

ORIGINAL ARTICLE

Basophil CD63 expression in chronic spontaneous urticaria: correlation with allergic sensitization, serum autoreactivity and basophil reactivity

Q. Chen,^{1,a} Z. Zhai,^{1,a} J. Xu,¹ W. Chen,^{2,3} S. Chen,¹ H. Zhong,¹ X. Huang,¹ F. Hao,^{1,*} Z. Song^{1,*}¹Department of Dermatology, Southwest Hospital, Third Military Medical University, Chongqing, China²IZZ Immunologie-Zentrum Zürich, Zürich, Switzerland³Department of Dermatology and Allergy, Technische Universität München, Munich, Germany

*Correspondence: Z. Song and F. Hao. E-mails: zhiqiang.song@hotmail.com; haofei62@medmail.com.cn

Abstract

Background The underlying causes and factors contributing to the disease severity of chronic spontaneous urticaria (CSU) are unknown.**Objective** Given the important role of basophils in the pathogenesis of urticaria and that CD63 serves as a useful marker for basophil activation and detecting, CD63 expression of basophils is a reliable tool for diagnosing allergy and hypersensitivity reactions to different allergens; the objective of this study was to investigate whether the level of basophil CD63 expression is correlated with allergen sensitization, serum autoreactivity and basophil reactivity in patients with CSU.**Methods** Basophil-enriched leucocytes were separated from the blood of 64 patients with chronic urticaria (54 CSU patients and 10 symptomatic dermatographism patients), 18 healthy control subjects and seven atopic donors without urticaria. Flow cytometry was then used to detect CD63 expression on the cell membrane of basophils from all samples. Analysis was also preformed on basophils incubated with sera from CSU patients with positive or negative autologous serum skin test (ASST).**Results** CD63 expression was significantly higher in the basophils from patients with CSU than in those from patients with symptomatic dermatographism and the healthy control group. The levels of CD63 expression in CSU patients with ASST⁺ and/or allergen sensitization were higher than those with ASST⁻ and/or no allergen sensitization patients. Incubation with ASST⁺ serum resulted in an increased expression of CD63 in the basophils of ASST⁺ CSU patients, whereas no such response was observed in healthy controls or ASST⁻ CSU patients.**Conclusion** The increased CD63 expression in basophils from CSU patients may correlate with allergen sensitization, autoreactivity of serum and basophil reactivity. Our results suggest that CD63 may contribute new insight into the pathogenesis of CSU.

Received: 13 January 2016; Accepted: 18 July 2016

Conflicts of interest

The authors declare that they have no conflict of interests.

Funding Source

Natural Science Foundation Project of Chongqing, cstc2015jcyjA10118; Clinical Research Funding of Southwest Hospital, SWH2013LC10.

Introduction

Chronic urticaria is a common skin disease that can be classified into spontaneous and inducible forms according to the 2014 European Academy of Allergy and Clinical Immunology (EAACI) guidelines.¹ Chronic spontaneous urticaria (CSU) is characterized by the spontaneous appearance of wheals and/or

angioedema for more than 6 weeks, and symptomatic dermatographism is characterized as wheals that can be induced by mechanical force. CSU and symptomatic dermatographism are the most common subtypes of chronic urticaria worldwide, including in the Chinese population.² Although the pathogenesis of chronic urticaria has not yet been clearly defined, previous studies have demonstrated that mast cells and basophils play important roles in inducing the symptoms by releasing inflammatory

^aThe first and the second author contributed equally to this work.

mediators and cytokines upon activation.³ Basophils are the rarest type of leucocytes in human peripheral blood, making up less than 0.5% of the total white blood cell count in healthy individuals. Decreased basophil numbers in patients with CSU indicate their role in the activation and function of the cells.^{4,5}

CD63 is a tetraspanin normally expressed only on the membrane of the lysosomes in the inactive basophils and mast cells. Upon activation, it moves to the surface of basophils along with degranulation.⁶ Therefore, CD63 is deemed a specific and reliable surface activation marker of basophils. The basophil activation test (BAT) by detection of basophil CD63 expression with flow cytometry after induction of patient sera has proven to be a useful tool for detection of functional autoantibodies relevant to autoimmune reactions in chronic urticaria⁷ and the diagnosis of allergy and hypersensitivity reactions to different allergens.^{8,9} CD63 expression has also been shown to be higher in IgE-mediated immediate allergic diseases such as asthma, rhinitis, anaphylaxis and atopic dermatitis,¹⁰ but its role in chronic urticaria remains unclear. One study showed that both allergic subjects and CSU subjects had increased basophil CD63 expression when compared with non-allergic subjects.¹¹

