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Induction of Basophil Desensitization in Physiological Medium: Enhancement after IgE-Dependent Upregulation of Surface IgE Binding on Basophils

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Key Words
Basophils · IgE · Anti-IgE antibody · Histamine release · Desensitization · FcεRI · Mite

Abstract

Background: Although the ability of basophils to release mediators, called releasability, may be an important aspect which influences the proinflammatory role of these cells, clinical approaches aiming at the depletion of the releasability have not been established. We examined whether the desensitization procedure in Ca^{2+}-containing physiological conditions can make basophils completely unresponsive to IgE-mediated stimulation, and whether basophil desensitization is affected by the surface IgE levels. ***Methods:*** Human peripheral blood basophils were cultured with low concentrations of anti-IgE antibody or recombinant mite allergen. Following culture, cells were stimulated and their histamine release was measured. ***Results:*** Culturing with mite allergen or anti-IgE antibody below threshold concentrations induced potent desensitization in basophils. The desensitizing effect of anti-IgE was dose- and time-dependent; IgE-dependent releasability was completely suppressed when basophils were incubated with a near-threshold concentration of anti-IgE for ≥4 h. In the continuous presence of subthreshold doses of anti-IgE, basophils remained desensitized even after 3 days. Basophils which had undergone an increase in surface IgE levels after 24-hour culture with IgE demonstrated enhanced desensitization. ***Conclusions:*** Near-threshold stimulation in physiological medium can affect basophils, thereby inducing complete and sustained deprivation of releasability without triggering degranulation. Basophil desensitization is regulated by their surface IgE levels. Induction of full desensitization may represent a potentially important therapeutic strategy for IgE-mediated allergic diseases in which basophils play pathogenic roles.

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Introduction

Activation of mast cells and basophils is a crucial event in IgE-mediated type I allergic reactions [1, 2]. Both types of cells abundantly express a high-affinity receptor for IgE (FcεRI) on their surface [3]. When the surface-bound IgE on these cells is cross-linked by specific antigens, the cells

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secrete a panel of bioactive mediators, including histamine. Thus, IgE-dependent mediator release from mast cells and basophils is thought to play a proinflammatory role in allergic diseases.

Several lines of evidence have shown that the severity of allergic reactions can be influenced, at least in part, by the extent of activation of mast cells and basophils in response to IgE-cross-linking stimuli. It has been reported that the lack of mast cell responses to IgE-mediated stimulation, as seen in FcεRI α-deficient mice, is directly linked to the inability of these mast cells to initiate allergic reactions even in the presence of antigen and antigen-specific IgE [4]. Recent studies by us and others have indicated that surface FcεRI expression is not only essential but also plays a regulatory role in the IgE-dependent effector functions of mast cells and basophils [5–9]. However, relatively little is known regarding the intrinsic mechanisms in these cells that regulate the magnitude of activation. Due to the difficulty of repeatedly obtaining tissue mast cells from humans, regulation of the extent of IgE-mediated degranulating ability, called releasability, has been analyzed mostly using blood basophils [10–12]. However, the precise mechanisms regulating basophil releasability have not been fully identified. Moreover, because it has been thought that basophil releasability involves 'black box' properties in nature, clinical approaches aiming at in vivo manipulation of their releasability have not yet been established.

The phenomenon of basophil desensitization has been known for more than 30 years [13]. In the presence of antigen, basophils are thought to generate not only activation signals but also IgE-mediated inhibitory changes that limit the extent of activation [14, 15]. Subthreshold doses of antigen are also reported to be able to desensitize basophils; slight to moderate suppression of basophil response to subsequent IgE-mediated stimulation can be obtained [16, 17], indicating an important aspect of desensitization, namely that it can take place even in the absence of apparent simultaneous activation. Usually induction of desensitization without degranulation is achieved by treating basophils with an optimal dose of antigen or anti-IgE in a buffer containing no Ca^{2+} or Mg^{2+} [14]. Although basophil desensitization may be an important phenomenon representing artificial downregulation of their releasability at least in vitro, induction of desensitization is not yet recognized as a clinically important approach for modulating the proinflammatory role of basophils in allergic diseases.

In this study, we examined the releasability of basophils following hours to days of culture with low levels of antigen or anti-IgE antibody in Ca^{2+}-containing physiological medium, and found that weak, even below-threshold treatment can result in full desensitization, i.e. complete and sustained deprivation of their degranulating capacity in response to subsequent IgE-mediated stimulation. Surface IgE levels on basophils were considered important in regulating desensitization.

Materials and Methods

Reagents

The following reagents were purchased as indicated: affinity-purified goat anti-human IgE antibody (IgG fraction; total IgG concentration 14 mg/ml; Medical and Biological Laboratory, Nagoya, Japan); normal goat IgG and piperazine-N,N′-bis-2-ethanesulfonic acid (PIPES; Sigma Chemical Co., St. Louis, Mo., USA); Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden); PBS, RPMI 1640 medium and fetal calf serum (Gibco, Grand Island, N.Y., USA); ionophore A23187 (Calbiochem-Behring, La Jolla, Calif., USA); human myeloma IgE (Biodesign International, Kennebunk, Me., USA), and recombinant Der f 2 (Asahi Breweries, Tokyo, Japan) [18]. Human IL-3 was kindly donated by Kirin Brewery (Tokyo, Japan).

Buffer

PIPES A buffer contained 25 m*M* PIPES, 119 m*M* NaCl, 5 m*M* KCl and 0.03% human serum albumin, and was adjusted to pH 7.4. For stimulation of basophils, PIPES A containing 2 m*M* Ca^{2+} and 0.5 m*M* Mg^{2+} (PIPES ACM) was used.

