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Relationship between changes in the 7-day urticaria activity score after treatment with omalizumab and the responsiveness of basophils to FcεRI stimulation in patients with chronic spontaneous urticaria

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ABSTRACT

Background: About one-half of all patients with chronic spontaneous urticaria have low or less reactivity of the basophils to FcεRI stimulation. However, the differences in the clinical characteristics between patients who show normal and attenuated basophil reactivities to FcεRI stimulation are still unclear. Furthermore, it also remains unknown as to what factors induce the poor reactivity of basophils to FcεRI stimulation.

Objective: The aim of the study is to investigate the differences in the clinical characteristics between patients who show normal and attenuated basophil reactivities to FcεRI stimulation.

Methods: We compared the clinical characteristics, including the autologous serum skin test-positive rates, serum concentrations of anti-IgE and anti-FcεRIα autoantibodies, and the FcεRI-crosslinking ability of these autoantibodies between patients with a negative basophil activation test (BAT) (≤10% CD203c^{high} basophils, n = 9) and positive BAT (>10% CD203c^{high} basophils, n = 13). We also monitored the changes in the 7-day urticaria activity scores after treatment with omalizumab, as compared to the score at the baseline, between the BAT-positive and BAT-negative patients.

Results: The BAT-negative patients showed a significantly higher urticaria control test score than the BAT-positive patients (p = 0.01). There were no significant differences in the autologous serum skin test-positive rates, concentrations of anti-IgE and anti-FcεRIα autoantibodies, and the FcεRI-crosslinking ability of these autoantibodies between the 2 groups. After treatment with omalizumab for 35 days, the score decreased to under 15 (corresponding to controlled or mild chronic spontaneous urticaria) in all of the BAT-negative patients, whereas in 6 out of the 13 BAT-positive patients, the scores remained over 16 (corresponding to moderate or severe chronic spontaneous urticaria).

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Conclusions: The weak reactivity of basophils to FcεRI stimulation may not be due to the desensitization of basophils by anti-IgE or anti-FcεRIα autoantibodies. The time to response to omalizumab might differ between BAT-negative and BAT-positive patients with chronic spontaneous urticaria.

Keywords: Autoantibody; Basophil; Basophil activation test: FcεRIα; Immunoglobulin E; Urticaria

INTRODUCTION

Chronic spontaneous urticaria (CSU) is characterized by the spontaneous occurrence of systemic daily wheals that persist for at least 6 weeks [1]. Basophils from patients with CSU have been shown to exhibit a bimodal response to anti-IgE activation [2]. About one-half of CSU patients show reduced anti-IgE-induced histamine release from basophils, and are designated as showing a negative result on the basophil activation test (BAT) (hereinafter, BAT-negative patients). Although an earlier study suggested a link between the serum histamine-releasing activity and the reduced response of basophils to anti-IgE stimulation in patients with CSU, evidence has also been accumulated to suggest that the reduced basophil response to anti-IgE stimulation can also be noted in subjects without serum histamine-releasing activity [2, 3]. Syk deficiency has been shown to be more common in BAT-negative patients than in BAT-positive patients, cold urticaria patients, atopic patients, or healthy donors ($p < 0.05$) [4]. It has been shown that in some patients with CSU, anti-IgE or anti-FcεRI α-chain (FcεRIα) autoantibodies (AAbs) induce downregulation of Syk in the peripheral blood basophils [4]. We previously demonstrated that the ability of the anti-IgE AAbs to induce FcεRI crosslinking differed significantly between nonatopic healthy subjects and CSU patients [5]. Yanase et al. [6] mentioned in their review, that basophils in CSU patients appear to be in a constant state of mild activation and continuously release small amounts of histamine.

It has been suggested that a positive basophil histamine release assay (BHRA) and positive autologous serum skin test (ASST) are predictive of a slow response to omalizumab in patients with CSU [7]. These assays are used to detect serum AAbs directed against either cell-bound IgE or unoccupied FcεRIα [7]. Furthermore, the inability of the serum from patients with CSU to upregulate CD203c expression on the peripheral blood basophils of healthy subjects has been reported to be correlated with the clinical response to omalizumab [8]. These findings suggest that patients with CSU who have some histamine-releasing factors, including AAbs against FcεRIα and IgE in their sera show a slow or weak response to omalizumab therapy. Therefore, we hypothesized that the low reactivity of basophils to FcεRI stimulation might be due to desensitization of the basophils by anti-IgE and/or anti-FcεRIα AAbs, and that BAT-negative patients may show a relatively slow or weak response to omalizumab.

MATERIALS AND METHODS

Ethical considerations
This study was approved by the Ethics Committees of the Nihon University School of Medicine (RK-15908-12). All the subjects provided written informed consent in accordance with the Helsinki Declaration of the World Medical Association.