In our previous study, we found that patients with a positive autologous serum skin test (ASST) and/or house dust mite sensitization had increased disease activity and a longer disease course compared to patients with a negative ASST and/or no allergen sensitization.¹² This suggests that there may be some subtle differences in the pathogenesis of CSU between ASST-positive and -negative patients, which may be relevant for determining a specific therapeutic schedule and prognosis judgment. This study was focused on the activation status of circulating basophils in subtypes of chronic urticaria, in particular, CSU patients showing different responses to ASST and skin prick test (SPT). By evaluating the expression of CD63 on the surface of basophils in patients with CSU and symptomatic dermographism, we aimed to explore the possible correlations among allergen sensitization, serum autoreactivity and basophil reactivity.

Materials and methods

Subjects

A total of 78 patients (30 males, 48 females; mean age, 36.82 years; age range, 5–77 years) with chronic urticaria who visited the dermatology clinic of Southwest Hospital (Chongqing, China) from April 2013 to March 2016 were randomly selected and enrolled in this study. All patients met the EAACI/GA2LEN/European Dermatology Forum guidelines on chronic urticaria (the disease duration between 6 weeks and 20 years) and provided fully informed consent. Among them, there were 68 patients with CSU (36 cases were ASST-positive and 32 cases were ASST-negative, nine cases with positive dermographism) and 10 patients solely with symptomatic dermographism. All of

the patients were being treated with non-sedating oral H1-antihistamines and the treatment was stopped at least 3 days before blood sample collection. Eighteen sex- and age-matched healthy subjects without any type of urticaria and being negative to both the ASST and SPT were enrolled as the normal control group. In addition, seven patients with atopic dermatitis and without any subtype of urticaria were enrolled as the atopic control group for reference. The study protocol was approved by the ethical committee of the Southwest hospital of Third Military Medical University, Chongqing, China.

SPT

The SPT was performed by placing a drop of allergen solution (Allergopharma™ Skin Prick Testing Kits, Reinbeck, Germany) on the inner side of the forearm, as described previously.¹³ The allergens included the house dust mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, cockroach allergen, mugwort pollen, humulus pollen, fish (catfish), shrimp, crab, egg, cow milk and peanut. Saline and histamine phosphate (10 mg/mL) were used as negative and positive controls respectively. Antihistamines were withdrawn at least 3 days before the test. The skin response was measured at 30 min after pricking, as previously described.¹² A patient with a positive SPT result to one or more common allergens was considered to show allergic sensitization (atopy).^{8–12}

ASST

The ASST was performed according to established methods.^{12,14} In brief, 0.05 mL of sterile autologous serum was injected intradermally, with 0.05 mL of 10 µg/mL histamine and normal saline as positive and negative control respectively. Skin areas involved in a spontaneous wheal reaction during the previous 24 h were avoided. The wheal size was recorded at 30 min. The ASST was considered positive, when the wheal size diameter was at least 1.5 mm greater than that of the negative control. Positive ASST was indicated as serum autoreactivity in the present paper.

Isolation of basophil-enriched leucocytes and serum collection

A 10-mL peripheral blood sample was collected by venipuncture in a collection tube with ethylenediaminetetraacetic acid, centrifuged at 440 g for 10 min, and then the supernatant was sucked off and the plasma was stored at –80°C for further use. To obtain the basophils, the same volume of free Ca²⁺ and Mg²⁺ D-Hanks' phosphate buffered saline (PBS) was added after removing the supernatants, and blended. The liquids were then carefully tiled beyond the Ficoll-Paque PREMIUM 1.084 separation medium (GE Healthcare, England) at a volume ratio of 2:1, and centrifuged at 420 g for 30 min. The basophil-enriched leucocytes at the interlayer were sucked up, aspirated and washed twice with free Ca²⁺ and Mg²⁺ D-Hanks' PBS. The cells were resuspended and then the

basophil-enriched leucocytes were isolated. Serum was collected with tubes without any anticoagulant.