Cell Preparation

Venous blood was drawn from healthy volunteers with no history of atopic diseases such as bronchial asthma, allergic rhinitis and atopic dermatitis after informed consent had been obtained. In some experiments, peripheral blood was drawn from consenting subjects with mite-sensitive allergic asthma, fulfilling the criteria for diagnosis of bronchial asthma [19].

In most experiments, basophil preparations were prepared by the dextran-glucose sedimentation procedure [20]. In brief, heparinized blood was allowed to sediment at room temperature for 90 min with 2.5 ml of 6% dextran (Pharmacia) and 3% glucose solution per 10 ml of blood. The plasma-leukocyte layer was transferred to another tube. After centrifugation, the cells were washed with PIPES A buffer containing 4 m*M* EDTA, washed again with PIPES A buffer, and then used for either culture or histamine release experiments. Basophils comprised ~ 1% of the total leukocytes.

For some experiments, basophils were highly purified by Percoll density centrifugation followed by an additional isolation procedure (purity >95%). Briefly, venous blood was anticoagulated with 10 m*M* EDTA and overlaid on Percoll solutions of different densities (1.079 and 1.070 g/ml). After centrifugation at 700 *g* for 15 min at room temperature, the cell bands between the two Percoll solutions were collected. Basophils were further separated by negative selection using an MACS Basophil Isolation Kit (Miltenyi Biotech, Bergisch-Gladbach, Germany) according to the manufacturer's instructions. The viability of cells in both crude and purified preparations, as assessed by the trypan blue exclusion test, was consistently >95%.

Histamine Release

Histamine release was performed as previously described [20]. Briefly, cells were resuspended in PIPES ACM buffer, and then stimulated at 37°C for 45 min in polystyrene tubes. The supernatants were stored at 4°C until histamine assay by an automated fluorometric technique. Histamine release was expressed as a percentage of the total cellular histamine after subtracting the spontaneous release (consistently less than 7%) in the absence of secretagogues, unless otherwise noted. Each experiment was performed at least in duplicate.

Cell Culture

Basophil preparations were resuspended in RPMI 1640 medium supplemented with 20% FCS, 2 m*M* *L*-glutamine (Gibco) and antibiotics (100 U of penicillin and 100 μg of streptomycin/ml, Gibco) and then cultured at 37°C in humidified air with 5% CO_2 in 25-cm^2 tissue culture flasks (IWAKI, Chiba, Japan) in a total volume of 5 or 10 ml. Desensitization of basophils was induced by culturing cells in the presence of either Der f 2 or anti-IgE. In some experiments, human myeloma IgE at 1 μg/ml was added to culture medium.

Analysis of Surface IgE, FcεRI and Goat Anti-IgE Ab on Desensitized Basophils

Following desensitization procedures, IgE remaining on the surface of basophils was stained with FITC goat IgG anti-human IgE (at 5.5 μg/ml, Biosource International, Camarillo, Calif., USA) for 30 min at 4°C. The cells were then resuspended in PBS containing 0.1% BSA and 0.1% sodium azide and analyzed by flow cytometry (EPICS XL System II, Coulter, Miami, Fla., USA). For a negative control stain, FITC control goat IgG (Jackson ImmunoResearch Laboratories, West Grove, Pa., USA) was used instead of FITC goat anti-human IgE. For staining of surface FcεRI on basophils, cells were incubated for 1 h at 4°C with 5 μg/ml of mouse IgG2b anti-human FcεRI α-chain mAb, CRA-1, which can bind to FcεRI α whether or not it is occupied by IgE [8]. After washing, cells were stained with FITC goat $F(ab')_2$ against mouse IgG (Jackson) at 7.5 μg/ml for 30 min. An isotype-matched mouse IgG2b mAb with irrelevant specificity (MOPC 141, Cappel, Aurora, Ohio, USA) was used as a negative control instead of CRA-1. Surface-bound goat anti-human IgE on basophils was stained with FITC rabbit anti-goat IgG (Jackson) at 10 μg/ml for 30 min.

Statistical Analysis

For analysis of statistical significance, data were compared by unpaired Student's t test. Data are expressed as the mean ± SEM.

Results

Induction of Full Desensitization of Basophils from Mite-Sensitive Asthmatics following Culture with Low Concentrations of Antigen or Anti-IgE

In a first series of experiments, fresh basophil preparations from mite-sensitive asthmatic subjects were stimulated with various concentrations of recombinant mite allergen, Der f 2. Although both the threshold and optimal doses of antigen varied among the donors, basophils in a representative experiment exhibited significant degranulation at as low as 0.1 ng/ml of Der f 2, whereas optimal release from the cells was observed at 1 ng/ml (fig. 1a). Overnight culture of the cells with very low concentrations (<0.01 ng/ml) of Der f 2 failed to induce apparent degranulation during culture, since the total amount of cellular histamine was essentially the same between basophils cultured with or without Der f 2 (fig. 1b). However, when the basophils were stimulated following culture for 24 h with subthreshold concentrations of Der f 2 or anti-IgE antibody, the magnitude of subsequent degranulation in response to the secretagogues was potently suppressed (fig. 1c), indicating that the basophils had been desensitized during the culture. The continuous presence of Der f 2 at as low as 0.003 ng (3 pg)/ml during overnight culture showed complete desensitization against subsequent stimulation with Der f 2 (1 ng/ml). Since similar desensitizing effects on IgE-dependent releasability of basophils were observed when subthreshold anti-IgE was included in the overnight culture (fig. 1c), nonspecific basophil desensitization by anti-IgE [16, 21, 22] was further examined in the following experiments using basophils from nonatopic donors.