Patient enrollment

Twenty-two consecutive patients with CSU who were receiving treatment with omalizumab (15 females and 7 males; age range, 24 to 87 years) were recruited for this study between April 2017 and August 2018. The characteristics of the patients are summarized in **Table 1**. The majority of patients were referred by doctors in private practice to the outpatient clinic of Nihon University Hospital. Of the 22 patients, 21 had already received treatment with high-dose H1-antihistamines. None of the patients had were received treatment with systemic corticosteroids or cyclosporine within the previous 3 weeks. We also enrolled 20 nonatopic healthy control (NC) subjects (8 females and 12 males; age range, 23 to 64 years) with no history of urticaria, asthma, allergic diseases, or autoimmune diseases as negative controls for the measurement of the anti-IgE and anti-FcεRIα AAbs concentrations, and also measurement of the ability of anti-FcεRIα and anti-IgE AAbs to induce FcεRI crosslinking in an *in vitro* elicitation test (**Table 1**).

Definitions and study protocol

The diagnosis of CSU was made according to the classification of the European Academy of Allergy and Clinical Immunology (EAACI), the Global Allergy and Asthma European Network (GA[2] LEN), the European Dermatology Forum (EDF), and the World Allergy Organization (WAO) [9]. The 7-day urticaria activity score (UAS7) is a patient-reported scoring system that captures the severity of pruritus and the number of hives appearing during one week [10]. The intensity of pruritus (range, 0 [none] to 3 [severe]) and the number of hives (range, 0 [none] to 3 [>50 hives]) were recorded daily (maximum, 6 points per day). The scores were then summed for 1 week to represent the UAS7 scale, (0–42). Urticaria control test (UCT) is a system that evaluates the status of urticaria for past 4 weeks by asking 4 questions. The score ranges from 0 to 16, and the larger the number, the better the state [11]. The serum IgE levels, basophil counts were checked by blood test.

Autologous serum skin test

The ASST was performed using 50 μL of the patient's own serum intradermally injected into the flexor aspect of the forearm; 50 μL of saline was injected 3–5 cm away as a control. The

Table 1. Characteristics of the all patients with CSU

Variable	NC subject (n = 20)	CSU (n = 22)
Age (yr)	35.5 (23–64)	47 (24–87)
Female sex	8 (42)	15 (68)
Disease duration (mo)	N/A	38 (4–216)
UAS7	N/A	31 (14–42)
UCT score	N/A	5 (0–8)
ASST-positive rate (n = 20)	ND	7 (35)
Presence of angioedema at baseline	0 (0)	3 (13.6)
Treatment		
H1 antihistamine at the conventional dosage	0 (0)	1 (4.5)
H1 antihistamine at high dose	0 (0)	21 (95.5)
Leukotriene receptor antagonists	0 (0)	20 (91.0)
Systemic corticosteroids	0 (0)	0 (0)
Cyclosporin	0 (0)	0 (0)
History		
Asthma	0 (0)	1 (4.5)
Allergic rhinitis	0 (0)	1 (4.5)
Atopic dermatitis	0 (0)	5 (22.7)
Pollinosis	0 (0)	3 (13.6)

Values are presented as number (%) or median (range) unless otherwise indicated.
NC, nonatopic control; CSU, chronic spontaneous urticaria; UAS7, 7-day urticaria activity score; UCT, urticaria control test; ASST, autologous serum skin test; N/A, not applicable; ND, not done.

results were measured after 30 minutes. If the serum-injected site manifested a wheal with a diameter of 1.5 mm greater than that of the saline-injected site, the result was considered positive [12]. According to the EAACI/GA(2) LEN task force consensus report [12], antihistamines were discontinued for 2–3 days prior to the ASST.

Basophil activation test

The Allergenicity Kit (Beckman Coulter Inc, Brea, CA, USA) was used for quantifying the expression of CD203c on the basophils. Heparinized whole blood was incubated with anti-FcεRI antibody (clone CRA1, BioAcademia, Osaka, Japan; 3 µg/mL), anti-IgE antibody (clone E124-2-8D, Beckman Coulter Inc.; 10 µg/mL), N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) (Sigma-Aldrich, Darmstadt, Germany; 1 µg/mL) or the negative control substance (phosphate-buffer saline), as indicated, for 15 minutes. PC7-conjugated anti-CD3, FITC-conjugated anti-CRTH2, and PE-conjugated anti-CD203c antibodies were also added during the reaction. The samples were analyzed using the Gallios flow cytometer (Beckman Coulter Inc., Brea, CA, USA) and FlowJo software (TreeStar, Woodburn, OR, USA). Basophils were identified on the basis of the forward and side scatter characteristics, negative expression of CD3 and positive expression of CRTH2 on the cells. Activated basophils were identified by the upregulated expression of CD203c as compared to that on the unstimulated cells (negative control). At least 500 basophils were analyzed in each assay.

