

# Brown Norway Rat Ovalbumin-Specific Immunoglobulin E Antibodies Increase the Human Basophil Expression of CD63 Marker

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## Abstract

Anaphylactic shock is an immunoglobulin E (IgE)-dependent hypersensitivity. Biological tests like leucocyte histamine release (LHR) and human basophil activation (HBA), frequently used in human allergy, reflect both the amount of IgE fixed on cells and the cellular reactivity. To assess whether serum-specific IgE from Brown Norway (BN) rats prepared for ovalbumin (OVA)-induced anaphylactic shocks can activate human basophils which has a potential interest in experimental allergy: such a test could rapidly assert an IgE sensitization in laboratory animals genetically T-helper 2 (Th2)-predisposed. Rats ( $n = 39$ ) were immunized three times (day 0, day 5 and day 21) with OVA injected subcutaneously. One week after the third immunization, a shock was induced with an intravenous (i.v.) bolus of OVA. Sensitization was assessed by passive cutaneous anaphylaxis (PCA) test and dosages of serum IgE antibodies anti-OVA by enzyme-linked immunosorbent assay. Blood basophils were counted before and during the shock. Before the shock induction (at day 21), an LHR test was performed on rat blood, and human basophils were sensitized with rat sera. HBA was demonstrated by the increase in the percentage of cells expressing CD63 antigen membrane, measured by flow cytometry. Twenty-one days after the first subcutaneous (s.c.) immunization, the rat serum induced a significant HBA. HBA was observed neither with the same serum previously heated nor with the serum from nonimmunized rats (NIRs). OVA-specific IgEs were significantly increased in immunized rat (IR) serum. The PCA test was negative when the serum was previously heated ( $56^{\circ}\text{C}$ ). We never observed any circulating basophils, and LHR test was negative. After OVA i.v. administration, all IRs died rapidly. HBA testing strongly suggests a mediation by specific IgE in the increase of CD63 in BN rats. Thus, HBA test seems useful in assessing whether an experimental allergy was induced in animals genetically predisposed to an immune response, Th2-mediated, like BN rat. We also conclude that rat basophil activation does not participate in the histamine release during anaphylactic shock in sensitized BN rats.

## Introduction

Allergy is a complex disease whose prevalence has dramatically increased in the recent years [1]. The growing incidence of food allergen exposure, resulting in food-induced anaphylaxis, is now a major medical issue [1]. Anaphylactic shock, the generalized form of immunoglobulin E (IgE) hypersensitivity, is characterized by complex

cardiovascular disorders including severe arterial hypotension [2]. Release of histamine and other inflammatory mediators from tissue mast cells and blood basophils, the primary event in anaphylactic reaction, is triggered by the aggregation of IgE bound to high-affinity receptors. This degranulation process can be measured by biological cellular tests like leucocyte histamine release (LHR) [3] or

basophil degranulation [4]. Human basophil sensitization with rodent IgE leads to histamine release, as demonstrated by Conrad *et al.* [5]. Recently, in human allergy, an increase of CD63 expression on basophils after *in vitro* allergen challenge was reported [6–10]. CD63 is located on the basophilic granule membranes in resting basophils, mast cells and platelets. Activation of basophils or mast cells induces the fusion of cytoplasmic granules with the plasma membrane and the release of mediators such as histamine. The increase of CD63 on cell surface after FCεRI cross-linking was detected by flow cytometry with anti-CD63 monoclonal antibodies [8] and seems to be sensitive and specific to basophil activation because of allergen-specific IgE.

Brown Norway (BN) rats are high IgE-responder strain and, up to a degree, resemble atopic humans in their predisposition to react to antigens with an overproduction of IgE [11–15]. The production of IgE in this strain of rat is a result of a mechanism triggered by T-helper 2 (Th2) activation [16–18]. The aim of our study was to investigate if specific IgE from BN rats prepared for ovalbumin (OVA)-induced anaphylactic shocks can activate human basophils which have a potential interest in experimental allergy. This test could be helpful in laboratory investigations by allowing an early assessment of an IgE sensitization.