Anti-IgE-Induced Full Desensitization of Basophils from Normal Subjects

Anti-IgE-induced immediate degranulation was assessed in freshly prepared basophils resuspended in either PIPES ACM or culture medium; minimal but significant histamine release was observed when cells were stimulated with anti-IgE antibody at 2–5 × 10^5 dilutions, which was regarded as the threshold, whereas maximal release occurred between 10^3 and 10^4 dilutions of anti-IgE (fig. 2a). The threshold and optimal concentrations of anti-IgE were always in a similar range in our preliminary experiments using basophils from various healthy subjects. On the other hand, as shown in figure 2b and c, basophils incubated for 24 h with subthreshold anti-IgE clearly demonstrated desensitization; basophil desensitization occurred dose-dependently below the threshold range of anti-IgE. Incubation with anti-IgE at 5–10 × 10^6 dilution significantly reduced the degranulating ability of basophils. IgE-dependent releasability was completely suppressed after basophils were incubated overnight with near-threshold doses of anti-IgE, and the complete loss of releasability was obtained in basophils cultured with anti-IgE at 0.4–2 × 10^6 dilution (fig. 2b, c). Anti-IgE-induced desensitization was not due to an effect on the general degranulating machinery, since the basophil response to a non-IgE-mediated secretagogue, A23187, was well preserved or even enhanced (fig. 2b, c) [23].

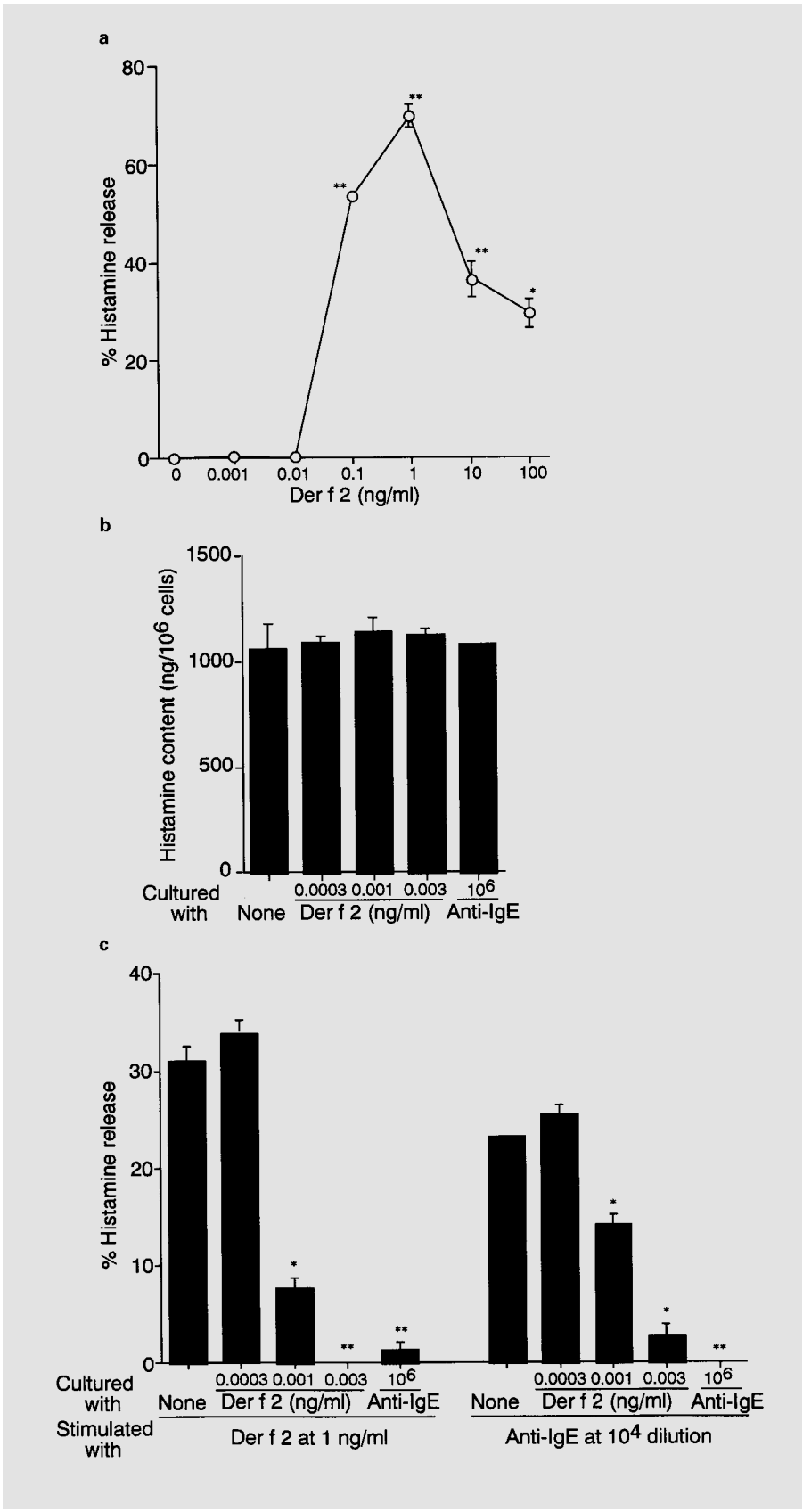

Fig. 1. a Der f 2-induced histamine release of basophils from a mite-sensitive asthmatic subject. Histamine content (b) and desensitization of basophils (c) of the same donor as in a, following overnight culture with subthreshold Der f 2 or anti-IgE. Basophils were cultured for 24 h with or without subthreshold concentrations of either Der f 2 (at 0.0003, 0.001 or 0.003 ng/ml) or anti-IgE (10^6 dilution). The cells were then washed and stimulated with various concentrations of Der f 2 (maximal release occurring at 1 ng/ml is shown) or an optimal dose of anti-IgE (10^4 dilution). Data are mean ± SEM of triplicate experiments. * $p < 0.005$; ** $p < 0.0001$ vs. spontaneous release (a) or the corresponding value of nondesensitized cells (c). Four other experiments showed similar results.