Isolation of IgG fraction from serum

IgG was purified from sera of patients with CSU and NC subjects using Ab-Rapid SPiN EX (ProteNova, Kagawa, Japan) according to the manufacturer's protocol. The purified IgG was reconstituted with phosphate-buffered saline (PBS) using Amicon centrifugal filter units (Merck Millipore, Darmstadt, Germany). The final concentrations of the purified IgG were diluted to one-sixth of the serum IgG concentrations.

Measurement of concentrations of anti-FcεRIα AAbs

The truncated human FcεRIα ectodomain (1–172 amino acids; rh soluble FcεRIα) was secreted from transfected Chinese hamster ovary cells as described [5]. Secreted rh soluble FcεRIα was purified from the culture supernatant of the cells using mouse anti-FcεRIα (clone CRA2, BioAcademia) [13]. An enzyme-linked immunosorbent assay (ELISA) for detecting anti-human FcεRIα AAbs was performed as previously described [5, 14-17] with the exception that a standard curve for IgG anti-FcεRIα was generated using humanized anti-FcεRIα mAb (clone CRA2, isotype human IgG1) [18]. Briefly, 1 µg/mL of purified recombinant soluble FcεRIα proteins [19] in sodium carbonate, pH 9.6 (Calbiochem, San Diego, CA, USA), were used to coat polystyrene microwells of an ELISA plate (Maxisorp; Nunc, Roskilde, Denmark) in duplicates and were incubated overnight at 4ºC. The plate was washed 4 times with PBS containing 0.05% Tween 20 (PBST) and then blocked with 10% fetal bovine serum (FBS) in PBS for 1 hour at room temperature. After washing, the purified IgG from the sera of patients with CSU or NC subjects were added at a 1:10 dilution with PBS to each well, then incubated for 2 hours at room temperature. After washing, horseradish peroxidase (HRP)-conjugated anti-human IgG mAb (clone G18-145; BD Pharmingen, Franklin, NJ, USA) was added to each well at a 1:10,000 dilution for measuring its concentration of total IgG, then incubated for 1 hour at room temperature. The reactions in the wells were developed with hydrogen peroxide plus 3,3',5,5'-tetramethylbenzidine in 100 mM citrate-phosphate buffer, pH 5.0 (KPL, Gaithersburg, MD, USA), and their absorbances were monitored at 450 nm and 570 nm using a Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific, Yokohama, Japan) after the addition of 2 N sulfuric acid. The linear region of the standard curve was used to quantify the anti- FcεRIα in the purified IgG.

Measurement of concentrations of anti-IgE AAbs

An ELISA was used to detect anti-IgE AAbs, as previously described [5, 20-23]. Briefly, the polystyrene microwells of an ELISA plate (Maxisorp) were coated with human myeloma IgE (1 μg/mL in sodium carbonate pH 9.6, Calbiochem) and incubated overnight at 4°C. Unbound protein was removed by 4 washes with PBST. Free binding sites were blocked by incubation with 10% FBS in PBS for 1 hour at room temperature. After washing, the purified IgG from the sera of patients with CSU or NC subjects were added at a 1:10 dilution with PBS to each well, which were then incubated for 2 hours at room temperature. After washing, HRP-conjugated anti-human IgG mAb (clone G18-145) was added to each well at a 1:10,000 dilution, which was then incubated for 1 hour at room temperature. The reactions in the wells were developed as mentioned above. The quantity of reactive IgG was calculated using standard amounts of purified human IgG (Jackson Immuno Research Laboratories, West Grove, PA, USA) ranging from 0.775 ng/mL to 50 ng/mL bound directly to the plates. To normalize the data among the plates, one specific donor's anti-IgE level was measured repeatedly on all of the plates.

Preparation of RS-ATL8 cells and RBL-NL4 cells

RS-ATL8 cells or RBL-NL4 cells (2.0×10^5 cells/1000 μL/well) [24] were cultured in MEM containing 10% FBS, 1% GlutaMAX-I, 100-U/mL penicillin, 100-μg/mL streptomycin, 0.5-mg/mL geneticin, and 0.2-mg/mL hygromycin B on a Costar 24-well clear tissue culture-treated multiple well plate (Corning Inc., Corning, NY, USA) at 37°C for 2 days in a 5% CO_2 incubator [5, 24]. The cells were replated and cultured as described above for an additional 2 days. The cells were then replated on a 96-well plate at 2×10^4 cells/50 μL/well and cultured at 37°C overnight in a 5% CO_2 incubator.

