

Chronic urticaria serum induces histamine release, leukotriene production, and basophil CD63 surface expression—Inhibitory effects of anti-inflammatory drugs

Bettina Wedi, MD, Vera Novacovic, cand. med. Michael Koerner, MD, and Alexander Kapp, MD, PhD Hannover, Germany

Background: A role of potential histamine-releasing autoantibodies against the high-affinity IgE receptor on the surface of basophils and mast cells is discussed in the pathogenesis of chronic urticaria. This so-called autoimmune urticaria may be diagnosed by a positive intracutaneous autologous serum skin test, which is found in about 30% of patients with chronic urticaria.

Objective: Our purpose was, first, to compare the effect of complement-inactivated sera of 20 patients with chronic urticaria and positive autologous serum skin tests, 20 patients with chronic urticaria and negative skin tests, and 20 control subjects without chronic urticaria (10 atopic and 10 nonatopic subjects) and, second, to analyze the effect of anti-inflammatory drugs on the serum activity.

Methods: The following assay systems were used: release of histamine in whole blood samples, surface expression of the activation marker CD63 on basophils, and sulfidoleukotriene de novo production in leukocyte suspensions. Whole blood, basophils, and leukocyte suspensions were obtained from a nonatopic and an atopic donor.

Results: Sera of patients with autologous serum skin test positive chronic urticaria resulted not only in significantly increased histamine release compared with skin test-negative chronic urticaria sera but also in a significant higher induction of basophil CD63 surface expression and sulfidoleukotriene de novo production. However, serum activity was neither characteristic for chronic urticaria nor for chronic urticaria with a positive autologous serum skin test. Preincubation with dapsone, chloroquine, and lidocaine dose dependently resulted in a significant reduction of all histamine release, CD63 expression, and sulfidoleukotriene production. In addition, mizolastine was able to inhibit serum-induced sulfidoleukotriene production.

Conclusion: Further studies investigating the in vivo effect of these drugs will have to clarify their role in the management of the subset of patients with chronic urticaria demonstrating serum-induced inflammatory effects. (*J Allergy Clin Immunol* 2000;105:552-60.)

Key words: Chronic urticaria, autoantibodies, histamine release, sulfidoleukotrienes, CD63

Chronic urticaria (CU) represents a common skin disease. It is defined by a period of whealing lasting for at least 6 weeks and often for decades. Urticarial symptoms are often associated with sometimes life-threatening angioedema. Because of pruritus and sleeplessness, life quality is significantly reduced.¹ Despite an exhaustive and expensive diagnostic approach, searches for etiologies of CU have been mostly frustrating.² CU can be triggered by infectious disease states, particularly *Helicobacter pylori*-associated gastritis,³⁻⁵ malignancy, or pseudoallergic reactions.^{6,7} However, in about 20% to 30% of subjects no cause can be identified.⁵ Emerging data give new evidence that autoantibodies to thyroid,⁸ to IgE,^{9,10} and especially to the high-affinity IgE receptor (FcεRI) may play an important role in the pathogenesis or the disease activity.¹¹⁻¹⁵ These autoantibodies are postulated to cross-link the FcεRIα on mast cells or basophils, resulting in histamine release. However, with use of an ELISA system for the measurement of anti-FcεR autoantibodies, these antibodies have also been demonstrated in other autoimmune disorders such as bullous pemphigoid, pemphigus vulgaris, dermatomyositis, systemic lupus erythematosus but also in atopic dermatitis.¹² In addition, basophil- and mast cell-stimulating serum activity was demonstrated to be present only in a subset of patients with CU¹⁶ but not in all patients with CU and a positive autologous skin test result. Recently Horn et al¹⁷ found anti-FcεRI autoantibodies lacking histamine-releasing (HR) capacity in multidonor intravenous Ig preparations. Interestingly, these autoantibodies showed strong cross-reaction with tetanus toxoid with an even higher affinity than for the FcεRIα. The authors deduced that the autoantibodies against FcεRIα may belong to the natural antibodies. The concept of natural self-reactive antibody repertoires being present in the serum of healthy individuals and reacting with one or more self-antigens has been described in detail.^{18,19}

Hence the role of these apparently nonspecific autoantibodies for the development or the maintenance of urticarial symptoms remains to be clarified. At present the only treatment strategies described so far for autoimmune urticaria are plasmapheresis²⁰ and high-dose intra-

From the Department of Dermatology and Allergology, Hannover Medical University, Hannover, Germany.

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Reprint requests: Bettina Wedi, MD, Department of Dermatology and Allergology, Hannover Medical University, Ricklinger Str 5, D-30449 Hannover, Germany.

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Abbreviations used

ASST:	Autologous serum skin test
AT:	Atopic controls
CU:	Chronic urticaria
D _{AT} :	Atopic donor
D _{NAT} :	Nonatopic donor
FcεRI:	α Chain of high-affinity IgE receptor
fMLP:	<i>N</i> -formyl-methionyl-leucyl-phenylalanine
HR:	Histamine release
NAT:	Nonatopic controls
SLT:	Sulfidoleukotriene(s)

venous Igs,²¹ therapeutic schedules that are not only expensive but also of temporary effect.²²

