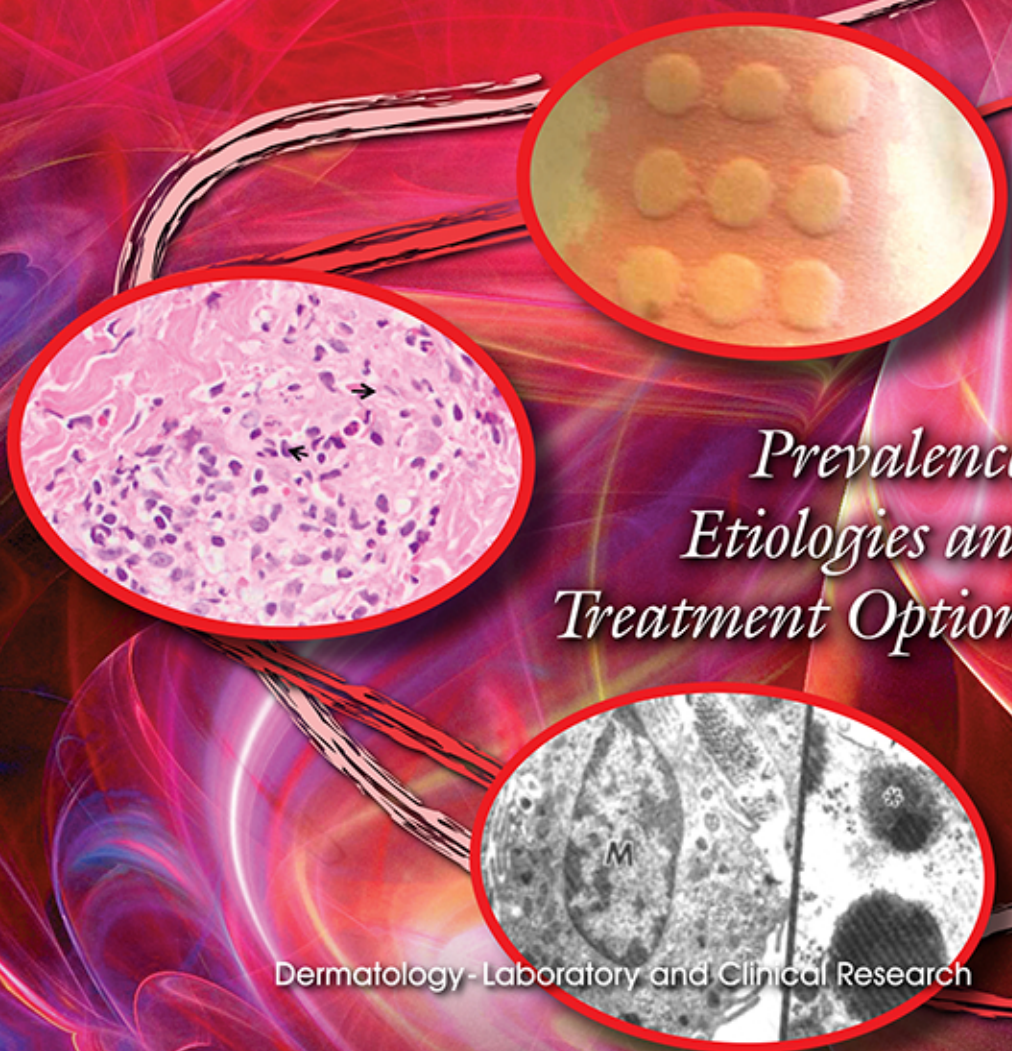
Suran Fernando
Editor

Urticaria



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URTICARIA

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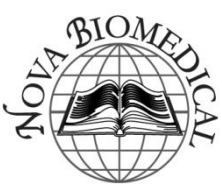
SURAN FERNANDO
EDITOR



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Contents

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Chapter 13

Basophil Activation Tests in Chronic Autoimmune Urticaria

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Diagnosis of Autoimmune Chronic Urticaria

A remarkable portion of chronic spontaneous urticaria (CSU) cases appear to have an autoimmune origin; about 35-45% of patients have IgG autoantibodies against the alpha chain of the high affinity IgE receptor (FcεRIα) and autoantibodies to the IgE molecule have been detected in 5-10% of the patients [1-4]. The activation of blood basophils and cutaneous mast cells by these autoantibodies has been presented in vitro, and this basophil activation was enhanced by complement activation [5]. In the case of these chronic autoimmune urticaria (CAU) patients, more severe and refractory symptoms emphasize the importance of a correct diagnosis [6, 7].

