

The Stripped Basophil Histamine Release Bioassay as a Tool for the Detection of Allergen-Specific IgE in Serum

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Key Words

IgE · Basophils · Histamine release bioassay ·
Radioallergosorbent assay

Abstract

Background: For the detection of allergen-specific IgE in serum, IgE-binding assays such as the radioallergosorbent assay (RAST) are commonly used. In this study, the applicability and sensitivity of the stripped basophil histamine release bioassay was investigated and compared to the RAST. **Methods:** Basophils were stripped of their IgE by an acidic buffer, sensitized by human serum and stimulated by allergen with or without interleukin (IL)-3. The histamine release was determined by fluorometric analysis. **Results:** We showed that for enhancement of the maximal histamine release and the sensitivity of the stripped basophil assay, the priming cytokine IL-3 can be added to the basophils simultaneously to the stimulus. Preincubation of the cells with IL-3, as described in other studies, was not necessary. The bioassay can be used to study the specificity of IgE-mediated reactions. Basophils sensitized by serum absorbed to a particular allergen did not respond to this allergen anymore. This method is very suitable to study cross-reactivity between allergens. The results obtained in the bioassay were comparable to

those obtained in the RAST. Using the RAST, lower concentrations of allergen-specific IgE were detected than in the bioassay. However, sera containing IgE against minor allergenic components were negative in the RAST, but strongly positive in the basophil assay. **Conclusions:** The stripped basophil histamine release bioassay is useful to complement and extend serological detection of allergen-specific IgE. Especially with sera containing IgE against minor components, this assay is more suitable than the RAST. Furthermore, in this assay, the dependency of IgE and of allergen-specific IgE in reactions can be studied in more detail.

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Introduction

To investigate IgE reactivity to histamine-releasing factor (HRF) [1, 2], our group used the stripped basophil histamine release assay [3]. In this assay, basophils from donors are stripped of their own IgE with an acidic buffer and sensitized by the IgE under investigation. By stimulating basophils with IgE-dependent HRF preparations, IgE reactivity can be investigated. The stripped basophil assay was found to be the only suitable test to detect HRF-reactive IgE. The HRF preparation could not be used in a

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skin prick test because of viral risk, since the preparation was produced using multiple cell donors. A direct basophil assay, in which basophils of patients themselves are used, was also not suitable because of the existence of non-releasable basophils in 20% of individuals [4]. The radioallergosorbent assay (RAST) could also not be used; extracts with histamine-releasing capacity were found to be negative with the RAST. The discrepancy between the RAST and the bioassay is one of the issues discussed in this paper.

The sensitivity of basophil bioassays can be increased by interleukin (IL)-3. IL-3 enhances the secretion of preformed mediators (e.g. histamine), newly synthesized lipid mediators (e.g. leukotriene C₄) and cytokines (e.g. IL-4) [5–9]. Besides upregulation, priming with IL-3 also shifts the dose-response curves of histamine release and leukotriene C₄ secretion to the left. IL-3 priming has been shown to affect the stimulation of basophils with all known IgE-dependent (e.g. anti-IgE, allergens) as well as IgE-independent agonists [e.g. f-Met-Leu-Phe, C5a (anaphylatoxin split product of complement factor C5)]. As standard procedure, basophils are preincubated with IL-3 for at least 5 min at 37°C to obtain short-term effects on the secretion of preformed and lipid mediators. For enhancement of leukotriene C₄ synthesis induced by the IgE-independent agonist C5a, preincubation of basophils with IL-3 is required [6]. To prime the histamine release in response to C5a [6] or anti-IgE [8], a preincubation period is not necessary; nevertheless, an optimal effect was reached after preincubating basophils for 5–10 min.

Because of the intensive use of the stripped basophil assay, we discovered the broad applicability of this assay. As control for sensitization of serum, in each experiment we performed, stimuli were tested, such as anti-IgE and allergens. In the current paper, these data were collected to describe how the bioassay can be used to detect allergen-specific IgE antibodies. First, the capacity of IL-3 to enhance the sensitivity of the assay was investigated. We analyzed if it was necessary to preincubate basophils with IL-3, or whether the priming effect was also obtained by adding allergen and IL-3 simultaneously to the basophils. Furthermore, it was investigated whether the results obtained in the histamine release assay were comparable with those in the RAST. Moreover, it was analyzed if the bioassay could be used to study the specificity of the IgE antibodies by specifically removing IgE from serum.