To further clarify the role of serum activity in CU we investigated the effect of sera from 20 patients with CU and positive autologous serum skin test (ASST) results, 20 patients with CU and negative ASST results, and 20 control subjects (10 nonatopic [NAT] and 10 atopic [AT] controls) by measuring not only HR but also basophil CD63 surface expression and leukocyte sulfidoleukotriene (SLT) de novo production. The surface marker CD63 (glycoprotein 53) is expressed with high density on activated basophils but only weakly expressed on resting basophils.²³ It is located in or on the granule membrane, belongs to the tetraspan family, and is of unknown function.^{24,25} The kinetics of CD63 up-regulation and HR were shown to be identical after stimulation with *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), grass pollen extract, or anti-IgE mAb.^{23,26,27} In addition, SLT production was assessed because previous studies have shown that SLT is approximately 100 times more potent on a molar basis compared with histamine in inducing wheal-and-flare reactions.²⁸ Furthermore, to find other treatment schedules in autoimmune urticaria than intravenous Ig or plasmapheresis, the effect of several anti-inflammatory drugs on serum activity was investigated. In our hands the standard nonsedating antihistamines were not very effective in the treatment of autoimmune urticaria. Therefore no antihistamines were used in the assay systems except for mizolastine, of which additional anti-inflammatory effects including leukotriene antagonistic properties have been shown.²⁹ Instead, we considered chloroquine and dapsone, which have been reported to be effective in urticaria vasculitis, hypocomplementemic urticarial vasculitis syndrome, and other antibody-mediated diseases.³⁰⁻³² Furthermore, the effect of lidocaine was investigated because very recently lidocaine showed promise as another class of immunomodulatory drug in allergic diseases.^{33,34}

MATERIAL AND METHODS

Drugs and stimuli

Chloroquine, dapsone (4,4'-diaminodiphenylsulfone), lidocaine, and goat polyclonal antihuman IgE mAb were purchased from Sigma Chemicals (Deisenhofen, Germany). Mizolastine was kindly provided by the Synthelabo Group (Tours, France). All drugs were diluted in water, except for dapsone and lidocaine, which were first

dissolved in dimethyl sulfoxide and further diluted with an appropriate medium to final working concentrations. The highest final concentration of dimethyl sulfoxide (0.1%) did not affect cellular function (not shown). Mouse anti-FcεRIα mAb (clone 22E7) was obtained from DPC Biemann, Bad Nauheim, Germany.

Patients, controls, and handling of sera

CU was defined as recurrent wheeling occurring at least twice a week for more than 6 weeks. In all patients with CU an intracutaneous test with autologous serum was carried out as described.¹⁴ Twenty patients with CU and positive ASST results and 20 patients with CU and negative ASST results were recruited consecutively and not screened as to severity. Ten nonatopic healthy subjects and 10 atopic controls with inhalant allergy or atopic dermatitis served as controls. All sera were stored at -20°C until investigation. For the HR and the CD63 surface expression experiments, sera were thawed to room temperature and heated at 56°C 30 minutes immediately before their use to denature IgE and the inactive complement. For the SLT de novo production heat-inactivated and noninactivated sera were compared.

Depending on the density of surface-bound IgE, 2 donors were selected for all experiments: one nonatopic healthy control (D_{NAT}) and one atopic donor (D_{AT}) with extrinsic atopic dermatitis (serum IgE 58.8 kU/L, specific IgE to seasonal allergens). Both donors did not receive any drugs. For HR assay and basophil CD63 expression whole blood was collected into tubes containing heparin, whereas for the measurement of SLT EDTA-anticoagulated blood was used.

Histamine release

HR was measured in whole blood cells from D_{NAT} and D_{AT} with use of an enzyme immunoassay (Histamine EIA, Immunotech, Marseilles, France). In detail, 200 μL of whole blood diluted 1:7 in HR buffer was challenged with 100 μL of sera diluted 1:2, 1:5, and 1:10 for 30 minutes at 37°C. Total cellular histamine was obtained by cell lysis induced by repeated freezing and thawing. After incubation, the supernatants were separated by centrifugation at 900g for 5 minutes at 4°C and acylated. HR was determined according to the manufacturer's instructions. All HR experiments were done in duplicate and net specific HR (in percent) was calculated with the following formula: [(Stimulated HR - Spontaneous HR) × 100] ÷ Total HR. On each microtiter plate, subjects of the different groups studied were mixed to avoid interassay variability. Anti-FcεRI antibodies (0.66 μg/mL) and anti-IgE (1:1000) served as positive controls.

Basophil CD63 surface expression

Surface expression of the basophil activation marker CD63 was measured with use of BASOTEST (Orpegen Pharma, Heidelberg, Germany). Briefly, 100 μL of whole blood was preincubated with 20 μL of wash buffer (containing IL-3 at a final concentration of 2 ng/mL) at 37°C for 10 minutes. Then stimulation with 100 μL of heat-inactivated serum (final dilution 1:2, 1:5, and 1:10) for 20 minutes at 37°C followed. Previous experiments revealed that this serum dilution gave optimal results. Degranulation was stopped by chilling on ice for 5 minutes. Thereafter phycoerythrin-conjugated anti-IgE and FITC-conjugated anti-CD63 mAb were added and incubated for 20 minutes on ice. Finally whole blood probes were lysed, fixed, washed, and analyzed within 2 hours on a FACScan flow cytometer (Becton-Dickinson Immunocytometry System, San Jose, Calif). While the threshold was set in fluorescence 2 (red fluorescence), only basophils with very bright IgE expression were "live gated." At least 1000 high IgE-expressing basophils per probe were acquired and the percentage of CD63-expressing cells was determined by measuring fluorescence 1 (green fluorescence) compared with the wash buffer serving as negative control (whose mean