Although the autologous serum skin test (ASST) is readily available for clinical practice, due to its moderate sensitivity and specificity, it is regarded solely as a screening test to specify this group of CAU patients through the detection of the autoreactivity of their sera. Binding assays such as western blotting and ELISA specifically detect autoantibodies, however, not all of these antibodies prove to be functional in CAU [8]. It has been demonstrated by ELISA that most anti-FcεRIα antibodies of CSU patients that activate complement belong to IgG1 and IgG3 subclasses [9]. Originally, the basophil histamine release (BHR) assay was accepted as the gold standard for the identification of functional autoantibodies. Recently, however, flow cytometric basophil activation tests (BAT) have emerged as alternative in vitro methods for detecting circulating autoantibodies in the sera of

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CAU patients. Moreover, accumulating evidence suggests that BAT tests can be an alternative gold standard to the BHR assay [10].

Basophils as Target Cells in Flow Cytometric Tests

Basophil granulocytes are the least abundant leukocytes in the blood (0.2-1% of total leukocytes). They play an important role in inflammatory reactions as effector cells [11]. Human basophils express the high affinity FcεRI which is the receptor that is most prominently involved in the activation of these cells [12]. Basophils can be activated through IgE receptor-mediated or non-IgE receptor-mediated pathways [13]. In the first case, allergens and autoantibodies against IgE or FcεRIα can trigger FcεRI crosslinking followed by the aggregation of these receptors and the stimulation of basophils. FcεRI activation results in a cascade of signal transduction events including the phosphorylation of Syk and Lyn kinases, and the activation of phospholipase C, p38 mitogen-activated protein kinase (MAPK), calcineurin pathways and a nuclear factor of activated T cells. Non-IgE receptor-mediated activation can be caused by endogenous (complement factors) and exogenous substances (bacterial peptides, drugs) affecting other receptors. The activation of basophils eventually leads to the release of several mediators such as histamine, leukotrienes and interleukins, as well as to the altered expression of various cell surface antigens [14].

Among the several cytokines that are capable of potentiating the activation of basophils, interleukin-3 (IL-3) is often used in BATs, as it is able to prime both IgE-dependent as well as IgE–independent agonists [15, 16]. Recently, several basophil-specific markers and activation markers have been identified which have contributed to the development of new flow cytometric BATs [17]. Basophils are more frequently identified by their expression of IgE and CD45, or by their strong expression of the IL-3 receptor alpha subunit (CD123), as well as by CCR3 eotaxin receptor that is a single selection marker for basophils [11, 18].

Phenotypic changes after activation were previously detected most frequently through CD63 and CD203c up-regulation, but there are several other activation markers that have also been identified (e.g., CD13, CD164, CD107a, CD107b, CD11b, CD11c,) [19-23, 14]. In addition, the intracellular marker p38 MAPK has proven to be a specific and sensitive marker for basophil activation in diagnosis of allergic disease [24]. The CD63 antigen, lysosome associated protein (LAMP-3), is a member of tetraspan family and it is known that this molecule is hardly detected on resting basophils and appears on the surface of basophils de novo after stimulation [25]. CD203c is an ecto-nucleotide pyrophosphatase/phosphodiesterase 3, belongs to the type II transmembrane protein family, and is selectively expressed on basophils and mast cells [26, 27]. As CD203c expression is restricted to basophils in peripheral blood, this single marker is suitable both for the identification of basophils and the detection of their activation. The up-regulation of CD63 and CD203c follow different mechanisms: CD63 mainly reflects the anaphylactic degranulation pathway that is associated with the release of preformed mediators, whereas CD203c shows piecemeal degranulation [23].