## Materials and methods

**Animals.** Four-week-old BN rats (223–330 g) (Janvier, Genest St Isle, France) were housed individually in a temperature- and light-controlled environment (14 h of light, 10 h of darkness) with *ad libitum* access to tap water and were given egg- or derivatives of egg component-free diet (UAR, Epinay sur Orge, France). All animals were acclimatized to these conditions for 1 week prior to the experiment. All experiments were conducted according to the Recommendations from the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals.

**Immunization protocol and sensitization assessment.** OVA (Sigma, Saint Quentin Fallavier, France) was dissolved in a suspension of aluminium hydroxide (Al(OH)<sub>3</sub>) (Sigma). The suspension (1 mg of OVA + 3.5 mg of Al(OH)<sub>3</sub> in 1 ml of 0.9% NaCl) was injected subcutaneously under the shoulder blades for the immunized group (IRs, *n* = 30) on day 0, 5 and 21. Nonimmunized rats (NIRs, *n* = 28) received only Al(OH)<sub>3</sub>. Passive cutaneous anaphylaxis (PCA) was induced on day 26, as previously described by Ovary [19] on guinea pig and Knippels *et al.* [11] on rat. Untreated BN rats (*n* = 4) were shaved on the back and flanks and injected intradermally with 0.1 ml of IR serum in serial dilutions (1/2, 1/4, 1/8, 1/16, 1/32 and 1/64), followed 48 h later by an intravenous (i.v.) injection of 1 ml of a mixture of OVA (1 mg/ml, Sigma) and Evans blue solutions (2% in sterile saline, Sigma). After 30 min, the animals were examined for positive responses. The

diameter of dye extravasation at the site of the serum injection was measured. The reaginic level was read as the reciprocal of the highest dilution giving a coloured spot of at least 5 mm in diameter. We performed PCA with serum of IRs (*n* = 4), serum of NIRs (*n* = 4) and with serum of IRs (*n* = 4), which was previously heated at 56 °C.

**Assays for anti-OVA-specific IgE.** Rat sera were tested for OVA-specific IgE by enzyme-linked immunosorbent assay (ELISA). For the detection of OVA-specific IgE, 96-well microtitre plates were coated overnight at 4 °C with 100 µl/well of mouse antirat IgE (MARE-1, Zymed, San Francisco, CA, USA) at a concentration of 1.5 µg/ml in carbonate buffer, pH 9.6. The plates were washed, and 100 µl/well of phosphate-buffered saline (PBS)/bovine serum albumin (BSA)–Tween 20 was added. After incubation for 1 h at 37 °C, the plates were washed, and diluted rat serum samples were added and incubated for two more hours at 37 °C. The plates were washed and subsequently, 100 µl/well of an 1 µg/ml solution of an OVA–digoxigenin (DIG) conjugate was added. The DIG was obtained from Boehringer (Mannheim, Germany), and a coupling to OVA was performed according to the manufacturer's instructions. The labelled OVA was separated on a sephadex G-25 column (Boehringer), and labelling efficiency was determined spectrophotometrically at 280 nm. Incubation with OVA–DIG was performed for 1 h at 37 °C. After washing, 100 µl/well of peroxidase conjugated sheep anti-DIG Fab fragments (Boehringer) diluted 1:3000 in PBS/BSA–Tween 20 was added. After incubation (1 h at 37 °C), the plates were washed again, and an enzyme substrate solution of tetramethylbenzidine (100 µl/well; 6 mg/ml of dimethyl sulphoxide, Sigma Chemicals Co., St Louis, MO, USA) was added. The plates were then developed at room temperature for 5–15 min. Finally, 100 µl/well of 2N H<sub>2</sub>SO<sub>4</sub> was added. Optical densities were read spectrophotometrically at 450 nm with an ELISA plate reader (Microplate Reader, Bio-Rad Laboratories, Richmond, CA, USA). A presera pool was used as a negative control. The pooled presera was measured at a 1:4 dilution. The average extinction in negative control wells, to which three times the standard deviation was added, provided the reference value taken for the determination of the titre in the sera. Each test serum was titrated starting at a 1:4 dilution to 1:512, and the reciprocal of the furthest serum dilution giving an extinction higher than the reference value was read as the titre. All analyses were performed in duplicate. The serum pool derived from the intraperitoneally (i.p.) Knippels immunized animals [12] was used as a positive control sample. Positive and negative control samples were incorporated for each 96-well plate.

