Basophils, high-affinity IgE receptors, and CCL2 in human anaphylaxis

Peter Korosec, PhD,[a]* Paul J. Turner, FRACP, PhD,[b]* Mira Silar, BSc,[a] Peter Kopac, MD,[a] Mitja Kosnik, MD, PhD,[a] Bernhard F. Gibbs, PhD,[c] Mohamed H. Shamji, PhD,[d] Adnan Custovic, MD, PhD,[b]‡ and Matija Rijavec, PhD[a]‡ *Golnik, Slovenia, and London and Chatham, United Kingdom*

Background: The role of basophils in anaphylaxis is unclear.
Objective: We sought to investigate whether basophils have an important role in human anaphylaxis.
Methods: In an emergency department study we recruited 31 patients with acute anaphylaxis, predominantly to Hymenoptera venom. We measured expression of basophil activation markers (CD63 and CD203c); the absolute number of circulating basophils; whole-blood *FCER1A*, carboxypeptidase A3 *(CPA3)*, and L-histidine decarboxylase *(HDC)* gene expression; and serum markers (CCL2, CCL5, CCL11, IL-3, and thymic stromal lymphopoietin) at 3 time points (ie, during the anaphylactic episode and in convalescent samples 7 and 30 days later). We recruited 134 patients with Hymenoptera allergy and 76 healthy control subjects for comparison. We then investigated whether the changes observed during venom-related anaphylaxis also occur during allergic reactions to food in 22 patients with peanut allergy undergoing double-blind, placebo-controlled food challenge to peanut.
Results: The number of circulating basophils was significantly lower during anaphylaxis (median, 3.5 cells/μL) than 7 and 30 days later (17.5 and 24.7 cells/μL, $P < .0001$) and compared with those in patients with venom allergy and healthy control subjects (21 and 23.4 cells/μL, $P < .0001$). *FCER1A* expression during anaphylaxis was also significantly lower than in convalescent samples ($P \leq .002$) and control subjects with venom allergy ($P < .0001$). CCL2 levels (but not those of other serum markers) were significantly higher during anaphylaxis (median, 658 pg/mL) than in convalescent samples (314 and 311 pg/mL at 7 and 30 days, $P < .001$). Peanut-induced allergic reactions resulted in a significant decrease in circulating basophil counts compared with those in prechallenge samples ($P = .016$), a decrease in *FCER1A* expression ($P = .007$), and an increase in CCL2 levels ($P = .003$).
Conclusions: Our findings imply an important and specific role for basophils in the pathophysiology of human anaphylaxis. (J Allergy Clin Immunol 2017;140:750-8.)

Key words: *Anaphylaxis, basophils, CD63 activation, FcεRI expression, CCL2, serum tryptase*

Anaphylaxis is a potentially life-threatening, rapidly progressing systemic allergic reaction that can lead to death caused by airway obstruction or vascular collapse after exposure to allergens, including insect venom, foods, and medication.[1] Mast cell activation is postulated to have a pivotal role in anaphylaxis,[2] and an increase in serum mast cell tryptase levels can confirm the diagnosis.[1] However, in subjects experiencing anaphylaxis, it is not unusual to find normal serum tryptase levels in the context of increased plasma histamine levels,[3-5] suggesting that anaphylaxis might also involve basophil activation. However, there are

From [a]University Hospital of Respiratory and Allergic Diseases, Golnik; [b]the Section of Paediatrics and MRC and Asthma UK Centre in Allergic Mechanisms of Asthma, Imperial College London; [c]Medway School of Pharmacy, University of Kent, Chatham; and [d]the Section of Allergy and Clinical Immunology, National Heart and Lung Institute and MRC and Asthma UK Centre in Allergic Mechanisms of Asthma, Imperial College London.

*These authors contributed equally to this work.

‡These authors contributed equally to this work.

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Corresponding author: Peter Korosec, PhD, CLG, University Clinic of Respiratory and Allergic Diseases, Laboratory for Clinical Immunology and Molecular Genetics, Golnik 36, 4204 Golnik, Slovenia. E-mail: peter.korosec@klinika-golnik.si.

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Abbreviations used
CPA3: Carboxypeptidase A3
CRTH2: Chemoattractant receptor–homologous molecule expressed on T_H2 lymphocytes
DBPCFC: Double-blind, placebo-controlled food challenge
ED: Emergency department
HDC: L-histidine decarboxylase
PMN: Polymorphonuclear leukocyte
ROC: Receiver operating curve
TSLP: Thymic stromal lymphopoietin

few published data demonstrating a direct contribution of basophils to IgE-mediated anaphylaxis in human subjects.

