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Elevated IgE to tissue factor and thyroglobulin are abated by omalizumab in chronic spontaneous urticaria

To the Editor,

Chronic spontaneous urticaria (CSU) is a common condition characterized by episodes of itchy wheals lasting more than 6 weeks.¹ Different biologic systems, that is, autoimmunity, coagulation, and autoallergy, concur to produce CSU symptoms.² The autoimmune mechanism is

based on circulating histamine-releasing IgG autoantibodies to the high-affinity IgE receptor (FcεRI) on both mast cells and basophils or membrane-bound IgE.³ Coagulation cascade is activated in CSU following the hyperexpression of tissue factor (TF) on activated skin eosinophils.^{4,5} The recently identified “autoallergic” mechanism is

mediated by specific IgE to different autoallergens.⁶⁻⁸ The recombinant anti-IgE humanized monoclonal antibody omalizumab was successfully used to treat CSU patients who exhibited IgE against thyroperoxidase⁹ and is nowadays the treatment of choice for CSU unresponsive to second-generation H1 antihistamines.¹ Its mechanism of action in CSU is not completely defined but is regarded to act by lowering free IgE levels and downregulating the FcεRI on mast cells and basophils. However, the frequent rapidity of response to omalizumab points toward an IgE-mediated pathomechanism.²

Here, we investigated the involvement of IgE to TF and thyroglobulin in 15 patients (mean ± SD age: 53 ± 14 years; M/F: 1/14) with severe CSU (urticaria activity score [UAS 7]: 32 ± 6; disease duration: 69 ± 91 months). Demographics and clinical and laboratory features of patients are reported in Table S1 (see: Data S1). A course of subcutaneous omalizumab 300 mg every 4 weeks was started in all patients who were reassessed by means of UAS 7 1 and 2 months later. The measurement of anti-TF and antithyroglobulin IgE in plasma and the cellular antigen stimulation test (CAST) on patients' basophils were performed at baseline and 1 week after omalizumab administration. Fifteen healthy subjects (1 man and 14 women, mean age 52 ± 16 years) undergoing routine check-up investigations served as normal controls for laboratory tests.

Anti-TF and antithyroglobulin IgE were measured by sandwich ELISA described for other molecules.¹⁰ CAST, which measures the amount of leukotriene C4 (LTC4) produced by blood basophils after in vitro stimulation by an allergen, was also performed as described.¹⁰ Data are reported as median values and interquartile ranges (25th and 75th percentiles). The between-group differences were analyzed by the Mann-Whitney test for independent samples, whereas the within-group differences were analyzed by the Wilcoxon test for paired samples with a significance level at $P < 0.05$. The good clinical response to omalizumab is reported in Figure 1. At baseline, CSU patients showed higher mean levels of IgE to TF (688 [415-815] OD) than normal controls (326 [129-424] OD; $P = 0.001$) and higher levels of IgE to thyroglobulin (520 [331-815] OD) than normal controls (184 [170-220] OD; $P = 0.001$) (Figure 2). For IgE to TF, using 475 OD as a cut-off level (resulting from the maximum level in normal subjects), nine of 15 patients (60%) showed elevated specific IgE. For IgE to thyroglobulin, using 278 OD as a cut-off level, thirteen of 15 patients (87%) showed elevated specific IgE. One week after omalizumab administration, the mean level of IgE to TF dropped to 421 (183-696) OD ($P = 0.005$) and the mean level of IgE to thyroglobulin dropped to 309 (268-506) OD ($P = 0.048$) (Figure 2). The 1-week drop of IgE to TF was directly correlated with the drop of UAS 7 at 2 months of omalizumab treatment ($r = 0.564$, $P = 0.028$), while the 1-week drop of IgE to thyroglobulin was directly correlated with the drop of UAS 7 both at one ($r = 0.600$, $P = 0.018$) and 2 months ($r = 0.667$, $P = 0.007$) of omalizumab treatment.