Materials and Methods

Materials

The following chemicals were used in this study: RPMI (Gibco BRL, Paisley, UK), fetal calf serum (FCS; Bodinco, Alkmaar, The Netherlands), streptokinase-streptodornase (SK-SD; Lederle Laboratories, Pearl River, N.Y., USA), Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden), HEPES (Sigma Chemicals Co., St. Louis, Mo., USA), human serum albumin (CLB, Amsterdam, The Netherlands), CNBr-activated Sepharose 4B (Pharmacia Biotech, Uppsala, Sweden), recombinant IL-3 (Pepro Tech Inc., Rocky Hill, N.J., USA) and sheep polyclonal anti-human IgE (SH25P01, CLB). *Dermatophagoides pteronyssinus* and *Dactylis glomerata* extract used in the histamine release assay were obtained from ALK (Copenhagen, Denmark). *D. glomerata* extract used for absorption studies was prepared as described previously [10]. Culture medium contained RPMI, 5% (v/v) heat-inactivated FCS, 50 µM 2-mercaptoethanol, 100 IU/ml penicillin, 100 µg/ml streptomycin, 300 µg/ml glutamine and 5.3 µl/ml SK-SD [2]. The culture medium was concentrated nine times using Amicon YM3 (3,000 MW) membranes (Amicon Inc., Beverly, Mass., USA). This preparation is referred to as culture medium FCS. Protein concentrations were determined using a BCA protein assay (Pierce, Ill., USA).

Study Subjects

Plasma samples were derived from volunteers (Haemonetics Plasma Collection System, Haemonetics Corporation, Braintree, Mass., USA), after informed consent was obtained. Plasma samples No. 163 and 151 were defibrinated by recalcification and dialyzed. Plasma sample No. 163 (total IgE 2,500 IU/ml) was positive for IgE to the common inhalant allergens house dust mite (HDM), grass pollen, birch pollen, cat dander, dog dander and *Alternaria*, as well as to bovine serum albumin (17 IU/ml). Plasma sample No. 151 (total IgE 1,600 IU/ml) was positive for IgE to the common inhalant allergens HDM, grass pollen, birch pollen, cat dander, dog dander, *Alternaria* and *Aspergillus*. Sera were obtained from atopic nonasthmatic and mild asthmatic patients as described elsewhere [11]. All subjects were allergic to *D. pteronyssinus*. The sera were tested for the presence of HRF-reactive IgE in the stripped basophil assay. Stimulation with *D. pteronyssinus* was used as control for sensitization. Sera from 38 out of 82 sera induced basophils to respond to HRF [Kleine Budde et al., unpubl. data]. These sera were tested in a second experiment for reactivity to the corresponding culture medium control in the presence of IL-3. *D. pteronyssinus* was again used as positive control, this time in the absence and the presence of IL-3. HRF-reactive plasma sample No. 151 was used as the reference plasma in these experiments.

Preparation of Basophils

Buffy coats were derived from selected donors (Haemonetics Plasma Collection System, Haemonetics Corporation), after informed consent was obtained, from the Department of Plasmapheresis of the CLB. Buffy coats were prepared from 1,000 ml of donor blood. The basophils were purified (2–5% purity) by Percoll centrifugation (1.078 g/cm³) as previously described [12, 13]. Approximately 500 tests can be performed with one buffy coat.

Indirect Histamine Release Assay

The stripped basophil histamine release bioassay was performed as described previously [2]. After washing partially purified basophils two times with 0.9% NaCl (w/v), IgE was removed from the surface

of the basophils by incubating the cells for 3.5 min with lactic acid buffer (13.4 mM lactic acid, 140 mM NaCl, 5 mM KCl, pH 3.9; 10 ml/1.5 × 10⁸ cells). Subsequently, the cells were washed with HEPES buffer [20 mM HEPES, 132 mM NaCl, 6 mM KCl, 1 mM MgSO₄, 1.2 mM K₂HPO₄, 5.5 mM glucose and 0.5% (w/v) human serum albumin, pH 7.4]. The basophils were passively sensitized [3] by incubation (37°C, 90 min) with the 'sensitization mixture' containing 150 µl of human plasma, 4 mM EDTA and 10 µg/ml heparin in a total volume of 1 ml. As a control, basophils were sensitized by serum with less than 1 IU/ml IgE. After sensitization, 2 ml of HEPES buffer was added to the cells, after which they were washed twice. Before incubation with stimuli, the cells were allowed to recover in HEPES buffer containing 1 mM CaCl₂ for 30 min at 37°C. Incubations (37°C, 60 min) were performed using 250 µl of cells (2.5 × 10⁶ cells) and 100 µl of allergen samples, diluted in HEPES buffer containing 1 mM CaCl₂. The reactions were stopped by the addition of 750 µl of ice-cold 0.9% NaCl (w/v), after which the cells were pelleted (3 min at 12,000 g), and 1 ml of supernatant was mixed with 200 µl of 12% (v/v) perchloric acid.

