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Establishment of the basophil activation test to detect photoallergens in solar urticaria

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Clinical Implications

We successfully detected a serum photoallergen using the basophil activation test in patients with solar urticaria. Additionally, we demonstrated that basophils from a patient with solar urticaria were activated in response to serum photoallergen by an IgE-mediated mechanism.

Solar urticaria (SU) is a form of physical urticaria that presents with wheals following light exposure. Although the responsible wavelengths responsible for SU vary among patients, including ultraviolet radiation B (UVB), UVA, and/or visible light (VL), most studies have cited action spectra (AS) between 300 and 500 nm.[1] Although the pathogenesis of SU remains largely hypothetical, the existence of a serum photoallergen (serum factor) has been suggested on the basis of intradermal tests using patient serum samples that were irradiated *in vitro* with AS.[2] In the last quarter of a century, intradermal tests that involved exposure of the patient to AS-irradiated patient serum or AS-irradiated healthy volunteer (HV) serum, and passive transfer tests using patient or HV serum were performed to verify the existence of a circulating photoallergen, although not yet fully identified.[2] However, intradermal tests using serum samples from other individuals are no longer ethically allowed. Thus, it is important to establish *in vitro* tests to replace the traditional *in vivo* test, to investigate endogenous serum photoallergens and select the appropriate therapeutic method. Therefore, we attempted to establish an alternative method to detect serum photoallergens in patients with SU *in vitro* by using the basophil activation test (BAT). In addition, we investigated whether basophils from patients were activated in response to a serum photoallergen involving an IgE-mediated mechanism.

We herein present two patients with SU in whom we successfully detected serum photoallergens using BAT.

Case 1 was a 22-year-old woman presenting with a 4-year history of SU. Serum total IgE was 213.7 IU/mL and she had atopic dermatitis and asthma in childhood. The minimal urticarial dose was positive at 3 J/cm^2 of UVA (Toshiba FL 32S BL lamps), and 7 mJ/cm^2 of UVB (Toshiba FL 32S E-30 lamps). No reaction to VL (slide projector; 15 cm distance, 15 minutes) was detected. Case 2, a 23-year-old man, had a 7-year history of SU, and serum total IgE was 105.4 IU/mL and he had no allergic past history or comorbidities. He revealed a positive phototest at 10 J/cm^2 of UVA and 10 minutes of VL. Intradermal injection with each autologous serum irradiated with UVA and UVB (case 1) or UVA and VL (case 2) was positive, but injection of non-irradiated autologous serum was negative, indicating the presence of an endogenous serum photoallergen.

Next, we confirmed whether an endogenous serum photoallergen could be detected using the BAT. The both patients were free of antihistamine and/or corticosteroids treatment at the time of sera and blood collection. During these procedures, serum samples and hematocytes were covered with aluminum foil to avoid natural light exposure. Serum samples were obtained from patients and HV. In case 1 we analyzed serum and hematocytes from different donors and in case 2, we used serum and hematocytes from the same subject; serum samples were used as

78 antigens immediately after irradiation and each sample was AS-irradiated or non-irradiated. We
79 adopted UVB-irradiated serum (case 1) and UVA-irradiated serum (case 2) as antigens in BAT
80 because the diameters of wheal was larger in intradermal injections with autologous serum
81 irradiated with UVB than with UVA (case 1), and larger with UVA than with VL (case 2).
82 For the BAT[4], a mixture of 50 μL of whole blood from the patient or HV with
83 phosphate-buffered saline 10 μL, anti-IgE antibody (0.09 μg/mL), and different volumes of
84 serum samples (1 μL, 3 μL, 10 μL, 30 μL) were incubated with 10 μL of staining reagent
85 consisting of CRTH2-FITC, CD203c-PE, and CD3-PC7 in 50 μL of activation buffer at 37°C
86 for 15 minutes. After blood samples were depleted of erythrocytes, remaining cells were
87 analyzed by flow cytometry (FACS verse, BD Biosciences, San Jose, CA, USA). Basophils
88 were gated by their specific forward and side scatter as well as CD3-negative and
89 CRTH2-positive properties as shown in Fig. E1 in the Online Repository. Results of serum or
90 IgE antibody stimulation were calculated as: (serum- or IgE antibody-induced CD203c
91 expression (MFI) – basal CD203c expression (MFI)). Each basal CD203c expression was
92 shown in Table E1 in the Online Repository.
93 In case 1, a concentration-dependent increase in CD203c expression was observed from a
94 mixture of the patient's hematocytes with UVB (AS)-irradiated HV serum as well as
95 UVB-irradiated autologous serum (Fig. 1A). No reaction was noted when the patient's

hematocytes were incubated with non-irradiated autologous serum and HV serum (Fig. 1A). In case 2, CD203c expression was increased when incubated with UVA (AS)-irradiated autologous and HV serum, but not with non-irradiated autologous and HV serum (Fig 1B). In contrast, no increase in CD203c expression on basophils was observed when HV hematocytes were incubated with any patient or HV serum samples (Fig. 1C, D). When the BAT was performed using UVA-irradiated HV serum as antigens in case 1, a concentration-dependent increase in basophil CD203c expression was observed as well as using UVB-irradiated serum (Table E1 in the Online Repository).

