

CORRESPONDENCE

Regarding Asero R, Lorini M, Chong SU, Zuberbier T & Tedeschi A. Assessment of histamine-releasing activity of sera from patients with chronic urticaria showing positive autologous skin test on human basophils and mast cells. Clin Exp Allergy 2004; 34:1111–14**Comments regarding 'Assessment of histamine-releasing activity of sera from patients with chronic urticaria showing positive autologous skin test on human basophils and mast cells' by Asero et al. A. P. Kaplan**

The article by Asero et al. is the first to try to compare an *in vivo* assay employing a skin test with *in vitro* methods for the diagnosis of chronic autoimmune urticaria. They emphasize the variability one finds, depending on the source of basophils and mast cells employed, and demonstrate that sera that give strongly positive skin tests may yield much lower percentage of positives when any one source of basophils or mast cells is employed. But, there was 95% positivity observed if one uses a panel of cells and considers a 'positive' if there is significant histamine release in any one of them.

Although I am in general agreement with these data, the conclusions are misleading. The authors selected 93 strongly positive patients by skin tests, while the other 141 weakly positive subjects are not included in the analysis, and no statistical methods are employed. These 141 other positives were the ones where we have questioned the utility and specificity of the skin test and agree that strongly positive tests are probably reliable [1]. The skin test is considered positive if the serum sample exceeds the control by 1.5 mm; a very small measurement. The control, however, is saline, whereas the proper control is a normal serum. When blood is clotted bradykinin is generated, and some proteolysis of C5 occurs so that C5a is released. These vasodilators plus the irritant effect of serum vs. saline can yield positives that may not be meaningful. Furthermore, the strongly positive 93 sera employed in these studies were used as the standard, i.e. set at 100% specificity for autoimmunity, so that any *in vitro* method could only equal this if perfect correlation is seen, and a lesser degree of correlation is of course likely, and was found. Most laboratories employing basophils choose a source from atopic subjects, and the cells can be primed with IL-3 to increase sensitivity [2]. A positive is set at greater than 15% histamine release, rather than 5% (when saline is used) to screen out non-specific effects. When selected donors of this sort are used there may not only be a strong correlation with sera that are clearly positive by skin test, but may discriminate between skin tests that are weakly positive because the antibody is present, from those in which a non-specific skin reaction is obtained. When *in vivo* methods are employed, it is the donor cells, not the sera, that need to be selected carefully. It is my contention that the basophil release assay done optimally is more quantitative, more sensitive (range 15–100% release) and more specific than the autologous skin test, and that only strongly positive skin

tests reliably distinguish chronic autoimmune urticaria from chronic idiopathic urticaria.

References

- 1 Kikuchi Y, Kaplan AP. A note for C5a in augmenting IgG dependent histamine release from basophils in chronic urticaria. *J Allergy Clin Immunol* 2002; 109:114–8.
- 2 Ferrer M, Luquin E, Kaplan AP. IL3 effect on basophils histamine release upon stimulation with chronic urticaria sera. *Allergy* 2003; 58:802–7.

A. P. KAPLAN

Division of Pulmonary and Critical Care Medicine,
Allergy and Clinical Immunology
Medical University of South Carolina
96 Jonathan Lucas Street
Suite 812 CSB
PO Box 250630
Charleston, SC 29425
USA
E-mail: kaplana@musc.edu

Comments regarding 'Assessment of histamine-releasing activity of sera from patients with chronic urticaria showing positive autologous skin test on human basophils and mast cells' by Asero et al. M. Ferrer

Each new contribution to the understanding of the aetiology of chronic urticaria is important and the article by Asero et al. is of considerable interest. I would, however, like to point out some methodological issues.

The authors based their conclusions on the ability to induce histamine release in basophils and mast cells employing selected sera from patients with chronic urticaria, all of which induced a strong autologous serum skin test (ASST). A subgroup of 93 patients from a total of 487 was studied and an additional 141 were ASST positive that were not included. It would have been more appropriate to perform the *in vitro* analysis on all the patients and compare the two methods using appropriate statistics. A healthy control group [1] is important since a true negative ASST control (skin test performed with normal sera) cannot be carried out for ethical reasons. ASST is a technique that has variability depending on the performer [2] and there is also considerable variation

among different mast cell and basophil donors [3–6]. Such variability could have been addressed from data derived from a healthy control group as well as basophils and mast cells responses of patients with weakly positive ASST. The authors do not explain why 5% was taken as the level to define positive histamine-releasing sera and they choose a different level of positivity for the mast cell studies because of 'high variability of different normal sera'. Here a control group was apparently utilized but those data were not included. Finally, no statistical methods and more than two-thirds of the ASST-positive subjects were excluded.

Thus, while some useful comparative data are obtained using strongly positive ASST sera as the standard, a real comparison of the two methods was not attempted.

References

- 1 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.
- 2 Sabroe RA, Grattan CE, Francis DM, Barr RM, Kobza BA, Greaves MW. The autologous serum skin test: a screening test for autoantibodies in chronic idiopathic urticaria. *Br J Dermatol* 1999; 140:446–52.
- 3 Biscoff SC, Zwahlen R, Stucki M et al. Basophil histamine release and leukotrienes production in response to anti-IgE and anti-IgE receptor antibodies. Comparison of normal subjects and patients with urticaria, atopic dermatitis or bronchial asthma. *Int Arch Allergy Immunol* 1996; 110:261–71.
- 4 Ferrer M, Luquin E, Kaplan AP. IL3 effect on basophils histamine release upon stimulation with chronic urticaria sera. *Allergy* 2003; 58:802–7.
- 5 Kopley CL, Youssef L, Andrews RP, Wilson BS, Oliver JM. Multiple defects in Fc epsilon RI signaling in Syk-deficient nonreleaser basophils and IL-3-induced recovery of Syk expression and secretion. *J Immunol* 2000; 165:5913–20.
- 6 Kopley CL, Youssef L, Andrews RP, Wilson BS, Oliver JM. Syk deficiency in nonreleaser basophils. *J Allergy Clin Immunol* 1999; 104:279–84.