**LHR test.** Blood (1 ml) was collected through the tail vein in eight IRs and six NIRs on D<sub>21</sub>, following immunization in accordance with the literature [20].

Histamine radioimmunologic assay (RIA) was performed using the method of Immunotech (Luminy, France, sensitivity 0.2 nM with a negligible cross-reaction with histamine metabolites and histidine). Fifty microlitres of whole blood was needed for the quantification of total histamine. Cell lysis was achieved by dilution of 1/20 in water and freeze-thawing. After dilution to 1/4 in the *N'*-2-hydroxyethylpiperazine-*N'*-2-ethane sulphonic acid-Tyrodé's buffer, the remaining blood was tested for histamine release in the presence of OVA allergen responsible for the shock. About 200 µl of the diluted blood was added to 100 µl of five dilutions of OVA, ranging from  $10^{-1}$  to  $10^{-6}$ . The samples were incubated for 30 min at 37 °C. The reaction was stopped by cooling the tubes down to 4 °C. After centrifugation at  $1000 \times g$  for 10 min at 4 °C, the supernatant was recovered and assayed for histamine. The histamine RIA (Immunotech) is based on the competition between acylated histamine for binding to monoclonal antibody-coated tubes. The radioactivity was counted in a gamma counter (Cobra II auto gamma, Packard, Canberra Compagny, Meriden, CT, USA) during 1 min. Results of LHR tests ( $H_i$ ) were expressed as a percentage of total histamine content ( $H_t$ ) of the sample after correction for spontaneous release ( $H_0$ ) from control tubes, using the following formula: histamine release  $H_R = [(H_i - H_0)/(H_t - H_0)] \times 100$ . The test was considered positive when two successive dilutions of the allergen induced a histamine release in excess of 5%.

*Sensitization of human basophils with BN rat serum.* A human leucocyte suspension, placed in 5 ml polystyrene tubes containing heparin (5 IU/ml) and ethylenediamine-tetraacetic acid (EDTA) (5 mM) from at least five donors with a proven basophil response to anti-IgE, was passively sensitized by rat serums and tested, as previously described [21, 22]. More specifically, the leucocyte suspension was centrifuged ( $500 \times g$ , 10 min) and washed twice with a HEPES calcium buffer (HCB) (HEPES 10 mM, NaCl 130 mM, KCl 5.6 mM, CaCl 5 mM, MgCl 4 mM, EDTA Na<sub>3</sub> 0.5 mM, pH 7.4) after the dissociation of IgE fixed on the receptors of basophils with acid pH (3.9). Cell pellets were pooled and incubated for 3 min at pH 3.7 in a lactic acid buffer (lactic acid 10 mM, NaCl 130 mM, KCl 5 mM, pH 3.7) and again washed twice with HCB. A centrifugation ( $500 \times g$ ) was performed for 5 min. The supernatant was discarded, and equal volumes of the washed leucocyte suspension and 2 ng/ml of interleukin-3 (IL-3) diluted in HCB were mixed and incubated for 15 min at 37 °C. This addition of IL-3 to the incubation medium serves as a preactivation or priming agent of human basophils and increases the sensitivity to agonists [23]. One hundred microlitres of human leucocyte suspension and 100 µl of rat serum were incubated for 90 min at 37 °C. Passively sensitized basophils were then ready for use.