To study whether basophil activation to serum photoallergen was mediated by IgE, we performed a passive sensitization assay using HV basophils and serum from case 1. The basophil donor's peripheral blood mononuclear cells (PBMCs) were incubated for 5 minutes on ice with 10 mM lactic acid (pH 3.9) to dissociate IgE from FcεRI on basophils. These acid-treated PBMCs were then incubated for 2 hours at 37°C in serum from a SU patient for passive sensitization[6]. To block IgE binding to basophils by passive sensitization, serum was pretreated for 30 minutes at room temperature with 30 μg/mL omalizumab, a monoclonal anti-IgE antibody (Novartis Pharma, Tokyo, Japan).[5] Basophil CD203c expression in HV increased when incubated with UVB (AS)-irradiated serum after sensitization by serum from case 1 but not from another HV (Fig. 2A). Moreover, omalizumab pretreatment of the serum for

114 passive sensitization blocked serum photoallergen-induced basophil activation (Fig. 2B). These
115 observations indicate that basophil activation by a serum photoallergen is a specific response in
116 patient serum and is mediated by IgE in the patient's serum in our case.
117 SU is a rare photodermatosis with a variety of symptoms that range from wheals and erythema
118 on sun-exposed skin to anaphylaxis.[6] Leenutaphong *et al.* classified patients with SU into two
119 subtypes depending on the characteristics of photoallergens: those with specific or nonspecific
120 photoallergens (universal chromophore), based on intradermal autologous or other individual's
121 serum test results.[3] Our BAT system allowed the classification while minimizing invasion of
122 individuals, and these two cases had nonspecific photoallergens because both patients'
123 basophils were activated by irradiated serum from the patient and HV. Moreover, the data of
124 omalizumab pretreatment in passive sensitization assay revealed that case 1 may have an IgE
125 antibody against a universal photoallergen.
126 Recently, most SU cases have been successfully treated with omalizumab[7] and the symptomatic
127 improvement during anti-IgE therapy supports the hypothesis that SU is mediated by IgE.[6]
128 However, failure of omalizumab for the treatment of a patient with SU was reported.[8] These
129 conflicting clinical outcomes of omalizumab therapy may be related to the heterogeneous nature
130 of SU. However, omalizumab might be a promising treatment option for cases of SU mediated
131 by IgE and the BAT system may predict the effectiveness of omalizumab in SU.

In summary, we have established the BAT with patients and HV serum irradiated with AS to assess serum photoallergens. Basophil activation in response to serum photoallergens was confirmed to be IgE-mediated. Although further studies are needed in BAT in more patients with SU, the BAT is promising for the detection of serum photoallergens.

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Figure Legends

Fig. 1

Basophil activation test (BAT) results. The values described “concentration of serum” were serum (μl) divided by buffer, staining reagent and whole blood (μl). BAT showed increased CD203c expression in patient hematocytes when mixed with action spectrum (AS)-irradiated patient or healthy volunteer (HV) serum, but not when mixed with non-irradiated patient or HV serum (A, B). There was no increase in CD203c expression in HV hematocytes when incubated with any patient or HV serum (C, D).

Fig. 2

Passive sensitization results. The values described “concentration of serum” were serum (μl) divided by buffer, staining reagent and whole blood (μl). The expression of CD203c in basophils from a healthy volunteer (HV) was upregulated using UVB-irradiated serum after sensitization by case 1 serum, but not HV serum (A). CD203c expression was not upregulated after passive sensitization with case 1 serum treated with omalizumab (B).

