

Significant correlation between the CD63 assay and the histamine release assay in chronic urticaria

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Summary

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Key words

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Conflicts of interest

None declared.

Background Antibodies directed to the α subunit of the high affinity IgE receptor and the IgE molecule are proposed to be of pathogenetic relevance in a group of patients with chronic urticaria (CU). The diagnosis of autoimmune chronic urticaria (ACU) is difficult; the autologous serum skin test (ASST) seems to be a useful screening test, but reliable, additional confirmatory methods are needed.

Objectives To assess the diagnostic value of a modified serum-induced basophil activation test, the CD63 expression assay, in the diagnosis of ACU by comparing the results of the CD63 assay with the results of the histamine release (HR) test, the ASST and serum levels of soluble CD40 ligand (sCD40L).

Methods Using basophils from an atopic (D_A) and a nonatopic (D_{NA}) donor the activity of sera of 72 patients with CU were measured in HR assay by enzyme-linked immunosorbent assay and in CD63 expression assay by flow cytometry. An ASST was carried out in all patients; in 30 of the 72 patients sCD40L was detected and correlations were derived between the different assays. Sera of 20 normal controls and 26 patients with systemic autoimmune diseases were also tested in the HR assay and in the CD63 expression assay.

Results Histamine-releasing activity was detected in the sera of 51% (D_A) and 32% (D_{NA}) of CU patients and 57% (D_A) and 28% (D_{NA}) of sera upregulated CD63 expression on the surface of basophils from the different donors. There was a significant correlation between the HR and the CD63 assays carried out on both donors, but the ASST showed a strong correlation with the HR assay only for basophils from the D_A . The serum level of sCD40L was significantly higher in patients with CU compared with controls, but the difference between the autoimmune and the nonautoimmune groups was not significant.

Conclusions The CD63 expression assay seems to be a reliable functional test in the diagnosis of ACU, particularly if highly sensitive donor basophils are used, but the determination of the sCD40L serum level was not sufficient to differentiate between the autoimmune and the nonautoimmune patient groups.

Chronic urticaria (CU) is characterized by the occurrence of widespread short-lived weals lasting for at least 6 weeks.^{1–3} There is now strong evidence for an autoimmune basis for a group of patients with CU, as currently it has become clear that 27–50% of them have functional autoantibodies against the α chain of the high affinity IgE receptor (Fc ϵ RI α) or the IgE molecule.^{4–8} It is difficult to distinguish the autoimmune chronic urticaria (ACU) group from the chronic idiopathic urticaria (CIU) group.⁹ In spite of the fact that the autologous serum skin test (ASST) does not identify patients with autoantibodies but rather with 'autoreactive' disease, the

ASST seems to be a useful screening test, which should be completed with confirmation by functional or binding assays.^{10–13} Functional tests detect biologically relevant mast cell/basophil-activating serum factors and the basophil histamine release (HR) assay is currently the 'gold standard' for detecting functional autoantibodies in sera of patients with CU, but functional tests do not identify directly the molecule of interest.¹¹ On the other hand, Western blot and enzyme-linked immunosorbent assay (ELISA) are not commercially available and false positive and negative results may occur.^{12,13}

The CD63 molecule is a tetraspan granule protein that is not expressed on resting basophils, but is upregulated with high density after activation.¹⁴ In our earlier study we were able to develop a modified basophil CD63 expression assay and demonstrated that basophils of highly sensitized atopic donors can be successfully used without priming with interleukin (IL)-3 for the *in vitro* flow cytofluorimetric diagnosis of ACU.¹⁵

We detected a strong correlation between the positive CD63 assay and the ASST. de Swerdt *et al.*¹⁶ also found that the combination of the ASST and the basophil CD63 expression assay might be the most useful for identification of patients with ACU. They proved that IgG antibodies are responsible for basophil activation which results in the CD63 expression. The aim of our present study was to investigate further the diagnostic reliability of the CD63 assay comparing the results with the HR assay.

CD40 ligand (CD40L) is a member of the tumour necrosis factor family of cell surface molecules, and mediates contact-dependent signals delivered by CD4+ T-helper cells to CD40+ target cells, such as macrophages, dendritic cells and B cells.¹⁷ In different autoimmune diseases, e.g. rheumatoid arthritis (RA), mixed connective tissue disease, systemic sclerosis and systemic lupus erythematosus (SLE), prolonged expression of CD40L on T cells, elevated levels of soluble CD40L (sCD40L) and its capacity to mediate excessive B-cell activation were demonstrated.^{18–21} A significantly higher expression of CD40L in activated T lymphocytes of patients with CU compared with patients with atopic dermatitis and controls was shown by Toubi *et al.*²² In our study the serum sCD40L level was also measured in the sera of patients with CIU to determine the value of this test in differentiating the ACU patient group from the non-ACU patient group.