Direct Basophil Histamine Release Assay

For the direct histamine release assay, basophils from allergic donors were used. After partial purification, the cells were resuspended in HEPES buffer containing 1 mM CaCl₂ and stimulated by allergens (50 µl for each test). The procedure was further performed as described for the indirect bioassay.

Histamine Analysis

Histamine was determined by fluorometric analysis as described by Siraganian [14]. Histamine release was expressed as the percentage of the total amount of histamine in the cells, determined by lysis of the cells by perchloric acid. The results were not corrected for spontaneous release. A histamine release of more than 5% was considered positive.

IL-3 Experiments

To study the effect of IL-3 on the histamine release, the allergen samples were diluted in HEPES buffer-1 mM CaCl₂ containing IL-3. IL-3 was directly added to undiluted allergen samples. After addition of the basophils to the stimuli, the final concentration of IL-3 was 600 pM.

Removal of Specific IgE from Sera

Allergen samples (5 mg of protein) were coupled to 300 mg of solid phase (CNBr-activated Sepharose 4B) as described before [15]. Allergen-specific IgE antibodies were removed from serum by absorption with an equal volume of Sepharose (50 mg/ml) for 4 h while rotating. To avoid complement activation, 4 mM EDTA was added. After the incubation, the Sepharose was spun down, and the absorbed serum was collected. The centrifugation step was repeated two times. As a control, the same serum was treated with glycine-Sepharose. To compensate for the dilution of serum due to the absorption procedure, 300 µl of absorbed supernatant instead of 150 µl was used for the sensitization of basophils.

RAST

Allergen samples (25 µg of protein for each test) were coupled to Sepharose. The RAST was performed as described previously [4, 15]. After correction for nonspecific binding to the allergosorbent, binding of more than 2% of added radioactivity was considered positive.

The concentration-specific IgE was determined using mouse/human chimeric IgE directed to Der p 2 (detection level 0.1 IU/ml) [16].

Statistics

Correlations were analyzed by the Pearson test. After logit transformation of histamine release, i.e. $\log[HR/(H_{r_{max}} - HR)]$, where HR stands for histamine release, linear regression was performed between histamine release and IgE to HDM. To investigate the effect of total IgE on the stripped basophil release assay, the obtained parameters were used to calculate the predicted histamine release. The influence of total IgE was tested by correlating the ratio of observed to predicted histamine release with the total IgE.

Results

The Stripped Basophil Histamine Bioassay

Before the stripped basophil assay was used for detection of IgE, we investigated whether every donor could be used for the stripped basophil assay. To study this, 40 basophil donors were tested. Stripped cells were sensitized by plasma sample No. 163 and stimulated by *D. pteronyssinus* protein (1.2 µg/ml) and IgE-dependent HRF (4 mg/ml). Stimulation of basophils from 16 donors induced a histamine release of more than 40% in response to *D. pteronyssinus* and/or HRF, basophils of 17 donors showed an average response (histamine release 20–40%) and 7 were nonreleasers (histamine release 0–20%). Basophils sensitized by serum from non-atopic subjects (n = 17) did not release histamine in response to allergen or HRF. In our experiments, basophil donors were used whose basophils had a histamine release of more than 40% in our preselection to ensure a high sensitivity and avoid interdonor variation.

After the stripping procedure, on average, 1.0–1.5 × 10⁹ cells were obtained (~2–5% basophils). In one incubation, 5.0–12.5 × 10³ basophils are stimulated. If reactivity to one allergen preparation is tested, approximately 50 sera can be tested in one experiment (10 stimuli: 1 diluent, 3 concentrations of anti-IgE, 5 concentrations of allergen and 1 IgE-independent stimulus, e.g. f-Met-Leu-Phe). In total, it is possible to measure approximately 500 incubations.