Figure No. 1- Unmarked

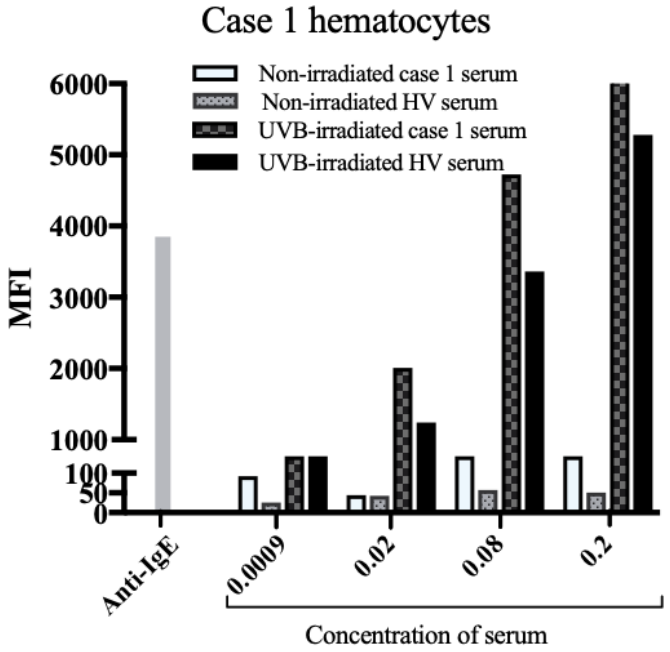


Fig 1, A

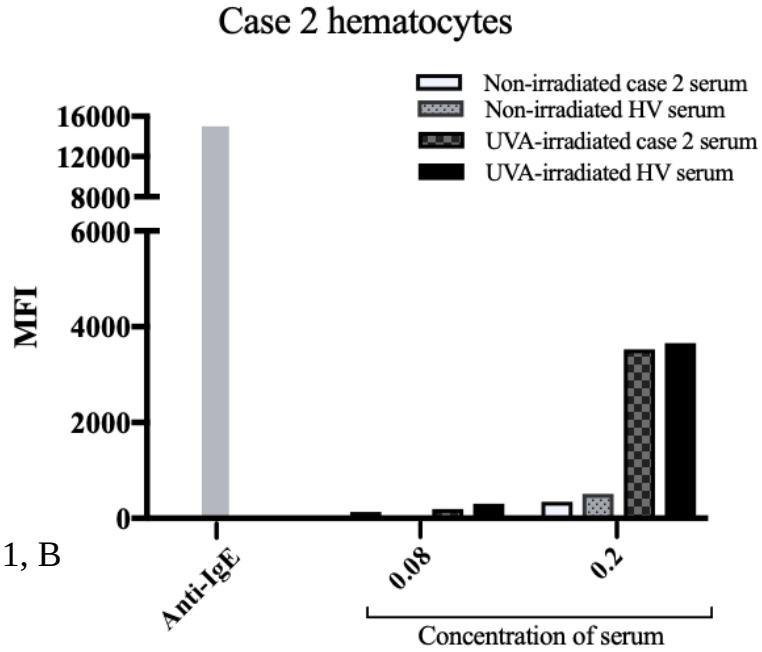
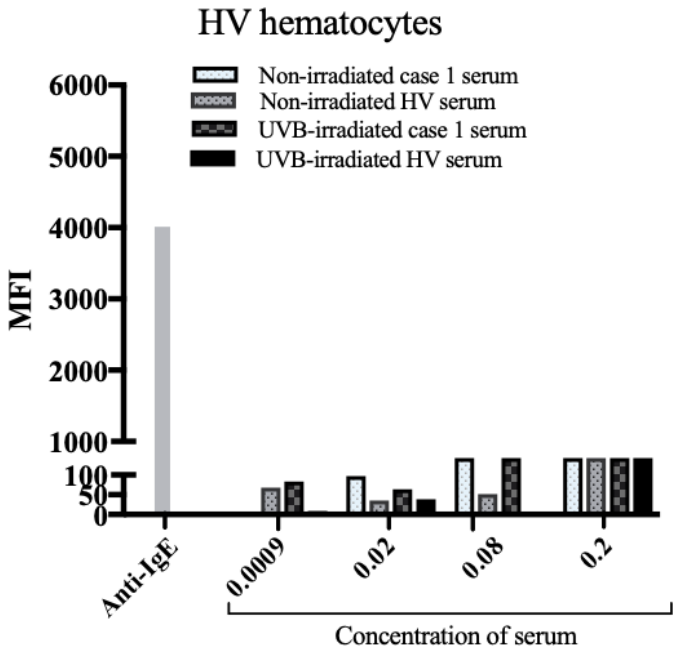