Patients and methods

Patients

Seventy-two patients (16 males, 56 females; mean age 40.4 years, range 13–74 years) with almost daily attacks of urticaria and with mean continuous disease duration of 8.4 months (range 3–32 months) were investigated. We excluded patients with lesions suspicious for urticarial vasculitis, patients with focal infection, physical urticarias, or food allergy or those who were taking drugs that could exacerbate CU. None of our patients took doxepin. Antihistamine treatment was stopped at least 4 days before, and corticosteroid or immunosuppressive therapy at least 2 months before serum samples were collected. All patients underwent the ASST, HR and CD63 expression assays. Serum sCD40L level was measured in 30 patients. Normal, nonatopic subjects ($n = 20$) served as controls. Sera of patients with dermatomyositis (DM, $n = 11$) and SLE ($n = 15$) were also tested in the HR and CD63 expression assays. Patients gave informed consent, which was in accordance with the guiding principles for human experimentation summarized in the Declaration of Helsinki.

The autologous serum skin test

The ASST was carried out according to the literature.¹² Positive reaction was defined when the serum-induced weal was red and had a diameter ≥ 1.5 mm than the saline-induced response at 30 min.

Stimulation of donor basophils with different agents

Cells from one highly sensitized atopic donor (D_A) and one nonatopic, healthy person (D_{NA}) (total serum IgE: 1468 and 1 kU L⁻¹, respectively) were used. The D_A was not taking antihistamines. The reactivity of the donor cells was tested by incubation with anti-IgE monoclonal antibody (mAb) (0.5 μ g mL⁻¹, Clone: D ϵ 2, Immunotech, Marseille, France), CU serum positive by immunoblotting (IB) for autoantibodies to the Fc ϵ RI α ¹⁵ and N-formyl-methionyl-leucyl-phenylalanine (FMLP) (10⁻⁵ mol L⁻¹; Sigma-Aldrich, Schnelldorf, Germany).

Histamine release from basophils

Peripheral blood leucocytes from two donors were separated as described²² and stimulated with the sera of patients. Fifty microlitres of cells were incubated with 50 μ L serum diluted 1 : 1 for 40 min at 37 °C. The reaction was terminated by the addition of 900 μ L of ice-cold buffer. Cells were sedimented and used for basophil surface CD63 expression assay whereas the supernatants were analysed for the histamine content by ELISA (Immunotech). For determining total histamine, cells were lysed by distilled water and repeated freezing and thawing twice. After subtraction of spontaneous release, the results were expressed as the net percentage of total histamine content. The cut-off values for different donors were determined as the 95th percentiles of HR induced by the sera of the controls (11.6% for D_A and 7.3% for D_{NA}).

CD63 surface expression assay

The CD63 expression on the surface of donor basophils was determined as published.¹⁵ Briefly, after incubation with CU sera the cells were labelled with goat antihuman IgE-FITC (fluorescein-isothiocyanate) and R-phycoerythrin-conjugated antihuman CD63 mAb (both from Caltag, Burlingame, CA, U.S.A.) and the percentage of IgE and CD63 double-positive basophils was measured using the Becton Dickinson FACS-Calibur flow cytometer (BD Biosciences, San Jose, CA, U.S.A.).

Values above the 95th percentile of CD63+ cells induced by the control sera were considered as positive (cut-off values for D_A : 5.1% and for D_{NA} : 2.2%).

Soluble CD40L determination

Venous blood was obtained from 30 patients with CU (mean age 36.5 years, range 21–56) and 15 age-matched control subjects. Serum was separated immediately and stored at -70 °C until assayed. Serum levels of sCD40L were quantitated using

an ELISA kit (Bender MedSystems, Vienna, Austria). The detection limit of the assay was 0.095 ng mL^{-1} and it was predicted to detect multimer forms of sCD40L by using the same antibody for coating and detection.

Applying some modifications to the method used by Vakka-lanka *et al.*¹⁸ we also measured the monomer, dimer and trimer forms of sCD40L (total sCD40L) by another ELISA with two antihuman sCD40L mAbs: clones TRAP-1 (BD Pharmingen, San Diego, CA, U.S.A.) and 24-31 (Ancell, Bayport, MN, U.S.A.), which detect different epitopes of sCD40L. After coating 100 ng TRAP-1 mAb onto the wells of the microtitre plate, the wells were blocked and incubated with sera diluted in phosphate-buffered saline or recombinant homotrimeric sCD40L ranging from 0.16 ng mL^{-1} to 50 ng mL^{-1} . Then biotin-labelled sCD40L mAb (24-31) was added (1 : 1000, 2 h at room temperature) followed by streptavidin-peroxidase (1 : 1000, 1 h, 37 °C; Amersham, Bucks, U.K.). The colour was developed with tetramethyl-benzidine peroxidase (Sigma-Aldrich, Steinheim, Germany).

Values above the 95th percentile of normal controls were considered as elevated (cut-off: 0.733 ng mL^{-1} for multimer sCD40L and 3.181 ng mL^{-1} for total sCD40L).

