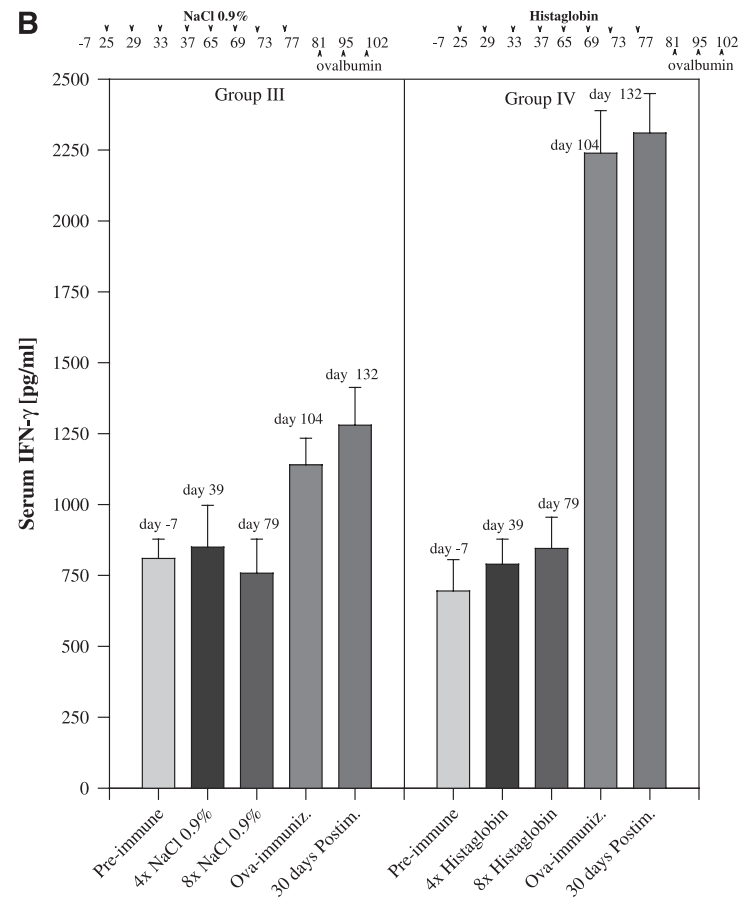
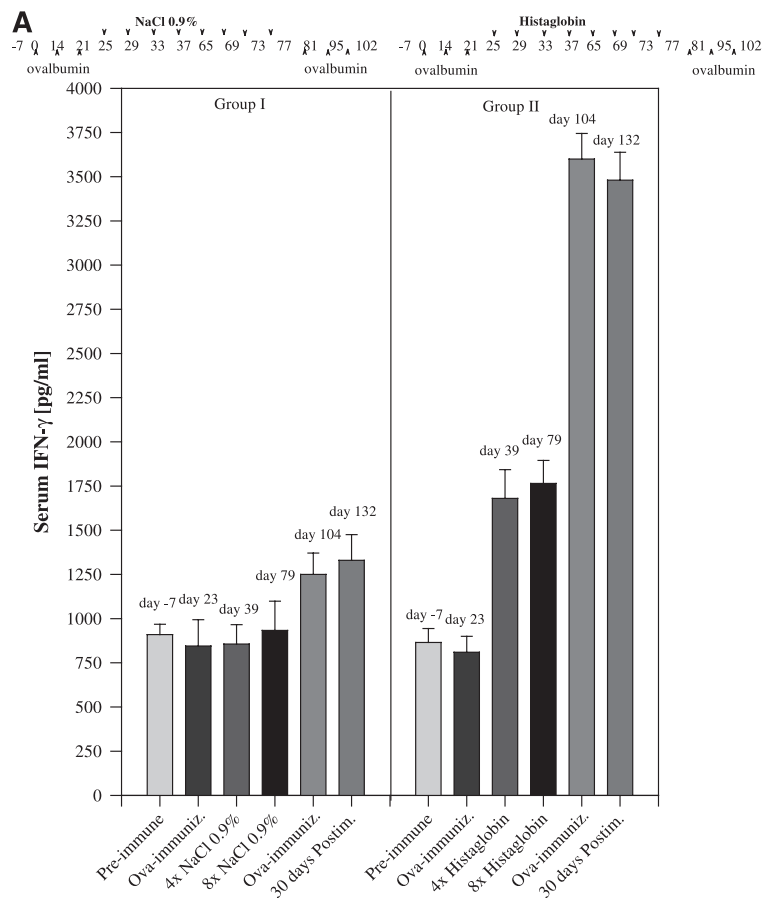


Fig. 8. (A) Determination of serum IFN- γ after OVA sensitization and Histaglobin treatment. (B) Determination of serum IFN- γ after Histaglobin treatment and subsequent OVA administration. BALB/c mice were either sensitized with OVA and subsequently treated with Histaglobin (A) or pretreated with Histaglobin and afterwards immunized with OVA (B). Time schedules for Histaglobin application, sensitization, and further treatment are indicated at the top of each graph. Control mice (A, group I; B, group III) received saline instead of Histaglobin (A, group II; B, group IV). Sera were taken on days indicated and IFN- γ was quantified by ELISA. Results are expressed as mean $\pm$ S.E.M. for $n = 5$ mice/group.