Fig 1, B



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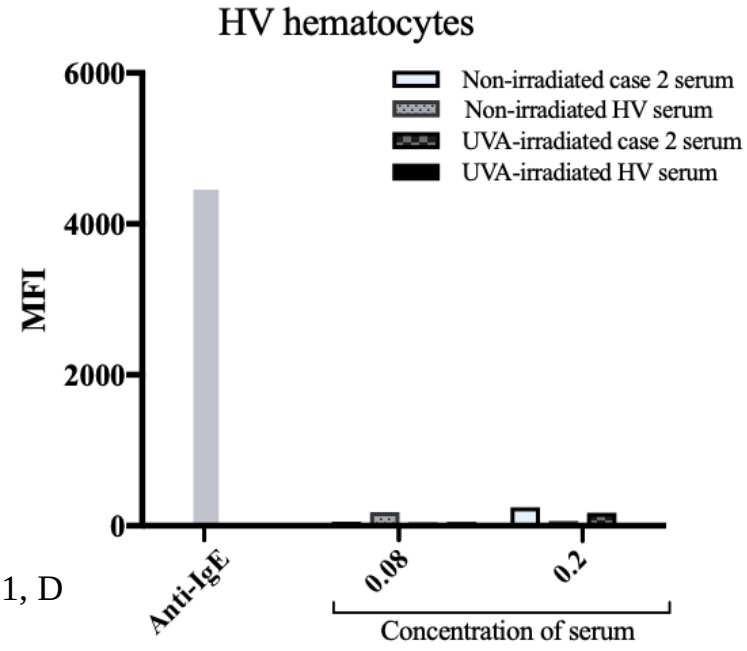