Measurement of the ability of anti-FcεRIα and anti-IgE AAbs to induce FcεRI crosslinking using an in vitro elicitation test (modified EXiLE test)

An *in vitro* elicitation test was modified from a previously described method reported by us [5, 24]. The test was performed by assessing an EXiLE test using human FcεRIαβγ$_2$ genes- and nuclear factor of activated T-cell-responsive luciferase reporter gene-introduced rat basophilic leukemia cells (RS-ATL8 cells) [25]. The purified IgG was replaced with MEM containing 10% FBS, 1% GlutaMAX-I, 100-U/mL penicillin, 100-μg/mL streptomycin, 0.5-mg/mL geneticin, and 0.2-mg/mL hygromycin B, using Amicon centrifugal filter units (Merck Millipore). To measure the ability of anti-FcεRIα AAbs to induce FcεRI crosslinking, 50 μL of purified IgG (equivalent to a 1:6 dilution of the serum IgG levels) obtained from CSU patients was added to each well and the samples were incubated at 37°C for 3 hours in a 5% CO_2 incubator. To measure the ability of anti-IgE AAbs to induce FcεRI-crosslinking, the purified IgG was preincubated with well-coated rh soluble FcεRIα (1 μg/mL), and the supernatants were sequentially incubated with well-coated rh soluble FcεRIα twice. Then, the supernatants were applied to IgE-sensitized RS-ATL8 cells. Fifty μL of luciferase substrate solution containing a cell lysis reagent (ONE Glo, Promega, Madison, WI, USA) was added to the cells, and the chemiluminescence was measured using a Centro LB 960 plate reader (Berthold Technologies, Bad Wildbad, Germany). Standard curves for the fold inductions in luciferase fluorescence were induced by mouse anti-human FcεRIα mAb (clone CRA1) or rabbit anti-human IgE Ab (Dako, Santa Clora, CA, USA). The luciferase expression levels were represented as the fold increase in light units compared with the results in nonstimulated cells. The minimum concentration of CRA1 or rabbit anti-human IgE Ab that induced a significantly higher intensity of luciferase fluorescence than that observed in the nonstimulated wells was used to define as the sensitivity of the EXiLE test.

Statistical analysis

We compared continuous variables between the 2 groups using the Mann-Whitney *U* test; to compare categorical variables, a 2-sided Fisher exact test was used. Basophil counts and BATs before and after omalizumab were compared using Wilcoxon matched-pairs signed-rank test. A *p* value was considered significant at $p < 0.05$. The data analyses were performed using Prism version 7 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

We enrolled 22 patients with CSU in this study. The clinical and laboratory data of the patients are shown in **Table 2**. The patients with CSU were divided into 2 groups according to the results on the BAT, namely, the basophil activation rates after stimulation with anti-FcεRIα monoclonal antibody (clone CRA1), as previously described [26]. Patients in whom the percentage of CD203c^{high} basophils in the BAT remained at ≤10% were defined as BAT-negative (n = 9), while those in whom the number of CD203c^{high} basophils increased to >10% were defined as BAT-positive (n = 13), as previously described [26, 27]. The response characteristics of the CSU patients are summarized in **Table 3**. We found that there was no significant difference in the UAS7 between the BAT-negative and BAT-positive patients, but that the UCT score was significantly higher in the BAT-negative than in the BAT-positive patients ($p = 0.010$). No significant differences were found in the age, sex, disease duration, positivity rates of ASST, peripheral blood basophil count, peripheral blood eosinophil count, serum total IgE levels, positivity rate of antinuclear antibody, concentrations of anti-IgE AAbs (**Fig. 1A**) and anti-FcεRIα AAbs (**Fig. 1B**) concentrations or in the FcεRI-crosslinking ability of these AAbs (**Fig. 1C, D**).