Basophil desensitization has previously been recognized in cells treated with an isotonic buffer containing either a subthreshold or optimal dose of stimuli in a simple buffer with or without Ca^{2+} [14–17, 24]. Our results above, however, indicated that subthreshold allergen or anti-IgE can also induce basophil desensitization effectively in Ca^{2+}-containing culture medium, in which cells can survive much longer. Thus, we employed RPMI-based medium for hours to days of desensitization procedure, and PIPES ACM buffer for the short-term stimulation of basophils throughout this study. Because the concentration of subthreshold anti-IgE required for full desensitization varied among the donors, and even among separate experiments using basophils from a single donor, the following studies using fully desensitized basophils were performed within 2 weeks of a preliminary experiment analyzing the adequate concentration of anti-IgE (usually in the range of 0.4–2 $\times$ 10^6 dilution for full desensitization without apparent loss of cellular histamine). Desensitized basophils in most of our experiments demonstrated a significantly enhanced histamine release to A23187 challenge compared to nondesensitized basophils (fig. 2b, 3a and b are examples), consistent with previous reports [23].

Slow and Stable Desensitization of Basophils

Whereas anti-IgE-mediated immediate degranulation is known to occur within 15 min, basophil desensitization during culture with subthreshold anti-IgE antibody took place much more slowly (fig. 3a). Preincubation of basophils for 20 min with subthreshold anti-IgE did not suppress the subsequent release, whereas continuous treatment for 2 or 4 h induced partial or full desensitization in basophils, respectively (fig. 3a). Although hours of incu-

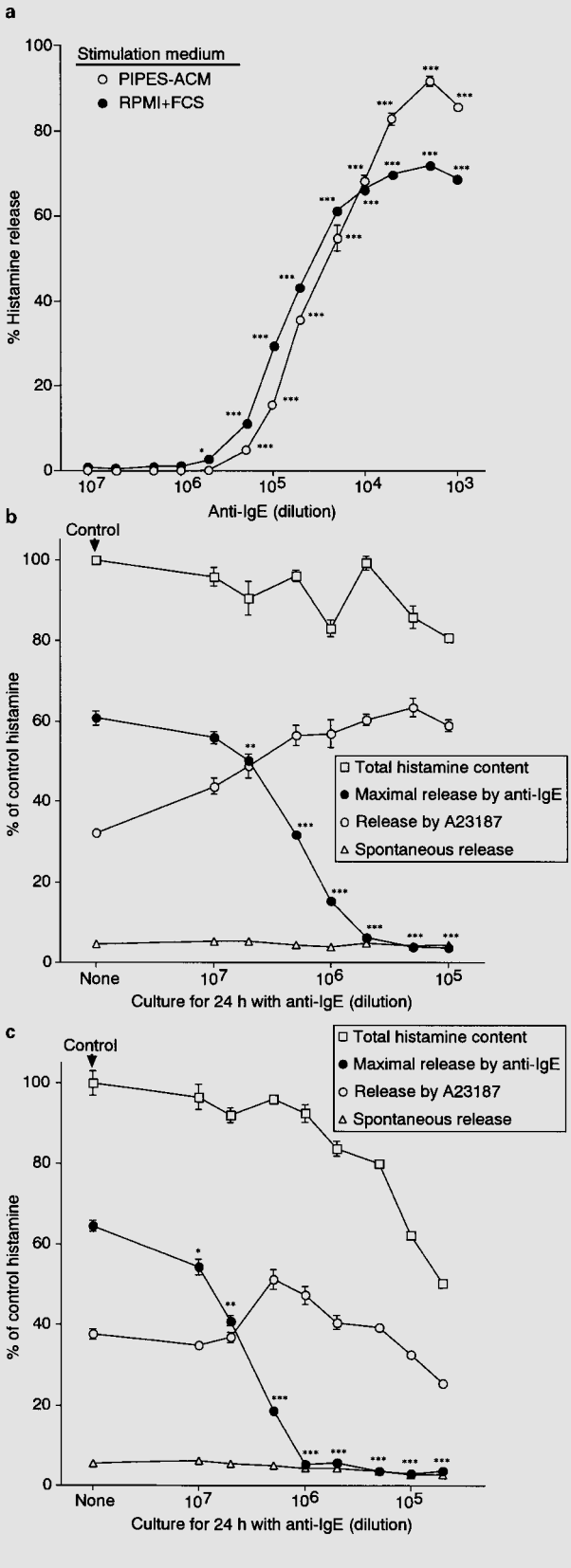

Fig. 2. a Anti-IgE-induced direct histamine release of basophils from a normal subject in either PIPES ACM buffer or culture medium. b, c Dose-dependent desensitizing effect of anti-IgE. The same basophil preparations (b) as in a or cell preparations in a separate experiment (c) were cultured for 24 h with or without various dilutions (x-axis) of anti-IgE. The cells were then washed and stimulated with either A23187 at 0.2 μg/ml or anti-IgE at 10^3, 3×10^3 or 10^4 dilution. The total amount of histamine, spontaneous release in the absence of stimuli, release by A23187 and maximal release by anti-IgE are indicated as percentages of the total histamine in nondesensitized cells (marked as 'Control'). * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0001$ vs. spontaneous release (a) or anti-IgE-induced maximal release of control cells (b, c). The data shown (mean ± SEM, n = 3) are representative of the results obtained in five separate experiments, all demonstrating similar results.