Statistical analysis

The results of normally distributed data were expressed as mean $\pm$ SD and the Student t-test was used for comparisons between two groups. Group data with non-normal distribution were expressed as medians and in interquartile ranges (IQ): 25th percentile and 75th percentile for variables and analysed by the Mann-Whitney rank sum test between two groups or by one-way ANOVA on ranks among several groups, as transformation failed to generate normally distributed data. All pairwise multiple comparisons were carried out with the post hoc Dunn's test. The correlations were assessed with Spearman correlation coefficients. A P-value of < 0.05 was regarded as significant.

Results

Characterization of donor basophils

Basophils from a D_A and a D_{NA} were characterized by their CD63 expression and by their HR in response to stimulation

with antihuman IgE antibody, CU serum-positive for anti-Fc ϵ RI α antibody confirmed by immunoblotting, and in response to FMLP. Basophils from the D_A showed high CD63 expression and HR after stimulation with all the three stimulatory agents, while basophils from the D_{NA} released only small amounts of histamine and demonstrated low-level CD63 expression in response to antihuman IgE antibody (Table 1). When stimulated with FMLP or anti-Fc ϵ RI α antibody containing serum the basophils from the D_{NA} expressed CD63 on their surface and produced HR, but in a smaller amount compared with basophils from the D_A .

Histamine release from basophils

Sera from 72 patients with CU were examined. Sera from 51% of the patients (37 of 72) released high amounts of histamine from basophils from the D_A , with cells from the D_{NA} doing likewise only in 32% of the cases (23 of 72) (Table 2). Using the cells from the D_A , sera of patients with CU showed significantly higher ($P < 0.01$) net HR compared with the amount obtained with the use of basophils from the D_{NA} . Ninety-two per cent (34 of 37) and 96% (22 of 23) of the sera, which released histamine in large amounts from basophils from the D_A and the D_{NA} , respectively, proved to be positive in the ASST (Table 2). In the ASST+ patient group, 85% (34 of 40) of the sera induced HR from cells from the D_A , while from the cells from the D_{NA} only 55% (22 of 40) induced HR. Sera of CU patients with positive ASST produced significantly higher ($P < 0.001$) net HR compared with sera of CU patients with negative ASST results, following the use of basophils from both donors (Fig. 1). Sera of patients with DM and SLE did not induce HR from basophils from either the D_A or from the D_{NA} .

CD63 expression on basophils

Sera from 57% (41 of 72) of CU patients resulted in the induction of the CD63 on the surface of basophils from the D_A , while on the cells from the D_{NA} only 28% (20 of 72) of the sera produced CD63 expression (Table 2). Significantly higher ($P < 0.001$) CD63 surface expression occurred on basophils from the D_A than on basophils from the D_{NA} , when incubated with sera of the patients (Fig. 2).

Table 1 Capacity of different agents to induce CD63 expression and HR from donor basophils. Cells from the D_A produced significantly higher CD63 expression and HR after stimulation with IgE antibody. Cells from both donors expressed CD63 and released histamine after stimulation with FMLP or IB+ CU serum, but the magnitude of the reactions was different. Student's t-test was used ($n = 5$ independent experiments)

	CD63 (%) (mean $\pm$ SD)		P-value	HR (%) (mean $\pm$ SD)		P-value
	D_A	D_{NA}		D_A	D_{NA}	
aIgE	40.26 $\pm$ 5.71	3.30 $\pm$ 0.83	< 0.001 s.	42.52 $\pm$ 3.19	5.80 $\pm$ 1.43	< 0.001 s.
FMLP	42.90 $\pm$ 4.35	35.64 $\pm$ 5.90	0.057 n.s.	52.95 $\pm$ 4.09	47.18 $\pm$ 6.82	0.143 n.s.
IB + CU se	67.86 $\pm$ 7.09	20.04 $\pm$ 4.35	< 0.001 s.	87.44 $\pm$ 6.57	61.64 $\pm$ 5.33	0.169 n.s.

HR, histamine release; aIgE, anti-IgE antibody; FMLP, N-formyl-methionyl-leucyl-phenylalanine; IB + CU se, serum positive by immunoblotting for antibodies to Fc ϵ RI α , D_A atopic donor; D_{NA} , nonatopic donor; s., significant; n.s., not significant

Table 2 Histamine production of basophils from the D_A and the D_{NA} after incubation with CU sera. Sera were also tested in the ASST and CD63 expression assay and the results were compared. CU sera and ASST+ CU sera induced significantly higher net HR from basophils from the D_A, compared with those from the D_{NA}. For calculation of statistical significance the Mann–Whitney rank sum test was used

	D _A				D _{NA}				D _A vs. D _{NA}
	HR n	HR (%) median (IQ)	HR+ n	HR– n	HR n	HR (%) median (IQ)	HR+ n	HR– n	HR P-value
Total CU	72	14.83 (3.54–66.90)	37	35	72	5.76 (3.15–18.11)	23	49	0.01 s.
ASST+ CU	40	57.59 (19.48–86.11)	34	6	40	10.89 (4.93–48.43)	22	18	< 0.001 s.
ASST– CU	32	3.49 (1.30–9.64)	3	29	32	3.75 (2.23–5.44)	1	31	0.74 n.s.
CD63+ CU	41	63.29 (20.34–86.63)	36	5	20	47.94 (26.83–78.41)	19	1	0.32 n.s.
CD63– CU	31	3.14 (1.27–7.16)	1	30	52	4.01 (2.33–6.38)	4	48	0.44 n.s.