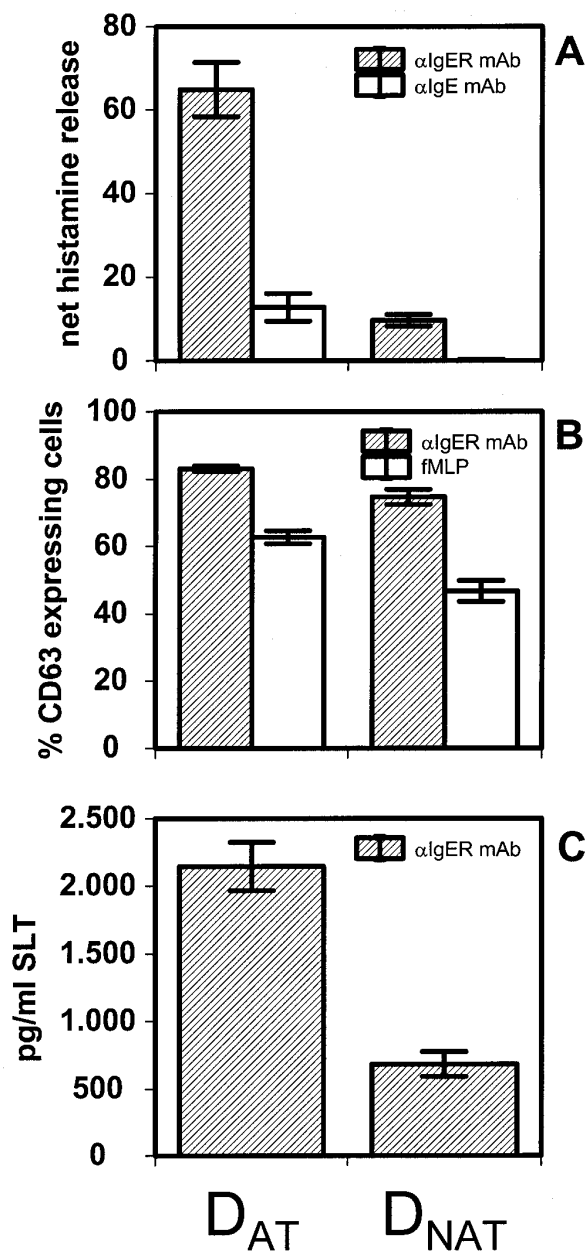


FIG 1. **A**, Net HR induced by mAb against FcεRIα (αIgER mAb, 0.66 μg/mL) and by anti-IgE mAb (αIgE mAb, 1:1000) was significantly higher in D_{AT} than in D_{NAT} ($P < .01$ and $P < .001$, respectively). **B**, Percentage of CD63-expressing cells was significantly higher in D_{AT} after stimulation with anti-FcεRI mAb (0.33 μg/mL, $P < .01$) and fMLP ($P < .001$) compared with D_{NAT}. **C**, In D_{AT} anti-FcεRI mAb (0.55 μg/mL) resulted in significantly higher production of SLT compared with D_{NAT} ($P < .05$).

± SD CD63 expression was 9.75 ± 0.725 for D_{AT} and 9.64 ± 1.33 for D_{NAT}, $n = 8$). The positive controls fMLP (1 μmol/L) and anti-FcεRI mAb (0.33 μg/mL) resulted in $62.63\% \pm 5.41\%$ and $83.1\% \pm 2.43\%$ CD63 expression for D_{AT} and $46.73\% \pm 3.08\%$ and $74.67\% \pm 5.96\%$ CD63 expression in D_{NAT} ($n = 8$).

SLT production

After being washed, leukocyte suspensions enriched by dextran sedimentation were resuspended in stimulation buffer containing 20

ng/mL IL-3. After 10 minutes of priming, heat-inactivated sera were added (final volume 1:5) and incubated for 40 minutes at 37°C. Anti-FcεRIα mAb (0.55 μg/mL) and anti-IgE mAb (1:1000) served as positive controls, whereas stimulation buffer served as a negative control. SLT de novo production was measured with use of the Cellular Antigen Stimulation Test (CAST-ELISA, Bühlmann Laboratories, Allschwil, Switzerland) according to the instructions of the manufacturer. Values for negative control (background) were subtracted.

Inhibition experiments with anti-inflammatory drugs

The following concentrations were used to inhibit serum activity of 4 ASST⁺ patients with CU with striking serum activity on D_{AT} cells, 10 μmol/L to 1 mmol/L dapsone, 100 μmol/L to 1 mmol/L chloroquine, 100 μmol/L to 1 mmol/L lidocaine, and 20 and 200 μg/mL mizolastine. For HR experiments D_{AT} cells were preincubated for 60 minutes with drugs before heat-inactivated sera diluted 1:2 were added and incubated for a further 30 minutes. For CD63 expression studies D_{AT} cells were preincubated for 60 minutes with drugs before sera diluted 1:2 were added and incubated for a further 20 minutes. For SLT production D_{AT} cells were preincubated for 60 minutes with drugs before sera diluted 1:5 were added and incubated for a further 40 minutes.

Statistical analysis

Depending on the type of distribution, the statistical significance between groups was determined by the Student *t* test or the Mann-Whitney rank sum test; a statistical software package (SigmaStat for Windows, Jandel Scientific, Erkrath, Germany) was used. A *P* value $< .05$ was considered statistically significant. Correlation coefficients were calculated with use of the Pearson product moment correlation. For computing the exact probability of a 2×2 contingency table, Fisher's exact test was used. Unless otherwise stated, means and SEM are shown in the figures.