Mast cells enter tissues as immature progenitors, where they undergo the final stages of their development and remain resident *in situ* for weeks or months. In contrast, basophils typically mature in hematopoietic tissues and subsequently circulate in the blood, with a half-life of less than 1 week.[6] Local allergen challenge studies in human subjects have demonstrated an influx of basophils to inflammatory sites within several hours of allergen exposure, demonstrating the existence of mechanisms for basophil recruitment from the circulation to the site of allergen exposure.[7-9] Both mast cells and basophils can rapidly secrete histamine and similar (but not necessarily identical) mediators and cytokines after IgE cross-linking.[2] In murine studies basophils contribute to IgG-mediated anaphylaxis.[10] In contrast, human basophils cannot be activated through IgG receptors, and their function is inhibited by IgG-mediated triggering through FcγRIIb receptors; moreover, they lack protease-activated receptors and antigen-presenting functions.[11,12]

We hypothesized that basophils play an important role in human anaphylaxis and specifically that (1) basophils are activated during human anaphylaxis, (2) there is a basophil migration during anaphylaxis, and (3) basophil-related biomarkers might be useful to confirm anaphylaxis. We addressed our hypotheses in a series of interlinked studies. First, in an emergency department (ED) study we investigated the upregulation of CD63 expression (the most commonly used basophil activation marker[13]) during and after anaphylaxis (predominantly caused by Hymenoptera venom allergy). We monitored the absolute numbers of circulating basophils; the corresponding whole-blood gene expression of *FCER1A*, carboxypeptidase A3 *(CPA3)*, and L-histidine decarboxylase *(HDC)*; and serum levels of the major basophil chemotactic factors, including the CCR2 ligand CCL2 and the CCR3 ligands CCL11 and CCL5.[14,15] We also measured levels of T cell–derived IL-3 (an important basophil priming and growth factor) and epithelial cell–derived thymic stromal lymphopoietin (TSLP), which promotes IL-3–independent basophil development and activation.[6,16,17] We then proceeded to assess whether the changes seen during venom-related anaphylaxis also occur during allergic reactions to food under the controlled setting of an oral double-blind, placebo-controlled food challenge (DBPCFC) in patients with peanut allergy.

METHODS

Study participants

ED study. We prospectively recruited 31 patients (13 female patients; age, 18-79 years) presenting with an acute episode of anaphylaxis to the ED of University Hospital Golnik, Slovenia (June-August 2011 and July-November 2013). Reaction severity was graded according to the Mueller criteria.[18] We collected blood samples during the reaction (at presentation to the ED) and in convalescent samples 7 and/or 30 days after the anaphylactic episode (see Table E1 in this article's Online Repository at www.jacionline.org).

Hymenoptera control subjects with venom allergy and healthy subjects. We recruited 2 groups of control participants for comparisons: (1) 134 patients (49 female patients; age, 23-67 years) with confirmed venom anaphylaxis from whom-blood samples were obtained at least 2 months after the last sting reaction and before initiation of venom immunotherapy and (2) 76 healthy control subjects (47 female subjects; age, 17-79 years).

Seventeen healthy subjects received a single dose of 64 mg of oral methylprednisolone and were monitored for up to 24 hours after the treatment to assess for possible confounding by treatment with corticosteroids and its effect on basophil activation, absolute cell count, *FCER1A* expression, and soluble markers (see Table E2 in this article's Online Repository at www.jacionline.org).

Peanut allergy study. We recruited 22 patients with peanut allergy (see Table E3 in this article's Online Repository at www.jacionline.org) in whom allergy was confirmed by using DBPCFCs (details are shown in the Methods section in this article's Online Repository at www.jacionline.org). Blood samples were collected before challenge, at cessation of challenge because of the onset of objective symptoms (but before administration of any treatment),[19] and 2 to 4 hours after challenge.

Ethical approval was obtained from the Slovenian National Medical Ethics Committee (ED study and control participants) and the London Central Research Ethics Committee (peanut allergy study). All subjects provided written informed consent.

Basophil activation, absolute cell count, gene expression, and serum markers

Detailed methodology is described in the Methods section in this article's Online Repository. Briefly, expression of CD63 and CD203c (markers of basophil activation) and enumeration of basophils ($CD123^+HLA\text{-}DR^-$ cells), lymphocytes, and polymorphonuclear leukocytes (PMNs) were determined by means of flow cytometry, as previously described.[20-22] In samples from patients with peanut allergy, we determined the absolute basophil count using a similar methodology, with basophils identified as chemoattractant receptor–homologous molecule expressed on T_H2 lymphocytes (CRTH2)–positive $CD303^-CD123^+$ cells.[23]

FCER1A, *CPA3*, and *HDC* gene expression was analyzed in whole-blood samples (PAXgene; PreAnalytiX, Hombrechtikon, Switzerland), as previously described.[22]

We measured serum concentrations of CCL2, CCL5, CCL11, IL-3, and TSLP by using ELISA, according to the manufacturers' instructions (Quantikine; R&D Systems, Minneapolis, Minn and Abcam, Cambridge, United Kingdom). For IL-3 measurements, we also performed spiking experiments (see the Methods section in this article's Online Repository). We measured serum total tryptase (α+β) levels with the ImmunoCAP 100 (Thermo Fisher, Uppsala, Sweden); tryptase concentrations that exceeded 11.4 μg/L were considered increased.

Statistical analysis

The distribution of data was assessed by using the D'Agostino and Pearson test. We used appropriate nonparametric and parametric tests for comparisons between groups, including the Wilcoxon signed-rank test, Mann-Whitney *U* test, *t* test with the Welch correction, and Pearson correlation. Data are expressed as medians unless otherwise stated. We compared the performance of basophil-related biomarkers in discriminating between patients with and without anaphylactic reactions using receiver operating characteristic (ROC) curve analysis. Analyses were performed with GraphPad Prism software (GraphPad Software, La Jolla, Calif).

RESULTS

Study participants

ED study and control subjects. Fig E1 and Table E1 in this article's Online Repository at www.jacionline.org show