CU, chronic urticaria; HR, histamine release; ASST, autologous serum skin test; IQ, interquartile ranges (25%–75%), D_A, atopic donor; D_{NA}, nonatopic donor; s., significant; n.s., not significant

When the sera were divided into two groups according to their reactivity in the HR assay, 97% (36 of 37) of HR+ sera resulted in CD63 induction on the surface of basophils from D_A, and 83% (19 of 23) resulted in CD63 induction if basophils from D_{NA} were used (Table 2). On cells from both donors incubation with HR+ CU sera caused a significantly higher ($P < 0.05$) CD63 expression compared with incubation with HR– CU sera. In the group of sera that caused a positive reaction in the ASST, 92.5% (37 of 40) was also positive for the CD63 assay if basophils from the D_A were used, while 50% (20 of 40) was positive if cells from the D_{NA} were stimulated. On the surface of basophils from both donors, stimulation with ASST+ CU sera resulted in significantly higher ($P < 0.05$) induction of the CD63 expression, compared with stimulation with ASST– CU sera (Fig. 2). Sera of patients with DM and SLE did not induce CD63 expression on donor cells.

Correlation of the histamine release assay, CD63 expression assay and autologous serum skin test

The results of the HR assay and the CD63 expression assay showed significant correlation (D_A: $r = 0.91$, D_{NA}: $r = 0.70$, $P < 0.001$ for both) when individual patients were assessed (Fig. 3). When the results of the HR assay and the ASST were compared, correlation was found only when basophils from the D_A were used [(D_A) positive HR, 51%; positive ASST, 55%; double positive, 92%; double negative, 83%; (D_{NA}) positive HR, 32%; positive ASST, 55%; double positive, 96%; double negative, 63%].

Measurement of soluble CD40 ligand

Serum sCD40L levels, either in the multimer (Fig. 4a) or total forms (monomer and multimer) (Fig. 4b) were significantly elevated in CU sera compared with healthy controls (median of multimer: 0.615 ng mL^{-1} vs. 0.23 ng mL^{-1} , IQ: $0.27\text{--}1.1 \text{ ng mL}^{-1}$ vs. $0\text{--}0.55 \text{ ng mL}^{-1}$, $P = 0.005$; and median of total: 1.515 ng mL^{-1} vs. 0.86 ng mL^{-1} , IQ: $1.14\text{--}6.01 \text{ ng mL}^{-1}$ vs. $0.652\text{--}1.122 \text{ ng mL}^{-1}$, $P < 0.001$).

Forty per cent (12 of 30) of CU sera were positive when multimer forms of sCD40L were measured and 27% (eight of 30) of CU sera were positive when total sCD40L were detected. Comparing the ASST+CD63+ CU group (ACU) with the ASST–CD63– CU group we found the sCD40L levels to be elevated in the former, although it did not reach statistical significance. Of the ACU sera, 31% (five of 16) were positive for multimer sCD40L, and 50% (eight of 16) positive for total sCD40L.

Discussion

The laboratory diagnosis of ACU is difficult, and the HR assay is taken as the 'gold standard' for the identification of patients with antibodies. In this study it was shown that a functional test, rarely used in the diagnosis of ACU, the basophil CD63 expression assay, has significant correlation with the HR assay. This correlation is found when the tests are carried out either with the use of basophils from atopic or from nonatopic donors.

To our knowledge, only Wedi *et al.*²³ have published a paper on the CD63 expression assay for the investigation of sera of patients with CU and compared the results with those of the HR test and sulfidoleukotriene (SLT) production following the use of basophils from two donors. In their experiments, similar to ours, higher capacity of surface CD63 induction and HR could be demonstrated by stimulation with ASST+ CU sera in D_A than in D_{NA}, and also in D_A, ASST+ CU sera resulted in a significantly higher release of histamine and induction of CD63 expression compared with ASST– sera. On the other hand, no significant correlation was found between HR, CD63 expression and SLT production. They proposed that CU serum activity inducing HR, CD63 expression and SLT production could not be used as a diagnostic marker for CU, but that it could identify a subset of CU patients with more severe disease and autoimmune pathogenesis. In our study we detected significant correlation between HR and CD63 expression assays when individual patients were assessed and on the basis of our results we suggest the application of the basophil CD63 expression assay in the diagnosis of ACU patients as an