Table. Identification of autoimmune chronic urticaria patients by in vitro basophil activation tests

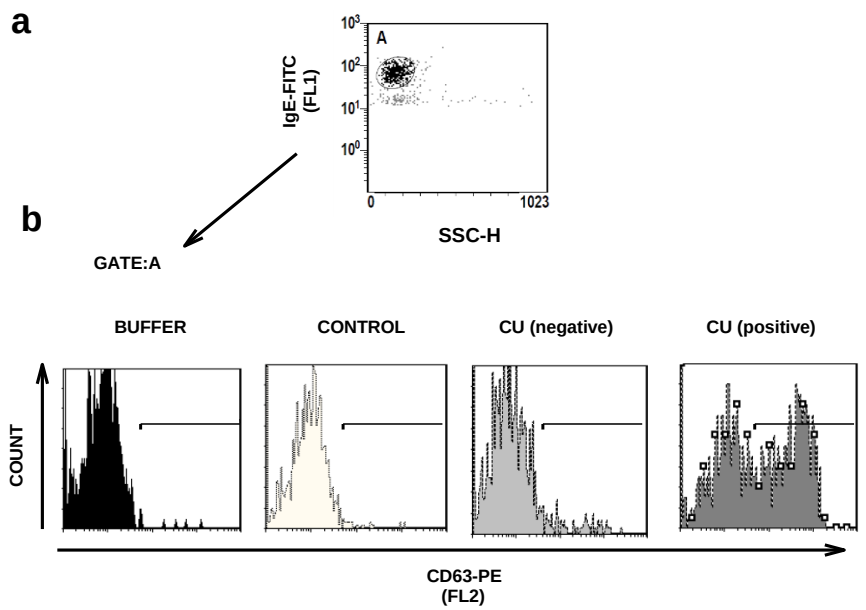
Reference	Number of CU Patients	Basophil Marker	Basophil Activation Marker	Il-3 Priming	Time of Incubation with CU Sera (Min)	Basophil Source	Donor Cell	
							ATOPIC D_A	NONA-TOPIC D_{NA}
[28]	40	IgE (BASOTEST	CD63 (BASOTEST)	+	20	whole blood	1 D_A	1 D_{NA}
[30]	30	IgE	CD63	–	40	leukocytes	2 D_A highly sensitized	1 D_{NA}
[38]	72	IgE	CD63	–	40	leukocytes	1 D_A h.s.	1 D_{NA}
[40]	109	IgE	CD63	–	40	leukocytes	1 D_A h.s.	nd.
[29]	23	IgE (BASOTEST)	CD63 (BASOTEST)	+	60	whole blood,	1D_A h.s.	nd.
[43]	64	CD123/HLA-DR	CD63	–	30	whole blood	1D_A h.s.	1D_{NA}
[46]	32.	IgE/CD45	CD203c	–	10	whole blood	1 D_A	nd.
[39]	101	IgE	CD63	+	30	buffy coat	nd.	1D_{NA}
[47]	110	CCR3	CD63 CD203c	+ (optimized)	30	PBMC	5 D_A	6 D_{NA}

Abbreviations: IL-3 – Interleukine-3; DA – atopic donor; h.s. – highly sensitized; DNA – non-atopic donor; PBMC – peripheral blood mononuclear cells, BASOTEST – Orpegen Pharma, Heidelberg, Germany; nd – not detected.

Performing functional BAT tests requires careful consideration of test parameters, including donor responsiveness, sample treatment, proper flow cytometric gating of basophils and the selection of activation markers, as described elsewhere [13, 22].

Detection of Functional Autoantibodies by Flow Cytometry

BAT tests aiming to demonstrate functional autoantibodies in CAU sera follow different methodological approaches. The tests detect different basophil cell surface and activation markers to identify basophils, apply different types of basophils (differently sensitized donor cells in whole blood or partly separated form, or cell lines), and either use IL-3 for priming or not (Table).



CU – chronic urticaria, control – non-atopic healthy control serum.