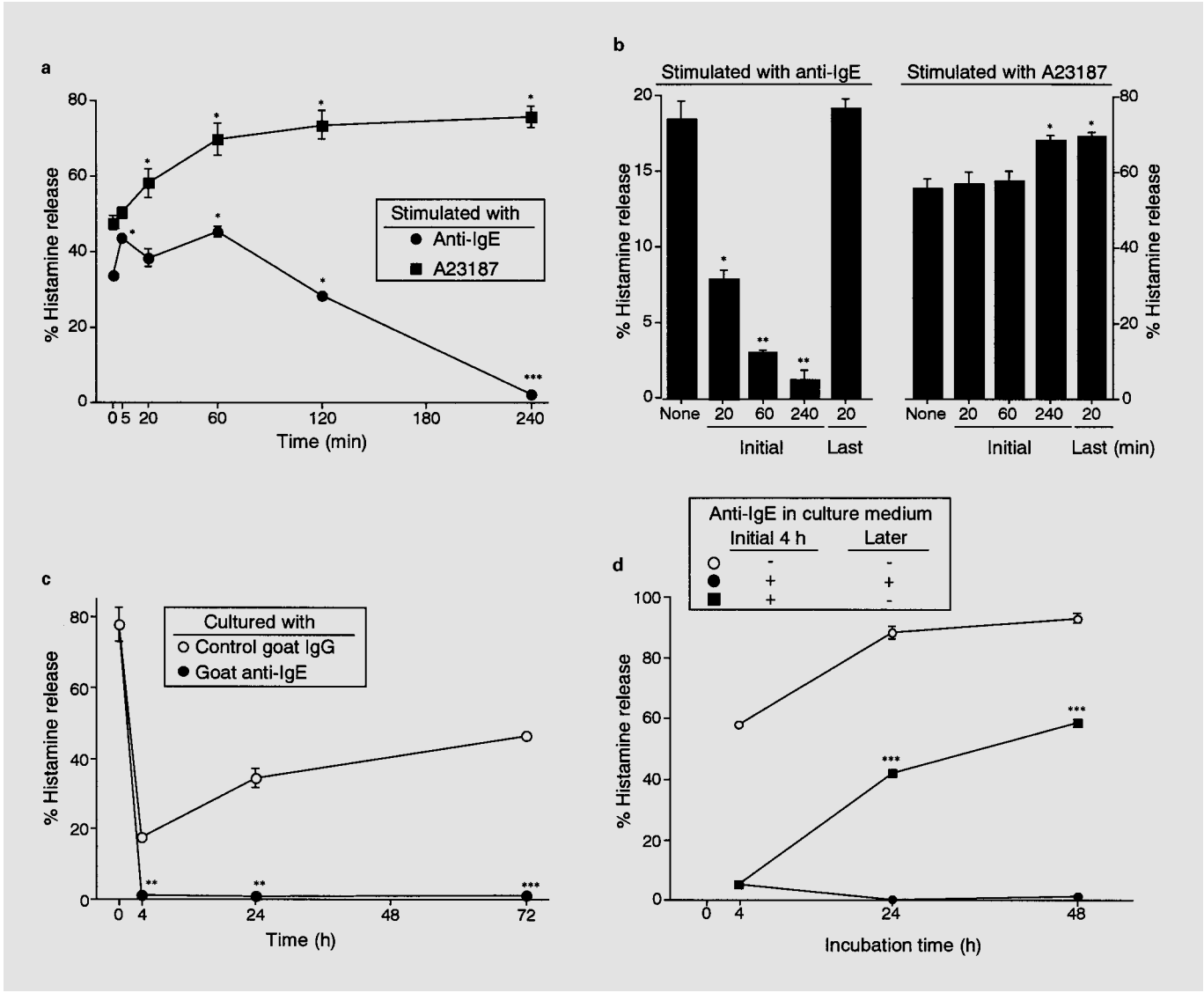

Fig. 3. Time-dependent basophil desensitization by subthreshold anti-IgE. a Basophils were incubated for 4 h in culture medium, and at various time points during the incubation a subthreshold anti-IgE at 5×10^5 dilution (stock concentration: 14 mg/ml) was added. After incubation, the cells were washed and stimulated with either anti-IgE at 10^4 dilution or A23187 at 0.2 μg/ml. The x-axis indicates the length of time in contact with subthreshold anti-IgE. Zero minutes correspond to control cells incubated without subthreshold anti-IgE. * $p < 0.05$; *** $p < 0.0001$ vs. percent release of control cells. b Basophils were incubated for a total of 4 h with or without subthreshold anti-IgE at 5×10^5 dilution; some preparations were treated only for the initial 20 or 60 min, or the last 20 min. Following incubation, the cells were washed and stimulated with either anti-IgE at 10^4 dilution or A23187 at 0.2 μg/ml. * $p < 0.05$; ** $p < 0.001$ vs. corresponding release of nondesensitized cells, indicated as 'None'. c Cells were incubated for 4, 24 or 72 h with subthreshold anti-IgE (at 5×10^5 dilution) or control goat IgG at 28 ng/ml. At each time point, the cells were stimulated with anti-IgE at 10^3 or 10^4 dilution, and the higher release is shown. ** $p < 0.001$; *** $p < 0.0001$ vs. release of control cells cultured with goat IgG. d Fully desensitized basophils by treatment with subthreshold anti-IgE at 5×10^5 dilution for 4 h were cultured for another 20 or 44 h with or without the same dose of anti-IgE. The cells were then washed and stimulated with anti-IgE at 10^4, 3×10^3 or 10^3 dilution; maximal release is shown. Histamine release of control nondesensitized cells was also analyzed. Data are the mean ± SEM (n = 3). *** $p < 0.0001$ vs. corresponding release of continuously desensitized cells. The data are representative of three or four separate experiments, all showing similar results.