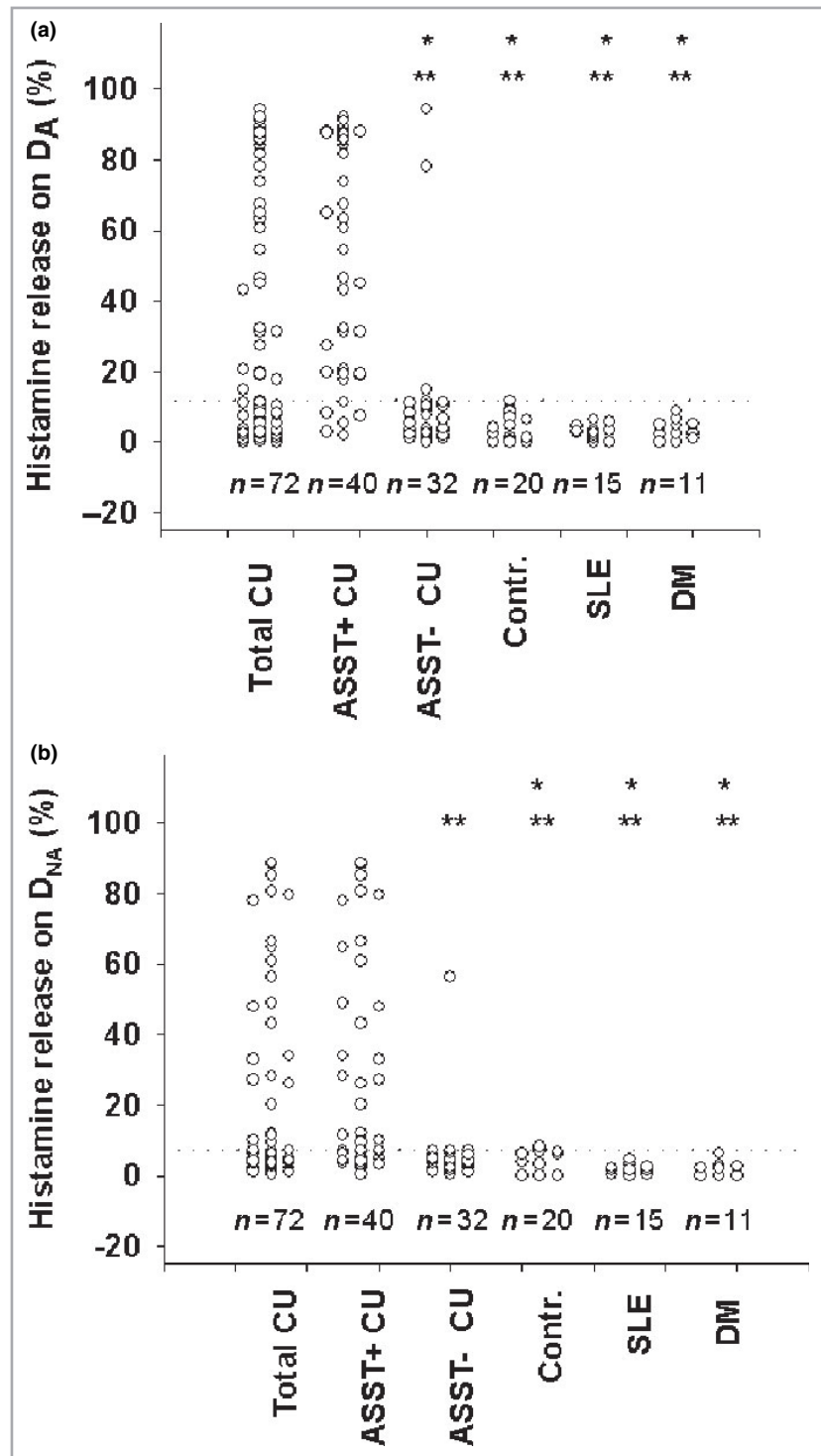


Fig 1. (a, b) Histamine release from basophils after challenge with sera from patients with CU, normal controls or patients with DM and SLE [(a) D_A, (b) D_{NA}]. A significantly higher amount of histamine was produced by basophils after stimulation with ASST+ CU sera, compared with ASST- CU sera (ANOVA, overall $P < 0.001$; * $P < 0.05$ vs. CU total, ** $P < 0.05$ vs. ASST+ CU, Dunn's test). CU, chronic urticaria; DM, dermatomyositis; SLE, systemic lupus erythematosus; D_A, atopic donor; D_{NA}, nonatopic donor; ASST, autologous serum skin test

alternative to the HR assay, and as a complementary tool to the skin test.