Figure. Detection of functional autoantibodies in chronic urticaria sera by two color flow cytometry. **a,** atopic donor leukocytes were stimulated with CU sera for 40 minutes at 37°C, and then labelled with IgE-FITC and R-phycoerythrin-conjugated anti-human CD63 monoclonal antibody. Basophil cells were gated (A) by their IgE-FITC positivity in a side scatter (SSC) (abscissa) vs, FL1 (ordinate) dot plot. The FL1 threshold was set to eliminate the irrelevant cell population. **b.** representative histograms presents surface expressions of CD63 activation marker on basophils after incubation with buffer, healthy control serum, and two different CU sera.

It was Wedi and colleagues who identified functional autoantibodies in heat-inactivated, diluted CSU sera for the first time through the use of two-colour BATs using whole blood and IL-3 stimulation [28]. A commercial test kit originally designed for the in vitro determination of allergen-induced basophil activation (Basotest, Orpegen Pharma, Germany) was adopted. In this test, anti-IgE was used to label basophils and the activation of basophils

was detected with an anti-CD63 monoclonal antibody. In their experiments, CSU sera with a positive ASST expressed the basophil activation marker CD63, produced sulphidoleukotriene de novo and demonstrated histamine-releasing activity. However, there was no correlation between the BHR and BAT in this system. Furthermore, the serum-induced basophil CD63 surface expression was also demonstrated in atopic and non-atopic control subjects without urticaria. As a consequence of the unsatisfactory specificity of this method, the authors did not consider the CAU serum-induced basophil CD63 expression as a diagnostic marker of CAU.

Similarly, Basotest was used to detect basophil activating autoantibodies in CSU sera by De Swerdt et al., who changed several parameters compared to the experiments described previously [29, 28]. In their experiment, using an enhanced concentration of IL-3, undiluted CSU sera and a longer incubation time for the activation of atopic donor cells resulted in a positive BAT in 51% of patients with chronic idiopathic urticaria [29]. It was also shown that decomplementation of CSU sera slightly reduced the basophil activating capacity of the sera and it was the IgG fraction that was responsible for this activity. The occurrence of other autoantibodies (anti-nuclear, anti-thyroglobulin, anti-thyroid peroxidase or anti-parietal cell antibodies) was significantly higher in patients with positive BAT results, supporting the autoimmune origin of this group. A significant correlation between the severity of urticaria and the degree of CD63 expression on basophils was also demonstrated.

There were some discordant but not overlapping results obtained when comparing ASST and BATs. The authors proposed that a positive BAT coupled with a negative ASST could be attributed to the higher sensitivity of the BAT as compared to the ASST or because desensitization of dermal mast cells could have occurred at the time of the ASST producing a negative result. A false-negative BAT can arise from the inhibition of anti-IgE antibody binding by fluorochrome-labelled anti-IgE used for the staining the basophils. Furthermore, the presence of mast-cell specific histamine releasing factors can also cause a positive ASST and negative BAT result [6, 29]. The authors suggested performing both the CD63 assay and ASST to detect CAU.

A modification of the two-colour BAT that also uses anti-IgE/anti-CD63 labelling of basophils has been introduced and validated by our group, and this method utilizes highly-sensitized atopic donor cells without IL-3 priming on dextran sedimented leukocytes [30].

The idea of using highly atopic donor cells emerged from the observation that allergic individuals have increased numbers of basophils and elevated levels of serum IgE, and the latter correlate with the number of FcεRI on basophils [31-33]. Moreover, basophils from highly sensitized allergic subjects are in a primed state and have an enhanced capacity to release mediators as well as increased sensitivity for activation via FcεRI or other receptors [34-36]. IL-3 is frequently used in in vitro basophil activation assays due to its capacity to potentiate or prime basophils [37]. Using basophils from highly-sensitized atopic donors prevents the preliminary priming of cells with this cytokine.

The utility of this assessment was corroborated by comparing the results of the BAT with those of the ASST and the serum BHR assay. There was a strong correlation between the results of the BAT and the ASST on two highly-sensitized atopic donor cells. In addition, significant correlations were found between the gold standard serum BHR assay and the BAT carried out on atopic donor cells [38]. The correlation between the serum BHR assay and the BAT was also proven by Altrich et al., but they found the serum BHR assay to be more sensitive [39]. The utility of the BAT was also demonstrated in our next study where physical

urticaria, CSU and CAU patients were evaluated, and as a result, the modified CD63 expression assay proved to be suitable for the diagnosis of the CAU group [40]. In this investigation, the CAU group had the more severe forms of urticaria as characterized by the urticaria activity score [41]. In another work, our results also provided supportive data for the statement by De Swerdt et al. that the degree of CD63 cell surface expression in the BAT is indicative of the severity of CAU [29, 42].