At baseline, basophils from CSU patients showed higher mean release of LTC4 (190 [111-541] pg/mL) than normal controls (120 [86-140] pg/mL; $P = 0.023$) following stimulation with TF and higher mean release of LTC4 (180 [100-475] pg/mL) than normal controls

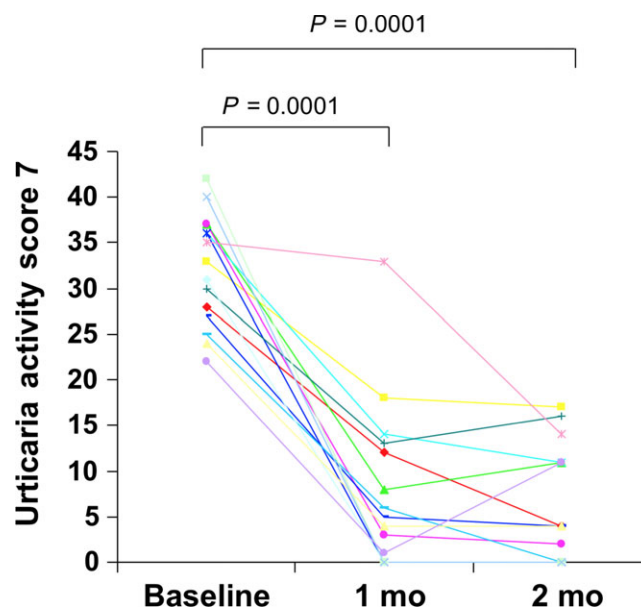


FIGURE 1 Urticaria activity score 7 (UAS7) in 15 patients with chronic spontaneous urticaria treated with omalizumab. Based on UAS7 values, 1 mo after omalizumab administration, 14 of 15 patients were classified as early responders. In one patient, UAS7 was unchanged. Nine patients showed a complete clinical response ($\text{UAS7} \leq 6$), while 5 patients showed a partial response (UAS7 from 8 to 18). At 2 mo of omalizumab treatment, all 15 were classified as responders; in 9, the response remained complete while in the other 6 it was partial [Colour figure can be viewed at wileyonlinelibrary.com]

(115 [74-135] pg/mL; $P = 0.013$) following stimulation with thyroglobulin. For stimulation with TF, using 212 pg/mL as a cut-off level, seven of 15 patients (47%) showed an increased release of LTC4, while for stimulation with thyroglobulin, using 180 pg/mL as a cut-off level, six of 15 patients (40%) showed an increased release of LTC4. One week after omalizumab administration, the mean release of LTC4 following stimulation with TF dropped to 98 (72-130) pg/mL ($P = 0.003$), while the mean release of LTC4 following stimulation with thyroglobulin dropped to 115 (85-135) pg/mL ($P = 0.008$) (Figure 2). The correlation between the 1-week drop of LTC4 following stimulation with thyroglobulin was directly correlated with the drop of UAS 7 both at one (0.609, $P = 0.047$) and 2 months ($r = 0.729$, $P = 0.011$) of omalizumab treatment.

Our data show that high levels of specific IgE to TF (a previously unreported autoallergen) and thyroglobulin (previously reported in one patient with CU^{11,12}) can be detected in a relevant proportion of patients with severe CSU, supporting the role of autoallergic mechanisms in the disease. Thus, it seems to be conceivable to add TF and thyroglobulin to the current short list of autoallergens described in CSU, such as double-stranded DNA,⁶ thyroperoxidase,⁷ and IL-24.⁸ Moreover, the observation that basophils of CSU patients may release LTC4 upon stimulation with TF and thyroglobulin provides further evidence for the involvement of the IgE-basophil system in CSU. CAST is a functional test based upon the determination of LTC4 produced by interleukin-3 (IL-3)-primed basophils