The cause of the differences between our results and those of Wedi *et al.*²³ may come from different laboratory techniques, such as avoidance of IL-3 priming and the whole blood system.¹⁵ In another investigation, when subjects allergic to grass pollen were selected, Sainte-Laudy *et al.*²⁴ found a good correlation between HR and CD63 expression assays

and concluded that flow cytometry (FCM) may represent a new alternative for allergy diagnosis. Indeed, for several types of allergy the diagnostic reliability of FCM has been emphasized.^{25–30} FCM is suitable for studying small cell populations such as activated basophils and is a convenient method in clinical laboratories and immunological centres. It is less expensive and less time consuming than the HR test.³⁰

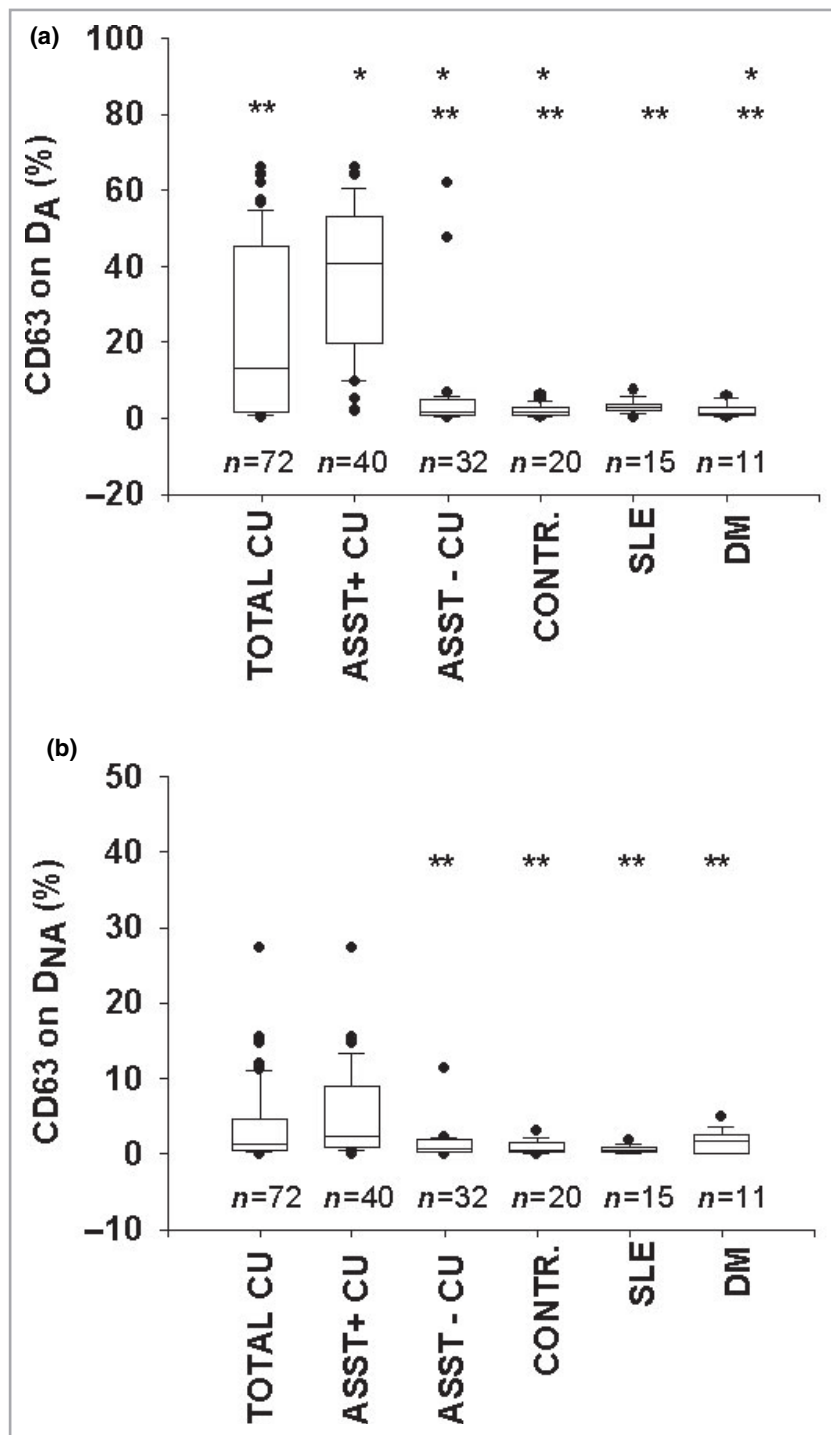


Fig 2. (a, b) CD63 expression of basophils from D_A (a) and D_{NA} (b) induced by sera from CU patients, normal controls or patients with DM and SLE. CU sera were divided into groups according to their reactivity in the ASST (ANOVA on ranks, overall $P < 0.001$; * $P < 0.05$ vs. total CU, ** $P < 0.05$ vs. ASST+ CU, Dunn's test). The lines inside the boxes represent the medians, the outer borders of the boxes indicate the 25th and 75th percentiles, the bars extending from the boxes designate the 10th and 90th percentiles. D_A , atopic donor; D_{NA} , nonatopic donor; CU, chronic urticaria; DM, dermatomyositis; SLE, systemic lupus erythematosus; ASST, autologous serum skin test