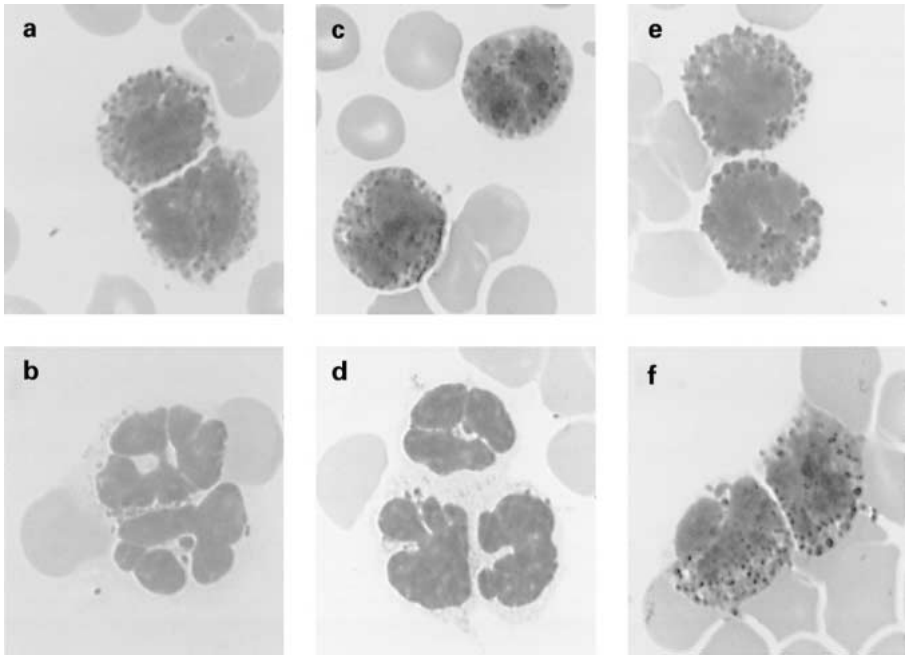

Fig. 4. Morphology of desensitized and nondesensitized basophils. Freshly isolated basophils (purity >95%) (a, b) or basophils cultured for 24 h with either control goat IgG at 28 ng/ml (c, d) or subthreshold anti-IgE at 5×10^5 dilution (e, f) were challenged for another 45 min with either goat IgG at 1.4 μg/ml (a, c, e) or anti-IgE at 10^4 dilution (b, d, f). May-Grünwald-Giemsa stains of cytospin preparations are shown. The viability of cells after culture was consistently >92% as assessed by trypan blue stain. Original magnification: × 1,000. Data shown are representative of two separate experiments showing similar results.

bation were necessary for basophils to reach full desensitization, contact with subthreshold anti-IgE during the initial part of incubation was especially important for inducing desensitization. As shown in figure 3a and b, basophils treated for 20 min with subthreshold anti-IgE did not show suppressed releasability to IgE-mediated stimulation unless the cells were further incubated for hours. However, the length of time required for induction of full desensitization varied greatly among the donors; basophils from some other donors showed a much faster time course, in which full desensitization occurred within only 1 h (data not shown).

As shown in figure 3c, desensitized basophils remained insensitive to IgE-mediated stimulation after culture for 3 days or even 7 days (in the presence of a basophil survival-prolonging cytokine, IL-3 at 300 p*M;* data not shown) without losing their histamine content, as long as a subthreshold dose of anti-IgE was present in culture medium. When basophils fully desensitized by 4-hour treatment with near-threshold anti-IgE were cultured for 1 or 2 days in the absence of anti-IgE, they showed apparent recovery from desensitization (fig. 3d). These results suggest that desensitized basophils are in a stable but mostly reversible state. However, it should be noted that control cells showed a significantly decreased response after hours of incubation with or without normal goat IgG (maximal release before culture in 11 experiments: 48.3 ± 5.8% vs. release after 4-hour culture without goat IgG: 18.0 ± 7.3%, $p < 0.01$; release after 4-hour culture with control goat IgG at 28 ng/ml: 19.1 ± 4.3%, $p < 0.01$), presumably due to a nonspecific effect during the initial hours of culture, from which cells later recover (fig. 3c, d).

Desensitization of basophils was also achieved in highly purified preparations (see fig. 4), indicating that subthreshold anti-IgE induces basophil desensitization, not via indirect effects involving contaminating cells.

Characterization of Basophils Fully Desensitized in Culture

Both freshly isolated basophils and basophils cultured overnight in medium without anti-IgE demonstrated typical basophilic granules in their cytoplasm and lobulated nuclei (fig. 4a, c); these cell preparations underwent morphological changes following challenge with a sufficient level of anti-IgE, showing loss of cytoplasmic granules (fig. 4b, d). Although basophils fully desensitized by overnight treatment with subthreshold anti-IgE showed well-granulated cytoplasm similar to fresh or nondesensitized basophils, they failed to demonstrate apparent morphological changes upon potent stimulation with anti-IgE (fig. 4e, f). As reported previously [25], the surface levels of IgE and FcεRI in desensitized basophils were comparable to those in nondesensitized basophils (fig. 5a, b). On the other hand, a considerable amount of goat anti-IgE

antibody was detected on the surface of desensitized basophils (fig. 5c), but the surface IgE was not saturated with anti-IgE antibody because incubation with an optimal dose of anti-IgE showed additional binding of the antibody to desensitized cells. These results collectively suggest that subthreshold anti-IgE antibody is definitely bound to the surface IgE on fully desensitized basophils, but that the antibody does not trigger the disappearance of surface IgE or FcεRI during desensitization.

Surface IgE Levels on Basophils Affect Desensitization

In the last series of experiments, we examined whether anti-IgE-induced desensitization is regulated by the surface IgE levels on basophils. After 24 h culture of basophils with human IgE at 1 μg/ml, the surface IgE or FcεRI levels were increased by approximately 50% compared to those of cells cultured without IgE (data not shown), consistent with previous studies [9]. After washing the cells, these cell preparations with different IgE densities were either directly stimulated or desensitized with anti-IgE antibody; the basophils with increased surface IgE showed not only upregulated degranulation in response to a low dose of anti-IgE (fig. 6a), but also enhanced and accelerated desensitization by subthreshold anti-IgE, as indicated in figure 6b and c. These results suggest that desensitization is regulated by the surface IgE levels on basophils.