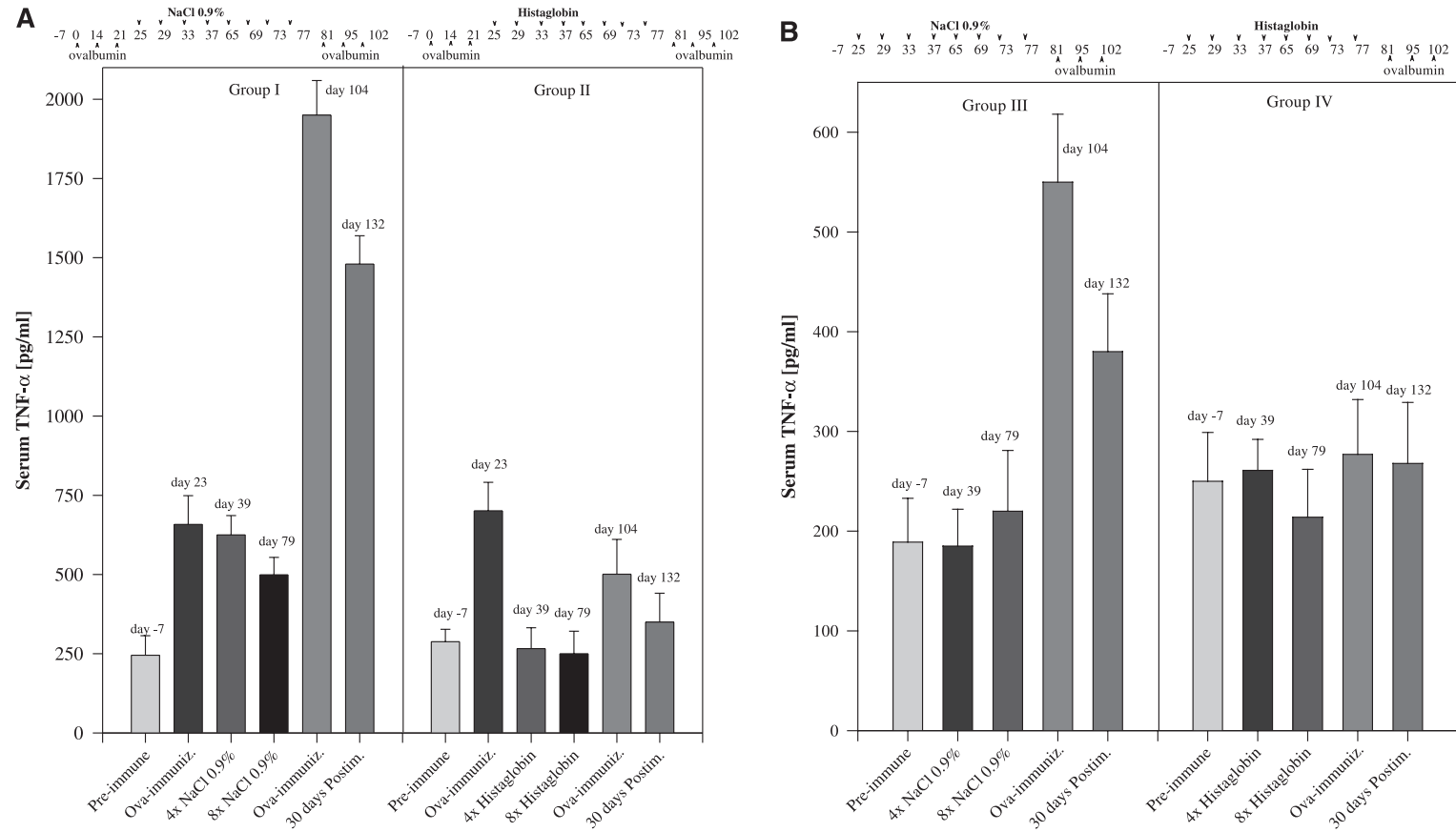


Fig. 9. (A) Determination of serum TNF- α after OVA sensitization and Histaglobin treatment. (B) Determination of serum TNF- α after Histaglobin treatment and subsequent OVA administration. BALB/c mice were either sensitized with OVA and subsequently treated with Histaglobin (A) or pretreated with Histaglobin and afterwards immunized with OVA (B). Time schedules for Histaglobin application, sensitization, and further treatment are indicated at the top of each graph. Control mice (A, group I; B, group III) received saline instead of Histaglobin (A, group II; B, group IV). Sera were taken on days indicated and TNF- α was quantified by ELISA. Results are expressed as mean $\pm$ S.E.M. for $n = 5$ mice/group.