A tricolour flow cytometric assay was performed to assess the diagnostic effectiveness of the BAT in discriminating between ASST+ and ASST– CSU patients [43]. Basophils were identified as CD123+HLA-DR– cells considering that the alpha chain of IL-3 receptor/CD123 is expressed mainly on basophils and monocyte-derived dendritic cells, and also the fact that basophils do not express HLA-DR antigen [44, 45]. The whole blood of one highly-sensitized atopic and one non-atopic donor was used without IL-3 priming and CD63 up-regulation was detected on basophils after incubation with CSU sera. Similar to our results, the ASST+ CSU sera induced significantly higher basophil CD63 expression compared to that of ASST– and healthy control sera. This tricolour system had higher sensitivity and specificity than our experiment, which applied whole blood without IL-3 priming as well, but with an anti-IgE monoclonal antibody for the purpose of labelling basophils. The fact that FcεRIα expression and the bound IgE on the cell surface of basophils can vary considerably, whereas the IL-3 receptor (CD123) is constitutively expressed on basophils independent of the allergy status of the donor [44, 45], can be one explanation for the higher specificity of this tricolour system. Monitoring cyclosporin therapy on three patients supported the usefulness of this method as well.

In another tricolour BAT performed by Yasnowsky et al., CD203c activation markers were measured instead of CD63, and labelling of basophils with anti-IgE was supplemented with anti-CD45 staining to identify CAU patients [46]. In this case, the ASST+ CSU sera triggered significantly higher basophil CD203c expression in comparison with ASST– CSU sera using whole blood of atopic donors without IL-3 priming. The enhancement of the CD203c expression correlated with the results of ASSTs and serum BHR assays. After the IgG depletion of representative CSU sera, the up-regulation of CD203c diminished, thereby providing supporting evidence that an IgG antibody can mediate CD203c expression. Interestingly, sera of patients with negative ASST induced a significantly higher CD203c expression than normal controls, although this was significantly lower than that of ASST+ patients. This observation suggests that there are undefined serum-factors present in CSU sera that can up-regulate CD203c. These results are similar to previous data, although Wedi et al. had a higher percentage of normal control inducing CD63 expression and Gyimesi et al. reported a higher percentage of ASST+ sera inducing CD63 expression [28, 30].

Discrepancies between the different BAT tests partly arise from using different sources of donor cells, which have individual features. The study conducted by Gentinetta et al. revealed that it is to what extent the basophil is primed rather than the mean IgE receptor density that influences the responsiveness of these cells in the BAT [47]. The IgE receptor density correlates instead with the degree of sensitization. To handle the differences in basophil reactivity obtained from different donors, these authors titrated the IL-3 concentration needed for optimal basophil activation. Measuring the CD63 expression on anti-CCR3 labelled basophils in 5 atopic and 6 non-atopic donors PBMC provided reproducible and comparable in vitro test results. However, as IL-3 alone is able to increase CD203c expression, this alternative basophil activation marker was not suitable in this case [48].

Perspectives

Very recently, a new multicolour flow cytometry method analysing BHR (Histaflow) was introduced which connects the detection of the basophil intracellular histamine content with CD63 and CD203c quantification [49]. Adaptation of this method would be a valuable new tool in the diagnosis of CAU. In a pilot experiment, the combined analysis of CD63 and CD203c activation markers and intracellular signal transduction pathways involved in basophil activation was assessed by flow cytometry [50]. Investigating the effect of specific kinase inhibitors on the phosphorylation of p38 MAPK and extracellular signal-regulated kinase (ERK)-1/2 revealed that different signalling pathways take part in the pathogenesis of CSU, and IgE-dependent and IgE-independent basophil activation can be distinguished after stimulation with CSU serum. Simultaneous analysis of basophil phenotype and intracellular signalling pathways might be a novel way to reveal the mechanisms that play a role in the pathogenesis and treatment of CAU.