Discussion

Although the extent of basophil degranulation in response to IgE-mediated stimulation varies greatly among individuals, it has been recognized that basophil releasability is modulated by many factors, including the genetic background [26], the presence of allergic diseases [27–29] and extracellular molecules such as IgE [9] and cytokines [30]. IgE-mediated stimulation itself can, under some conditions, reduce the degree of degranulation, which is known as desensitization. Numerous studies have so far focused on the phenomenon of desensitization using either basophils or mast cells [10, 14–17, 24, 31, 32], but the precise mechanism accounting for desensitization has remained unclear.

In this study, we have shown that, using antigen or anti-IgE at near-threshold concentrations, full desensitization of basophils can be consistently obtained without affecting their granule contents. By using a physiological culture medium for desensitization procedures, we were able to maintain desensitized basophils in vitro for days.

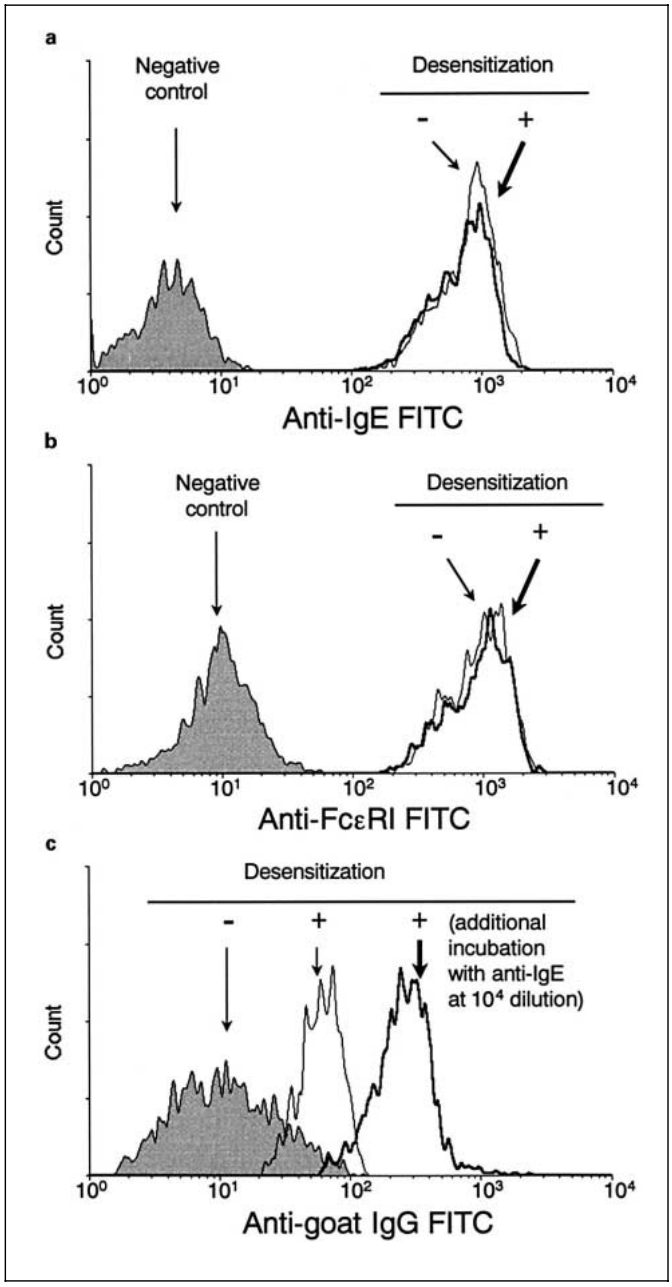

Fig. 5. Flow-cytometric analysis of surface IgE (a), FcεRI (b) and goat anti-IgE (c) on desensitized basophils. Purified basophils (purity >95%) were cultured for 24 h with either desensitized anti-IgE at 10^6 dilution or control goat IgG at 14 ng/ml, and then analyzed by flow cytometry. Negative controls in a and b correspond to nondesensitized cells cultured with control IgG, followed by negative control staining. c Desensitized basophils further incubated at 4°C for 1 h with a 10^4 dilution of anti-IgE are also indicated. Data shown are representative of three separate experiments demonstrating similar results.