*Human basophil activation test.* Prepared sensitized cells were washed once with HCB. About 20 µl of these cells was mixed with three dilutions of OVA (1, 0.2 and

0.04 µg/ml) in plate wells. After incubation at 37 °C for 30 min, 150 µl of HCB was added, and the plates were centrifuged ( $500 \times g$ , 10 min, 4 °C). Following the allergen stimulation step and removal of 150 µl of the supernatant for histamine dosage, 40 µl of HCB was added to the cell pellets, and the plates were centrifuged ( $500 \times g$ ) for 5 min at 4 °C. About 40 µl of supernatants was discarded, and 20 µl of cell suspensions was mixed with 50 µl of the two diluted polyclonal antibodies: fluorescein isothiocyanate (FITC)-conjugated antihuman IgE (dilution 1/8 of a 0.5 mg/l solution, Caltag, H15701, Leperray en Yvelines, France) and phycoerythrin (PE)-conjugated antihuman CD63 (Immunotech, 1914).

After the suspension was incubated for 20 min at room temperature, the remaining erythrocytes were lysed by a 3 min incubation with a lysing buffer (1 ml of whole blood lysing solution, Ortho Diagnostics, Mahwah, NJ, USA), and 250 µl of formol was added to preserve the cells. After 3 min at room temperature, 100 µl of HCB was added, and plates were centrifuged ( $500 \times g$ ) for 5 min. Leucocytes were finally diluted in 250 µl of HCB and analysed by flow cytometry using a bi-colour flow cytometer (Cytoron absolute, Ortho diagnostics). Basophils were gated in the lymphocyte region of the side scatter region, which relates to the cell's granularity and is fluorescent. Percentage of basophil activation (CD63-positive, anti-human IgE FITC bright cells/antihuman IgE FITC bright cells  $\times 100$ ) was calculated by the cytometer. The test was considered positive when two successive dilutions of the allergen induced an activation of at least 5% in excess of what was observed in the controls. This test was performed on D<sub>0</sub>, D<sub>5</sub> and D<sub>21</sub> in six NIRs and eight IRs, and on D<sub>0</sub>, D<sub>7</sub>, D<sub>21</sub> and D<sub>26</sub> for one NIR and one IR. We performed human basophil activation (HBA) test with serum preheated at 56 °C from four IRs on D<sub>21</sub>. After CD63 expression assessment, we studied human basophil histamine release to demonstrate the presence of OVA-specific antibodies in rat sera. Supernatants (40 µl) were stored at -70 °C for histamine analysis (Immunotech, RIA).

*Specificity of anti-IgE and CD63 labelling.* To determine the phenotype of anti-IgE-positive populations, the suspensions were labelled with polyclonal FITC-conjugated antihuman IgE and a second antibody: anti-CD14 PE (which is expressed on cells of the monocyte/macrophage lineage), anti-CD19 PE (which is expressed on all normal B cells) or anti-CD45 PE (which is expressed on all human leucocytes). The specificity of the PE-conjugated antihuman CD63 labelling was examined by the activation of cell suspensions with anti-IgE and incubated with PE-conjugated antihuman CD63 antibody in the presence of anti-CD14 PE or anti-CD19 PE.

*Protocol of anaphylactic shock.* At least 1 week after the last immunization, rats were anaesthetized with sodium thiopental (Rhône-Poulenc-Mérieux, Lyon, France) (62.5 mg/kg i.p.) and artificially ventilated with a constant

volume through an endotracheal tube. Catheters (PE10 tubing, Becton Dickinson, Mountain View, CA, USA) were inserted in the jugular vein and carotid artery. The carotid catheter was connected to a probe (5266-137-1F, Ohmeda, Singapore), which was used to record mean arterial blood pressure (MABP) and heart rate (HR) on an oscillograph (Hewlett Packard, Orsay, France). Once the baseline values were established, 1 ml of OVA (1 mg) was given intravenously to all rats through the jugular catheter. The hemodynamic parameters were measured at 1, 3, 5, 15, 30, 60, 120 min after the challenge dose of OVA.