Conclusion

The EAACI task–force position paper published recently in *Allergy* acknowledges the utility of the basophil CD63 expression assay in diagnosis of CAU in addition to the BHR assay, ASST and western blot/ELISA [10]. On the basis of the presented data, the future of flow cytometric BATs is promising and they may become crucial tools in the diagnosis of CAU and in the monitoring of response to therapy.

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References

[1] Hide M, Francis DM, Grattan CE, Hakini J, Kochan JP, Greaves MW. Autoantibodies against the high affinity IgE receptor as a cause of histamine release in chronic urticaria. *N. Engl. J. Med.* 1993; 328: 1599-1604.

[2] Kaplan AP. Chronic urticaria: Pathogenesis and treatment. *J. Allergy Clin. Immunol.* 2004; 114: 465-74.

[3] Gruber BL, Baeza ML, Marchese MJ, Agnello V, Kaplan AP. Prevalence and functional role of anti-IgE autoantibodies in urticarial syndromes. *J. Invest. Dermatol.* 1988; 90: 213-217.

[4] Grattan CE, Francis DM, Hide M, Greaves MW. Detection of circulating histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria. *Clin. Exp. Allergy* 1991; 21: 695-704.

[5] Kaplan AP, Greaves M. Pathogenesis of chronic urticaria. *Clin. Exp. Allergy* 2009; 39: 777-787.

[6] Sabroe RA, Fiebiger E, Francis DM, Maurer D, Seed PT, Grattan CE, Black AK, Stingl G, Greaves MG, Barr RM. Classification of anti-Fc epsilon RI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J. Allergy Clin. Immunol.* 2002; 110: 492-499.

[7] Kaplan AP, Finn A. Pathogenesis and treatment of chronic urticaria. *Postgrad. Med.* 1999; 106: (7 Suppl) 12-17.

[8] Fiebiger E, Hammerschmid F, Stingl G, Maurer D. Anti-FcεRIα autoantibodies in autoimmune mediated disorders. Identification of a structure function relationship. *J. Clin. Invest.* 1998; 101: 243-251.

[9] Sopundararajan S, Kikuchi Y, Kusumam DVM, Kaplan AP. Functional assessment of pathogenic IgG subclasses in chronic autoimmune urticaria. *J. Allergy Clin. Immunol.* 2005; 115: 815-821.

[10] Konstantinou GN, Asero R, Ferrer M, Knol EF, Maurer M, Raap U, Schmid-Grendelmeier P, Skov PS, Grattan CE. EAACI taskforce position paper: evidence for autoimmune urticaria and proposal for defining diagnostic criteria. *Allergy* 2013; 68: 27-36.

[11] Valent P. The phenotype of human eosinophils, basophils, and mast cells. *J. Allergy Clin. Immunol* 1994; 94: 1177-1183.

[12] Falcone FH, Haas H, Gibbs BF. The human basophil: a new appreciation of its role in immune responses. *Blood* 2000; 96: 4028-4038.

[13] Kleine-Tebbe J, Erdmann S, Knol EF, McGlashan DW Jr., Poulsen LK, Gibbs BF. Diagnostic tests based on human basophils: potentials, pitfalls and perspectives. *Int. Arch. Allergy Immunol.* 2006; 141: 79-90.

[14] Bochner BS. Systemic activation of basophils and eosinophils: markers and consequences. *J. Allergy Clin. Immunol.* 2000; 106: S292-302.

[15] Kleine Budde I, de Heer PG, van der Zee JS, Aalberse RC. The stripped basophil histamine release bioassay as a tool for the detection of allergen-specific IgE in serum. *Int. Arch, Allergy Immunol.* 2001; 126: 277-285.

[16] Ferrer M, Luquin E, Kaplan AP. IL-3 effect on basophils histamine release upon stimulation with chronic urticaria sera. *Allergy* 2003; 58: 802-807.

[17] Florian S, Sonneck K, Czerny M, Hennersdorf F, Hauswirth AW, Bühring HJ, Valent P. Detection of novel leukocyte differentiation antigens on basophils and mast cells by HLDA8 antibodies. *Allergy* 2006; 61: 1054-1062.

