

Recombinant *Dirofilaria immitis*-Derived Antigen Can Suppress Passive Cutaneous Anaphylaxis Reactions

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Key Words

IgE · Mast cells · FcεRI · *Dirofilaria immitis* · Passive cutaneous anaphylaxis

Abstract

Background: High levels of antigen-nonspecific IgE are produced in animals infected with helminth parasites. Generally, the increase in IgE is thought to exacerbate allergic reactions. However, high levels of antigen-nonspecific IgE may alter some features of anaphylactic reactions. To investigate the molecular mechanisms of antigen-nonspecific IgE production induced during filarial infections, we previously constructed rDiAg (recombinant *Dirofilaria immitis*-derived antigen) in *Escherichia coli*. In the present study, we examined the effect of rDiAg on the production of antigen-nonspecific IgE and on allergic cutaneous reactions in rats. **Methods:** Osmotic pumps filled with 200 µg of rDiAg or with 200 µl of PBS (control) were subcutaneously implanted in Wistar rats, and plasma samples were collected weekly thereafter. IgE levels were determined by ELISA. Homologous passive cutaneous anaphylaxis (PCA) reactions with anti-DNP-As IgE were examined 21 days after implantation. ¹²⁵I-IgE binding assays were examined on peritoneal mast cells from rDiAg-infused rats and control rats. **Results:** Antigen-nonspecific IgE production was induced in rDiAg-infused rats. PCA reactions were suppressed in rDiAg-infused rats in spite of high levels of IgE

and a markedly increased expression of FcεRI. ¹²⁵I-IgE did not bind to mast cells derived from rDiAg-infused rats, but it bound dose dependently to mast cells derived from control rats. **Conclusion:** The present data support the hypothesis that antigen-nonspecific IgE might protect against antigen-specific IgE by means of competition for mast cell receptors. rDiAg is an essential factor to induce antigen-nonspecific IgE in helminth infections.

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Introduction

Parasitic helminth infections are accompanied by host responses that are dominated by a T-helper type 2 (Th2) profile and the emergence of parasite-specific immunosuppression [1–4]. The increased production of IgE is especially characteristic in animals infected with parasites. These infections result not only in the production of parasite-specific IgE but also in the production of a large amount of parasite-nonspecific IgE. However, there is little information available on the nature of the factors that positively modulate the antigen-nonspecific IgE response in helminth infections. We previously purified the excretory and secretory antigen (ES) derived from *Dirofilaria immitis* [5]. We found that purified ES composed of *D. immitis*-derived antigen (DiAg) enhanced CD23 expression and potentiated antigen-nonspecific IgE synthesis through increased generation of Th2-related cytokines

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1018–2438/01/1252–0144\$17.50/0

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[6]. To further understand the inflammatory reactions of the host-parasite relationship at a molecular level, we investigated the molecular structure of the DiAg. In our preliminary experiments, a DNA sequence analysis showed that DiAg is 387 bp in length and consists primarily of neutrophil chemotactic factor (DiNCF), polyprotein antigen (DiPA), and cuticular antigen (Di5) from the *D. immitis* adult worm [7–9]. The DiAg is composed of 129 amino acid residues, has a molecular weight of about 15,000 kD and behaves as a positively charged protein. Previously, we used *Escherichia coli* to construct recombinant DiAg (rDiAg). In addition, we found that rDiAg corresponded approximately to a monomer unit of the nematode polyproteins and induced antigen-nonspecific IgE production through proliferation and IL-10 production in B cells [unpubl. observation]. IgE and antigen-dependent activation of mast cell mediator secretion is thought to be an important factor in the pathogenesis of asthma and other allergic diseases [10]. Some reports have shown a strong positive correlation between serum IgE and clinical symptoms in allergic patients [11–13]. These data suggest that the increase in IgE may exacerbate allergic reactions. However, high levels of antigen-nonspecific IgE might alter the features of anaphylactic reactions. There are at least two possibilities. First, high levels of antigen-nonspecific IgE may deteriorate the allergic disease by actively stimulating the production of Th2-related cytokines and the high-affinity IgE receptor FcεRI. Second, high levels of antigen-nonspecific IgE may protect against unrelated allergies by means of competition for mast cell receptors.

In the present study, we investigated the effect of rDiAg on passive cutaneous anaphylaxis (PCA) reactions in rats, and sought to identify the mechanism of suppression in PCA reactions.

Material and Methods

Rats

Five-week-old Wistar rats were obtained from CLEA Japan (Tokyo, Japan) and were maintained under conventional conditions. Osmotic pumps (Alza, Palo Alto, Calif., USA) were filled with either 200 µl of phosphate-buffered saline (PBS, as a control) or 200 µg of rDiAg in 200 µl of PBS, which were subcutaneously infused at a continuous pumping rate of 0.5 µl/h during 14 days. All rats were sacrificed 21 days after pump implantation.