Blood samples for histamine dosages were collected before OVA injection and at 1, 5 and 15 min intervals thereafter. The samples were placed in tubes containing EDTA and were cooled on ice bath. Within 2 min of drawing, they were centrifuged (500 *g*) for 15 min at 4°C. The plasma was then removed and stored at -70°C for histamine analysis (Immunotech, RIA, sensitivity 0.2 nM with negligible cross-reaction with histamine metabolites and histidine). An autopsy was performed to verify that catheter insertion was correct.

**Cytologic study.** The determination of the blood leucocyte differential including basophil and platelet counts was performed using an automatized analyser (Technicon, H2 type, Bayer, Munich, Germany) and was controlled by a microscopic counting of the blood smears after staining with May Grünwald Giemsa and toluidine blue in four NIRs and four IRs. This study was performed 21 days after the first immunization. The microscopic counting was also realized prior and during the shock: blood samples were taken before i.v. injection of OVA and at intervals of 1, 5 and 15 min after the injection.

**Statistics.** Statistical analyses were performed with an SAS software (Chicago, IL, USA). Data are presented as mean  $\pm$  standard error of the mean (SEM) in both IR and NIR groups. To compare the variability between the two groups at each time, a two-way (group and time) analysis of variance (ANOVA) and ANOVA with repeated measures were applied. The Bonferroni procedure was used to determine the significance at a  $P < 0.05$  level for hemodynamic parameters.

## Results

### PCA test with and without previously heated serum

The PCA test was positive with IR serum ( $n=4$ ): the reaction was noted for 1/64 dilution of serum, and a circular blue spot (over 5 mm) appeared 30 min after the challenge. This reaction was not observed with NIR serum ( $n=4$ ) or with previously heated serum from IRs ( $n=4$ ).

### OVA-specific IgE response

Pre-study blood samples for each animal were tested for OVA-specific IgE. No anti-OVA IgEs were detected in

NIRs. These sera were pooled and used as negative control in the ELISA test. OVA-specific IgEs were present in the sera pooled from the Knippels-positive control animals (17). This pool serum was subsequently used as positive control in the ELISA's test. Rats exposed to OVA subcutaneously on day 0, day 5 and day 21 developed OVA-specific IgE responses. The results are presented in Fig. 1 and show that OVA-specific IgEs were present in the serum of all animals on day 21. OVA-specific IgEs were detected on day 0 in four out of 30 animals and on day 5 in three out of 30 animals.

### *In vitro* histamine release and cytologic study

In IRs, the lack of histamine release was noted after leucocyte challenge with different OVA concentrations. Basophils were never observed in any group before and during the shock. Large basophilic lymphocytes were observed only after OVA i.v. administration for the shock induction but not before.

### HBA with BN rat serum

The flow cytometric method was carried out in three steps: first, a gating lymphocyte population was performed, followed by a gating of brightly fluorescent FITC (IgE-positive) cells, and finally the percentage of fluorescent CD63-positive cells was calculated before and after activation with OVA. A high-gain photomultiplier was used to differentiate the three cell populations within the cells gated in the lymphocyte region. Before activation, no CD63-positive cells were detected with anti-IgE amongst the auto-fluorescent (0.04%) or intermediate cells (0%). However, CD63-positive cells were detected, mainly amongst the intermediate cells after activation. Neither IgE-positive nor CD63-positive cells were seen in the platelet population. No CD63-positive cells were observed in either group (IRs and NIRs), before or 7 days after the first immunization with OVA. Activation of human basophils was evident on day 21 with serum from all IR animals, as indicated by the rise in CD63 expression on basophil membrane to  $14.7 \pm 10.2\%$  (see Table 1). Conversely, HBA remained lower than 5% in NIR group. No basophil activation was seen with the heated serum of IRs.

### Anaphylactic shock features

The onset of anaphylactic shock in IRs was monitored by the change in blood pressure. A dramatic arterial hypotension and a delayed HR decrease were observed in every IR, 3 min after OVA i.v. administration (Fig. 2): MABP fell from  $129.8 \pm 22.1$  to  $23.2 \pm 9.1$  mmHg and HR from  $377.7 \pm 34.2$  to  $187.2 \pm 170$  beats/min within 30 min after OVA administration ( $P < 0.05$ ). No hemodynamic variations were observed in NIRs.