Preincubation and Simultaneous Addition of IL-3

We investigated whether preincubation of basophils with IL-3 was required to obtain enhancement of the allergen histamine release. Basophils from a *D. pteronyssinus*-allergic donor (RAST 3 IU/ml) were stimulated by HDM *D. pteronyssinus* protein. The histamine release from basophils preincubated with 600 and 120 pM IL-3 for 10 min at 37°C was compared to the histamine release

from basophils directly stimulated with allergen samples diluted in IL-3. In figure 1a, it is shown that priming by IL-3 is more pronounced in the case of simultaneous addition of IL-3 and allergens to basophils. It must be emphasized that the final concentrations of IL-3 during the allergen incubations were not the same in both conditions: after preincubation with 600 and 120 pM IL-3, the cells were diluted in the stimuli, resulting in a concentration of 500 and 100 pM IL-3, respectively.

In the stripped basophil bioassay, the same enhancing effect of IL-3 was found as in the direct assay. Stripped basophils were sensitized by serum from atopic subjects and stimulated by HDM protein with or without IL-3. Again, it was demonstrated that subthreshold concentrations of allergen induced histamine release if IL-3 was simultaneously added to the basophils (fig. 1b).

Comparison of Basophil Assay and RAST

To compare the basophil assay with the RAST, basophils were sensitized by serum from atopic subjects and stimulated by 540 ng/ml *D. pteronyssinus* protein. Moreover, the concentration of HDM-specific IgE in these sera was determined using the RAST. In figure 2a–f, six independent experiments with different donors and sera are shown. The correlation between the histamine release in the bioassay and the RAST obtained with same batch of HDM is depicted. The minimal concentration of specific IgE that could be detected (histamine release >5%) in the bioassay was 2.7 IU/ml IgE to HDM (ranging from 1.2 to 4.2 IU/ml).

Thirty-three sera were tested in a second experiment in the absence and the presence of IL-3. The relationship between the histamine release with or without IL-3 and the concentration of IgE against HDM is shown in figure 2g. The detection limit was higher than in the six other experiments shown: a histamine release of >5% could be induced with 9 IU/ml IgE to HDM in the absence of IL-3, and with 2.6 IU/ml in the presence of IL-3.

Influence of Total IgE

To determine whether the sensitivity of the bioassay was dependent on the total IgE in tested sera, the histamine release with or without IL-3 (fig. 2g) was predicted on basis of IgE to *D. pteronyssinus* by linear regression. If total IgE influenced the results of the histamine release assay, then at high IgE concentrations, the ratio of observed to predicted histamine release would be lower than at low IgE concentrations. No correlation between the ratio of observed to predicted histamine release and total IgE of sera was found (fig. 2h).