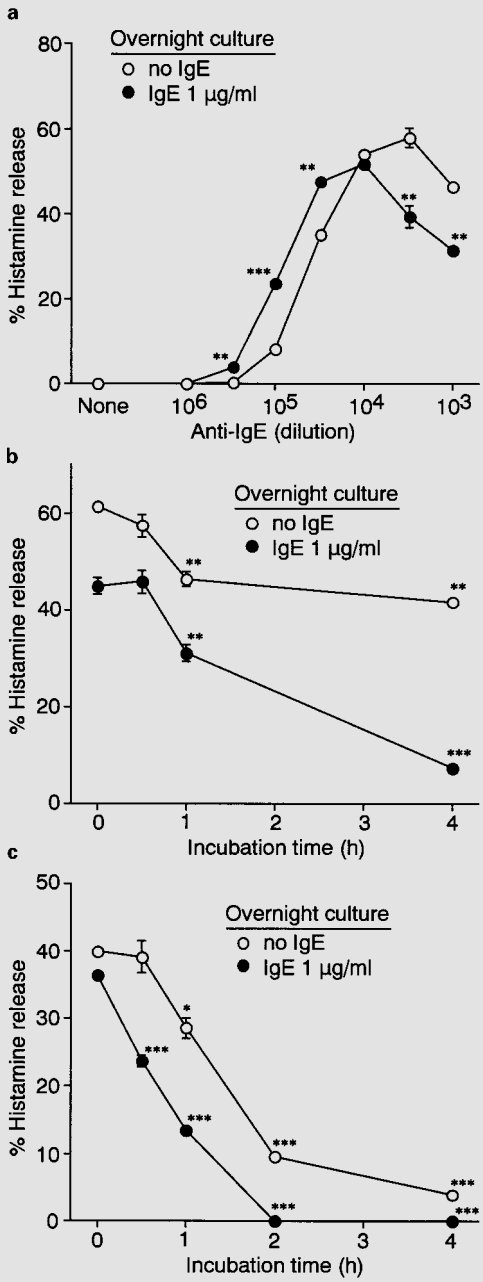

Fig. 6. Surface IgE levels on basophils affect IgE-dependent degranulation (a) and desensitization (b, c). a Basophils were cultured for 24 h with or without IgE to modify their surface IgE levels, and then stimulated with various dilutions of anti-IgE. Cultured basophils (b) as in a or cultured basophils in a separate experiment (c) were desensitized for the indicated times with subthreshold anti-IgE at 5×10^5 dilution. Cells were then stimulated with an optimal dose of anti-IgE. Data are mean ± SEM (n = 3). ** $p < 0.005$; *** $p < 0.0001$ vs. corresponding value of cells cultured without IgE (a) or corresponding release of nondesensitized cells (0 h) (b, c). The data are representatives of five separate experiments showing similar results.

The results of this study and previous reports collectively suggest that the phenomenon of desensitization could be regarded as a condition of basophils, which is probably separate from cellular activation in several ways. First, the range of concentrations of anti-IgE or antigen required for dose-dependent desensitization was completely different from that required for cell activation. Second, the subthreshold desensitization process took place much more slowly than the activation process [16, 17]. Third, basophil desensitization appeared to be a mostly reversible, stable condition, whereas cell activation consists of a transient event associated with apparent morphological changes. In addition, the required dose of anti-IgE and the time course for basophil desensitization seemed highly variable among individuals and even among experiments using cells from a single subject. This was in clear contrast to anti-IgE-induced basophil activation, which showed only minimal variation in terms of the threshold and time course of histamine release reaction among donors. In some subjects, basophil releasability declined rapidly during several hours of in vitro incubation at 37°C even without anti-IgE, thus making further experiments analyzing desensitization impossible. In addition, many of the tested subjects demonstrated obviously decreased degranulating ability after several hours of incubation without antigen, anti-IgE or goat IgG, as shown in figure 3c, presumably due to the culturing procedure itself. However, we believe that the machinery for subthreshold desensitization is intrinsic in basophils from all subjects because we have never seen any subjects whose basophil releasability remained intact and resistant to treatment with subthreshold anti-IgE. Our findings support the notion that desensitization is a unique state of basophils, which can be induced stably by IgE-cross-linking molecules.

Our results clearly indicate that complete desensitization can occur without changes in the levels of surface IgE or FcεRI, confirming the previous reports [25]. In addition, basophils with increased surface IgE-binding ability showed enhanced desensitization, suggesting that surface IgE levels are an important factor regulating desensitization. Although the responsible site(s) leading to desensitization obviously reside upstream of the degranulating machinery because A23187-induced degranulation occurred well in desensitized basophils [23], we are not sure to what extent intracellular reactions such as tyrosine kinase phosphorylation are central in desensitization, since very low concentrations of antigen or anti-IgE were sufficient for induction of stable and complete desensitization. On the other hand, several reports have addition-

ally demonstrated that activation of basophils and mast cells with optimal concentrations of antigen or anti-IgE can induce rapid desensitization [14, 32], thereby restricting the intensity of ongoing activation by a self-limiting process. A very recent report has indicated that the desensitization process occurring under optimal stimulation is dependent on the activation of src family kinases, but not of protein kinase C [33]. Although we did not examine the details of desensitization occurring under optimal challenge with a high dose of anti-IgE, it may be interesting to see whether acute desensitization induced under such potent stimulation is mediated by similar mechanism(s) to that of slow desensitization induced by subthreshold treatment.

Recently, we and others have already reported that IgE upregulates surface FcεRI expression on mast cells and basophils in vitro and in vivo, and that these cells with upregulated FcεRI expression exhibit enhanced activation to IgE-mediated stimulation [5–8, 34]. The results of the present study are in line with those reports and provide additional evidence that a relatively small (~ 50%) difference in surface FcεRI expression can be functionally important especially when cells are stimulated with a lower dose of stimuli. However, it may even be possible that the functional relevance of FcεRI upregulation may depend on the cells' environment and/or IgE-mediated stimuli, as suggested by the recent finding of MacGlashan and Schroeder [9] that basophils cultured with specific IgE for a week with IL-3 showed functional enhancement in response to antigen challenge but not to anti-IgE. Thus further studies will be necessary to elucidate what mechanisms modulate the relationship between changes in surface FcεRI expression on basophils (and mast cells) and the cellular functional consequences to various stimuli. In addition, enhancement of both immediate degranulation and late desensitization in basophils after IgE-induced increase in surface FcεRI levels (fig. 6) may enlighten a complex aspect of basophil biology.

Efficient induction of desensitization in vitro in a physiological medium containing Ca^{2+} suggests that desensitization may play a more important role in vivo than previously recognized. The results generated in this study will at least contribute to a better understanding of the mechanisms of drug desensitization, a clinical procedure known for many years. Our findings will further suggest that approaches to manipulating desensitization may be potentially important for the therapeutics of allergic diseases in which basophils play proinflammatory roles. Because recent clinical trials using nonanaphylactogenic anti-IgE antibody have confirmed that IgE is an especially important target molecule for treating allergies [35, 36], induction of desensitization may be an alternative way to block IgE-mediated downstream events. Thus, future studies analyzing the detailed mechanisms of basophil desensitization in addition to a comparison of desensitization between basophils and mast cells may allow us to address to what extent induction of desensitization is useful as a therapeutic strategy for clinical allergy.

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