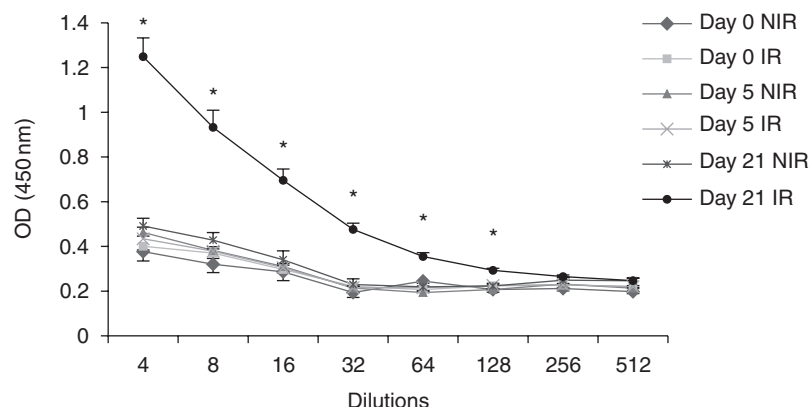


**Figure 1** Ovalbumin (OVA)-specific immunoglobulin E (IgE) titres in serum from immunized Brown Norway rats (IRs,  $n=30$ ) and from nonimmunized rats (NIRs,  $n=6$ ) on day 0, day 5 and day 21 after the first injection (1 mg of OVA or  $\text{Al}(\text{OH})_3$  subcutaneously, see *Materials and methods*). Optical density (OD) from enzyme-linked immunosorbent assays (ELISAs) was measured at different dilutions (1:4 to 1:512). The data are presented as mean OD  $\pm$  standard error of the mean (SEM). \* $P < 0.05$ , level of OVA-specific IgE in IRs on day 21 versus level of OVA-specific IgE in IRs on day 0 and 5 or level of OVA-specific IgE in NIRs whatever the day.

### *In vivo* histamine release after OVA challenge

Histamine release was measured before and at intervals of 1, 5 and 15 min after OVA challenge. Peak plasma histamine levels are presented in Fig. 3 and show a significant increase in IRs at 1 and 5 min after allergen injection, as compared with prechallenge values. No changes of histamine levels were detected in NIRs.

### Discussion

Anaphylactic shocks were induced in BN rats immunized with OVA, classically responsible for food allergy. Hypersensitivity was induced by repeated subcutaneous (s.c.) OVA administration over a period of 4 weeks before a shock was triggered by i.v. OVA challenge. BN rats developed hypersensitivity reactions that are close to human anaphylaxis, and circulating antibodies seen after immunization were identified as Igs analogous to human IgE [11–15]. The functional split of Th lymphocytes into cells primarily secreting IL-2 and interferon- $\gamma$  that induce classical cell-mediated immune responses (Th1), or IL-4 that direct the immune system towards the production of

specific Ig isotypes (Th2) [24]. Th2 cells are involved in the induction and maintenance of IgE antibody responses, facilitating the development and expression of allergic sensitization [24]. BN rat is a highly polarized Th2 model [16, 17]. The tissue inflammation is induced by Th2-type inflammatory cells producing a variety of inflammatory mediators, oxygen radicals and Th2-type cytokines such as IL-4, IL-5 and IL-13 [18]. BN rats produce high levels of IL-4 as compared with that of other strains; this could explain the important production of OVA-specific IgE [17].

IRs were able to release histamine *in vivo*: hypotension and a significant increase in plasma histamine levels were observed within 1 and 5 min following OVA i.v. administration. This confirms that the BN rat is a suitable model for anaphylaxis studies. However, the occurrence of shock after OVA injection is not sufficient proof that production of specific IgE was induced by OVA immunization.