[18] Hausmann OV, Gentinetta T, Fux M, Ducrest S, Pichler WJ, Dahinden CA. Robust expression of CCR3 as a single basophil selection marker in flow cytometry. *Allergy* 2011; 66: 85-91.

[19] Boumiza R, Debard AL, Monneret G. The basophil activation test by flow cytometry: recent developments in clinical studies, standardisation and emerging perspectives. *Clin. Mol. Allergy* 2005; 3: 9.

[20] Chirumbolo S, Vella A. Ortolani R, De Gironcoli M, Solero P, Tridente G, Bellavite P. Differential response of human basophil activation markers: a multi-parameter flow cytometry approach. *Clin. Mol. Allergy* 2008; 6: 12.

[21] McGlashan D Jr. Human basophil phenotypes and the associated signalling mechanisms. *The Open. Allergy J.* 2010, 3: 60-72.

[22] de Weck AL, Sanz ML, Gamboa PM, Aberer W, Bienvenu J, Blanca M, Demoly P, Ebo DG, Mayorga L, Monneret G, Sainte-Laudy J. Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls. *Int. Arch. Allergy Immunol.* 2008; 146: 177-189.

[23] Hennersdorf F, Florian S, Jakob A, Baumgärtner K, Sonneck K, Nordheim A, Biedermann T, Valent P, Bühring HJ. Identification of CD13, CD107a, and CD164 as novel basophil-activation markers and dissection of two response patterns in time kinetics of IgE-dependent up-regulation. *Cell Res.* 2005; 15: 325-335.

[24] Aerts NE, Dombrecht EJ, Bridts CH, Hagendorens MM, de Clerck LS, Stevens WJ, Ebo DG. Simultaneous flow cytometric detection of basophil activation marker CD63 and intracellular phosphorylated p38 mitogen-activated protein kinase in birch pollen allergy. *Cytometry B Clin. Cytom.* 2009, 76: 8-17.

[25] 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.

[26] Bühring HI, Simmons PJ, Pudney M, Müller R, Jarrossay D, van Agthoven A, Wilheim M, Brugger W, Valent P, Kanz L. The monoclonal antibody 97A6 defines a novel surface antigen expressed on human basophils and their multipotent and unipotent progenitors. *Blood* 1999; 94: 2343-2356.

[27] Bühring HI, Seiffert M, Giesert C, Marxer A, Kanz L, Valent P, Sano K. The basophil activation marker defined by antibody 97A6 is identical with the ectonucleotide pyrophosphatase/phosphodiesterase 3. *Blood* 2001; 97: 3303-3305.

[28] Wedi B, Novakocic V, Koerner M, Kapp A. Chronic urticaria serum induces histamine release, leukotriene production, and basophil CD63 surface expression – Inhibitory effects of anti-inflammatory drugs. *J. Allergy Clin. Immunol.* 2000; 105: 552-560.

[29] De Swerdt A, Van den Keybus C, Kasran A, Cadot P, Neyens K, Coorevits L, Kochuyt AM, Degreef H, Ceuppens JL. Detection of basophil–activating IgG autoantibodies in chronic idiopathic urticaria by induction of CD63. *J. Allergy Clin. Immunol.* 2005; 116: 662-667.

[30] Gyimesi E, Sipka S, Dankó K, Kiss E, Hídvégi B, Gál M, Hunyadi J, Irinyi B, Szegedi A. Basophil CD63 expression assay on highly sensitized atopic donor leukocytes – a useful method in chronic autoimmune urticaria. *Br. J. Dermatol.* 2004; 151: 388-396.

[31] Saini SS, Klion AD, Holland SM, Hamilton RG, Bochner BS, McGlashan DW Jr. The relationship between serum IgE and surface levels of FcεR on human leukocytes in various diseases: correlation of expression with FcεRI on basophils but not on monocytes or eosinophils. *J. Allergy Clin. Immunol.* 2000; 106: 514-520.

[32] Reilly KM, Yap PL, Dawes J, Barnetson RS, MacKenzie F, Allan TL. Circulating basophil counts in atopic individuals. *Int. Arch. Allergy Appl. Immunol.* 1987; 84: 424-426.