RESULTS

Histamine-releasing activity of whole blood

Net HR induced by anti-FcεRI mAb and anti-IgE mAb was significantly higher in the atopic donor (D_{AT}) compared with the nonatopic donor (D_{NAT}) ($P < .01$ and $P < .01$, respectively, Fig 1, A). Use of the high-releasing donor D_{AT} sera of CU patients with positive ASST results (ASST⁺ CU) resulted in a significantly higher net HR compared with CU patients with negative ASST results (ASST⁻ CU) as well as with NAT and atopic controls (AT) (Fig 2, A). Serum activity was dose dependent with maximal induced net HR at a dilution of 1:2 (Fig 2, A). However, serum-induced HR was not able to differentiate between CU and NAT or AT. With use of the nonatopic donor (D_{NAT}) ASST⁺ CU sera mediated net HR was higher compared with ASST⁻ CU sera and sera of controls but not to statistical significance (Fig 2, B). In D_{NAT}, only ASST⁺ CU sera diluted 1:10 resulted in a significantly higher net HR compared with ASST⁻ CU sera. ASST⁺ CU serum activity was about 2.7-fold lower in D_{AT} and 2-fold lower in D_{NAT} compared with the effects of anti-FcεRI mAb (0.66 μg/mL) and about 1.4-fold higher in D_{AT} and 4-fold higher in D_{NAT} compared with anti-IgE mAb (1:1000).

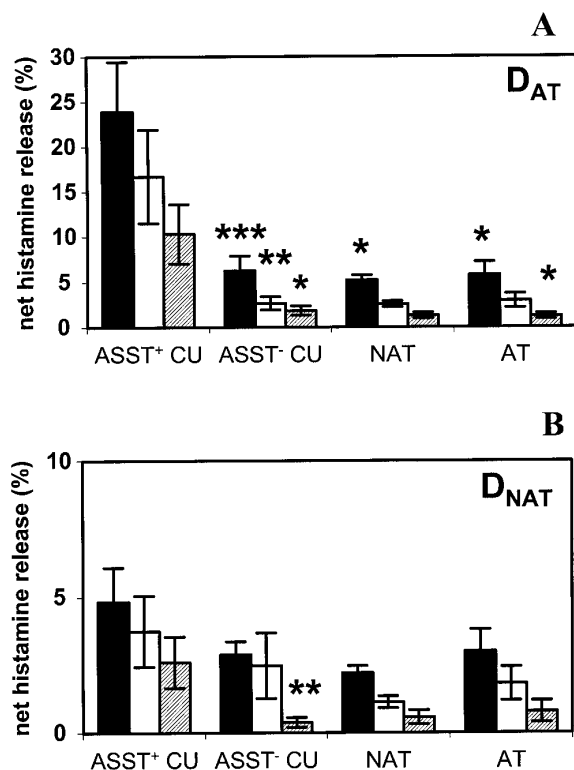


FIG 2. Net HR induced by heat-inactivated sera of CU patients with positive (ASST⁺ CU) and negative ASST (ASST⁻ CU) and of nonatopic (NAT) and atopic controls (AT). Serum dilution 1:2 (solid bars), 1:5 (open bars), and 1:10 (hatched bars). **A**, Atopic donor, D_{AT}. **B**, Nonatopic donor, D_{NAT}. Asterisk, $P < .05$; two asterisks, $P < .01$; three asterisks, $P < .001$ compared with ASST⁺ CU group.

Induction of basophil CD63 surface expression

The percentage of CD63-expressing cells was significantly higher in D_{AT} after stimulation with anti-FcεRI mAb ($P < .01$) and fMLP ($P < .001$) compared with D_{NAT} (Fig 1, B). This higher capacity of surface CD63 induction could be demonstrated also by stimulation with ASST⁺ CU sera resulting in a CD63 expression in D_{AT} that was about 2-fold higher than in D_{NAT} (Fig 3, A and B). In D_{AT} ASST⁺ CU sera resulted in a significantly higher induction of the basophil activation marker CD63 compared with ASST⁻ CU sera, as well as to the NAT and AT controls (Fig 3, A). However, in D_{NAT} ASST⁺ CU sera-induced effects reached significance only when compared with ASST⁻ CU sera but not with NAT or AT (Fig 3, B). In both donors serum activity was dose dependent, showing the highest CD63 expression with a dilution of 1:2. ASST⁺ CU serum activity was about 1.6-fold lower in D_{AT} and 3.3-fold lower in D_{NAT} compared with the effects of anti-FcεRI mAb (0.33 μg/mL) and about 1.2-fold lower in D_{AT} and 2.5-fold lower in D_{NAT} compared with fMLP (1 μmol/L).

SLT de novo production

Stimulation with anti-FcεRI mAb resulted in a significantly higher SLT release in D_{AT} compared with D_{NAT} ($P < .05$, Fig 1, C). This striking difference between the atopic and nonatopic donor was not so evident when serum activ-

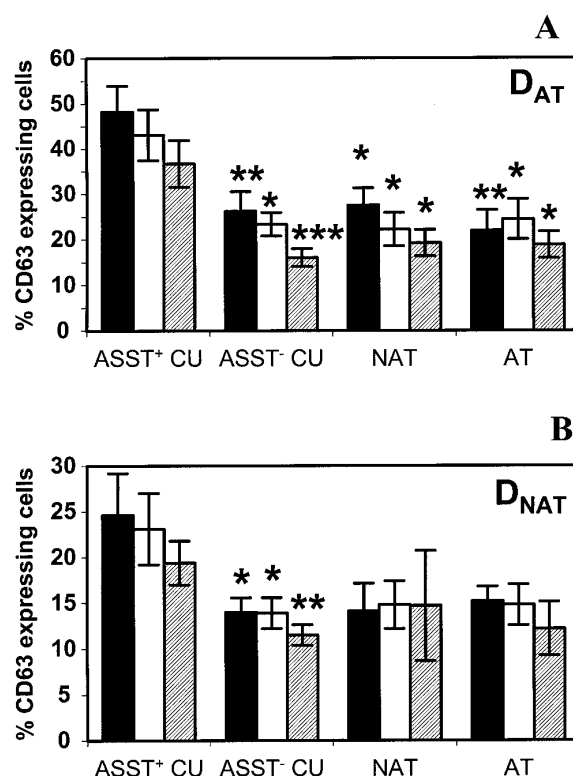


FIG 3. Basophil activation determined by percentage of surface CD63-expressing peripheral blood leukocytes life-gated according to their bright surface IgE expression. Stimulation with heat-inactivated sera of CU patients with positive (ASST⁺ CU) and negative ASST (ASST⁻ CU) and of nonatopic (NAT) and atopic controls (AT). Serum was diluted 1:2 (solid bars), 1:5 (open bars), and 1:10 (hatched bars). **A**, Atopic donor, D_{AT}. **B**, Nonatopic donor, D_{NAT}. Asterisk, $P < .05$; two asterisks, $P < .01$; three asterisks, $P < .001$ compared with ASST⁺ CU group.