IgE Assay

Blood was obtained weekly from each group of rats following pump implantation. After centrifugation, the plasma was collected and stored at –80 °C until analyzed. Total IgE concentrations were

determined by ELISA. IgE ELISA was performed as described [6]. In brief, 96-well plates (Nunc, Roskilde, Denmark) were coated overnight at 4 °C with 5 µg/well of mouse anti-rat IgE (Zymed, San Francisco, Calif., USA) in 100 µl/well of 1% (w/v) bovine serum albumin (BSA) in PBS. Plasma samples diluted (1:20) in 1% BSA/PBS were incubated for 1 h at 37 °C. After washing, 100 µl of horseradish-peroxidase (HRP)-conjugated goat anti-rat IgE [diluted 1:10,000; GARa/IgE(Fc)PO; Nordic Immunology, The Netherlands] was added and incubated at 37 °C for 1 h. After washing, all bound Abs were detected using an *o*-phenylenediamine substrate (Wako Pure Chemicals, Japan), and the colorimetric reaction was stopped with 2 N H₂SO₄. The optical density values were determined at 492 nm.

To assess rDiAg-specific IgE levels, 100 µl of biotinylated rDiAg (5 µg/ml) was added into avidin-coated plates and incubated for 30 min at room temperature. Diluted plasma samples were applied to each well. After incubation for 1 h at 37 °C, the plasma samples were aspirated and applied to mouse anti-rat IgE monoclonal antibody (Zymed)-coated plates. The subsequent procedures were performed as described above. IgE concentrations were determined in comparison to a standard curve generated by serial dilution of monoclonal rat IgE (Zymed). The lower limit of detection in the ELISA was 100 pg/ml for IgE.

Detection of Specific DiAg Antibody by Western Blotting

DiAg were detected by immunoblotting with the rat plasma and human serum. Rat plasma samples were collected 21 days after pump implantation. Briefly, 2 µg of rDiAg and 0.4 µl of plasma from rDiAg-infused rats (positive control) were run on 20% sodium dodecylsulfate (SDS) polyacrylamide gels. Following transfer to polyvinylidene difluoride (Millipore, Bedford, Mass., USA) membrane, the immunoblots were blocked by incubating with Block Ace (Dainihon Pharmaceuticals, Osaka, Japan) and probed for overnight at 4 °C with plasma from rDiAg-infused and control rats or serum from a *D. immitis*-infected patient (male, 42 years old) diluted (1:100) in 0.1% (w/v) BSA in PBS. The immunoblots were then probed for 2 h at room temperature with HRP-conjugated anti-rat or anti-human IgE diluted 1:500 in 0.1% BSA/PBS.

PCA Reactions

PCA reactions with rat anti-2,4-dinitrophenylated *Ascaris suum* extract polyclonal IgE (anti-DNP-As IgE; Cosmo Bio, Tokyo, Japan) were carried out on rats 21 days after pump implantation. The PCA reactions were performed as previously described [14]. Briefly, rats were injected intradermally with 100 µl of a serial dilution of anti-DNP-As IgE into dorsal skin sites that had been shaved 48 h earlier. After a 48-hour sensitization period, the rats were challenged intravenously with 1 mg of DNP-As in 1 ml of 0.5% Evans blue in saline. The reactions were examined 30 min after the challenge. The activity of PCA was determined by measurement of the cutaneous concentration of Evans blue. The amount of dye was determined colorimetrically after extraction with 1 ml of 1 N KOH and 9 ml of a mixture of acetone and phosphoric acid (5:13), according to the method of Katayama et al. [14]. The absorbance intensity of the extraction was measured at 620 nm using a spectrophotometer, and the amount of dye was calculated using the Evans blue measuring line.

Rat Mast Cell Preparations

Twenty-one days after pump implantation, male Wistar rats were anesthetized with ether and 20 ml of Tyrode buffer (NaCl, glucose, NaHCO₃, KCl, NaH₂PO₄) containing 0.1% gelatin (Sigma, St. Louis,

Mo., USA) was injected into the peritoneal cavity. The abdomen was then gently massaged for 2 min. The peritoneal cavity was carefully opened, and the buffer containing suspended cells was recovered by aspiration with a siliconized pipette and was placed in tubes. Thereafter, the cell suspension was centrifuged at 50 *g* for 5 min at room temperature, and the cell pellet was resuspended in 2 ml Tyrode buffer. The mast cells were purified by centrifugation at room temperature in a BSA density gradient according to the method of Sullivan et al. [15]. In brief, 2 ml of cell suspensions was layered over 4 ml of a solution of 38% BSA (w/v) in medium in 50-ml centrifuge tubes. The cells were allowed to settle for 25 min and then centrifuged at 450 *g* for 20 min. Under these conditions, pure mast cells are obtained in the BSA layer. Mast cell preparations were 95% pure as assessed by toluidine blue staining. More than 97% of cells were viable as judged by trypan blue uptake.