Fig 1, D

Figure No. 2- Unmarked

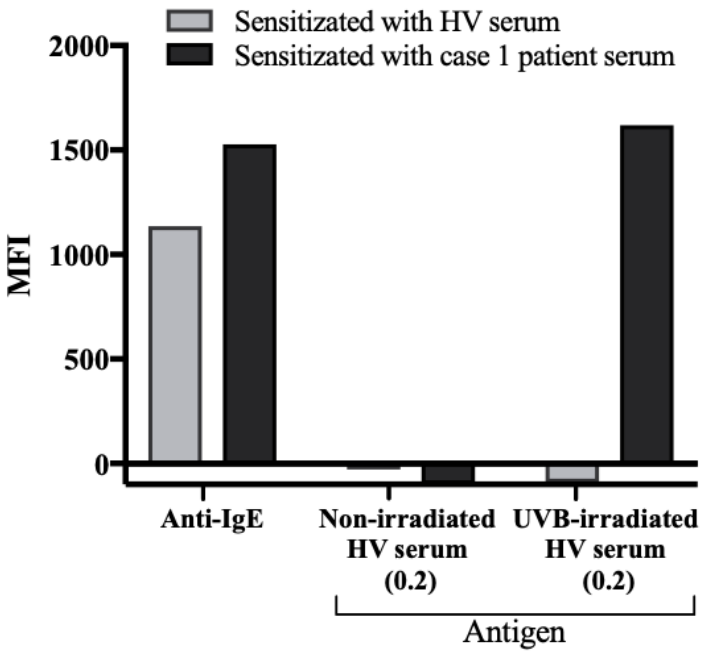

Fig 2, A

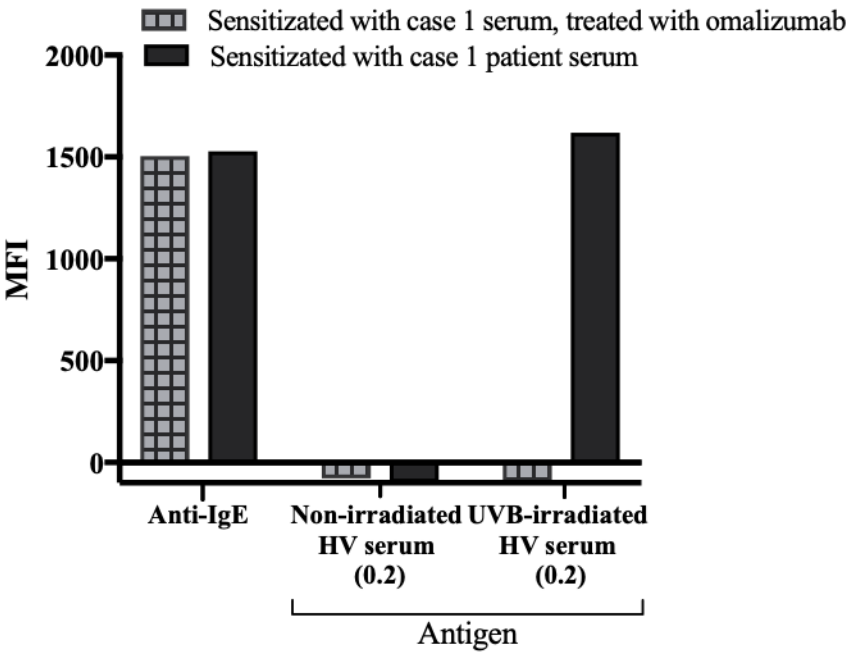

Fig 2, B

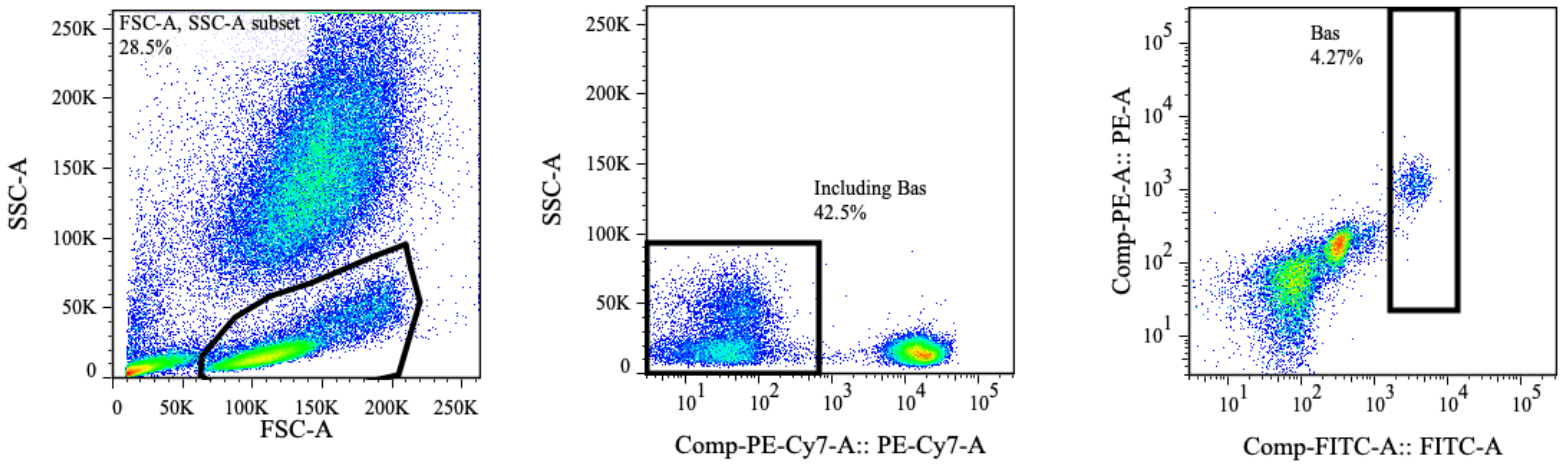

Fig E1

Table E1 Basal CD203c expression in each assay (upper) and basophil activation test using UVA-irradiated HV (healthy volunteer) serum as antigens in case 1 (lower)

			Basal CD203c expression (MFI)
Basophil activation test	case 1 assay	case 1 hematocytes	888
		HV hematocytes	1152
	case 2 assay	case 2 hematocytes	2293
		HV hematocytes	1051
Passive inhibition assay	Sensitizated with HV serum		1428
	Sensitizated with case 1 patient serum		2298.5
	Sensitizated with case 1 serum, treated with omalizumab		2194

		CD203c expression (MFI)	
	Concentration of HV serum	Case 1 hematocytes	HV hematocytes
Non-irradiated HV serum	0.0009	4	-93
	0.02	-4	-91
	0.08	35	-55
	0.02	-21	-66
UVA-irradiated HV serum	0.0009	57	441
	0.02	128	265
	0.08	805	320
	0.02	1679	202
UVB-irradiated HV serum	0.0009	629	144
	0.02	2441	109
	0.08	4641	208
	0.02	6697	562
Anti-IgE antibody		4936	3509

The values described “concentration of serum” were serum (μl) divided by buffer, staining reagent and whole blood (μl). BAT showed increased CD203c expression in patient hematocytes when mixed with UVA-irradiated HV (healthy volunteer) serum as well as with UVB-irradeated HV serum, but not when mixed with non-irradiated HV serum. There was no increase in CD203c expression in HV hematocytes when incubated with any HV serum.

MFI, mean fluorescence intensity; HV, healthy volunteer

Fig. E1 Flow cytometry data analysis

On the FSC/SSC plot (left), the basophil scatter gate and leukocyte gate are defined. On the PE-Cy7/SSC plot (middle), the CD3 negative population is defined. On the FITC/CD203c plot (right), both CRTH and CD203c positive groups are defined as basophils.

FITC, fluorescein isothiocyanate; PE, phycoerythrin; PECy7 PE-cyanine 7; FSC, forward scatter; SSC, side scatter.