OVA sensitization was confirmed by PCA tests, which is an *in vivo* cellular test developed in animals by Ovary [19]. In order to produce the PCA reaction, antibodies must be fixed to the mast cells of the skin; this process is termed sensitization. In rat, two antibody classes are important for

**Table 1** Human basophil activation (HBA) as percentage (%) of cells expressing CD63 marker in presence of serum from immunized rats (IRs,  $n=8$ ), serum preheated at  $56^\circ\text{C}$  ( $n=4$ ) from IR or serum from nonimmunized rats (NIRs,  $n=6$ ) on days 0, 5 and 21 following subcutaneous ovalbumin immunization

| Rats   | HBA (%) with IR serum |    |    |   |   |    |    |     | HBA (%) with heated IR serum (rats 1–4) | HBA (%) with NIR serum (rats 1–6) |
|--------|-----------------------|----|----|---|---|----|----|-----|-----------------------------------------|-----------------------------------|
|        | 1                     | 2  | 3  | 4 | 5 | 6  | 7  | 8   |                                         |                                   |
| Day 0  | <5 all rats           |    |    |   |   |    |    |     | NP                                      | <5                                |
| Day 5  | <5 all rats           |    |    |   |   |    |    |     | NP                                      | <5                                |
| Day 21 | 20                    | 35 | 10 | 6 | 7 | 22 | 10 | 7.5 | <5                                      | <5                                |

NP, not performed.

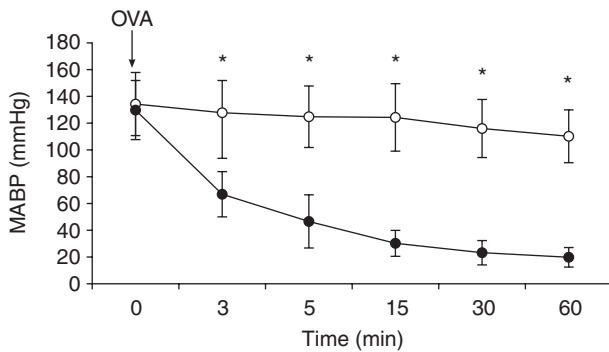


**Figure 2** Mean arterial blood pressure (MABP, mmHg) in rats immunized (IRs) with ovalbumin (OVA) (●,  $n=39$ ) and control rats (○,  $n=28$ ) after intravenous injection of OVA (arrow). Data are presented as mean  $\pm$  standard error of the mean (SEM). \* $P < 0.05$ , MABP in nonimmunized rats versus MABP in IRs.

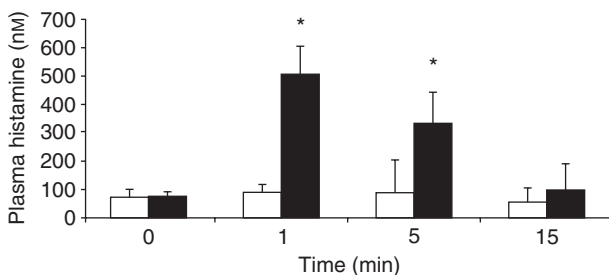


**Figure 3** Histamine plasma levels (nM) before and after (1, 5 and 15 min) intravenous administration of ovalbumin (OVA) in immunized (IRs,  $n=6$ , ■) and nonimmunized rats (NIRs,  $n=6$ , □). Values are mean  $\pm$  standard error of the mean (SEM) for each group before and after OVA challenge. \* $P < 0.05$  versus before ovalbumin administration.

PCA: IgGa, which requires a sensitization time of 4 h and IgE which requires 2 days or longer [25]. The presence of OVA-specific IgE in IRs is heat sensitive, which was demonstrated by negative PCA tests with serum previously heated at 56 °C. Specific IgE anti-OVA in IRs was characterized 21 days after the first immunization using ELISA methodology.