Stimulation of basophils

To evaluate the responses of basophils to different types of triggering factors, the basophils were equally divided into six tubes. One tube of cells was used for detection of CD63 expression immediately after basophil isolation. Three tubes of cells were incubated with sera from ASST-positive CSU patients, sera from ASST-negative CSU patients, and sera from healthy donors respectively. The other two tubes of cells were incubated with free Ca^{2+} and free Mg^{2+} D-Hanks' PBS as the negative control, and with 10^{-6} mol/mL of the non-specific cell activator N-formyl-methionyl-leucyl-phenylalanine (BD FastImmune; Becton-Dickinson, San Jose, CA, USA) as the positive control respectively. All tubes were incubated for 30 min at 37°C . The reaction was stopped by cooling the tubes on ice.

Detection of CD63 expression

After the reaction was stopped, the cells were stained with 15 μL of CD63-FITC/CD123-PE/ anti-HLA-DR-PerCP antibody cocktail (BD FastImmune; Becton-Dickinson, San Jose, CA, USA) and incubated for 30 min at 4°C . The samples were lysed, washed and resuspended in 300 μL of 0.5% paraformaldehyde, and were then measured in a flow cytometer (FACScan; Becton-Dickinson Immunocytometry System, San Jose, CA, USA) within 1 h. For analysis, a first gate using the forward-scattered/side-scattered light characteristics of leucocytes was defined, and then CD63^{+} CD123^{+} HLA-DR-basophils were identified as described previously.^{15,16}

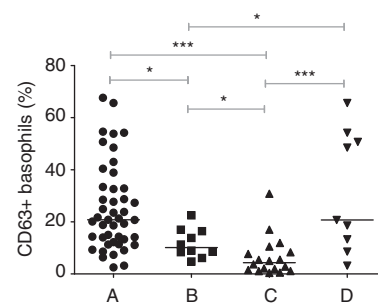
Statistical analysis

Statistical analysis was performed using the GraphPad Prism version 5.01 software (GraphPad Software Inc.: San Diego, CA, USA, 2007). The Mann-Whitney *U*-test or the Wilcoxon matched-pairs test was used, as appropriate, to determine significant differences between groups. All data are shown as medians and the difference was considered significant at a *P*-value of less than 0.05.

Results

CD63 expression is increased in CSU

The expression of CD63 was detected on the cell membrane of basophils collected from subjects with CSU ($n = 45$), CSU with positive dermographism ($n = 9$), purely symptomatic dermographism ($n = 10$) and the normal controls ($n = 18$). As shown in Fig. 1, the mean percentage of CD63^{+} basophils was significantly higher in the CSU than in the symptomatic dermographism samples (both $P < 0.05$) and normal controls (both $P < 0.001$), and the expression level in the symptomatic dermographism samples was also significantly higher than that in the



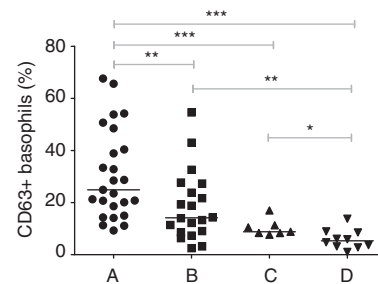
A, CSU
B, Symptomatic dermographism
C, Normal controls
D, CSU with positive dermographism

Figure 1 Comparison of basophil CD63 expression between chronic urticaria patients and normal controls. Peripheral blood samples from CU patients and healthy subjects were collected and basophil-enriched leucocytes were isolated. The CD63 expressions on basophils were measured in a flow cytometer by gating with CD63^{+} CD123^{+} HLA-DR-. Mann-Whitney test, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

normal controls ($P < 0.05$). However, there was no significant difference between the CSU and CSU with positive dermographism samples ($P = 0.70$).

CD63 expression is increased in CSU patients with allergic sensitization

CD63 expression was also compared among the atopic patients ($n = 7$), normal controls ($n = 11$) and CSU patients with ($n = 30$) and without ($n = 24$) allergic sensitization. As shown in Fig. 2, CD63 expression of the atopic patients was significantly higher than that of the normal controls ($P < 0.05$).