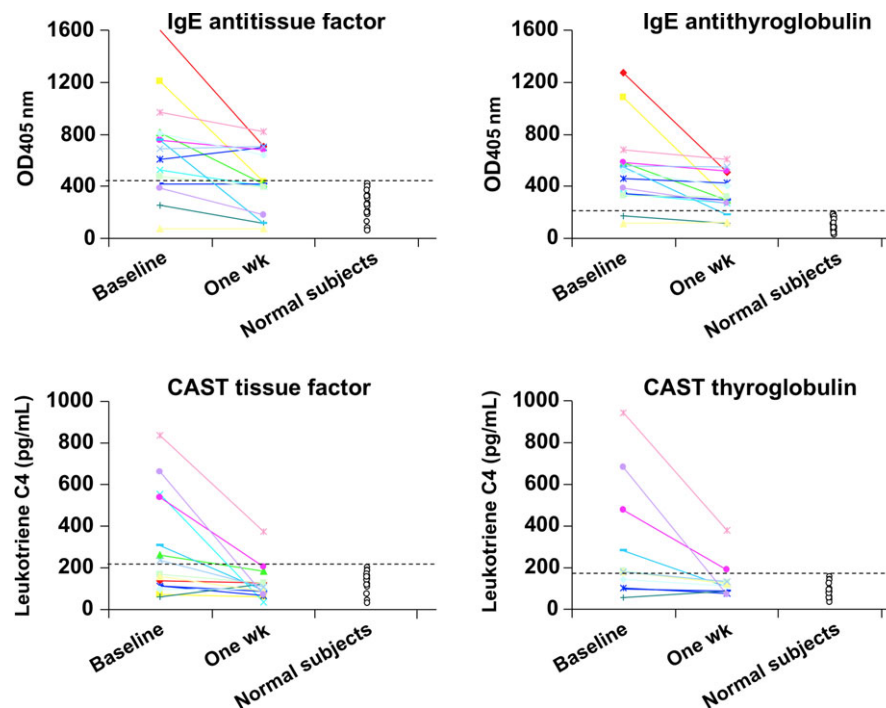


FIGURE 2 Plasma levels of IgE to tissue factor and thyroglobulin (upper panels) and cellular antigen stimulation test (CAST) (lower panels) in 15 patients with chronic spontaneous urticaria treated with omalizumab and in 15 healthy controls. Levels of IgE to tissue factor and thyroglobulin and stimulation-induced release of leukotriene C4 by tissue factor and thyroglobulin were evaluated at baseline in both patients with chronic spontaneous urticaria and controls, and re-evaluated 1 week after omalizumab administration in the patients. Dashed lines represent the upper limit of normal subjects [Colour figure can be viewed at wileyonlinelibrary.com]

stimulated in vitro by allergens¹⁰ and has been used to diagnose allergy to drugs, foods, and occupational allergens. Here, it was used for the first time in CSU. Our data show that CSU patients have elevated specific IgE antibodies to TF, and these antibodies are functionally able to mediate the release of LTC₄ by TF-stimulated peripheral basophils. TF, the main initiator of coagulation, is overexpressed in CSU lesional skin,⁵ where it can activate coagulation and is also accessible to mast cell-bound IgE. IgE-anti-TF could cause "autoallergic" mast cell degranulation, similarly to previously reported IgE to thyroperoxidase and IL-24^{7,8} and IgE to thyroglobulin seen in the present study. In particular, thyroglobulin, that circulates in peripheral blood,¹³ may interact with IgE-anti-thyroglobulin bound to mast cells and basophils throughout the body inducing their degranulation. Autoreactive IgE against several targets in patients with CSU might explain, at least in part, the clinical success of the anti-IgE omalizumab. Indeed, another noteworthy finding of our study is just the decrease in specific IgE to TF and thyroglobulin induced by omalizumab. This fall paralleled the clinical response to omalizumab in our patients who had severe CSU, supporting the view that omalizumab acts as specific IgE-neutralizing agent in this disease. The possibility that specific IgE occurred in the context of a concomitant IgG autoimmune response was unlikely because only two of 15 CSU patients had positivity for antithyroglobulin IgG, and in these two cases, levels of specific IgE to thyroglobulin (475 OD and 331 OD [Table S2]) were only slightly higher than normal controls and below the 50th percentile of CSU patients.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Demographics, clinical and laboratory features of patients with chronic spontaneous urticaria.

Patient	Sex	Age (yr)	Disease duration (mo)	UAS7	Angioedema	ASST	Total IgE (KU/L)
1	F	72	28	28	No	Pos	153
2	F	55	8	33	No	Neg	90
3	F	42	48	37	No	Neg	34
4	F	53	140	36	Yes	Neg	186
5	F	80	5	36	No	Neg	607
6	F	63	84	37	No	Neg	1052
7	F	56	12	30	No	Neg	2000
8	F	31	84	27	No	Neg	89
9	F	44	10	25	No	Neg	45
10	F	50	10	31	No	Neg	78
11	F	54	8	42	No	Neg	139
12	F	24	360	24	Yes	Neg	432
13	M	48	48	40	Yes	Neg	81
14	F	73	96	35	No	Neg	1270
15	F	54	96	22	Yes	Neg	48

UAS7: urticaria activity score 7 days; ASST: autologous serum skin test. Normal values of total IgE are < 100 KU/L.

Table S2. Autoantibodies to thyroglobulin divided into two classes (IgG and IgE) in patients with chronic spontaneous urticaria at baseline.

	Anti-thyroglobulin autoantibodies	
Patient	IgG	IgE
1	Neg	1203,0
2	Neg	519,8
3	Neg	814,5
4	Neg	613,4
5	Neg	256,3
6	Pos	474,5
7	Neg	1597,0
8	Pos	330,7
9	Neg	521,5
10	Neg	433,8
11	Neg	155,7
12	Neg	536,3
13	Neg	353,1
14	Neg	125,5
15	Neg	957,8

IgG anti-thyroglobulin values are merely reported as negative or positive.
IgE anti-thyroglobulin values are reported as OD.