In different publications the percentage of CU sera with positive results in the HR assay has varied between 25% and 72%, according to the selected patient population and donor basophils.^{13,31} In this study, basophils from D_A showed much more activation than basophils from D_{NA} following incubation with CU sera both in the HR and CD63 assays. This difference in the reactivity of atopic and nonatopic donors was also observed in the study of Wedi *et al.*²³ If we take into consideration the fact that IgE poorly sensitized cells from D_{NA}

respond to sera containing IgE competitive and noncompetitive anti-FcεRI antibodies, while IgE highly sensitized basophils react both with anti-IgE and with noncompetitive anti-FcεRI antibodies, the pronounced differences between the results obtained on the two donors may be explained by two facts. The first is that basophils from D_A are in a primed state. These basophils have an increased capacity for the release of histamine and other factors and an increased sensitivity for activation through FcεRI or other traditional receptor-dependent

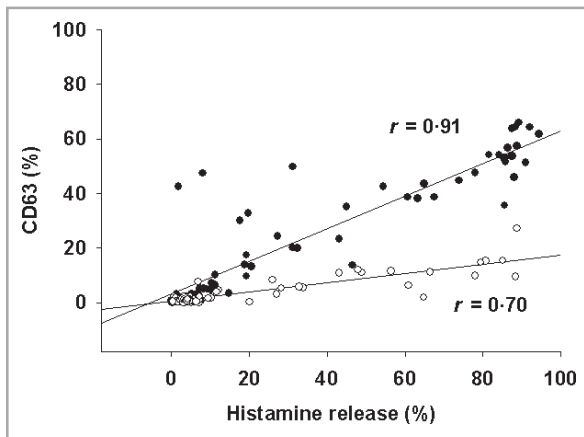


Fig 3. Correlation between the HR and the CD63 expression assays. There was a significant correlation between the HR assay and the CD63 expression assay when individual patients were assessed on both donors. (Black circle D_A : $r = 0.91$, open circle D_{NA} : $r = 0.70$; $P < 0.001$ for both, Spearman rank correlation). HR, histamine release; D_A , atopic donor; D_{NA} , nonatopic donor

mechanisms.^{32–34} The second explanation could be the high amount of anti-IgE antibodies in the sera of our CU patients, although this does not seem probable, as different studies have shown that only about 9% of patients with CU have functional anti-IgE antibodies, and most autoantibodies are directed against the high affinity IgE receptor.³¹

In our study ASST showed a correlation with the HR test only if sensitized basophils were used. The results of studies investigating the correlation between HR assay and ASST are inconclusive. Some authors agree that only a proportion of sera from ASST+ patients are able to induce HR and that this may be due to false positivity in the ASST.^{1,6,31} Another reason also may be the decreased sensitivity of functional tests. In this study we were able to reach good correlation between HR assay and ASST if we improved the sensitivity of the *in vitro* test by utilization of basophils from one highly sensitive atopic donor. Asero *et al.*³⁵ also increased the sensitivity of the HR test by another method. They used six different nonatopic donor cells (basophils and mast cells) and also detected correlation between ASST and HR assay.

Not only have increased levels of membrane and soluble forms of CD40L been detected in several autoimmune diseases, but serum levels also correlated with clinical disease activity in SLE patients and RA patients with vasculitis, with significantly higher values for sCD40L in serum compared with RA patients without vasculitis.^{19,36} To our knowledge only Garmendia *et al.*³⁷ reported measurements on sCD40L levels in CU patients, using an ELISA, which detected only the multimer form of sCD40L. We used for the first time two different types of ELISA to detect sCD40L in sera of CU patients. Besides the ELISA, which detected only the multimer form of sCD40L, we applied another two-site ELISA that detected the monomer and the multimer forms (total) of sCD40L together. Significantly increased sCD40L levels in patients with CU com-

pared with controls were detected, and also higher values were found in the group of patients with positive ASST than in patients with negative ASST, although the differences between the ASST positive and negative groups were not significant. The results obtained with the two-site ELISA assay might be more sensitive in differentiating between ACU and non-ACU patients because all samples with positive total sCD40L values were positive in both the ASST and CD63 assays and the differences between sCD40L levels measured in ASST+ and ASST- CU groups were more pronounced using total sCD40L ELISA compared with ELISA detecting only the multimer form of sCD40L.

In CU patients, Toubi *et al.* reported increased expression of CD40L in activated T cells, and Henz *et al.* found that mast cells were capable of expressing CD40L and different cytokines.^{22,38} The increased expression of CD40L in activated T cells and also the high levels of the soluble form might play a role in the increased B-cell proliferation of CU patients.²² On the other hand, the sCD40L level may also be indicative of the degree of mast cell and T-lymphocyte activation and may be related to the inflammatory processes generated by these cells.³⁸ CU patients with autoantibodies usually have more severe disease than patients without autoantibodies and a higher sCD40L serum level may indicate a more severe inflammation, but differences in the values of the sCD40L are not sufficient to make a clear distinction between ACU and non-ACU groups.