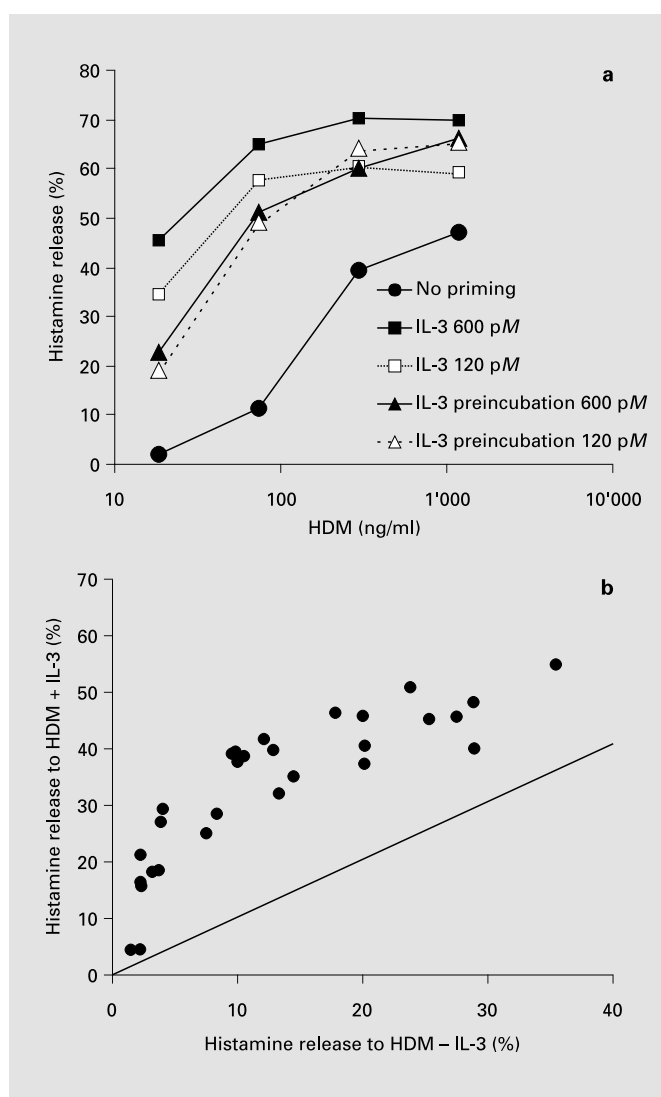


Fig. 1. Priming of allergen-induced histamine release of basophils by IL-3. **a** The priming capacity of IL-3 with and without preincubation of basophils. Basophils from an HDM-allergic donor were either preincubated with 0, 120 or 600 pM IL-3 for 10 min at 37 °C, or IL-3 was added to the cells simultaneously with the allergens. **b** Effect of IL-3 on the histamine release in the stripped basophil bioassay. The histamine release of basophils sensitized by serum from 29 donors in response to 540 ng/ml HDM protein was compared to that if IL-3 (600 pM) was simultaneously added to the cells. The histamine release of stripped basophils was less than 2%. Spontaneous release with and without IL-3 was below 3%.

Stripped Basophil Assay Is More Sensitive than RAST

In some cases, the histamine release bioassay was found to be more sensitive than the RAST. Sera from two allergic subjects were negative on RAST to culture medium FCS and to the individual components (FCS, SK-