Reverse Transcription Polymerase Chain Reaction

Total RNA was isolated from a known number of peritoneal mast cells (5×10^5) derived from control and rDiAg-infused-rats, using the RNeasy Mini Kit (Qiagen, Chatworth, Calif., USA). cDNA synthesis was performed using a single-strand cDNA synthesis kit, RT-PCR high (Toyobo, Osaka, Japan) according to the manufacturer's instruction. Briefly, 1 μ g of total RNA was dissolved in 20 μ l of RTase buffer containing 1 mM dNTPs, 125 pmol random primer, 10 U RNase inhibitor, 20 U of RNase H and incubated at 30°C for 10 min and 42°C for 20 min. The reaction was terminated by heating to 99°C for 5 min, then chilled at 4°C for 5 min using GeneAmp PCR system 9700 (PerkinElmer, Foster City, Calif., USA). The above cDNA products were used for PCR at a final volume of 25 μ l containing 1 $\times$ Plus buffer (rTaq), 0.5 U of rTaq DNA polymerase and 0.4 pmol/ml of each primer. The primer sequences used were as follows: Fc ϵ RI β -subunit sense chain, 5'-AAAGTTGATAGAGTGCGGAG-3'; anti-sense chain, 5'-ATGGTTGCTTCGTGACTTCTT-3'. PCR was performed under the following conditions: denaturation at 95°C for 1 min, annealing at 54°C for 30 s and extension at 72°C for 30 s. The final extension was at 72°C for 9 min.

Semi-Quantitative RT-PCR

To assess the relative levels of Fc ϵ RI β -subunit mRNA transcripts in the rDiAg-infused rats and control rats semi-quantitative RT-PCR on RNA isolated from mast cells was performed. Total RNA (1 μ g) was reverse transcribed, and the resulting cDNA was amplified as described above. Aliquots of each PCR reaction were removed at 2-cycle intervals by electrophoresis on 1.5% agarose gels and stained with 0.5 μ g/ml of ethidium bromide (Sigma). The intensity of the ethidium bromide fluorescence was measured densitometrically and plotted as a function of cycle number to generate amplification curves for the Fc ϵ RI β -subunit PCR products. Adequate PCR cycles to generate the same fluorescence intensity of rDiAg-infused and control Fc ϵ RI β -subunit PCR fragments were determined within the linear amplification phases of the function curves (relative level: rDiAg infused, 32 cycles, and control, 44 cycles). These RT-PCR conditions were measured by template dilution method. cDNA resulting from reverse transcription was serially diluted by 4-fold dilutions from 40- to 2,560-fold and from 1- to 3-fold for rDiAg-infused and control rats, respectively. After 42 cycles, the PCR product from each reaction was electrophoresed and stained with 0.5 μ g/ml of ethidium bromide. The fluorescence intensities of the resulting PCR fragments were plotted as a function of dilution to determine the relative ratio of the expression of these mRNAs.

Assay of Histamine Release

Histamine release was quantified as described by Chen and Enerbäck [16]. In short, 100 μ l of mast cell suspensions (5×10^5 cells) derived from naive rats were incubated with 10 μ l of an emulsion containing 10 μ g of ultrasonically emulsified *L*- α -phosphatidyl-*L*-serine from bovine brain (Sigma) for 5 min in a water bath at 30°C after which 10 μ l of anti-DNP-As IgE (1 mg/ml, Cosmo Bio) was added, and incubation was continued for 1 h. The cells were preincubated without or with 50 μ l of rDiAg (10 or 30 μ g/ml) at 37°C for 30 min before challenge with 20 μ l of DNP-As (1 mg/ml). Controls used for the calculation of spontaneous release were obtained by adding 10 μ l of PBS without antibody. The reaction was stopped by placing the tube in an ice bath for at least 5 min. The cells were separated from the histamine released by centrifugation at 400 *g* for 5 min at 4°C. The histamine content was determined using a histamine Elisa kit (ICN, Costa Mesa, Calif., USA), according to the manufacturer's instructions, and the concentration of histamine was calculated by comparison with a standard curve provided by the manufacturer. The net histamine release was obtained by subtracting the spontaneous release in control samples.

Binding Assays

Rat polyclonal IgE (Cosmo Bio) was radiolabeled with ^{125} I to a specific activity of 3.3×10^7 cpm/mg protein using Bolton-Hunter reagent (Di-iodinated NEN, Boston, Mass., USA). 1×10^5 washed peritoneal mast cells from both rDiAg-infused and control rats were incubated for 1 h at 25°C in culture medium with serially diluted ^{125} I-IgE (12,500–200,000 cpm) with or without 100-fold molar excess of unlabeled IgE in a total volume of 100 μ l. The incubated cell mixtures were placed on the 200- μ l oil mixture of 80% dibutylphthalate and 20% olive oil in a microcentrifuge tube, and were then centrifuged at 5,000 *g* for 2 min at 4°C. The precipitated cells and upper layer of non-binding ^{125} I-IgE, which was separated by cutting off the microcentrifuge tube, were placed in a glass tube. The radioactivity of the glass tube was counted using a γ -counter (Aloka, Japan). The equilibrium dissociation constant, *K*_d, and the number of receptors were determined according to the methods of Kulczycki and Metzger [17].