ity was analyzed (Fig 4, A and B). In contrast, with use of non-heat-inactivated sera, SLT production in D_{NAT} was slightly higher with ASST⁻ CU and control sera. On the whole, sera that were not heat inactivated showed significantly higher SLT de novo production compared with heat-inactivated sera. This may imply participation of complement components or of anti-IgE autoantibodies in the sera. However, in D_{AT} heat-inactivated, like noninactivated ASST⁺ CU, sera resulted in a significant higher SLT production compared with ASST⁻ CU sera, NAT, and AT sera (Fig 4, A). In D_{NAT} serum-induced SLT production did not reach statistical significance except for non-heat-inactivated ASST⁺ CU sera compared with AT sera (Fig 4, B). In comparison to the effects of anti-FcεRI mAb (0.55 μg/mL) ASST⁺ CU serum activity was about 11.7-fold lower in D_{AT} and 8.8-fold lower in D_{NAT} with use of heat-inactivated sera and about 4.7-fold lower in D_{AT} and 1.7-fold lower in D_{NAT} when sera were not heat inactivated.

Comparison of HR, CD63 expression, and SLT production

Not all ASST⁺ CU sera showed activity in all the assay systems tested, namely HR, CD63 expression, or SLT

production (Table I). A significant number of sera demonstrated only one or two of these activities. Taken together, 25% of sera did not have any activating effect in the D_{AT} and 30% in the D_{NAT} , although ASST was positive in all these patients. There was no significant correlation between HR and CD63 expression, HR and SLT production, or CD63 expression and SLT production. However, serum activity in ASST⁺ CU sera seemed to be associated with higher urticarial activity, defined by a higher frequency of daily symptoms and the occurrence of angioedema (Tables I and II). Seventy-five percent of ASST⁺ CU patients showed daily symptoms and 45% had concurrent angioedema compared with 45% and 15% in ASST⁻ CU, respectively. Duration of CU (40 ± 57 months in ASST⁺ CU and 41 ± 14 months in ASST⁻ CU) was not associated with serum activity or positive ASST. Interestingly, we found a higher but nonsignificant prevalence of antibodies to thyroid in patients demonstrating serum activity (35% in ASST⁺ CU vs 15% in ASST⁻ CU). In ASST⁺ CU sera median total serum IgE levels were lower (68 kU/L) compared with ASST⁻ CU sera (119 kU/L), but the difference did not reach statistical significance ($P = .0742$).

Inhibitory effect of anti-inflammatory drugs

A short preincubation period with chloroquine (1 mmol/L), dapsone (1 mmol/L), and lidocaine (1 mmol/L) significantly and dose dependently inhibited HR (Fig 5, A), CD63 expression (Fig 5, B), and SLT de novo production (Fig 5, C). However, mizolastine (200 μ g/mL) only induced a significant inhibition of SLT production. These inhibitory drug concentrations were not cytotoxic as assessed by trypan blue dye exclusion (not shown). In an additional experiment it could be demonstrated that the above-mentioned drugs were also able to inhibit anti-Fc ϵ RI mAb-induced HR, basophil 63 expression, and SLT production in D_{AT} (not shown).

DISCUSSION

Our data confirm previous published results demonstrating that not in all patients with CU,³⁵ and even not in all patients with CU and positive ASST results, serum shows HR activity.¹⁵ Hide et al¹⁶ showed 65% of CU sera with positive ASST inducing HR when human basophils were used. Fiebiger et al¹² demonstrated anti-Fc ϵ RI α mAb in 37% of chronic urticaria sera but ASST was not done. They found 66% histamine-releasing activity in anti-Fc ϵ RI-autoantibody⁺ CU sera and 17% in autoantibody⁻ CU sera. With use of their criterion (HR >15% of anti-Fc ϵ RI mAb-positive control), in this study we found 55% sera with HR activity among the ASST⁺ CU patients, 15% among the ASST⁻ CU patients, 30% among the atopic controls, and 10% among the nonatopic controls (analyzing donor D_{AT}). Thus the frequency of serum HR activity in CU patients was comparable to that of previously published data, although in our study whole blood was assessed, in contrast to basophil-enriched leukocyte suspensions.