A, CSU with allergic sensitization
B, CSU without allergic sensitization
C, Atopic patients
D, Normal controls

Figure 2 Different CD63 expressions on basophil among chronic spontaneous urticaria (CSU) patients with allergic sensitization, CSU patients without allergic sensitization, atopic patients and normal controls. Mann-Whitney test, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

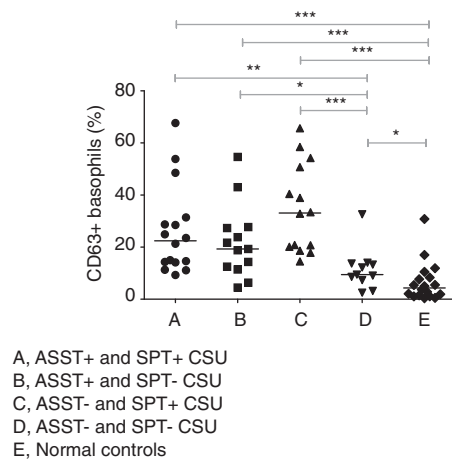


Figure 3 Comparison of basophil CD63 expression between different subtypes of CSU patients based on the allergen sensitization and serum autoreactivity. Mann-Whitney test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. CSU, chronic spontaneous urticaria

Furthermore, CD63 expression of the CSU with allergic sensitization patients was significantly higher than that of the other three groups (all $P < 0.01$), and the CSU without allergic sensitization patients also showed higher CD63 expression than the normal controls ($P < 0.01$).

Higher CD63 expression in CSU is related to both allergen sensitization and serum autoreactivity

We then divided the CSU patients into four groups according to the results of the ASST and SPT, as ASST⁺SPT⁺ ($n = 16$), ASST⁺SPT⁻ ($n = 13$), ASST⁻SPT⁺ ($n = 14$) and ASST⁻SPT⁻ ($n = 11$), for further analyses. As shown in Fig. 3, the CD63 expression levels of four subtypes of CSU patients were significantly higher than that of the normal controls (all $P < 0.05$). Furthermore, the CD63 expression of the ASST⁺SPT⁺, ASST⁻SPT⁺ and ASST⁺SPT⁻ groups were all significantly higher than that of the ASST⁻SPT⁻ group (all $P < 0.05$).

Both serum autoreactivity and basophil reactivity contribute to the higher CD63 expression in CSU

To further investigate the possible roles of sera autoreactivity and basophil reactivity on CD63 expression, the basophils of CSU patients ($n = 22$) and normal controls ($n = 10$) were incubated with ASST⁺ serum or ASST⁻ serum respectively. As shown in Fig. 4a, the basophils of CSU patients incubated with ASST⁺ serum showed significantly higher CD63⁺ expression than those incubated with ASST⁻ serum ($P < 0.05$). Such differences could not be observed on the basophils collected from the normal controls ($P = 0.4675$) (Fig. 4b).

Next, we divided the basophils of CSU patients into ASST⁺ basophils (basophils from ASST⁺ patients, $n = 11$) and ASST⁻ basophils (basophils from ASST⁻ patients, $n = 11$) groups