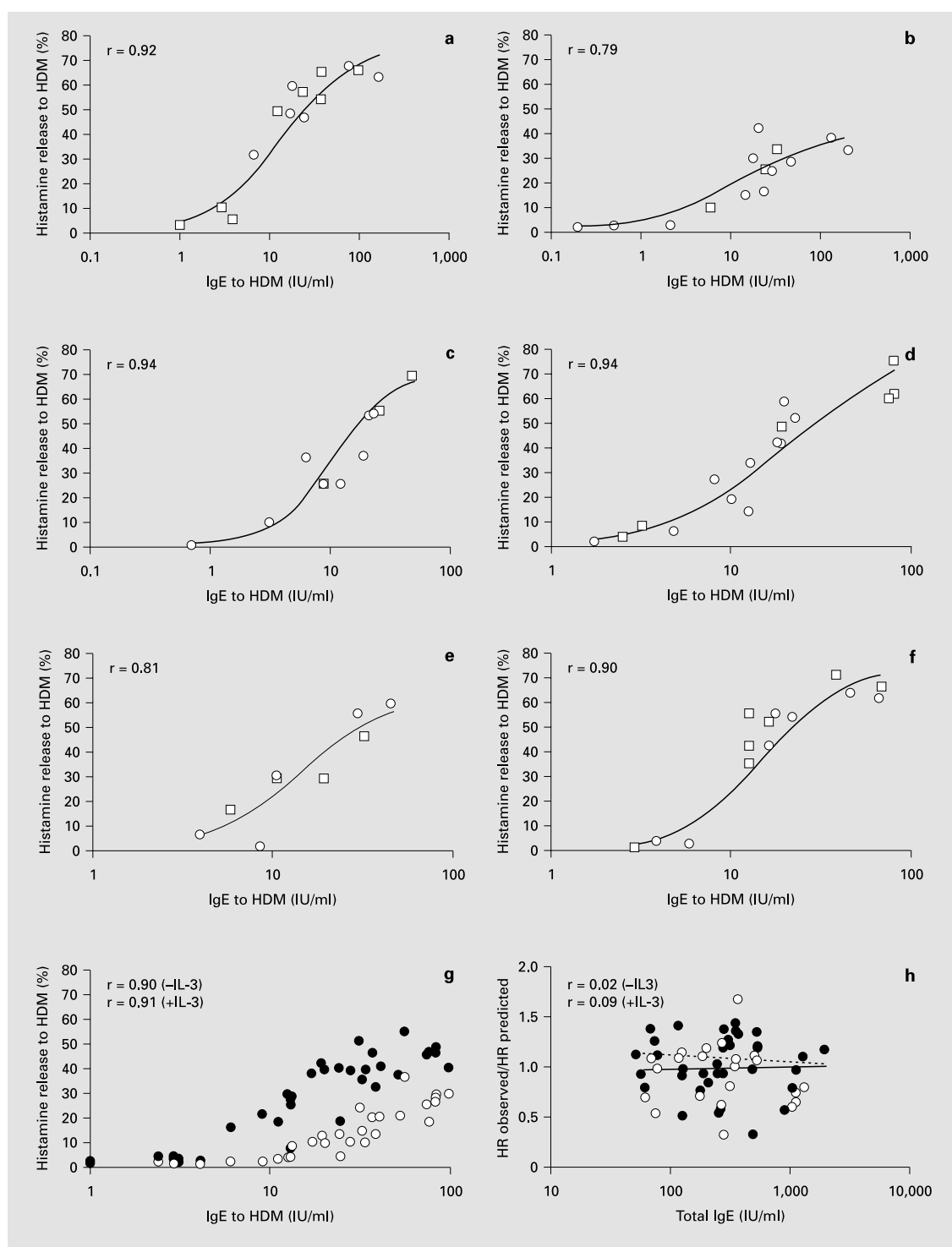


Fig. 2. Comparison between the stripped basophil histamine release assay and RAST. **a–f** The relationship between histamine release in response to HDM versus HDM-specific IgE in six independent experiments using different basophil donors and sera is shown. The histamine release of basophils sensitized by reference plasma No. 151 in response to HDM was 51, 18, 39, 44, 39 and 53%, respectively. **g** Thirty-three sera, indicated by squares in **a–f**, were tested in a

second histamine release experiment in the absence (○) and presence (●) of 600 pM IL-3. **h** The correlation between total serum IgE and the ratio of observed to predicted histamine release (HR) was determined in the absence (○) and presence (●) of IL-3. The histamine release of stripped basophils was less than 2%. Spontaneous release with and without IL-3 was below 3% in all experiments.

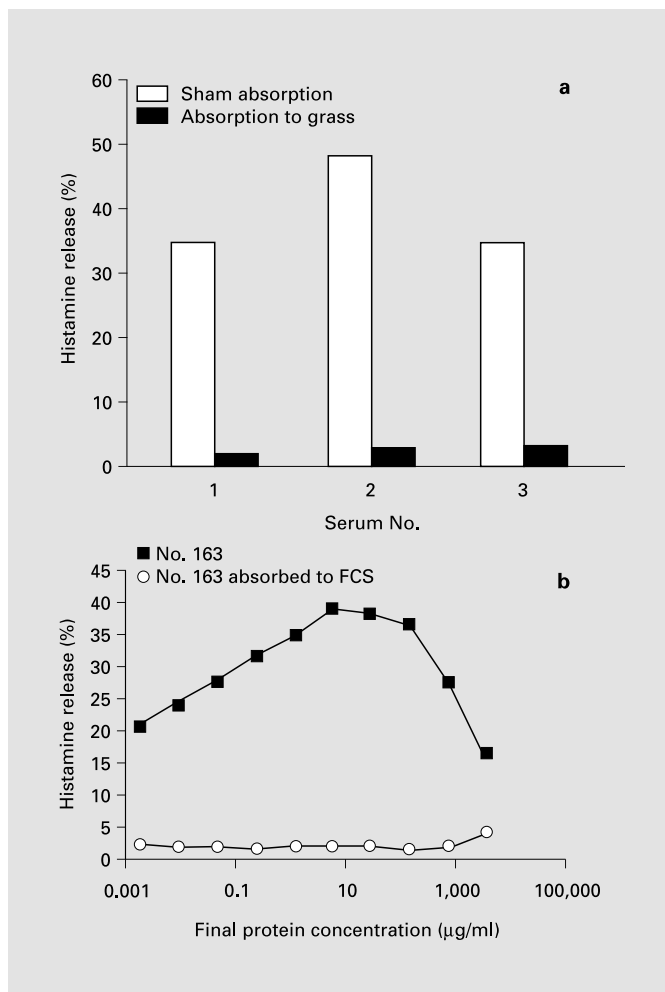


Fig. 3. Specificity of allergen-induced histamine release. **a** IgE to grass pollen was removed from three sera by incubating them with grass pollen coupled to solid phase. The histamine release of stripped basophils sensitized by absorbed sera in response to 1.3 μg/ml grass pollen protein was compared to a control absorption to glycine-Sepharose. **b** Dose-response analysis of basophils sensitized by depleted serum. IgE against FCS was removed from plasma sample No. 163 using the absorption method. Stripped basophils sensitized by absorbed serum were stimulated by different concentrations of culture medium and compared to basophils sensitized by plasma sample No. 163. Spontaneous release as well as release of stripped basophils was below 2%.

SD, penicillin, streptomycin), whereas in the histamine release bioassay, a positive IgE-dependent response was found. The histamine release of basophils sensitized by these sera in response to 3.6 and 0.7 mg/ml culture medium FCS was 48 and 14%, respectively, in the case of serum 1, and 33 and 5%, respectively, in the case of serum 2.