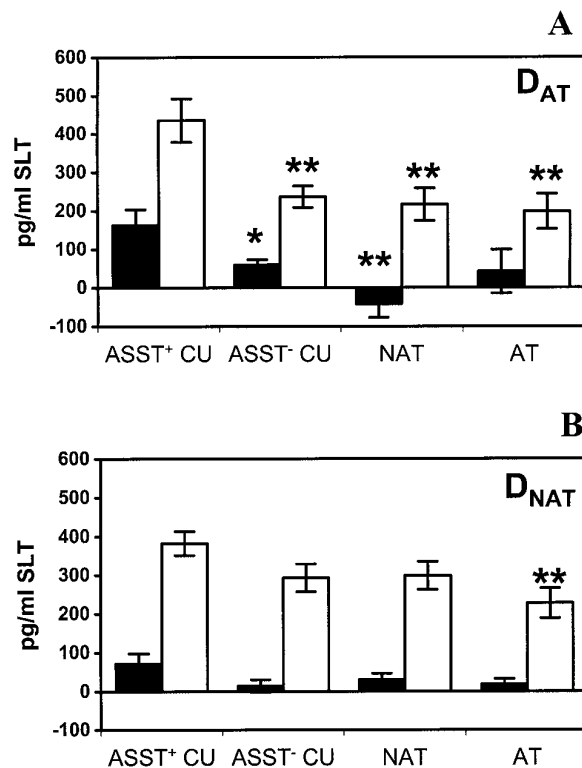


FIG 4. SLT de novo production of leukocyte suspensions stimulated with heat-inactivated (solid bars) and non-heat-inactivated sera (open bars) of CU patients with positive (ASST⁺ CU) and negative ASST (ASST⁻ CU) and of nonatopic (NAT) and atopic controls (AT) (dilution 1:5). Asterisk, $P < .05$; two asterisks, $P < .01$ compared with ASST⁺ CU group.

For the first time, we clearly demonstrated that the serum of a subgroup of CU patients with positive ASST does not only show histamine-releasing activity but also induction of the basophil activation marker CD63 and SLT de novo production. Compared with HR, even more ASST⁺ CU sera induced CD63 expression or SLT production (Table I). With use of the atopic donor, increased CD63 expression was found in 70% of ASST⁺ CU patients and 45% of ASST⁻ CU patients, but only in 35% of controls. Interestingly, HR and CD63 surface expression did not show a significant correlation, although this was indicated by previous studies with fMLP, anti-IgE, or pollen antigens as stimuli.^{26,27,36} Similarly, there was no significant correlation between HR and SLT production or SLT production and CD63 surface expression. This fits with other studies concluding that leukotriene de novo production and the release of preformed histamine are regulated differentially.^{37,38} After heat inactivation serum activity significantly declined regarding induction of SLT production, suggesting that the classic complement pathway may be involved in this serum activity. Another possibility might be the presence of anti-IgE autoantibodies in the sera.

Confirming previous results,^{35,39} our data indicate the presence of histamine-releasing serum activity particularly in subjects with more severe and active urticaria

TABLE I. Effect of individual ASST⁺ CU sera by means of HR, basophil CD63 expression, and SLT production in the atopic donor (D_{AT}) and the nonatopic donor (D_{NAT})

Sex	Duration of CU (mo)	Daily urticae	Recurrent angioedema	IgE (kU/L)*	Elevated levels of Ab to thyroid	D _{AT} †			D _{NAT}		
						HR	CD63	SLT	HR	CD63	SLT
F	120	Yes	Yes	19	No	++	++	++	++	++	+
F	96	Yes	Yes	30	Yes	++	++	++	++	++	+
F	12	Yes	Yes	11	Yes	++	++	+	++	++	++
M	12	Yes	Yes	167	No	++	++	++	+	+	+
F	6	Yes	Yes	17	No	++	++	++	+	++	—
F	9	Yes	No	58	Yes	++	++	++	+	—	++
M	11	Yes	Yes	413	No	+	+	++	+	—	++
F	72	Yes	Yes	23	Yes	++	++	++	—	—	—
F	24	Yes	No	108	Yes	—	++	++	+	—	++
F	12	Yes	Yes	208	Yes	—	++	+	—	—	++
F	40	No	No	9	Yes	—	++	+	—	—	—
F	7	No	No	152*	No	—	+	+	+	—	—
F	7	No	No	73	No	+	+	—	—	—	—
M	4	Yes	No	62	No	—	—	++	—	—	++
F	13	Yes	No	137	No	—	+	—	—	—	—
M	48	Yes	Yes	115*	No	—	—	+	—	—	—
F	36	No	No	243	No	—	—	—	—	—	—
M	240	Yes	No	447*	No	—	—	—	—	—	—
F	9	No	No	39	No	—	—	—	—	—	—
F	13	Yes	No	33	No	—	—	—	—	—	—

Ab, Antibody; F, female; M, male.

*Atopic constitution by means of positive Phadiatop/Sx1.

†Net HR of ≤10% was —, of >10% up to ≤20% was +, and >20% ++. CD63 expression of ≤25% expressing cells was —, of >25% up to ≤40% was +, and >40% was considered ++. SLT release of ≤100 pg/mL was —, of >100 pg/mL up to ≤150 pg/mL +, and >150 pg/mL ++.

TABLE II. Effect of individual ASST[–] CU sera by means of HR, basophil CD63 expression, and SLT production in the atopic donor (D_{AT}) and the nonatopic donor (D_{NAT})

Sex	Duration of CU (mo)	Daily urticae	Recurrent angioedema	IgE (kU/L)*	Elevated levels of Ab to thyroid	D _{AT} †			D _{NAT}		
						HR	CD63	SLT	HR	CD63	SLT
M	2	No	No	292	No	++	++	++	—	—	—
M	2	Yes	Yes	8	Yes	++	+	—	—	—	—
F	2	Yes	Yes	195	No	+	+	+	—	—	—
F	220	No	Yes	163	No	—	+	+	—	—	—
F	96	Yes	No	512*	No	—	+	—	—	—	—
F	24	No	No	76	No	—	+	—	—	+	—
M	8	No	No	73	No	—	+	—	—	+	—
F	12	Yes	No	38	No	—	+	—	—	—	—
F	6	Yes	No	879	Yes	—	+	—	—	—	—
F	7	Yes	No	91	Yes	—	—	++	—	—	+
F	26	No	No	479	No	—	—	—	—	—	+
F	9	No	No	265	No	—	—	—	—	—	—
F	60	Yes	No	234*	No	—	—	—	—	—	—
F	196	No	No	474	No	—	—	—	—	—	—
M	80	Yes	No	53	No	—	—	—	—	—	—
F	4	No	No	101*	No	—	—	—	—	—	—
F	24	No	No	78	No	—	—	—	—	—	—
F	190	No	No	112	No	—	—	—	—	—	—
F	120	Yes	No	44	No	—	—	—	—	—	—
F	4	No	No	126*	No	—	—	—	—	—	—

Ab, Antibody; M, male; F, female.