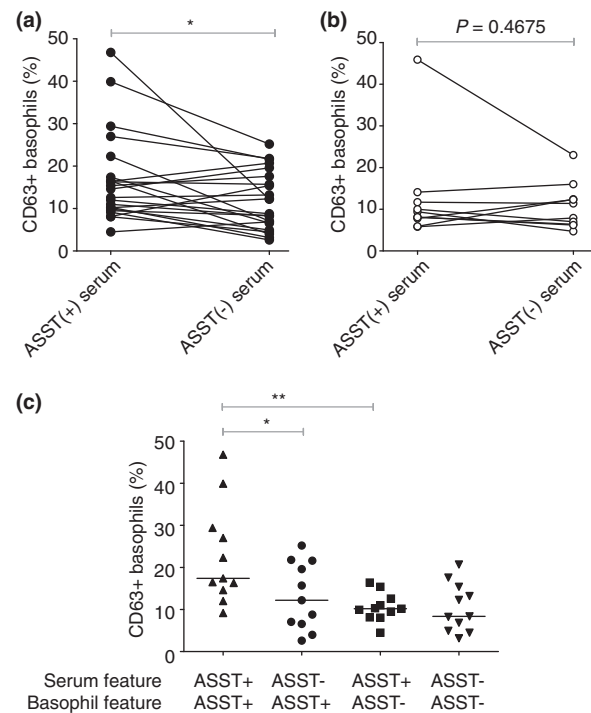


Figure 4 The impacts of sera histamine releasing factors and intrinsic potential of basophils on the CD63 expression. ASST: autologous serum skin test. (a) The basophils of chronic spontaneous urticaria (CSU) patients ($n = 22$) incubated with the mixed sera from five ASST⁺ CSU patients showed significantly higher CD63⁺ expression than those incubated with the mixed sera from five ASST⁻ CSU patient. The experiment shown is representative of three experiments. (b) Basophil from the normal controls ($n = 10$) incubated with ASST⁺ serum did not show any differences in CD63⁺ expressions compared to those incubated with ASST⁻ serum. The experiment shown is representative of three experiments. (c) ASST⁺ basophils (basophils from patients with positive ASST, $n = 11$) and ASST⁻ basophils were incubated with serum with ASST⁺ or ASST⁻ respectively. The CD63 expression of on ASST⁺ basophils were significantly higher when incubated with ASST⁺ serum than when incubated with ASST⁻ serum ($P < 0.01$), whereas there was no significant difference in CD63 expression between ASST⁻ basophils incubated with ASST⁺ and with ASST⁻ serum ($P = 0.9658$).

according to the patient's response to the ASST. We found that CD63 expression of ASST⁺ basophils was significantly higher when incubated with ASST⁺ serum than that incubated with ASST⁻ serum ($P < 0.05$). There was no significant difference in CD63 expression between ASST⁻ basophils incubated with ASST⁺ and with ASST⁻ serum ($P = 0.9658$) (Fig. 4c). As shown in Fig. 4c, the ASST⁺ basophils expressed higher CD63 than ASST⁻ basophils when they were both incubated with ASST⁺ serum ($P < 0.01$). We further confirmed this using the other seven sets of sera to incubate one set of basophils, and we got similar results (data was not shown).

Discussion

This study showed that CD63 expressions on the surface of circulating basophils are increased in CSU patients compared to that in any of the other subtypes, which was in line with earlier reports,^{11,22} and the levels of CD63 expression in CSU patients with ASST⁺ and/or allergen sensitization were higher than those with ASST⁻ and/or no allergen sensitization patients.

The allergic sensitization in chronic urticaria patients has been found to be higher than that in healthy subjects.^{12,13,18,19} However, the significance of allergic sensitization in the pathogenesis of CSU is still a matter of debate,^{13,18–20} and there has been no experimental study to directly address this question. In line with the findings of Vasagar *et al.*, our results also showed a significant increase in CD63 expression on the surface of circulating basophils in both the CSU and atopic groups.¹¹ CD63 expression in the CSU patients with allergic sensitization was significantly higher than that without sensitization. It has been proposed that a positive SPT to common allergens indicates allergic reaction or sensitization.¹⁷ Since CSU patients with SPT⁺ had a higher urticaria activity score than those with a SPT⁻,¹² our results about a higher level of spontaneous basophil activation in CSU patients with allergic sensitization suggested that allergic sensitization may be an underlying factor contributing to the pathogenesis of CSU.

In about half of CSU cases, a positive reaction to autologous serum test implying histamine releasing factors in serum, is found, which is termed as autoreactive chronic urticaria. Autoantibodies to either the α -chain of high-affinity IgE receptor (Fc ϵ RI α) or IgE itself in ASST-positive serum may be the main factor responsible for the spontaneous activation of basophils in patients with CSU. Studies showed that sera from CSU patients with a positive ASST result could induce higher CD63 expression on the basophils of atopic donors *in vitro*.^{21–23} In our previous study, we observed that CSU patients who were positive for ASST had greater scores of disease activity and dermatology QoL index (DLQI), higher doses of total antihistamine medication required every month and higher frequencies of angioedema compared with patients with negative ASST.^{2,12} Present results indicated that both serum autoreactivity and basophil reactivity contribute to the higher CD63 expression in CSU. Further investigation is needed to see whether CD63 expression on basophils is correlated with the disease severity.

Luquin *et al.*²⁵ reported that basophils from chronic urticaria patients were hyper-responsive to serum either from healthy individuals or from patients with CSU. We found that basophils from CSU patients were hyper-responsive to sera from CSU patients with ASST⁺ but not to those with ASST⁻. And only basophils from ASST⁺ patients but not basophils from ASST⁻ patients showed a significantly higher CD63 expression after incubation with ASST⁺ sera. These results imply that both histamine releasing factors in sera and the intrinsic potential of

basophils may contribute to the expression of CD63 and basophil activation.