[33] McGlashan DW Jr, Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, Sterbinsky SA, Hamilton RG, Lichtenstein LM. Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J. Immunol.* 1997; 158: 1438-1445.

[34] Lie WJ, Knol EF, Mul FP, Jansen HM, Roos D, Van der Zee JS. Basophils from patients with allergic asthma show a primed phenotype. *J. Allergy Clin. Immunol.* 1999; 104: 1000-1007.

[35] Casoralo V, Spadaro G, Marone G. Human basophil releasability. VI. Changes in basophil releasability in patients with allergic rhinitis or bronchial asthma. *Am. Rev. Respir. Dis.* 1990; 142: 1108-1111.

[36] Abrahamsen O, Haas H, Schreiber J, Schlaak M. Differential mediator release from basophils of allergic and non-allergic asthmatic patients after stimulation with anti-IgE and C5a. *Clin. Exp. Allergy* 2001; 31: 368-378.

[37] Sainte-Laudy J, Sabbah A, Vallon C, Guerin JC. Analysis of anti-IgE and allergen induced human basophil activation by flow cytometry. Comparison with histamine release. *Inflamm. Res.* 1998; 47: 401-408.

[38] Szegedi A, Irinyi B, Gál M, Husz J, Dankó K, Kiss E, Sipka S, Szegedi G, Gyimesi E. Significant correlation between the CD63 assay and histamine release assay in chronic urticaria. *Br. J. Dermatol.* 2006; 155: 67-75.

[39] 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.

[40] Irinyi B, Széles Gy, Gyimesi E, Tumpek J, Heredi E, Dimitrios G, Ádány R, Hunyadi J, Szegedi A. Clinical and laboratory examinations in the subgroups of chronic urticaria. *Int. Arch. Allergy Immunol.* 2007; 144: 217-225.

[41] Breneman D, Bronsky EA, Bruce S, Kalivas JT, Klein GL, Roth HL, Tharp MD, Treger C, Soter N. Cetrizine and asternizole therapy for chronic idiopathic urticaria: a double-blind, placebo-controlled, comparative trial. *J. Am. Acad. Dermatol.* 1995; 33: 192-198.

[42] Irinyi B, Gyimesi E, Garaczi E, Bata ZS, Kemény L, Zeher M, Remenyik E, Szegedi A. Extended diagnostic value of autologous serum skin test and basophil CD63 expression assay in chronic urticaria. *Br. J. Dermatol.* 2013; 168: 656-658.

[43] Frezzolini A, Provini A, Teofoli P, Pomponi D, De Pita G. 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.

[44] Moretti S, Lanza F, Dabusti M, Tieghi A, Campioni D, Dominici M, Castoldi GL. CD123 (Interleukin 3 receptor alpha chain). *J. Biol. Requl. Homeost Agents* 2001; 15: 98-100.

[45] Varro R, Chen CH. A no-wash 3-colour basophil degranulation assay using the CD123+ HLA-DR phenotype for basophil classification. *Cytometry* 2000; 10: S116.

[46] Yasnowski KM, Dreskin SC, Efaw B, Schoen D, Vedanthan PK, Alam R, Harbeck RJ. Chronic urticaria sera increase basophil CD203c expression. *J. Allergy Clin. Immunol.* 2006; 117: 1430-1434.

[47] Gentinetta T, Pecaric-Petkovic T, Wan D, Falcone FH, Dahinden CA, Pichler WJ, Hausmann OV. Individual IL-3 priming is crucial for consistent in vitro activation of donor basophils in patients with chronic urticaria. *J. Allergy Clin. Immunol.* 2011; 128: 1227-1234.

[48] Hauswirth AW, Sonneck K, Florian S, Krauth MT, Bohm A, Sper WR, Valenta R, Schemthaner GH, Printz D, Fritsch G, Buhning HJ, Valent P. Interleukin-3 promotes the expression of E-NPP3/CD203c on human blood basophils in healthy subjects and in patients with birch pollen allergy. *Int. J. Immunopathol. Pharmacol.* 2007; 20: 267-278.