Environmental Health Unit, University of Toronto, Toronto, Ontario, Canada. E-mail: vadasp@smh.ca.

Disclosure of potential conflict of interest: P. Vadas has provided expert testimony in medical legal cases of injury due to anaphylaxis and owns US patent no. 8257697 ("Use of platelet activating factor acetylhydrolase as a biomarker for anaphylaxis"); however, no money was issued to him or his institution. G. Liss declares that he has no relevant conflicts of interest.

REFERENCES

1. Brown SGA, Stone SF, Fatovich DM, Burrows SA, Holdgate A, Celenza A, et al. Anaphylaxis: clinical patterns, mediator release, and severity. J Allergy Clin Immunol 2013;132:1141-9.e5.
2. Vadas P, Gold M, Perelman B, Liss GM, Lack G, Blyth T, et al. Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. N Engl J Med 2008;358:28-35.
3. Brown SG. Clinical features and severity grading of anaphylaxis. J Allergy Clin Immunol 2004;114:371-6.

Available online October 30, 2013.
http://dx.doi.org/10.1016/j.jaci.2013.09.012

Reply

To the Editor:

Vadas and Liss[1] have restated their previous finding[2] that severe anaphylaxis is associated with low activity of the enzyme that deactivates the platelet-activating factor (PAF), PAF-acetylhydrolase (PAF-AH). They conclude that low PAF-AH activity should be used to identify at-risk individuals for targeted interventions. This assumes that low PAF-AH activity will identify those most likely to benefit. However, most people experiencing severe anaphylaxis do not have low PAF-AH activity,[3] yet may equally benefit from the same interventions. Another assumption is that low PAF-AH activity is a driver of severity rather than a consequence of it. Both Vadas et al[1,2] and our group[3] used samples collected during anaphylaxis, which may alter PAF-AH activity through oxidative stress or consumption/deactivation of the enzyme by the PAF. It is possible that PAF-AH activity might then return to normal in many cases. Bansal et al[4] highlighted this potential problem and found no difference in PAF-AH activity between 40 controls and 59 people who had previously experienced severe anaphylaxis.

In our article, we reported PAF-AH activity from the first sample taken on arrival in the emergency department, because this was the only one that was reliably collected across all sites.[3] However, in 26 cases with severe anaphylaxis, we were also able to measure PAF-AH activity in blood samples taken 1 hour after arrival and at discharge. There was substantial variability over time, with some cases showing a normalization of PAF-AH activity by the time of discharge (Fig 1). Unfortunately we did not obtain convalescent samples for comparison.

There are also potential problems with assay variability and standardization across sites. Vadas et al[2] used a cutoff value of 20 nmol/mL/min or less, we[3] used a cutoff of 12.4 nmol/mL/min or less to define low PAF-AH activity on the basis of the first centile of a panel of 30 healthy controls, and Bansal et al[4] found even lower PAF-AH activity in healthy controls (mean 9 ± 5 nmol/mL/min).

Before we entertain the use of PAF-AH activity to target people for interventions to prevent anaphylaxis, we first need to improve assay standardization and confirm that convalescent measurements taken weeks to months after a reaction still correlate with reaction severity. We then need to determine whether interventions based on this result can indeed influence outcomes.

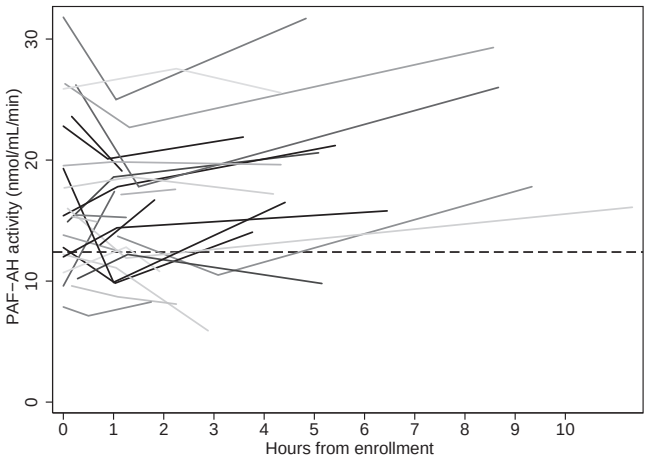

FIG 1. Changes in PAF-AH activity over time in 26 patients presenting to the emergency department with severe anaphylaxis. The lower limit of healthy normal range for our laboratory (12.4 nmol/mL/min) is represented by the *dashed horizontal line*.