In conclusion, the CD63 expression assay seems to be a reliable functional assay in the diagnosis of ACU, particularly if highly sensitive donor basophils are used, and this test shows good correlation with the HR assay. Numerous attempts have been made to develop specific and sensitive binding assays for ACU, including ELISA and immunoblotting assays, but the results have been disappointing. Kikuchi and Kaplan¹³ and Greaves³ also concluded that functional assays are required. Our opinion is that the CD63 expression assay is convenient, but it has to be underlined that basophils from both atopic and nonatopic donors are important and need be obtained to make this functional test sensitive enough. Determination of sCD40L serum levels was not sufficient to differentiate between the ACU and the non-ACU patient groups, although ACU patients produced higher sCD40L levels, indicative of more severe inflammatory processes, which are also manifested by a more severe clinical picture.

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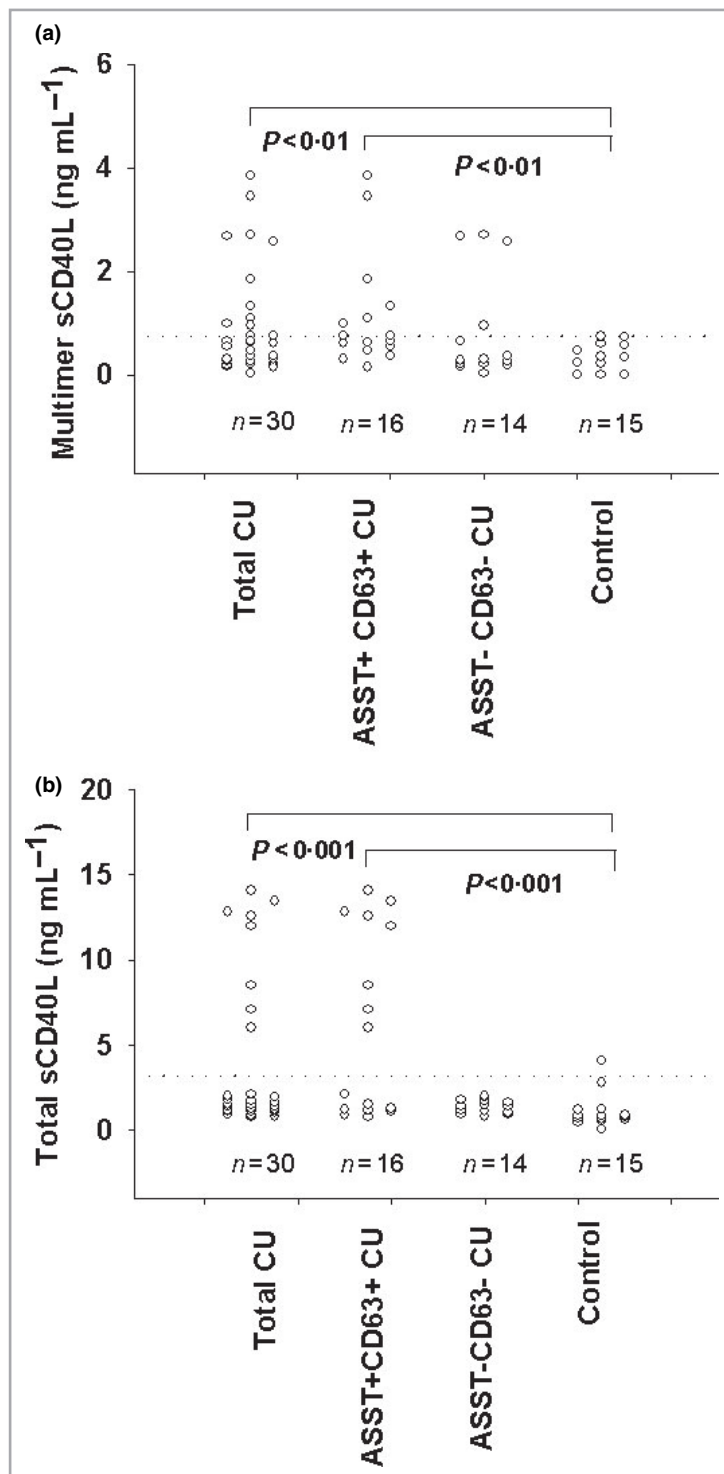


Fig 4. (a, b) Serum concentrations of sCD40L in healthy subjects and CU patients. (a) Serum sCD40L in multimer forms was measured by a one-site commercial ELISA. (ANOVA on ranks, overall $P = 0.005$). (b) Serum total sCD40L was detected by specific two-site ELISA using two different monoclonal antibodies against sCD40L (ANOVA; overall $P < 0.001$; only significant Dunn's multiple comparison post-test P -values between groups are shown). Dotted line represents the cut-off level for sCD40L positivity. sCD40L, soluble CD40 ligand; CU, chronic urticaria; ELISA, enzyme-linked immunosorbent assay

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