4. Discussion

The regulation of IgE production is of clinical importance since this antibody plays a major role in type I allergic responses. Many approaches have been used to prevent or inhibit the generation of IgE responses in experimental models of allergy [24–30]. In this study we demonstrate, using a murine *in vivo* model system, that Histaglobin treatment causes a reduction in total and allergen-specific serum IgE. Moreover, Histaglobin is capable of down-regulating an established IgE response. Also, a strong inhibition of the IgG1 production was observed in mice pretreated with Histaglobin, or treated after OVA immunization. This reduction of OVA-specific IgE and IgG1, in addition to the enhancement of IgG2a, suggests that Histaglobin modulates the Th1/Th2 balance in favour of a Th1-type response.

The Th2 immune response is characterized by the development of IL-4 and IL-5-producing effector T cells [31]. These cells contribute to the allergic response in several aspects. They control production of IgE and IgG1 [32], up-regulate endothelial adhesion molecules necessary for the recruitment of inflammatory cells into the airways [33,34], and promote development, maturation, and recruitment of eosinophils [35] which are to a great extent responsible for tissue damage during allergic reactions [36,37]. Histaglobin preferentially inhibits allergen-induced peritoneal accumulation of eosinophils in BALB/c mice [20]. There are numerous examples of experimental models in which modulation of the Th1/Th2 balance by administration of recombinant cytokines or cytokine antagonists (e.g. IL-12, IFN- γ , IL-1 antagonist, and IL-5 antagonist) alters the outcome of diseases. For instance, IL-12 converts an established Th2 response to Th1 dominance in some situations, suggesting its possible application in the treatment of allergy [38,39]. IFN- γ or antibody against IL-4 or IL-5 inhibits pulmonary eosinophilia in allergic mice [40]. However, direct administration of such cytokines causes undesirable side effects and has limited therapeutic use. Among the many physiological responses mediated by IL-4 is its ability to promote the differentiation of T helper precursors toward the Th2 lineage while inhibiting Th1 cell development [41–43]. IL-4 has been shown to be essential for IgE production *in vivo* [41–44]. IFN- γ counteracts the IgE-promoting activity of

IL-4 and induces a marked enhancement of the IgG2a isotype in mice *in vitro* and *in vivo* [14,44–46]. Different strategies have also been used to limit the production of IgE by interfering with the functional activities of IL-4. One approach for inhibiting IgE production has been the use of drugs which inhibit IL-4 activity; *in vitro* and *in vivo* studies in both humans and mice demonstrated that IFN- γ is a potent inhibitor of IgE production by interfering with the IL-4-mediated switch to ϵ -gene expression [47–49]. A different way has been to block IL-4 or its specific receptor: These approaches were performed by treating mice with a monoclonal anti-IL-4 antibody [49,50] or an antibody directed against IL-4R [49]. Also, soluble IL-4R (sIL-4R) molecules were recently shown to suppress IL-4-mediated T- and B-cell function [49,51]. In this study we found that Histaglobin specifically down-regulates the induction of serum IL-4 in response to OVA immunization. Pretreatment or administration of Histaglobin after OVA immunization both markedly inhibited the generation of IL-4. Our results suggest that Histaglobin could exert its anti-allergic function by the induction of IFN- γ production and thus through inhibition of IL-4.

TNF- α is implicated as an important proinflammatory cytokine in asthma [52]. In OVA-sensitized BALB/c mice, a marked increase in eosinophils, lymphocytes, and neutrophils in bronchoalveolar lavage (BAL) fluids was paralleled with elevated production of IL-4, IL-5, and TNF- α in the lung [22]. Our data revealed that Histaglobin in both human (*in vitro*) and murine systems (*in vivo*) [18] effectively suppresses the induction of TNF- α in response to different stimuli. In addition to the well-known antiallergic effect of Histaglobin, our findings may be of clinical importance since TNF- α has been found to be implicated in different diseases, for example, rheumatoid arthritis, multiple sclerosis, and other autoimmune diseases.

Taken together, our investigations in a mouse model system indicate that Histaglobin is effective in inhibiting the IgE response when administered prior to allergen sensitization (prophylactic treatment), and reduces an established, allergen-induced IgE response (therapeutic treatment). Histaglobin can be easily generated at low costs and its therapeutical effects—documented over the past 25 years—clearly indicate that this drug is able to treat many allergic diseases without any known side effects.

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