Table 2. Clinical and laboratory data of the patients with CSU

Patient	No.	Age (yr)	Sex	DD (mo)	UAS7	UCT	ASST	Basophil count (/μL)		Eosinophil count (/μL)	Total IgE (IU/ml)	Serum concentration, anti-IgE AAbs (μg/mL)	Anti-IgE AAbs (FI)	Serum concentration, anti-FcεRIα AAbs (μg/mL)	Anti-FcεRIα AAbs (FI)	Proportion (%) of CD203c^{high} basophil	
								Pre	Post							Anti-FcεRIα Ab	Anit-IgE Ab
Negative BAT patients	1	38	M	14	21	8	+	38.5	50	259	2,129	1.83	1.15	1.59	1.06	1.19	11.4
	2	35	F	14	35	7	–	31.5	30	119	79	1.53	1.42	1.18	1.03	1.51	4.93
	3	41	F	36	28	7	–	42.4	49	111	478	0.50	1.30	0.48	1.00	1.79	30.2
	4	31	F	216	23	8	ND	42.4	37.8	545	69	ND	ND	ND	ND	2.56	1.8
	5	47	F	18	35	6	+	37.2	53	211	83	1.32	1.10	1.08	0.97	2.8	13.7
	6	49	F	60	32	6	+	11.8	ND	0	1	2.11	1.55	1.44	1.95	3.12	1.59
	7	41	M	8	42	4	+	18	41	41	340	1.54	ND	0.29	ND	5.64	47.9
	8	33	M	84	28	5	–	19.4	35.7	155	562	1.30	1.31	2.09	1.08	6.04	23
	9	82	F	24	30	3	–	12.4	50.4	50	180	0.64	1.42	0.42	1.10	7.38	34.1
Positive BAT patients	10	71	M	17	42	1	–	54	37.2	90	585	0.98	1.14	0.29	1.06	10.5	29.9
	11	68	M	40	35	3	–	8.8	ND	70	1,149	1.35	1.22	1.81	1.03	10.6	32.1
	12	64	F	4	35	6	+	33.2	63	108	413	0.99	1.09	1.89	0.97	13.6	31.8
	13	24	F	12	20	6	–	38.5	68.4	424	946	0.81	1.58	0.97	0.58	17.1	69.8
	14	39	F	48	28	0	+	10.6	21.6	81	29	0.92	1.29	0.71	1.65	17.3	42.2
	15	47	F	96	42	0	–	9	ND	81	272	0.95	1.10	0.56	1.12	18.8	59.4
	16	51	M	24	28	7	ND	69.3	70	122	318	1.07	1.16	0.31	1.00	20.8	35.9
	17	48	F	180	42	0	+	32.7	69.3	142	243	2.89	1.79	5.35	1.06	29	60.1
	18	67	M	72	42	0	–	18.2	41	400	190	0.95	1.13	0.20	0.96	31	39.1
	19	44	F	168	22	6	–	29.6	28.8	118	72	1.32	ND	0.50	ND	38.5	74
	20	60	F	30	42	1	–	11.4	81.9	0	830	0.80	1.30	1.18	0.87	41.2	48.5
	21	87	F	60	14	5	–	31.8	60	297	8	2.05	1.96	4.22	13.00	58.1	38
	22	27	F	60	14	4	–	19.2	28.2	90	1,595	1.21	1.40	1.80	1.40	61	74.9

CSU, chronic spontaneous urticaria; BAT, basophil activation test; AAbs, autoantibodies; DD, disease duration; UAS7, 7-day urticaria activity score; UCT, urticaria control test; ASST, autologous serum skin test; Ab, antibody; FI, fold increase; ND, not done.

Table 3. Comparison of patient characteristics between CSU patients with responders and nonresponders

Variable	Negative BAT patients (n = 9)	Positive BAT patients (n = 13)	p value
Age (yr)	41 (24–87)	51 (24–87)	0.149[†]
Female sex	6 (66.7)	9 (69.2)	>0.999[#]
Disease duration (mo)	24 (8–216)	42 (4–180)	0.480[†]
UAS7	30 (21–42)	35 (14–42)	0.753[†]
UCT score	6 (3–8)	3 (0–7)	0.01[*,†]
ASST-positive rate	4/8 (50)	3/12 (25)	0.356[#]
blood basophil count (/μL)	28.1 ± 12.8	28.2 ± 18.0	0.544[†]
blood eosinophil count (/μL)	166 ± 165	156 ± 132	0.909[†]
Total IgE (IU/mL)	436 ± 665	512 ± 486	0.471[†]
Antinuclear antibody positive rate	2 (22.2)	2 (15.4)	>0.999[#]

Values are presented as median (range), number (%), or mean ± standard deviation.
CSU, chronic spontaneous urticaria; BAT, basophil activation test; UAS7, 7-day urticaria activity score; UCT, urticaria control test; ASST, autologous serum skin test.
[*]Statistical significance between CSU patients with responders and nonresponders. [#]Fisher exact test. [†]Mann-Whitney *U* test.
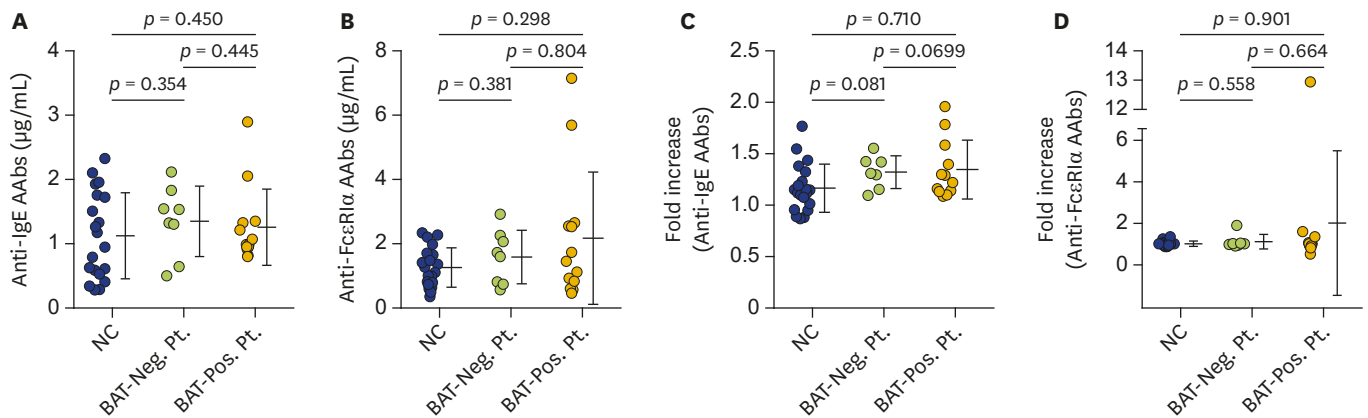