*Atopic constitution by means of positive Phadiatop/Sx1.

†Net HR of ≤10% was —, of >10% up to ≤20% was +, and >20% ++. CD63 expression of ≤25% expressing cells was —, of >25% up to 40% was +, and >40% was considered ++. SLT release of ≤100 pg/mL was —, of >100 pg/mL up to ≤150 pg/mL +, and >150 pg/mL ++.

TABLE III. Effect of individual control sera (first 10 were nonatopic donors, next 10 atopic donors) by means of HR, basophil CD63 expression, and SLT production in the atopic donor (D_{AT}) and the non-atopic donor (D_{NAT})

Sex	D_{AT}^*			D_{NAT}		
	HR	CD63	SLT	HR	CD63	SLT
F	-	+	-	-	-	-
F	-	-	-	-	-	-
F	-	+	+	-	-	-
F	-	+	+	-	+	-
F	-	+	-	-	-	+
M	-	++	-	-	+	-
F	-	-	-	-	-	-
F	-	-	-	-	-	-
M	-	-	-	-	-	-
F	-	+	-	-	-	-
M	-	-	+	-	-	-
F	-	++	++	-	-	+
M	-	-	++	-	-	-
M	-	-	++	-	-	-
F	-	-	+	-	-	-
F	-	-	++	-	-	-
F	+	-	-	-	-	-
M	-	-	-	-	-	-
M	-	-	-	-	-	-

F, Female; M, male.

*Net HR of $\leq 10\%$ was -, of $>10\%$ up to $\leq 20\%$ was +, and $>20\%$ ++. CD63 expression of $\leq 25\%$ expressing cells was -, of $>25\%$ up to 40% was +, and $>40\%$ was considered ++. SLT release of ≤ 100 pg/mL was -, of >100 pg/mL up to ≤ 150 pg/mL +, and >150 pg/mL ++.

including concurrent angioedema and daily symptoms. Moreover, serum-induced basophil CD63 expression and SLT production were also found to be more frequent in subjects with severe urticaria. Nevertheless, although serum-induced HR, CD63 expression, and SLT production were more frequent in ASST⁺ CU patients, these activities were neither characteristic for ASST⁺ CU patients nor for CU at all.

Moreover, the ubiquitous presence of anti-Fc ϵ RI autoantibodies in human serum has been demonstrated.¹⁷ These autoantibodies seem to be nonpathogenic with the exception of chronic urticaria. Until now, anti-Fc ϵ RI autoantibodies with histamine-releasing activity were considered to be specific for chronic urticaria. This is also confirmed by our data (Tables II and III).

However, our data show that serum-induced basophil CD63 surface expression and de novo SLT release can also be found in atopic and nonatopic control subjects without chronic urticaria.

Taken together, CU serum activity inducing HR, CD63 expression, and SLT production cannot be used as a diagnostic marker for CU but may identify a subset of CU patients with more severe disease and autoimmune pathogenesis. In this regard it is noteworthy that we found a higher prevalence of antibodies to thyroid in ASST⁺ CU sera demonstrating serum activity. Thirty-five percent of ASST⁺ CU patients had autoantibodies to thyroid, in contrast to 15% of ASST⁻ CU patients.