LHR tests performed prior to shock induction showed that no *in vitro* histamine release was detected in either IRs or NIRs. A plausible explanation for this negative result resides in the lack of circulating basophils, as these cells were never observed in BN rat blood despite numerous microscopic investigations and specific staining with toluidine blue. It is well known that the amount of basophils in rat blood is very low compared with that in guinea pigs or humans [26]. In humans, basophils, which are not found in normal noninflammatory tissues [26] have high-affinity receptors for IgE and can release preformed mediators such as histamine. Basophils are seen at sites of allergic inflammation, especially in the late-phase reaction [27]. Ogilvie [28] showed that blood levels of basophils and eosinophils

were increased in PVG and August rats 3 days after nematode infections. In our study, the pathophysiological changes occurred very quickly after allergen challenge. It is conceivable that longer delays would probably have resulted in blood basophil observation in our tests.

*In vitro* OVA administration induced an HBA, whatever the IR serum tested. Results were negative in both the groups before the first immunization and even 2 days after the second immunization with OVA. After day 21, HBA tests were strongly positive in all IRs and negative in all NIRs. In human basophils, CD63 protein is present on the stroma of intracytoplasmic granules [6]. After basophil activation, the granule membranes fuse with the cell plasma membrane; CD63 is translocated, and its plasma membrane expression increases [6]. This suggests that CD63 expression seems to be a highly reliable means for the determination of allergy, as positive results were observed only in the IR group. The flow cytometric method we used was based on the double anti-IgE FITC, anti-CD63 PE labelling and allowed us to calculate the percentage of IgE-positive and CD63-positive basophils. The evidence for the IR serum antibodies being OVA-specific IgE was provided by the absence of positive PCA or HBA test after heating the serum at 56 °C. HBA tests were performed in the presence of IL-3, a cytokine known to enhance basophil response measured by the release of histamine or leukotriene C4 [23] without an increase in the spontaneous CD63 expression [9]. Such an addition of IL-3 to the incubation medium acts as a preactivation or priming agent for human basophils and increases the sensitivity to OVA. Recently, several peptides have been identified as regulators of human basophils or mast cells. Basophils are responsive to multiple cytokines [29, 30] and chemokines [31]. Füreder [32] found no action for several recombinant human chemokines on the expression of surface antigens (CD11b, CD63, IgER and IL-3R). Moreover, cytokines and chemokines were produced only in pathological conditions *in vivo* (such as allergic or inflammatory reactions), and relatively high concentrations were required for basophil activation [32]. These thermolabile factors are not allergen-specific as compared with specific IgE. HBA tests were negative when we tested NIR serum and heated IR serum. Thus, specific OVA IgG antibodies seem not to be involved in the HBA because these antibodies are not thermosensitive [25]. Also, HBA test was performed 1 week before the induction of anaphylactic shock, and we can assume that rat serum cytokines and chemokine levels were very low. The fact that human basophils bear not only IgE receptors but also IgG receptors on their surface [33] lead us to believe that IR serum antibodies may bind to other cell-surface receptors than those for homologous IgE. However, passive sensitization of human basophils with mouse IgE antibodies was inhibited by human IgE or rat IgE but not by human IgG [5]. Furthermore, the binding of  $^{125}$ I-human IgE to human

basophilic leukemia cells was inhibited by both mouse and rat IgE [5]. These experimental results indicate that rodent IgE bind to the same receptors as human IgE.

The sudden onset of the anaphylactic shock, the absence of circulating basophils and the rise of blood histamine levels are in favour of mast cell involvement. Mast cells usually distributed throughout normal connective tissue, mainly adjacent to blood and lymphatic vessels, are also seen in cardiac tissue [26]; thus a local activation of cardiac mast cells might contribute to anaphylactic shock.

In conclusion, BN rats are a good strain for anaphylactic shock studies and can release histamine *in vivo* after allergen challenge. We demonstrated an OVA-induced sensitization by PCA test and the production of specific IgE anti-OVA. The serum from IR sensitized human basophils, their activation being assessed by the increase of CD63 expression and histamine release. We have not observed any histamine release in rat blood after *in vitro* challenge with different concentrations of OVA. Therefore, our results indicate that basophil activation does not seem to participate in the histamine release observed in the anaphylactic shock of sensitized BN rats. They also underline the possible usefulness of HBA test for the assessment of experimental allergy in BN rats, a strain which is mainly characterized by Th2-type immune responses.

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