Fig. 1. Comparison of the serum concentrations of anti-IgE AAbs (A) and anti-FcεRIα AAbs (B), and the FcεRI-crosslinking ability of these AAbs (C, D) among nonatopic control (NC) subjects (n = 20), BAT-negative patients (n = 9), and BAT-positive patients (n = 13). The luciferase expression levels are represented as the fold increase in light units as compared to the results from the nonstimulated cells. Statistical analyses were performed using the Mann-Whitney *U* test. AAbs, autoantibodies; BAT, basophil activation test. BAT-Neg. Pt., BAT-negative patients; BAT-Pos. Pt., BAT-positive patients.

Omalizumab 300 mg was administered by subcutaneous injection 3 times at 4-week intervals (arrows in **Fig. 2A**). The first day on which a response was detected was defined as the first of 7 continuous days on which the UAS7 score was 6 or less [7]. Venous blood samples were collected before the start of omalizumab treatment and on day 84 after the start of omalizumab treatment. At the end of 84 days of treatment, the UAS7 was ≤6 in 16 patients (73%) (**Fig. 2A**). The UAS7 was significantly decreased on days 7, 35, and 84 as compared to that on day 0 in both the BAT-negative and BAT-positive patients (**Fig. 2B, C**). Furthermore, the UAS7 was significantly lower on days 35 and 84 as compared to that on day 7 in the BAT-negative patients (**Fig. 2B**), whereas in the BAT-positive patients, a significant difference in the UAS7 score was only observed between day 7 and day 84 (**Fig. 2C**). After omalizumab treatment for 35 days, the UAS score decreased to under 15 (corresponding to controlled or mild CSU; **Fig. 2B**) in all of the BAT-negative patients, whereas in 6 out of the 13 BAT-positive patients, the scores remained over 16 (corresponding to moderate or severe CSU; **Fig. 2C**). Significant increase in the peripheral blood basophil counts after treatment with omalizumab was found in both the BAT-negative and BAT- positive patients (**Fig. 3A**, *p* = 0.0391; **Fig. 3B**, *p* = 0.0186; respectively). Significant increase of the percentages of CD203c^{high} basophils in response to FcεRI stimulation was seen after treatment with omalizumab in both the BAT-negative and BAT- positive patients (**Fig. 3C**, *p* = 0.0156; **Fig. 3D**, *p* = 0.0156; respectively).
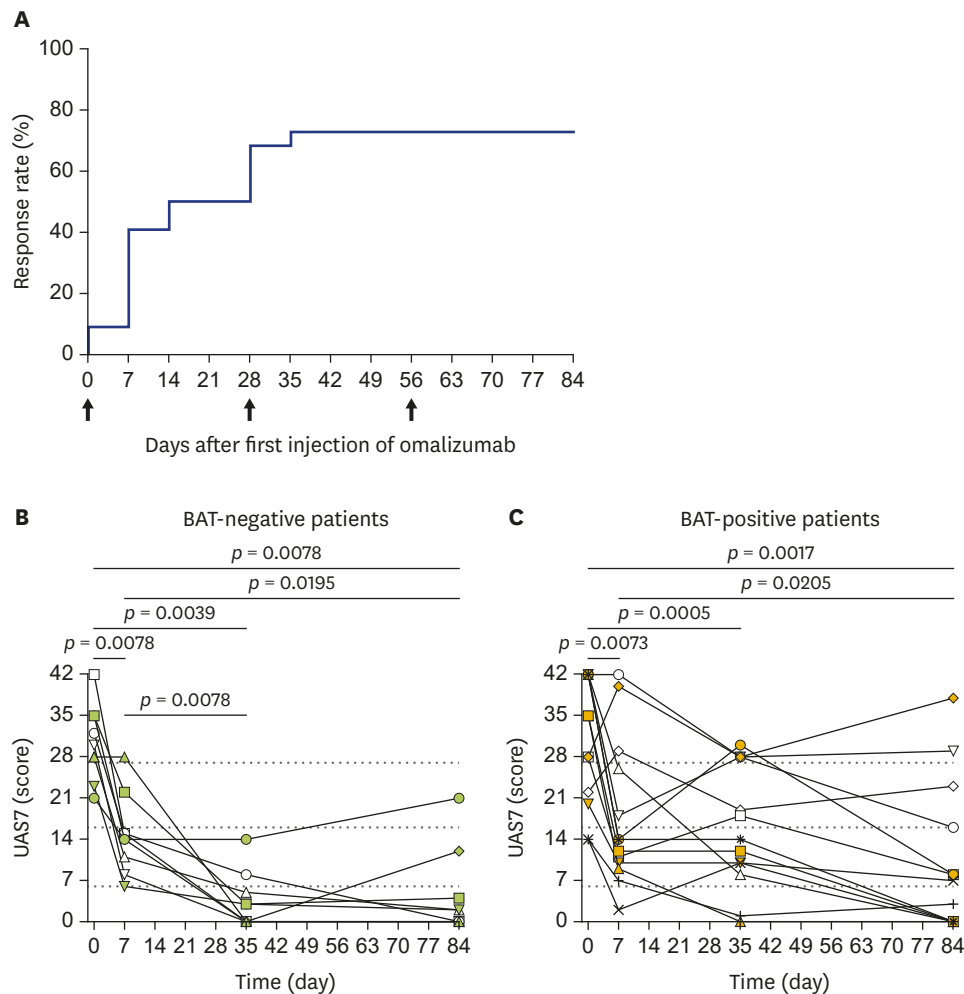
Fig. 2. Efficacy of omalizumab in the BAT-negative (n = 9) and BAT-positive patients (n = 13). (A) The percentage of all patients (n = 22) in whom UAS7 ≤ 6 was achieved, plotted against the day of response to omalizumab therapy. The arrows indicate the days of omalizumab injections. (B) Changes in the UAS7 score in the BAT-negative patients before (day 0) and after treatment with omalizumab (days 7, 35, and 84; n = 9). (C) Changes in the UAS7 score in the BAT-positive patients before (day 0) and after treatment with omalizumab (days 7, 35, and 84; n = 13). Dotted lines show the boundaries of the disease severity. Statistical analyses were performed using the Mann-Whitney *U* test. BAT, basophil activation test; UAS7, 7-day urticaria activity score.