Simon G. A. Brown, MBBS, PhD, FACEM[a,b]
Shelley F. Stone, PhD[a]
Daniel M. Fatovich, MBBS, PhD, FACEM[a,b]
Geoffrey K. Isbister, MBBS, MD, FACEM[c]

From [a]the Centre for Clinical Research in Emergency Medicine, Western Australian Institute for Medical Research and the University of Western Australia, and [b]the Department of Emergency Medicine, University of Western Australia, Royal Perth Hospital, Perth, Australia; and [c]the Department of Clinical Toxicology and Pharmacology, Calvary Mater Hospital and the Discipline of Clinical Pharmacology, University of Newcastle, Newcastle, New South Wales, Australia. E-mail: simon.brown@uwa.edu.au.

Disclosure of potential conflict of interest: S. Brown has received grants from the National Health and Medical Research Council, the Royal Perth Hospital Medical Research Foundation, the Fremantle Hospital Medical Research Foundation, and the Food Allergy and Anaphylaxis Network. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Vadas P, Liss G. Anaphylaxis: clinical features and mediator release patterns. J Allergy Clin Immunol 2013;132:1456-7.
2. Vadas P, Gold M, Perelman B, Liss GM, Lack G, Blyth T, et al. Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. N Engl J Med 2008;358:28-35.
3. Brown SGA, Stone SF, Fatovich DM, Burrows SA, Holdgate A, Celenza A, et al. Anaphylaxis: clinical patterns, mediator release, and severity. J Allergy Clin Immunol 2013;132:1141-9.e5.
4. Bansal AS, Chee R, Sumar N. Platelet-activating factor, PAF acetylhydrolase, and anaphylaxis. N Engl J Med 2008;358:1515-6; author reply 1516-7.

Available online October 30, 2013.
http://dx.doi.org/10.1016/j.jaci.2013.09.013

Human basophils lack the capacity to drive memory CD4$^+$ T cells toward the IL-22 response

To the Editor:

Gaudenzio et al[1] reported that human mast cells drive memory CD4$^+$ T cells toward IL-22 producers. They found that mast cells form synaptic-like contacts with CD4$^+$ T cells and promote

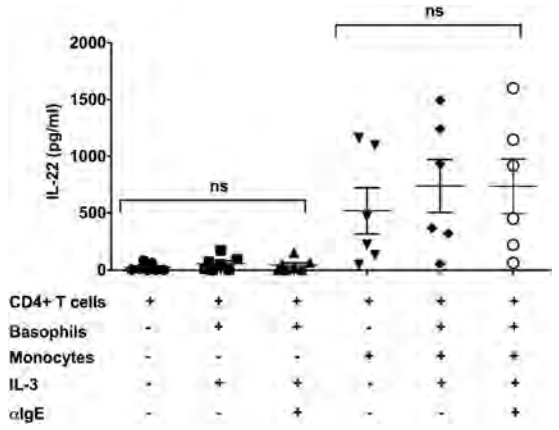

FIG 1. Human basophils lack the capacity to drive memory $CD4^+$ T cells toward the IL-22 response. Secretion of IL-22 (in picograms per milliliters, n = 6-7) by memory T cells that were cultured alone or with various combinations of activated basophils and peptidoglycan-stimulated monocytes is shown. Statistical analysis was done by using 1-way ANOVA, and comparison between sets of results was assessed by using the Tukey post-test. *ns*, Not significant.

IL-22–producing $CD4^+$ T cells through a TNF-α/IL-6–dependent mechanism. Thus these results point toward a role for mast cells in driving T_H responses in inflammatory conditions.