Statistical Analysis

All values are expressed as means $\pm$ SEM. The results were examined using Student's *t* test. A *p* value < 0.05 was considered to be statistically significant.

Results

rDiAg Induces Antigen-Nonspecific IgE Production in vivo

To examine whether rDiAg induces IgE in plasma in the same manner as in ES or parasite helminth infections, we compared IgE production in rDiAg-infused rats and control rats. The rDiAg-infused rats were subcutaneously implanted with osmotic pumps filled with 200 μ g of rDiAg/200 μ l of PBS. In the controls, the osmotic pump was filled with 200 μ l of PBS only. Plasma samples were collected weekly after pump implantation. As shown in

figure 1, IgE production was significantly increased in rDiAg-infused rats, and the peak of IgE was on day 14.

rDiAg-specific IgE was not detected during any of the periods by ELISA. In addition, specific anti-DiAg antibody was not detected in plasma from rDiAg-infused rats by Western blotting (fig. 2b: 2). As a positive control,

serum from a Di-infected patient was subjected to Western blotting. A 14.4-kD DiAg band was detected (fig. 2: 4). To study HRP anti-rat IgE antibody, plasma from rDiAg-infused rats was run on SDS-PAGE and IgE was detected by Western blotting. The 75-kD IgE ϵ heavy chain band was detected.

These results suggest that rDiAg may induce antigen-nonspecific IgE in rats.

rDiAg Inhibits PCA Reaction

We examined the effect of rDiAg on the in vivo model of IgE-dependent PCA reactions in rats. In the rDiAg-infused group, the PCA reaction activated by anti-DNP-As IgE was significantly inhibited compared with the control group (fig. 3). However, the number and viability of peritoneal mast cells were not different between the two groups. We next examined the effect of rDiAg on mast cells in vitro. Histamine contents in the culture supernatants in the absence or presence of 10 or 30 μ g/ml of rDiAg were 66.3 ± 18.25 , 68.1 ± 19.02 and 67.8 ± 18.16 ng/ml, respectively. Values are means $\pm$ SE of 8 rats. These results show that the levels of histamine release were not significantly different. Thus, rDiAg does not directly inhibit histamine release from peritoneal mast cells.

Fc ϵ RI Expression Is Enhanced in rDiAg-Infused Rats

We showed that total IgE production was significantly increased in rDiAg-infused rats and investigated the density of Fc ϵ RI on mast cells. Fc ϵ RI contains four polypeptide subunits: an α -subunit that binds to the Fc portion of the IgE molecule, a β -subunit and two γ -subunits [18]. In

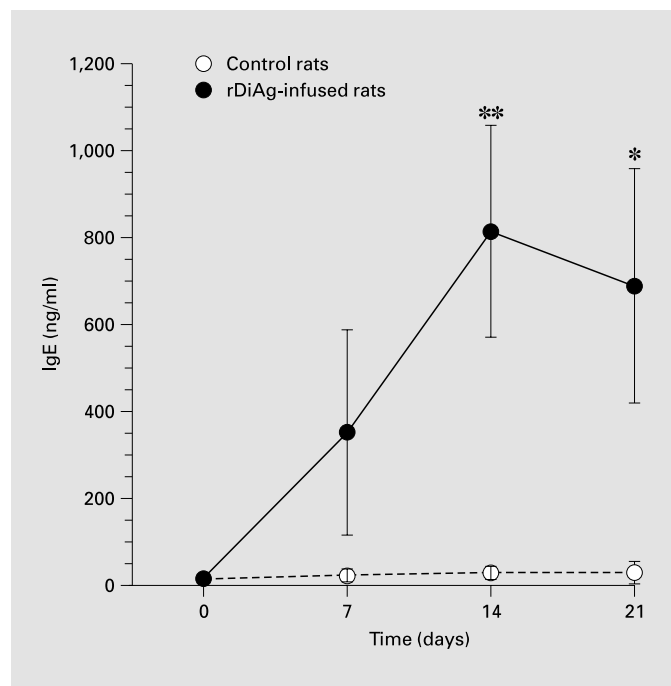
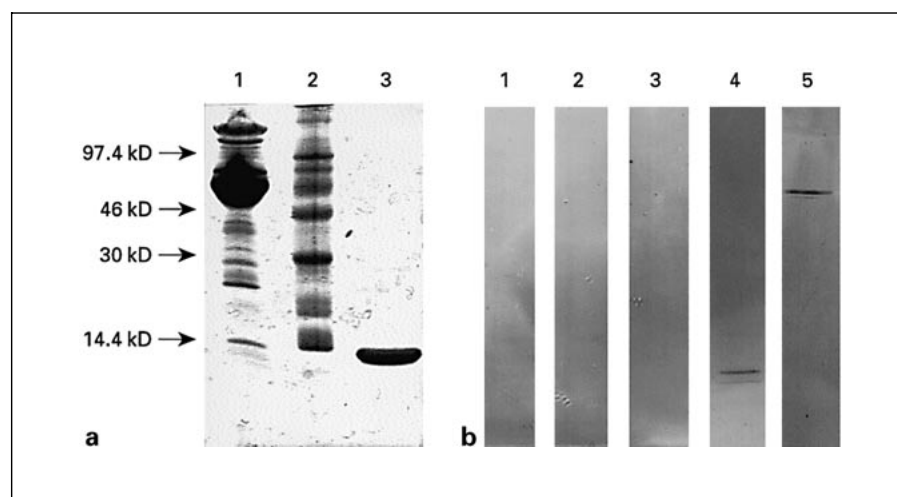