Although a significant increase in the percentage of CD203c^{high} basophils in response to fMLP stimulation was seen after treatment with omalizumab in the BAT-positive patients (**Fig. 3F**, $p = 0.0313$), no such difference was observed in the BAT-negative patients (**Fig. 3E**).

DISCUSSION

We demonstrated that after treatment with omalizumab for 35 days, all of the BAT-negative patients showed a decrease of the UAS corresponding to a controlled or mild severity state (**Fig. 2B**), while 6 out of the 13 BAT-positive patients (46%) still showed scores corresponding to moderate or severe CSU (**Fig. 2C**). Although the precise reason(s) is unclear, the significantly lower UCT score in the BAT-positive patients as compared to the BAT-negative patients (**Table 3**) could be one possible reason. The findings must be verified in a larger population. Palacios et al. [8] and Gericke et al. [7] reported that CSU patients with basophil-activating serum activity are less likely to show a complete and rapid response
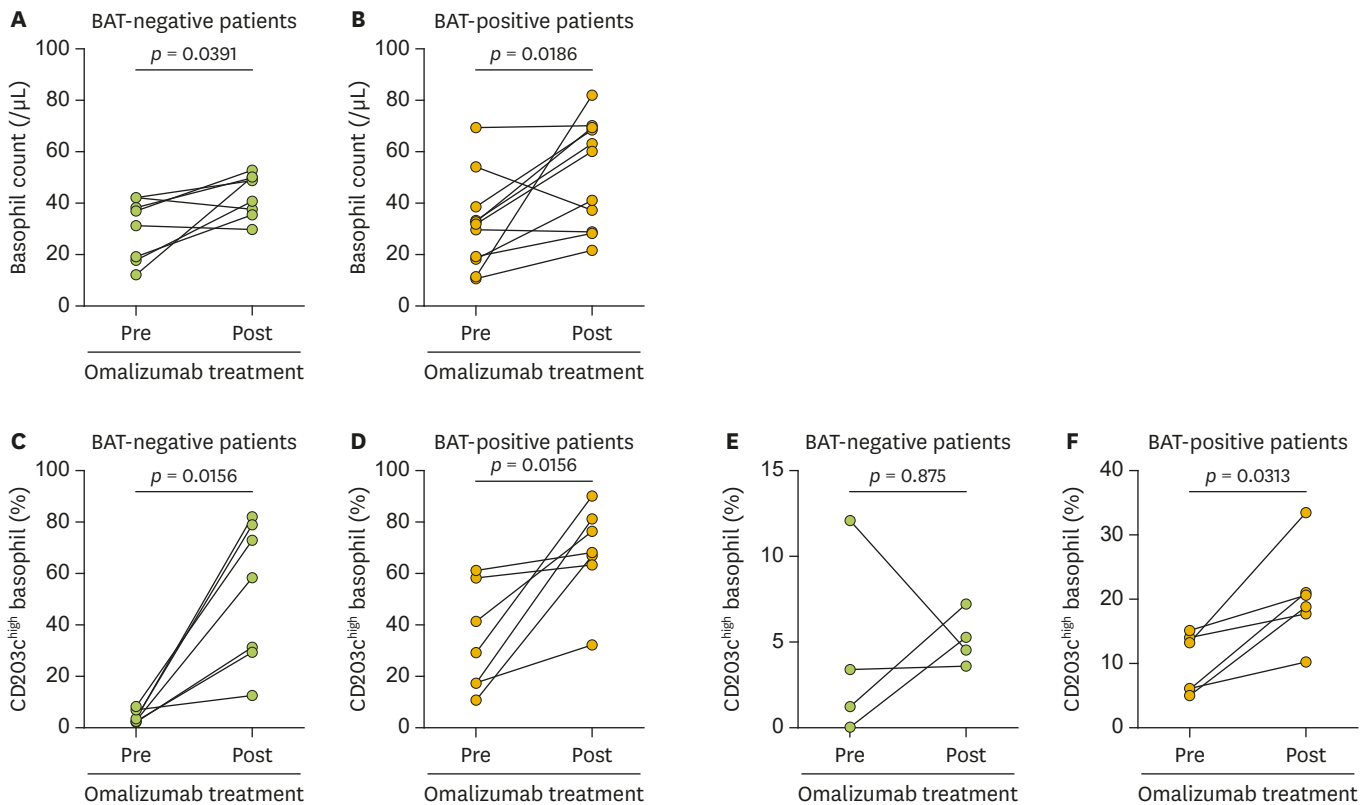
Fig. 3. Comparison of the peripheral blood basophil counts (A, n = 8; B, n = 11), proportion (%) of CD203^{high} basophils after FcεRI aggregation (C, n = 7; D, n = 7), and proportion (%) of CD203^{high} basophils following N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) stimulation (E, n = 4 ; F, n = 6) in the BAT-negative and BAT-positive patients before (day 0) and after treatment with omalizumab (day 84). Statistical analyses were performed using the Mann-Whitney U test. BAT, basophil activation test;