Tissue-resident mast cells share several common features with basophils.[2,3] Both types of cells release various cytokines on activation, including IL-4, IL-6, and IL-13; express common receptors, such as FcεRI, CD200R3, C3aR, C5aR, IL-3 receptor, IL-18 receptor, and IL-33 receptor; and release several inflammatory mediators, such as histamine and leukotrienes. In addition, basophils are also known to recruit to the inflamed tissues. In view of these common features, similar activation mechanisms, and cytokine profiles, we explored whether human basophils, similar to mast cells, possess the capacity to promote IL-22 responses from $CD4^+$ T cells.

First, we examined the direct effect of basophils on IL-22 responses from $CD4^+CD45RO^+CD25^-$ memory T cells. Basophils were activated either by IL-3 alone or through IL-3 and FcεRI cross-linking (see the Methods section in this article's Online Repository at www.jacionline.org). We found that neither IL-3–primed nor FcεRI-activated basophils alone could promote IL-22 from memory $CD4^+$ T cells (Fig 1). These results imply that unlike mast cells, basophils are poor inducers of IL-22 responses from $CD4^+$ T cells.

Activation and expansion of $CD4^+$ T cells implicate coordination of 4 different signals, including activation of professional antigen-presenting cells (APCs) through pattern-recognition receptors (signal zero), interactions of antigen-loaded HLA-DR with T-cell receptor–CD3 complexes (signal 1) and costimulatory molecules (signal 2), and signaling events mediated by T cell–polarizing cytokines (signal 3). Several reports, including ours, have recently demonstrated that circulating human basophils lack the features of APCs to mediate T-cell responses. This inability of basophils to promote T-cell responses was mainly due to the absence of HLA-DR and the costimulatory molecules CD80 and CD86. Thus the lack of T-cell receptor– and costimulatory molecule–mediated signals might explain the inability of human basophils to mediate IL-22 responses.[4,5]

However, the above results did not provide clues on the indirect effect of basophils in promoting APC-mediated IL-22 responses. Therefore by using Toll-like receptor (TLR)–activated monocytes, we determined whether circulating human basophils are capable of enhancing IL-22 from $CD4^+$ T cells. Monocytes were pulsed with the TLR2 agonist peptidoglycan and subsequently cocultured with memory $CD4^+$ T cells either in the presence or absence of activated basophils. We found that TLR-activated monocytes promoted IL-22 from memory $CD4^+$ T cells. However, IL-3–treated basophils did not further amplify monocyte-mediated IL-22 responses (Fig 1). Similar results were also obtained in the presence of FcεRI-activated basophils. These results suggest that circulating human basophils lack the ability to augment APC-mediated IL-22 responses.

In summary, our data indicate that although basophils share several common properties with mast cells, unlike these cells, basophils do not possess the capacity to drive memory $CD4^+$ T cells toward IL-22 producers, either directly or through APCs.

Meenu Sharma, MSc[a,b]
Srini V. Kaveri, DVM, PhD[a,c,d,e]
Jagadeesh Bayry, DVM, PhD[a,c,d,e]

From [a]Unité 872, Institut National de la Santé et de la Recherche Médicale, Paris, France; [b]Université de Technologie de Compiègne, Compiègne, France; [c]Centre de Recherche des Cordeliers, Equipe 16–Immunopathology and Therapeutic Immunointervention, Université Pierre et Marie Curie–Paris 6, Paris, France; [d]Université Paris Descartes, Paris, France; and [e]International Associated Laboratory IMPACT (Institut National de la Santé et de la Recherche Médicale, France–Indian Council of Medical Research, India), National Institute of Immunohaematology, Mumbai, India. E-mail: jagadeesh.bayry@crc.jussieu.fr.

Supported by Institut National de la Santé et de la Recherche Médicale, Université Pierre et Marie Curie, Université Paris Descartes, European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement HEALTH-2010.2.4.5-2 ALLFUN (to J.B.).