It is unclear why ASST⁺ and ASST⁻ basophils have different response to ASST⁺ serum. Human basophil reactivity can be modulated by different factors such as activation of immunoreceptor tyrosine-based inhibitory motifs by IgE/IgG/allergen complexes, downregulation of Fc ϵ RI α , inhibitors such as IgG and IgA immunocapture and activators in the presence of priming cytokines IL3 and IL5.²⁵ For a given immunological status, basophil reactivity may also depend on the concentrations of the allergen and autoantibodies, affinity of the specific IgE to the allergen, inter-epitope distance, contact delay between the allergen and related membrane receptor-bound specific IgE and intrinsic basophil reactivity.^{26–28} (Fig. 5).

In summary, our data showed higher but different CD63 expressions on the surface of circulating basophils from patients with different subtypes of chronic urticaria based on ASST and SPT response. The expression level of CD63 on basophils from

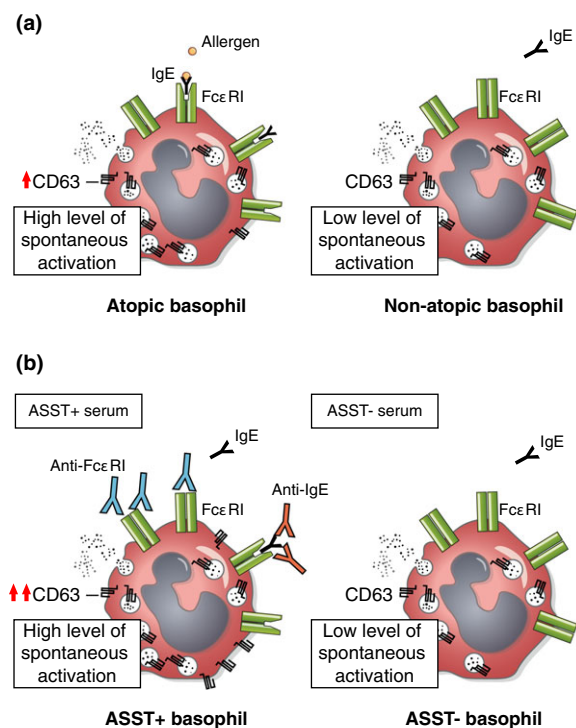


Figure 5 (a) The concentrations of the allergen, or affinity of the specific IgE to the allergen may contribute to higher CD63 expression and high level of spontaneous activation of basophils in chronic spontaneous urticaria (CSU) with allergen sensitization. (b) The concentration of autoantibodies to Fc ϵ RI, IgE or other histamine releasing factors in the ASST⁺ serum, and the intrinsic reactivity of ASST⁺ basophil lead to a higher CD63 expression and higher level of spontaneous activation of basophils in CSU with positive ASST.

CSU patients are correlated with allergic sensitization, serum autoreactivity and intrinsic potential of the basophil, which may provide new clues to understand the underlying pathogenesis of CU.

Acknowledgements

We thank Mr. Baiyu Zhong and Mrs. Xiaofang Zhou for the technical preparation of blood samples.