Fig. 1. Time course of IgE levels in rDiAg-infused rats and control rats. Means $\pm$ SE of 8 rats. $p < 0.05$, vs. control.

Fig. 2. Specific anti-DiAg antibody was not detected in plasma from rDiAg-infused rats by Western blotting. **a** 2 μ g of rDiAg (3) and 0.4 μ l of plasma from a rDiAg-infused rat (positive control; 1) were run on 20% SDS-polyacrylamide gels. The gels were stained with Coomassie brilliant blue. 2 = Molecular weight marker. **b** Immunoblots were probed overnight at 4°C with plasma from rDiAg-infused (lanes 1 and 2) and plasma from control rats (lane 3) and serum from a Di-infected patient (lane 4) diluted to the concentration in 0.1% BSA/PBS. The immunoblots were then probed with HRP-conjugated anti-rat (lanes 1–3 and 5) or anti-human IgE (lane 4) in 0.1% BSA/PBS.



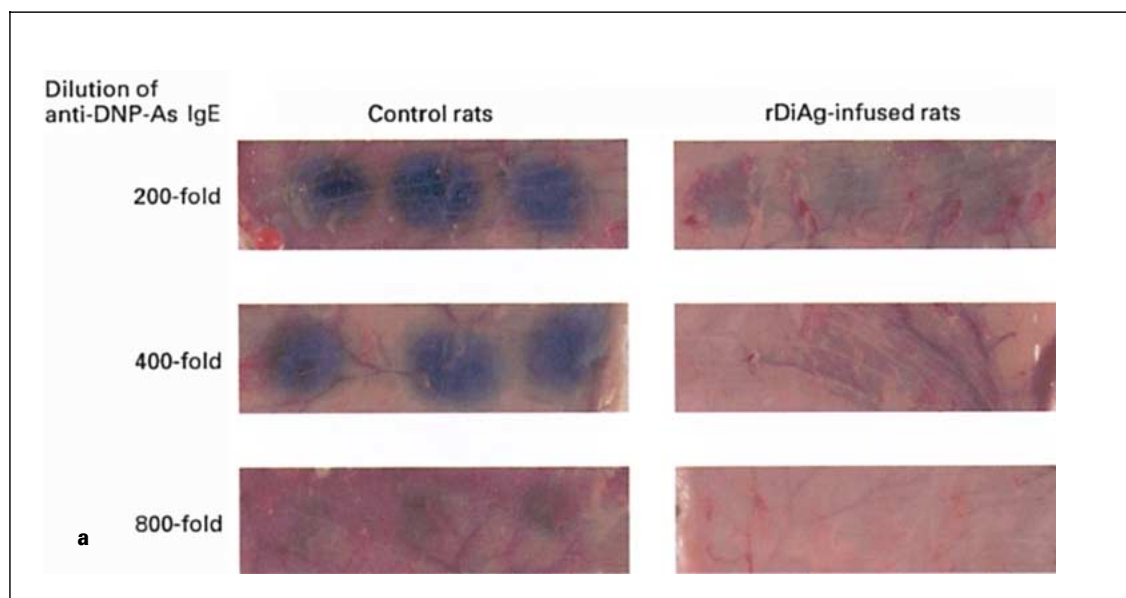
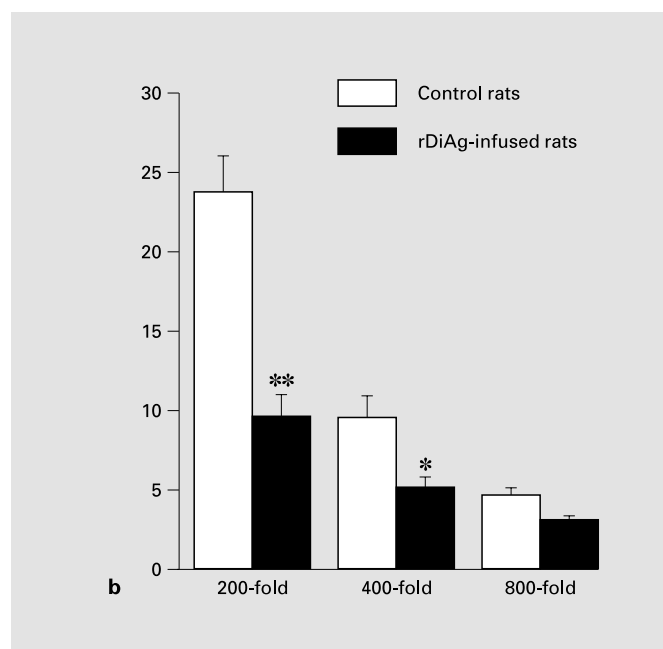


