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Letter to the Editor

Basophil count is a sensitive marker for clinical progression in a chronic spontaneous urticaria patient treated with omalizumab

Dear Editor,

Omalizumab is a monoclonal anti-IgE antibody that has been approved to treat antihistamine-refractory, chronic spontaneous urticaria (CSU). By blocking circulating IgE, omalizumab can prevent IgE attaching to the high-affinity IgE receptor (FcεRI) on mast cells, basophils, and probably other FcεRI-expressing cells, including antigen-presenting cells. Because peripheral IgE levels are highly associated with FcεRI cell surface expression, the efficacy of treatment with omalizumab may also be due to secondary suppression of cell function by reducing this receptor's expression. However, the precise mechanism of how omalizumab improves CSU remains largely unclear.^{1,2}

A 67-year-old male with a 6-year history of severe CSU had undergone treatment with antihistamines, then up-dosing of antihistamines alone, and finally switched to combination with H2 blockers and cyclosporine. Due to being refractory to all previous treatments, the patient was started on omalizumab therapy at a dose of 300 mg every 4 weeks, which showed a curative effect after only one dose in the first week. Since wheals appeared on his body ten weeks after the fourth treatment, we restarted omalizumab at the same dose and his symptom was relieved again within 3 days.

During omalizumab treatment, we followed basophil behavior by flow cytometric analysis after obtaining informed consent, which was approved by the IRB of Kansai Medical University. One hundred microliters of whole EDTA blood were incubated with CRA1-Billiant violet (BioLegend, San Diego, CA), CRA2-biotin (Bio Academia, Osaka, Japan), streptavidin-APC (BD, San Jose, CA) and antibodies for CRTH2-FITC, CD203c-PE and CD3-PECy7 (Allergenecity kit, Beckman Coulter, Brea, CA) for 15 min. Cells were then lysed and fixed for 10 min, and analyzed on a FACS Canto II (BD). Forward scatter (FSC) and side scatter (SSC) were used to select the cell population, and then basophils were identified as CD3-negative and CRTH2-positive cells (Fig. 1(a)).

Both CRA1 and CRA2 are anti-FcεRI antibodies.³ Since CRA1 reacts with a region other than the IgE binding site, we can evaluate total expression levels of IgE receptor by CRA1. One week after the initiation of omalizumab, his symptoms were relieved with an accompanying dramatic decrease of the cell surface CRA1 levels on peripheral basophils. After 4 doses of treatment, the mean fluorescent intensity (MFI) of CRA1 remained low (Fig. 1(b),(c)). In parallel with his CSU symptoms, 10 weeks after ceasing omalizumab

treatment, the MFI of CRA1 increased with the relapse of wheals, and decreased again once the treatment was restarted. Conversely, CRA2 recognizes the IgE binding site and competes with IgE; thereby, we can estimate the free receptor not bound to IgE by CRA2 expression.³ Before omalizumab treatment, we did not find any CRA2-positive basophils, indicating that all FcεRI on the circulating basophils were occupied with IgE. We can observe CRA2-positive basophils only after the initiation of omalizumab, clearly demonstrating that omalizumab treatment sufficiently neutralized circulating IgE in this patient (Fig. 1(d)).

CD203c, a multifunctional ectoenzyme involved in the clearance of extracellular nucleotides, has been used as an activation marker for basophils, and in this study we used this activation marker for the evaluation of peripheral basophil condition without any stimuli. CD203c intensity increased after the initiation of omalizumab (Fig. 1(e)), even though CSU eruptions were dramatically improved after omalizumab treatment. This paradoxical phenomenon suggests that peripheral circulating basophils seem to be activated after omalizumab treatment, which has also been reported by others.⁴

Interestingly, the peripheral basophil count had been persistently low at almost 0 cells/μl for at least 1 year prior to omalizumab treatment. One week after omalizumab injection, basophil numbers increased to 8.4 cells/μl. During treatment with omalizumab, the number of basophils was between 8.4 cells/μl and 18.8 cells/μl. Ten weeks after stopping omalizumab, wheals appeared again and basophil numbers returned to 0 cells/μl. Three days after resuming omalizumab, the basophil count once again increased to 9 cells/μl (Fig. 2). Thus, basophil count was well correlated with the clinical course of CSU, and restoring basophil numbers may indicate that CSU is under control. The underlying mechanism may be explained by the recruitment of peripheral basophils into the tissue, which evokes hives reactions during the active phase of CSU. We believe in this situation that activated basophils migrate into local sites and only non-activated basophils remain in the peripheral blood when the patient shows CSU symptoms. This may explain the paradoxical phenomenon that CD203c expression actually increased after omalizumab treatment when hives were under control and basophil numbers remained normal in peripheral blood. Our findings are consistent with other previous reports showing an increase in the basophil count in peripheral blood during treatment with antihistamines.⁵ We should point