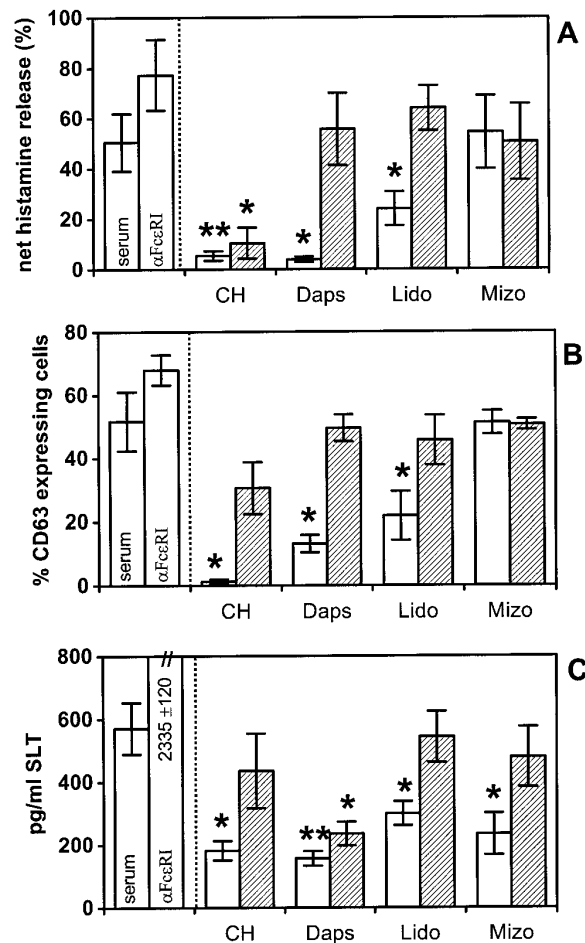


FIG 5. Inhibitory effect of anti-inflammatory drugs on net histamine release (A), percent CD63-expressing basophils (B), and SLT production (C) induced by heat-inactivated sera of 4 ASST⁺ CU patients with high serum activity. Serum, incubation with serum alone (dilution 1:2); α Fc ϵ RI, incubation with α Fc ϵ RI mAb alone (for mAb concentrations see legend to Fig 1). Open bars, Serum coincubated with concentration 1 (ie, 1 mmol/L chloroquine [CH], 1 mmol/L dapsone [Daps], 200 μ g/mL mizolastine [Mizo], and 1 mmol/L lidocaine [Lido]). Hatched bars, Serum coincubated with concentration 2 (ie, 100 μ mol/L chloroquine [CH], 100 μ mol/L dapsone [Daps], 20 μ g/mL mizolastine [Mizo], and 100 μ mol/L lidocaine [Lido]). In A preincubation with drugs was for 60 minutes, sera were diluted 1:2; in B drug preincubation was for 30 minutes, sera were diluted 1:2; and in C drug preincubation was for 40 minutes, and sera were diluted 1:5. Asterisk, $P < .05$; two asterisks, $P < .01$ compared with serum.

Although this difference was not statistically significant, it is notable that thyroid autoimmunity occurs in less than 6% of the general population.⁸ Thus in the ASST⁺ subset chronic urticaria symptoms may express a latent autoimmune disease.

Previous attempts to treat these patients consisted of plasmapheresis or high-dose intravenous Ig and were of temporary success. In this study we found significant inhibitory effects of 4 anti-inflammatory drugs on serum-induced inflammatory effects, namely, chloroquine, dapsone, lidocaine, and mizolastine. However, mizolastine resulted in a significant inhibition of serum-induced SLT

production only. Mizolastine represents a nonsedating second-generation antihistamine with additional anti-inflammatory effects, including the inhibition of neutrophil migration, and leukotriene antagonistic properties that may be mediated by inhibition of 5-lipoxygenase.²⁹ Mizolastine has been shown to be effective in the treatment of allergic rhinitis⁴⁰ and CU.⁴¹ Apart from mizolastine, lidocaine was shown to significantly inhibit serum activity. In a preliminary in vivo study lidocaine showed promise as another class of immunomodulatory drugs that can be substituted for glucocorticoids in the treatment of asthma.³³ This was confirmed by recent studies pointing to lidocaine as a first nontoxic steroid alternative for patients with severe steroid-dependent asthma.³⁴ Up to 1 mmol/L lidocaine was demonstrated to inhibit activation and survival of eosinophils in a noncytotoxic manner.⁴² The mechanism of lidocaine-mediated immunomodulation may involve leukocyte Na⁺ channels⁴³ or the modulation of intracellular pH.⁴² Our data point to a further immunomodulatory effect of lidocaine, namely, the inhibition of HR, basophil CD63 expression, and SLT production. In addition, dapsone showed marked inhibitory effects on serum-induced basophil activation. For many years dapsone has been the drug of choice for the treatment not only of malaria and leprosy but also a number of noninfectious inflammatory conditions associated with neutrophil infiltration such as rheumatoid arthritis,⁴⁴ bullous pemphigoid,⁴⁵ leukocytoclastic vasculitis, and dermatitis herpetiformis.^{46,47} However, the exact mechanism of its anti-inflammatory action is not fully understood.⁴⁸ Dapsone inhibits neutrophil adhesion, chemotaxis, lipoxygenase activity, and the cell's ability to generate reactive oxygen species.⁴⁹⁻⁵² Although CU is sometimes associated with a neutrophilic infiltrate and is then termed neutrophilic urticaria, most lesions are not characterized by neutrophil predominance.^{53,54} Hence dapsone may have additional anti-inflammatory effects on other leukocyte types, such as basophils. Interestingly, in 1991 Cox and Friedmann⁵⁵ observed urticated papules and plaques 18 to 24 hours after performance of autologous serum skin tests. In this aspect it is noteworthy that dapsone treatment leads to a dramatic improvement of dermatitis herpetiformis. Thus dapsone may inhibit serum-mediated pathogenic effects in dermatitis herpetiformis as well as in autoimmune urticaria.

Moreover, chloroquine showed significant inhibitory effects on serum activity. Chloroquine and hydroxychloroquine have been used for many years in the treatment of lupus erythematosus, rheumatoid arthritis, and malaria. Chloroquine is thought to have immunosuppressive effects by inhibition of MHC class II antigen presentation and the production of inflammatory cytokines such as TNF- α , IL-1 β , and IFN- γ .^{56,57} Chloroquine raises the pH of phagolysosomes and may induce basophilic differentiation of HL-60 cells by increasing the intracellular pH.⁵⁸ In neutrophils and monocytes hydroxychloroquine reduced superoxide production and release,⁵⁹ and a significant decrease on neutrophil lysosomal enzyme activity has been shown,⁶⁰ raising the possibility that

such effects extend to other leukocytes. In a double-blind placebo-controlled study treatment of moderate asthmatics, 30 weeks of hydroxychloroquine resulted in a significant improvement of airflow and a significant fall of serum IgE levels.⁶¹ These data point to a modulation of Ig levels.

Taken together, this study clearly demonstrates that a subset of patients with CU and positive ASST is not only characterized by serum activity resulting in HR but also in the induction of basophil CD63 expression and SLT de novo production. However, the clinical relevance of this CU serum activity and the reason for targeting the skin but not the nose or the lungs are far from being clear. Nevertheless, apart from dapsone, mizolastine, and lidocaine, chloroquine and hydroxychloroquine may be interesting drugs for the treatment of autoimmune urticaria particularly because of their remarkably benign toxicity profile. Further controlled studies are needed to elucidate a potential beneficial effect of these anti-inflammatory drugs in the treatment of a subset of patients with CU showing serum activity by means of HR, induction of basophil CD63 surface expression, and SLT production.

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