Fig. 3. Effect of rDiAg on PCA reactions. **a** Photograph of the PCA reaction sites. **b** Amount of Evans blue dye on PCA reaction sites. Reactions were examined 21 days after osmotic pump implantation. The anti-DNP-As IgE was diluted in saline (200-, 400- and 800-fold). The rats were injected intradermally with 100 μ l of anti DNP-As IgE into each skin site. After 48 h, the rats received an injection of 1 mg of DNP-As. 30 min after challenge, the dorsal skin was removed. Values are means $\pm$ SE of 8 rats. $p < 0.05$, vs. control.



rats, transfection studies have shown that the β -subunit of Fc ϵ RI is necessary for the expression of the α -subunit and γ -subunit on the cell membrane [13]. Here, we examined mRNA levels of the β -subunit to investigate the density of Fc ϵ RI on the peritoneal mast cells derived from control or rDiAg-infused rats on day 21 after pump implantation.

To compare the expression mRNA levels of the β -subunit of Fc ϵ RI in peritoneal mast cells from rDiAg-infused rats and control rats, we analyzed RT-PCR results by tem-

plate dilution method (fig. 4). 1 μ g of total RNA extracted from mast cells of rDiAg-infused rats or control rats was reverse-transcribed and then amplified by PCR. Aliquots of PCR products were removed at 2-cycle intervals and electrophoresed on agarose gels stained with 0.5 μ g/ml of ethidium bromide (fig. 4a). By plotting the ethidium bromide fluorescence as a function of cycle number, the β -subunit of Fc ϵ RI amplification curves were generated (fig. 4b). To obtain the same fluorescence intensity of the