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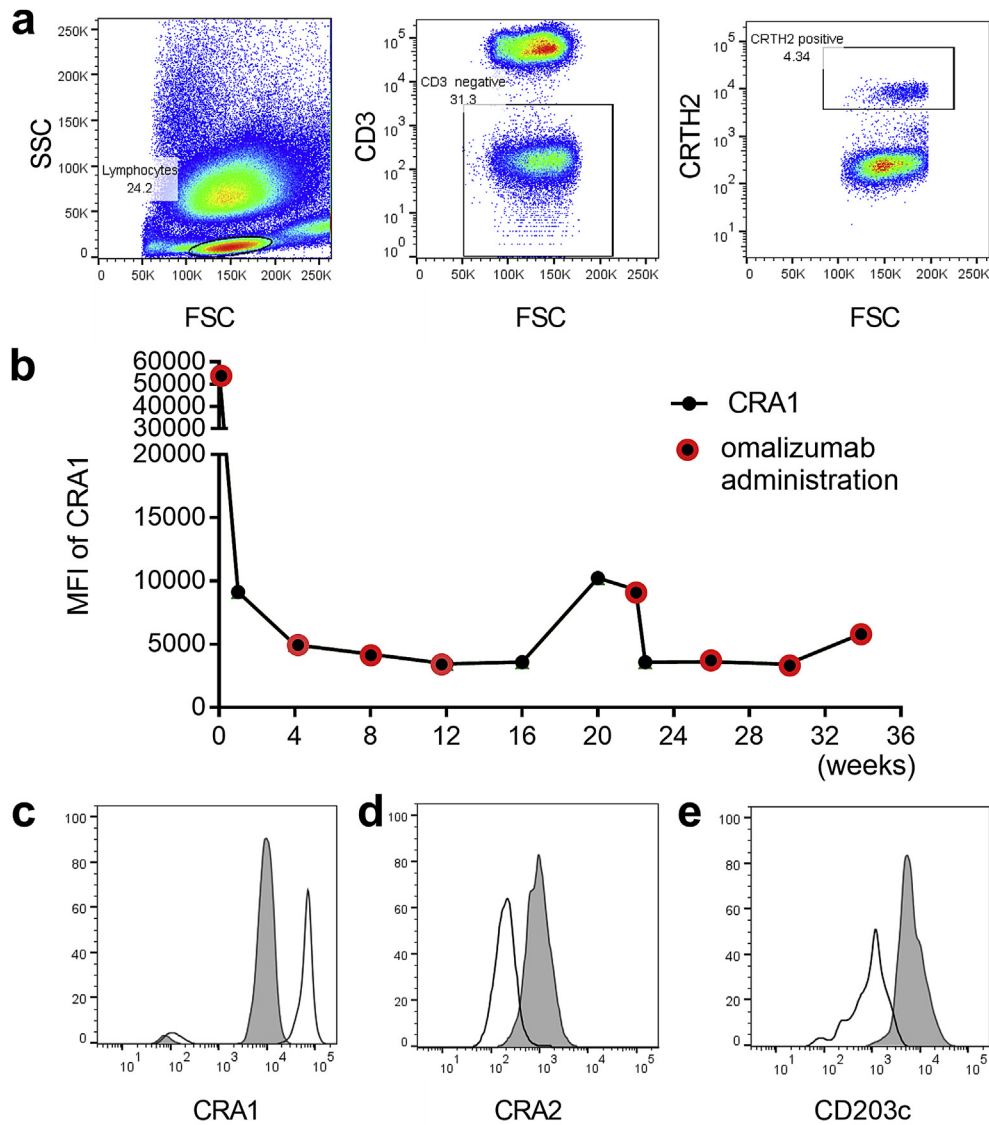


Fig. 1. Flow cytometric analysis of peripheral basophils. (a) Gating for the identification of basophils. From the lymphocyte population gated by FSC and SSC, we identified basophils as CD3-negative and CRTH2-positive. (b) Expression of IgE receptor by CRA1, and the dynamic of CRA1 expression during treatment. (c) CRA1 expression, (d) CRA2 expression and (e) CD203c expression before (white) and 1 week after (gray) treatment with omalizumab.

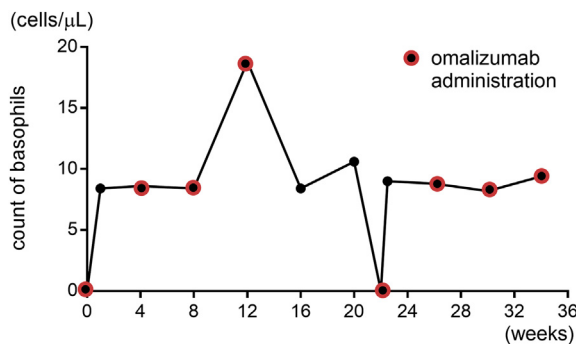


Fig. 2. Peripheral basophils count during omalizumab treatment. While using omalizumab, the patient's basophil count increased and was maintained within normal range. Once the treatment stopped, the basophil count dropped to 0 cells/μL.

out that there is a limitation in our studies because we substitute peripheral basophils instead of dermal mast cells or local basophils migrated into the tissue in order to evaluate the effectiveness of omalizumab.

In conclusion, we believe that peripheral basophil count may be a potential and easy marker for assessing the condition of CSU. We hope our findings aid in the discovery of more insights into the underlying mechanisms of how omalizumab actually works in treating this challenging disease. Further long-term follow-up investigations including a large number of patients will be required in order to validate the value of basophil counts in clinical practice.

Conflict of interest

The authors have no conflict of interest to declare.

Izumi Kishimoto ^{a,b}, Naotomo Kambe ^{a,b,*}, Nhung Thi My Ly ^a,
Chuyen Thi Hong Nguyen ^c, Hiroyuki Okamoto ^a

^a Department of Dermatology, Kansai Medical University, Hirakata, Japan

^b Allergy Center, Kansai Medical University, Hirakata, Japan

^c Department of Dermatology and Venereology, University of Medicine and Pharmacy, Ho Chi Minh City, Viet Nam

* Corresponding author. Department of Dermatology, Kansai Medical University, 2-5-1 Shin-machi, Hirakata, Osaka 573-1010, Japan.

E-mail address: nkambe@hirakata.kmu.ac.jp (N. Kambe).

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