Specificity of the Stripped Basophil Assay

To analyze the applicability of the stripped basophil assay for the allergen-specific IgE, it was determined if IgE reactivity could be specifically abolished. This procedure would also be very suitable to study cross-reactivity between allergens. IgE molecules against grass pollen were removed from sera, then stripped basophils were sensitized by the absorbed sera and stimulated by grass pollen extract. The histamine release was compared to that from sera absorbed to inactivated control Sepharose. In figure 3a, it is shown that for three sera, the histamine release to grass pollen was totally inhibited by the removal of specific IgE. Absorption did not significantly change the histamine release to anti-IgE. Histamine release in response to 100 ng/ml anti-IgE after absorption to control and grass pollen was 13 and 16%, respectively, for serum 1 (RAST score >15 IU/ml), 12 and 10%, respectively, for serum 2 (RAST score >15 IU/ml) and 14 and 10%, respectively, for serum 3 (RAST score >35 IU/ml). The experiment was repeated using another basophil donor, and similar results were obtained.

Removal of IgE against a particular allergen might not be complete, and observed effects might just be caused by a shifting of the dose-response curves. To study this, plasma sample No. 163 was absorbed to FCS-Sepharose. After sensitization of basophils with the absorbed serum, the cells were stimulated by different concentrations of culture medium FCS. The histamine release in response to FCS was totally abolished over the total range of allergen concentrations (fig. 3b). Priming the response with IL-3 also did not result in a histamine release by culture medium FCS.

However, sera positive in the histamine release bioassay, but without detectable IgE antibodies to a particular allergen in the RAST, were difficult to absorb. Removal of IgE to the allergen was not possible, because the binding to Sepharose was too low. Absorbing sera from the above-described sera 1 and 2 to FCS did not change the histamine release in response to culture medium. The histamine release in response to 3.6 mg/ml RPMI-FCS of sera absorbed to control and FCS, respectively, was 48 and 44% (serum 1) and 33 and 31% (serum 2).

Discussion

The applicability of the histamine release bioassay for the serological detection of IgE antibodies and allergens was discussed in this paper. We confirmed that the sensitivity of both the direct and the stripped basophil bioassay

could be increased by the cytokine IL-3. The enhancing effect of IL-3 on the maximal histamine release induced by allergens as well as on the sensitivity of basophil assays has been described before [5, 17]. In most previous studies, the basophils were preincubated with IL-3 for 5–10 min before the stimulus was added [5]. We showed that the priming effect was also obtained by adding the allergen and IL-3 to the basophils simultaneously. Allergen samples were diluted in IL-3-containing buffer, which makes the bioassay less laborious. Since IL-3 itself does not induce histamine release at the concentration used in this study (600 pM), the addition of this concentration of IL-3 as a standard procedure is recommended to ensure a high sensitivity of the assay.

In this study, we also showed that the bioassay could be used to analyze IgE-mediated reactions in more detail. Allergen-specific IgE can be removed from sera by incubating with allergen bound to a solid phase. The removal of IgE was specific and complete, and even in the presence of IL-3 and with a full dose-response analysis, no histamine release was induced. Using this absorption procedure, cross-reactivity between allergens can be studied. For instance, cross-reactivity between latex and grass pollen [18] could be demonstrated using this method. Cross-reactivity between allergens might be studied by sensitizing basophils with serum depleted of IgE to a particular allergen and analyzing the histamine release in response to another allergen. If two allergens are cross-reactive, basophils sensitized by the depleted serum will also not respond to the cross-reactive allergen anymore. However, with sera negative in the RAST but positive in the bioassay, the batch-wise absorption method was not effective. In this case, the absorption might be performed with more absorbent than in RAST; using an allergen Sepharose column, IgE against that allergen is most likely removed. The specificity of this absorption procedure should be checked carefully, particularly with large amounts of absorbent.

Total IgE might influence the sensitization of basophils by competition with specific IgE for the receptor. We did not find a relationship between the total IgE and the ratio of observed to predicted histamine release. If total IgE had an effect on the sensitivity of the bioassay, the ratio of observed to predicted histamine release should decrease with increasing total IgE concentrations. The absence of the interference of total IgE might be caused by the fact that the amount of serum used in our experiments did not saturate the basophils [19].

Usually, IgE-binding assays such as the RAST are used for the detection of specific IgE in serum. We showed that the results obtained in the RAST and in the stripped his-

tamine release assay were in general well correlated. The detection limit in our RAST is 0.1 IU/ml IgE [16]. In the stripped bioassay, we showed that in the absence of IL-3, 2.7 IU/ml IgE (ranging from 1.2 to 4.2 IU/ml) to HDM was the lower detection limit. In another experiment with less responsive basophils, it was shown that the presence of IL-3 lowers the detection limit for IgE, in this case from 9 to 2.6 IU/ml. However, in our experiments, only one concentration of allergen was used, which might not induce the maximal histamine release for each serum. Comparing the two assays, the RAST is in general more sensitive than the basophil assay.