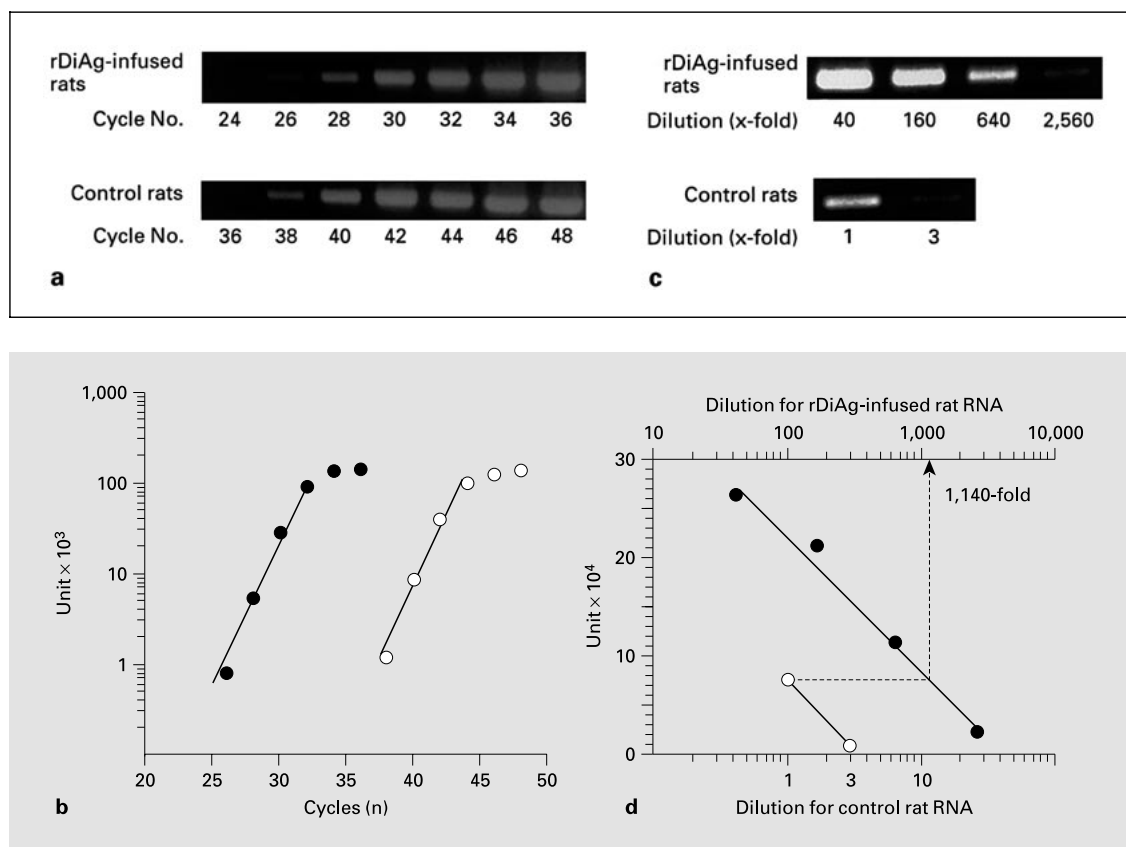


Fig. 4. rDiAg induces expression of FcεRI β-subunit mRNA in peritoneal mast cells. Total RNA was extracted from peritoneal mast cells (5×10^5) from control and rDiAg-infused rats. **a** RNA from mast cells was reverse-transcribed, and cDNA was amplified using FcεRI β-subunit-specific primers. Aliquots of the PCR reaction were removed at 2-cycle intervals and electrophoresed. **b** After staining with ethidium bromide, fluorescence intensity of each lane was measured and plotted to generate amplification curves for amplified fragments. 42 cycles were required to amplify the same amount of PCR product from control rats (○) as PCR product on 31 cycles from rDiAg-infused rats (●). **c** Template cDNA was sequentially diluted (for rDiAg-infused rats, 40, 160, 640, and 2,560; for control rats, 1 and 3) to analyze the FcεRI β-subunit mRNA ratio. PCR products at 42 cycles of each reaction were electrophoresed and stained by ethidium bromide. **d** Fluorescence intensity of PCR from control rats (○) and rDiAg-infused rats (●) were measured and plotted as a function of dilution. The FcεRI β-subunit mRNA ratio was determined to be 1,140-fold by this plot.

rDiAg-infused rats and control rats, 31 cycles for the rDiAg-infused rat and 42 cycles for the control rat were needed. This result indicates that FcεRI β-subunit mRNA content in mast cells from control rats is far less than that from rDiAg-infused rats. To determine the approximate FcεRI β-subunit mRNA ratio by using these PCR cycles, the template RNA was then diluted and amplified by RT-PCR (fig. 4c). Fluorescence intensities of the FcεRI β-subunit products were plotted as a function of dilution (fig. 4d). Through these experiments, the FcεRI β-subunit mRNA expression ratio was determined to be 1,140-fold (control rat/rDiAg-infused rat ratio, 0.088%).

This result was comparable to the results obtained from protein levels of FcεRI from mouse mast cells, as reported by Yamaguchi et al. [12].

Binding Assays

To clearly demonstrate that FcεRI expressed in peritoneal mast cells from rDiAg-infused rats is saturated with nonspecific IgE, ^{125}I -IgE binding assays were examined. As shown in figure 5a, ^{125}I -IgE bound to peritoneal mast cells from control rats. The level of ^{125}I -IgE binding to the mast cells increased with increasing IgE concentration. In contrast, ^{125}I -IgE did not bind to peritoneal mast cells