M. FERRER

Department of Allergy and Clinical Immunology
Clínica Universitaria
Universidad de Navarra
Pamplona, Spain
E-mail: mferrerp@unav.es

Response by authors: Assessment of histamine-releasing activity of sera from patients with chronic urticaria showing positive autologous skin test on human basophils and mast cells. R. Asero and A. Tedeschi

Dr Kaplan's and Dr Ferrer's letters raise a number of interesting issues. The following considerations reply to comments made in both letters.

Autologous serum skin test

We certainly agree that the utility and specificity of the autologous serum skin test (ASST) may be questioned in patients showing weak skin responses. We also agree that considering the ASST positive if the weal exceeds by 1.5 mm, the saline control is probably a small measurement although this cut-off has been clearly established by authoritative authors [1]. Our study stemmed from previous observations that sera from patients showing an unequivocally positive response on ASST are frequently not able to induce histamine release (HR) from basophils *in vitro* [2, 3]. Thus, as we clearly declared at the end of the introduction section, our aim was to assess whether such 'low sensitivity' of *in vitro* assays could be overcome by the use of both basophils and mast cells from different donors. To this end there was a need to select patients showing a strong reactivity on ASST; the weal induced by the intradermal injection of autologous serum exceeded 10 mm in all our patients, with an evident flare. We can conclude that the study confirmed our hypothesis: sera from nearly all these patients were finally found to release histamine from basophils and/or mast cells *in vitro*.

We agree that bradykinin as well as C5a may be generated during the clotting process; nonetheless, in our hands, ASST has always shown excellent specificity: in a previous study [3] 0/20 atopic subjects with chronic urticaria showed a positive ASST, and no skin reactivity has been observed so far by Dr Tedeschi in a number of normal subjects during recent, further investigations (unpublished). Further, in a recent paper Sabroe et al. found that ASST was positive only in 1/40 normal subjects [4]. Finally, we have carried out ASST in about 1000 patients so far, and the intradermal injection of saline induced some weal-and-flare reaction only in very few patients with severe dermographism.

About the use of a positive ASST as a 'gold standard' for the detection of auto-reactivity, we agree that this might be somewhat questionable; however, the same holds true also for *in vitro* HR assays (HRAs) as a number of patients negative on HRA show a markedly positive ASST. In view of recent studies showing that patients with autoimmune chronic urticaria have a more severe disease than patients with idiopathic chronic urticaria, we think that the ASST remains the only easily accessible diagnostic tool in normal clinical settings.

It is possible that a proper control for ASST is normal serum, but this cannot be used for ethical reasons.

In vitro assays

As far as we know, priming basophils with IL-3 enhances HR but does not change the sensitivity of the assay (in fact the cut-off must be set at 15% rather than at 5%). Moreover, it has been shown that IL-3 *per se* may induce HR from human basophils from atopic subjects [5, 6], which may add a further confounding factor to the picture.

We agree that *in vitro* assays may be helpful in discriminating between skin tests that are weakly positive but, as observed before, this was not the aim of the study and patients showing weak skin responses were not included.

As to the sensitivity of *in vitro* tests, the fact remains that sera from only a proportion of patients strongly positive on ASST induce HR *in vitro* if cells from a single donor are used and this clearly represents a further obstacle to the routine use of these assays.

We used a 5% cut-off for basophil assays because HR +2SD induced by a large number of normal sera did not exceed this level. Similarly, the cut-off in mast cell experiments was set on the basis of the results of HR+2SD induced by normal sera as clearly stated in the Methods section of our paper.

In conclusion, perhaps Dr Kaplan's and Dr Ferrer's criticisms of our paper stem from a misinterpretation of the aim of our study.

References

- 1 Sabroe RA, Grattan CE, Francis DM et al. The autologous serum skin test: a screening test for autoantibodies in chronic idiopathic urticaria. *Br J Dermatol* 1999; 140:446–52.
- 2 Greaves MW. Chronic urticaria. *J Allergy Clin Immunol* 2000; 105:664–72.
- 3 Asero R, Tedeschi A, Lorini M et al. Chronic urticaria: novel clinical and serological aspects. *Clin Exp Allergy* 2001; 31:1105–10.
- 4 Sabroe RA, Fiebiger E, Francis DM et al. Classification of anti-FcεpsilonRI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J Allergy Clin Immunol* 2002; 110:492–9.
- 5 Miadonna A, Roncarolo MG, Lorini M, Tedeschi A. Inducing and enhancing effects of IL-3, -5, and -6 and GM-CSF on histamine release from human basophils. *Clin Immunol Immunopathol* 1993; 67:211–15.
- 6 Haak-Frendscho M, Arai N, Arai IK, Baeza ML, Finn A, Kaplan AP. Human recombinant granulocyte-macrophage colony stimulating factor and interleukin-3 cause basophil histamine release. *J Clin Invest* 1988; 82:17.

R. ASERO* AND A. TEDESCHI†

*Ambulatorio di Allergologia

Clinica San Carlo

Paderno Dugnano and

†Unità Operativa di Medicina Interna I

IRCCS Ospedale Maggiore Policlinico

Milan

Italy

E-mail: r.asero@libero.it