References

- Zuberbier T, Aberer W, Asero R *et al.* The EAACI/GA(2) LEN/EDF/WAO Guideline for the definition, classification, diagnosis, and management of urticaria: the 2013 revision and update. *Allergy* 2014; **69**: 868–887.
- Zhong H, Song Z, Chen W *et al.* Chronic urticaria in Chinese population: a hospital-based multicenter epidemiological study. *Allergy* 2014; **69**: 359–364.
- Saini SS. Chronic spontaneous urticaria: etiology and pathogenesis. *Immunol Allergy Clin North Am* 2014; **34**: 33–52.
- Passante E. Mast cell and basophil cell lines: a compendium. *Methods Mol Biol* 2014; **1192**: 101–113.
- Vonakis BM, Saini SS. Basophils and mast cells in chronic idiopathic urticaria. *Curr Allergy Asthma R* 2005; **5**: 270–276.
- Knol EF, Mul FP, Jansen H, Calafat J, Roos D. Monitoring human basophil activation via CD63 monoclonal antibody 435. *J Allergy Clin Immunol* 1991; **88**: 328–338.
- Gyimesi E, Sipka S, Danko K *et al.* Basophil CD63 expression assay on highly sensitized atopic donor leucocytes—a useful method in chronic autoimmune urticaria. *Br J Dermatol* 2004; **151**: 388–396.
- Tammaro A, Narcisi A, Amodeo R *et al.* CD63 cell expression detected by flow-cytometric determination of basophil activation in allergic patients. *Int J Immunopath Ph* 2012; **25**: 1143–1147.
- Hausmann OV, Gentinetta T, Bridts CH, Ebo DG. The basophil activation test in immediate-type drug allergy. *Immunol Allergy Clin* 2009; **29**: 555–566.
- Erdmann SM, Heussen N, Moll-Slowsky S, Merk HF, Sachs B. CD63 expression on basophils as a tool for the diagnosis of pollen-associated food allergy: sensitivity and specificity. *Clin Exp Allergy* 2003; **33**: 607–614.
- Vasagar K, Vonakis BM, Gober LM, Viksman A, Gibbons SP Jr, Saini SS. Evidence of in vivo basophil activation in chronic idiopathic urticaria. *Clin Exp Allergy* 2006; **36**: 770–776.
- Song Z, Zhai Z, Zhong H, Zhou Z, Chen W, Hao F. Evaluation of autologous serum skin test and skin prick test reactivity to house dust mite in patients with chronic spontaneous urticaria. *PLoS ONE* 2013; **8**: e64142.
- Kulthanan K, Jiamton S, Rutnin NO, Insawang M, Pinkaew S. Prevalence and relevance of the positivity of skin prick testing in patients with chronic urticaria. *J Dermatol* 2008; **35**: 330–335.
- Kulthanan K, Jiamton S, Gorvanich T, Pinkaew S. Autologous serum skin test in chronic idiopathic urticaria: prevalence, correlation and clinical implications. *Asian Pac J Allergy Immunol* 2006; **24**: 201–206.
- De Swert A, Van Den Keybus C, Kasran A *et al.* Detection of basophil-activating IgG autoantibodies in chronic idiopathic urticaria by induction of CD 63. *J Allergy Clin Immunol* 2005; **116**: 662–667.
- Chirumbolo S. Monitoring of CD63% in basophil activation test and suggested new parameters for allergy diagnosis. *Inflamm Res* 2012; **61**: 171–176.
- Yocum MW, Khan DA. Assessment of patients who have experienced anaphylaxis: a 3-year survey. *Mayo Clin Proc* 1994; **69**: 16–23.
- Caliskaner Z, Ozturk S, Turan M, Karaayvaz M. Skin test positivity to aeroallergens in the patients with chronic urticaria without allergic respiratory disease. *J Investig Allergol Clin Immunol* 2004; **14**: 50–54.
- Gecer E, Erdem T. Aeroallergen prick skin test and autologous serum skin test results in patients with chronic urticaria and their comparison. *Ann Dermatol* 2012; **24**: 472–474.
- Augey F, Gunera-Saad N, Bensaid B, Nosbaum A, Berard F, Nicolas JF. Chronic spontaneous urticaria is not an allergic disease. *Eur J Dermatol* 2011; **21**: 349–353.
- Ebo DG, Bridts CH, Hagendorens MM, Aerts NE, De Clerck LS, Stevens WJ. Basophil activation test by flow cytometry: present and future applications in allergology. *Cytometry B Clin Cytom* 2008; **74**: 201–210.
- Kikuchi Y, Kaplan AP. Mechanisms of autoimmune activation of basophils in chronic urticaria. *J Allergy Clin Immunol* 2001; **107**: 1056–1062.
- Altrich ML, Halsey JF, Altman LC. Comparison of the in vivo autologous skin test with in vitro diagnostic tests for diagnosis of chronic autoimmune urticaria. *Allergy Asthma Proc* 2009; **30**: 28–34.
- Frezzolini A, Provini A, Teofoli P, Pomponi D, De Pita O. Serum-induced basophil CD63 expression by means of a tricolour flow cytometric method for the in vitro diagnosis of chronic urticaria. *Allergy* 2006; **61**: 1071–1077.
- Luquin E, Kaplan AP, Ferrer M. Increased responsiveness of basophils of patients with chronic urticaria to sera but hypo-responsiveness to other stimuli. *Clin Exp Allergy* 2005; **35**: 456–460.
- Knol EF. Requirements for effective IgE cross-linking on mast cells and basophils. *Mol Nutr Food Res* 2006; **50**: 620–624.
- Lourenco FD, Azor MH, Santos JC *et al.* Activated status of basophils in chronic urticaria leads to interleukin-3 hyper-responsiveness and enhancement of histamine release induced by anti-IgE stimulus. *Brit J Dermatol* 2008; **158**: 979–986.
- Sainte-Laudy J, Touraine F. Use of basophil sensitivity not reactivity as a good marker for allergy diagnosis. *Inflamm Res* 2009; **58**(Suppl 1): 28–29.