to omalizumab, respectively. The discrepancy between the findings of the aforementioned authors [7, 8] and our results suggests that the presence of histamine-releasing activity in the serum of BHRA-positive and ASST-positive patients might not induce desensitization of basophils to IgE-mediated stimulation, resulting in the weak activation of basophils in response to FcεRI stimulation.

In our study, no significant differences in the positivity rate of ASST were found between the BAT-negative and BAT-positive patients (**Table 3**). Thus, the positivity rate of ASST showed no correlation with the reactivity of basophils to FcεRI stimulation in the BAT. Furthermore, the serum concentrations of anti-IgE and anti-FcεRIα AAbs, and the FcεRI-crosslinking ability of these AAbs were not significantly different between the BAT-negative and BAT-positive patients (**Fig. 1**). Thus, anti-IgE and anti-FcεRIα AAbs might not be necessary or sufficient to induce weak reactivity of the basophils to FcεRI stimulation. Another serologic factor may be autoreactive IgE [28, 29] and/or the so-called cytokinergic IgE [30, 31]. In our study, a significant increase in the percentages of CD203c^{high} basophils in response to FcεRI stimulation after treatment with omalizumab was seen in both the BAT-negative and BAT-positive patients (**Fig. 3C**, p = 0.0156; **Fig. 3D**, p = 0.0156; respectively). Furthermore, most BAT-negative patients converted to BAT-positive after treatment with omalizumab. The basophil surface expression levels of FcεRIα has been reported to decrease significantly after treatment with omalizumab [32]. Thus, omalizumab increases the reactivity of basophils to FcεRI stimulation by changing intracellular signaling. While a significant difference in the

percentage of CD203c^{high} basophils following fMLP stimulation between before and after the treatment with omalizumab was seen in the BAT-positive patients (**Fig. 3F**), no such difference was seen in the BAT-negative patients (**Fig. 3E**), A previous study demonstrated that utilization of fMLP did not reveal any differences in basophil activation between the BAT-negative and BAT-positive patients [26]. Further studies are therefore required. Omalizumab binds to free IgE and may also bind to autoreactive IgE. If autoantigens were present in the skin tissues of patients with CSU, but not in healthy donors, the suggestion by Vonakis et al. [2] that the anti-IgE response of the basophils from patients with CSU is not related to the presence of serologic factors would be reasonable.

Limitations of the study are that all the data are derived from an open observational study without controls. Another weakness is that the number of patients is small. The findings must be confirmed in a sufficiently large population. Furthermore, the majority of patients with CSU (91%) receive treatment with high doses of H1 antihistamines (**Table 1**); the possible influence of high-dose H1 antihistamine therapy on the efficacy of omalizumab cannot be excluded.

In conclusion, we demonstrated that the time to response to omalizumab might differ between BAT-negative and BAT-positive patients with CSU. Further studies are required to identify factors that induce weak FcεRI-mediated basophil activation.

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