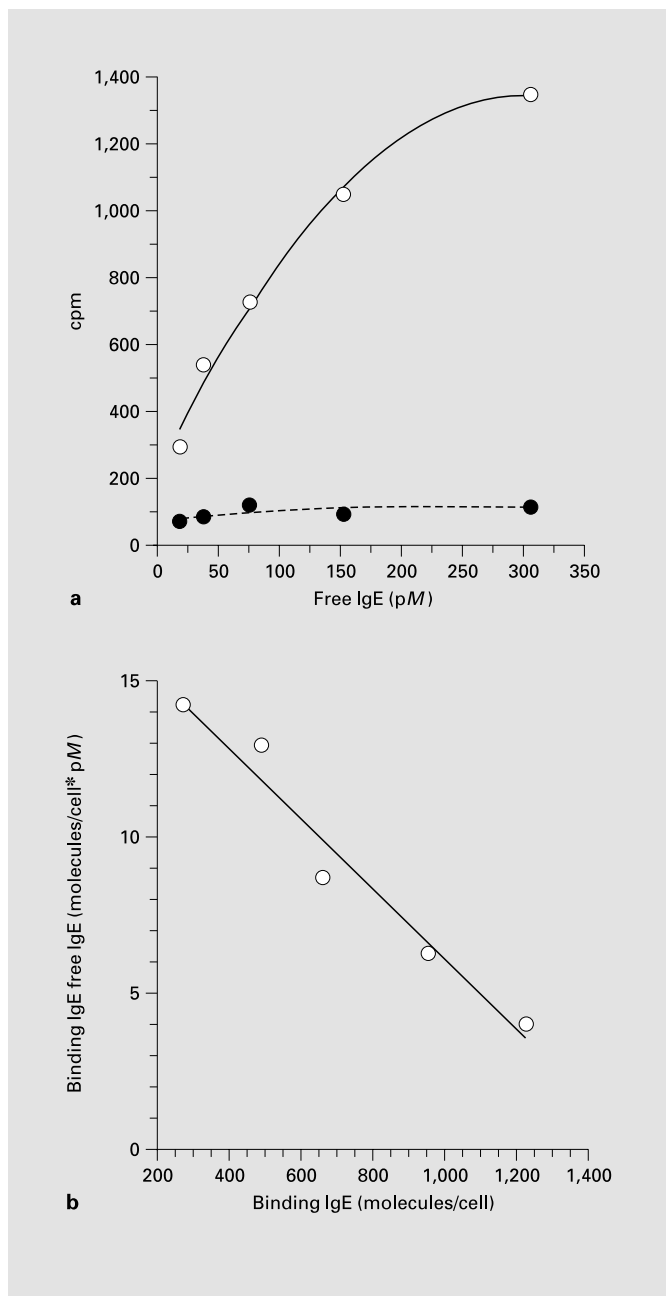


Fig. 5. **a** Binding of ^{125}I -IgE to peritoneal mast cells from rDiAg-infused and control rats. **b** Scatchard plot of ^{125}I -IgE binding to peritoneal mast cells from control rats. Peritoneal mast cells from an rDiAg-infused rat (●) and a control rat (○) were incubated in culture medium with serial dilution of ^{125}I -IgE with or without 100-fold molar excess of unlabeled IgE. Nonspecific IgE binding was subtracted from total binding at each IgE concentration point.

from rDiAg-infused rats. ^{125}I -IgE binding to peritoneal mast cells from control rats suggests the presence of a very small number of high-affinity receptors ($K_d = 3\text{D}86.5\text{ pM}$; 1,500 sites/cell), according to a Scatchard plot analysis (fig. 5b). The K_d of IgE binding to the mast cells is similar to that of other mammalian FcεRI [19].

Discussion

The present study demonstrated that rDiAg induced high levels of antigen-nonspecific IgE production in rats similar to those seen at early-stage parasitic helminth infections.

IgE plays a key role in the physiopathology of allergic disorders through its binding to high-affinity IgE receptors (FcεRI) on mast cells and basophils [10]. Previously, Pastorello [11] reported a strong positive correlation between serum IgE and clinical symptoms in allergic patients. Furthermore, Yamaguchi et al. [12] reported that IgE-dependent upregulation of FcεRI expression could significantly increase the ability of mouse mast cells to release mediators in response to challenge with specific antigens. These data indicate that IgE itself might regulate the ability of mast cells to bind IgE and to release histamine, which may lend support to the concept that the increase in IgE exacerbates allergic reactions. However, PCA reactions were suppressed in spite of both high levels of antigen-nonspecific IgE and enhanced FcεRI β-subunit mRNA expression in rDiAg-infused rats. A recent study by Chen and Enerbäck [20] demonstrated that IgE-mediated histamine release of mast cells in athymic rats was significantly lower than that in euthymic rats, even though IgE receptors and IgE levels were similar. This suggests that the putative T-cell-independent IgE response in vivo and the IgE-mediated histamine releasability of mast cells is largely T cell dependent. However, we cannot rule out a role for T-cell-derived cytokines in this study. We suggest an alternative mechanism of PCA suppression. If the majority of IgE receptors are occupied with antigen-nonspecific IgE, newly synthesized antigen-specific IgE must compete for binding sites with antigen-nonspecific IgE on the mast cells. This hypothesis is supported by previous studies. For example, IgE-mediated anaphylactic reactions were not elicited in E myeloma bearing humans [21], and systemic anaphylaxis was suppressed in *Nippostrongylus brasiliensis* (Nb)-infected rats [22]. In addition, passive sensitization with E myeloma protein was inhibited in peritoneal mast cells from Nb-infected rats [23], and PCA reactions were suppressed in

Nb-infected rats [24]. Consistent with this hypothesis, ¹²⁵I-IgE did not bind to mast cells derived from rDiAg-infused rats, but dose-dependently bound to mast cells derived from control rats. This observation indicates that the suppression of PCA reactions in rDiAg-infused rats can be explained by antigen-nonspecific IgE molecules occupying mast cells and thereby inhibiting the binding of anti-DNP-As IgE.

In conclusion, rDiAg can induce antigen-nonspecific IgE in rats as well as Nb infection. Furthermore, rDiAg can inhibit PCA reactions through increasing antigen-nonspecific IgE production. These results suggest that rDiAg might be the essential factor to induce antigen-nonspecific IgE production in helminth infection.

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