Disclosure of potential conflict of interest: J. Bayry has received a grant from the European Community's Seventh Framework Programme. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Gaudenzio N, Laurent C, Valitutti S, Espinosa E. Human mast cells drive memory $CD4^+$ T cells toward an inflammatory IL-22^+ phenotype. J Allergy Clin Immunol 2013;131:1400-7.e11.
2. Voehringer D. Protective and pathological roles of mast cells and basophils. Nat Rev Immunol 2013;13:362-75.
3. Karasuyama H, Mukai K, Obata K, Tsujimura Y, Wada T. Nonredundant roles of basophils in immunity. Annu Rev Immunol 2011;29:45-69.
4. Kitzmuller C, Nagl B, Deifl S, Walterskirchen C, Jahn-Schmid B, Zlabinger GJ, et al. Human blood basophils do not act as antigen-presenting cells for the major birch pollen allergen Bet v 1. Allergy 2012;67:593-600.
5. Sharma M, Hegde P, Aimanianda V, Beau R, Mohan MS, Senechal H, et al. Circulating human basophils lack the features of professional antigen presenting cells. Sci Rep 2013;3:1188.

Available online November 4, 2013.
http://dx.doi.org/10.1016/j.jaci.2013.09.024

Reply

To the Editor:

We thank Sharma et al[1] for their interest in our recently published article.[2] In our study we demonstrate that primary human mast cells can express MHC class II and costimulatory molecules upon treatment with IFN-γ and can form functional immunologic synapses with previously activated T cells. We also show that cognate interaction between mast cells and freshly isolated memory $CD4^+$ T cells leads to the generation of T_H22 and IL-22/IFN-γ–producing T_H cells.[2] In addition, we previously demonstrated that IFN-γ–primed mouse mast cells express MHC class II and costimulatory molecules and form immunologic synapses with

METHODS

Isolation of circulating human basophils, monocytes, and memory T cells

Buffy bags from healthy donors were obtained from Centre Necker-Cabanel, Etablissement Français du Sang, Paris, France. Ethics committee permission was obtained for the use of these buffy bags (no. 12/EFS/079). Basophil-rich fractions of PBMCs were obtained by using Percoll density gradient centrifugation. Basophils from these basophil-rich PBMCs were isolated by using the Basophil Isolation Kit II (Miltenyi Biotec, Paris, France). Monocytes were isolated from PBMCs by using CD14 magnetic microbeads (Miltenyi Biotec). For isolation of memory T cells, untouched total $CD4^+$ T cells were purified by means of negative selection with the $CD4^+$ T Cell Isolation Kit II (Miltenyi Biotec). Furthermore, untagged memory ($CD45RO^+$) T cells were subsequently isolated from total $CD4^+$ T cells by positively selecting naive ($CD45RA^+$) T cells with CD45RA microbeads (Miltenyi Biotec). Finally, $CD4^+CD45RO^+CD25^-$ memory T cells were obtained by depleting $CD25^+$ cells with CD25 microbeads (Miltenyi Biotec). The purity of various cellular populations was greater than 95%.

Coculture of basophils and monocytes with $CD4^+CD45RO^+CD25^-$ memory T cells

$CD4^+CD45RO^+CD25^-$ memory T cells were cultured in U-bottom, 96-well plates (0.1×10^6 cells/200 μL per well) in X-vivo-10% AB serum and IL-2 (100 IU/mL) either alone or with IL-3 (100 ng/10^6 cells)–primed basophils, with IL-3 and anti-IgE (10 ng/0.1 million cells)–treated basophils, with monocytes stimulated with peptidoglycan (5 μg/0.5 million cells), with peptidoglycan-stimulated monocytes and IL-3–primed basophils, or with peptidoglycan-stimulated monocytes along with IL-3 and anti-IgE–treated basophils. The ratio of T cells and monocytes or basophils was maintained at 5:1. After 4 to 5 days of culture, cell-free culture supernatants were collected for analysis of IL-22.

IL-22 analysis

Levels of IL-22 (ELISA Ready-SET-Go; eBioscience, San Diego, Calif) in cell-free culture supernatants were quantified by means of ELISA. The detection limit was 8 pg/mL.

Statistical analysis

The significance of differences was assessed by using 1-way ANOVA, and comparison between sets of results was assessed by using the Tukey post-test. *P* values of less than .05 were considered statistically correlated. Prism 5 software (GraphPad Software, La Jolla, Calif) was used for statistical analyses.