In some cases, the basophil assay was more sensitive than the RAST. We described in this study two sera that were negative in RAST to the individual components of the culture medium FCS. In the histamine release bioassay, in contrast, a positive IgE-dependent reaction was found even in the absence of IL-3. IgE molecules might have escaped detection in RAST either because they were present in very low concentrations in serum or directed against minor allergenic components. Bovine serum albumin, the main component of the culture medium, might interfere with the binding of a minor component to the solid phase. Discrepancies between RAST and histamine release tests have been described before. In 66% of allergic patients selected for a positive intracutaneous skin test and a negative RAST, the histamine release test was positive [4]. That the reaction was indeed IgE dependent was proved by the fact that purified IgE antibodies were positive in the RAST.

The major advantage of basophil assays is that the biological activity of IgE is determined. Basophils are stimulated by allergen, and as a consequence of cross-linking of IgE molecules by allergen, mediators are released. The histamine release can be analyzed [14], or alternatively, the leukotriene release [20]. A RAST gives no information about the biological activity of IgE. For instance, IgE molecules directed to cross-reactive carbohydrate determinants have poor biological activity [21]. Thus, in a RAST, this will lead to false-positive results. On the other hand, in direct basophil bioassays, false-negative results are obtained by the existence of so-called 'nonreleasers' [22]. Approximately 20% of patients are 'nonreleasers', i.e. despite a positive skin prick test and RAST, no histamine release can be induced from basophils by allergen or anti-IgE [22]. It has been shown that basophils from such 'nonreleasers' have a defect in the signaling pathway [23, 24]. Normal levels of IgE receptors are present on the basophils, but after IgE cross-linking, no extracellular calcium influx or protein kinase C activation are induced. Using

the stripped basophil release assay, problems with nonreleasing basophils can be circumvented. In this assay, basophils from nonallergic donors are used which are selected for their capability to release histamine. These basophils are stripped of their own IgE with an acidic buffer and sensitized by the patient serum to be investigated. By stimulating basophils with an allergen, IgE reactivity can be investigated. By using a reference serum, interdonor variation is avoided and individual tests can be directly compared with each other.

A disadvantage of the stripped basophil histamine assay compared to the RAST is the fact that a basophil donor is required. Furthermore, the bioassay is more laborious than the RAST. To investigate 500 data points (50 sera, 10 stimuli/sera), it would take 9–10 h to perform the basophil experiment. The time needed for histamine release analysis depends on the method used. In our study, the histamine release was determined by fluorometric analysis. It is possible to measure 15 histamine samples in 1 h; thus, it would take 5–6 days to measure all incubations. However, serological analysis using a RAST takes only 4–8 h. However, the stripped basophil assay takes less time than direct basophil assays, since only one basophil experiment needs to be performed instead of with all patients individually. In one stripped basophil experiment, approximately 50 sera can be tested. There is no need for the patients themselves to be present, which is another advantage over the direct bioassay. Additionally, comparison between the IgE reactivity of different patients is independent of basophil reactivity.

Another advantage of the stripped histamine release assay over RAST, skin prick tests and direct basophil assays is that the involvement of IgE in allergen-induced

responses can be studied in various ways. If histamine release is induced from basophils sensitized by an IgE-deficient serum, the reaction is IgE independent. The response might, for instance, be mediated by chemokines like MCP-1 [25]. Alternatively, IgE dependency can also be analyzed by heat inactivation of serum before sensitization of basophils [13]. Another possibility is to preincubate the serum to be investigated by blocking anti-IgE antibodies [13]. Moreover, as shown in the current study, dependency on allergen-specific IgE can be investigated by specifically removing these IgE antibodies.

Overall, it can be concluded that the stripped histamine release bioassay is not suitable as a routine diagnostic test for allergy, since it is too laborious. However, the assay is very useful to complement and extend allergy diagnostics. The bioassay should be used especially if there is a discrepancy between the allergic symptoms of patients and the results obtained by RAST. The sensitivity of the assay is enhanced by adding the cytokine IL-3 directly to the allergen samples. Moreover, the stripped basophil assay is very suitable to study the involvement of IgE and allergen-specific IgE in allergic reactions. In this bioassay, cross-reactivity between allergens can be studied by the removal of allergen-specific IgE.

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