Peripheral basophil reactivity, CD203c expression by Cryj1 stimulation, is useful for diagnosing seasonal allergic rhinitis by Japanese cedar pollen

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Introduction
Seasonal allergic rhinitis (SAR) by Japanese cedar (Cryptomeria japonica) pollen (SAR-JCP), the most common allergic disease in Japan, is an important public health issue because of its high prevalence, its effects on the general public, and its associated medical expenses [1]. We reported previously that the prevalence of AR was 44.2% in adults, and that the most common allergen in AR is JCP (89.6%) in Japan [2]. The diagnostic tools used for AR are positive history of nasal symptoms by allergen exposure, positive reaction of skin test, and nasal provocation test [3]. Although skin tests are useful and easy to perform, they sometimes cause an anaphylactic reaction, and/or a false positive or negative reaction. Specific IgE in serum is also useful to clarify the immunological reaction with accuracy and objectivity, but the specific IgE in serum and allergic symptoms are not always detected together. Therefore, SAR-JCP diagnosis requires the development of more accurate and reliable examination tools.

Although basophils exist as only 1% of circulating whole blood cells, they play fundamentally important roles in allergic reactions. They express high-affinity IgE receptors (FcεRI) as well as mast cells. IgE binds to FcεRI, which is present on the surface of basophils. This complex can bind to an antigen, leading to basophil activation and degranulation, releasing histamine, and producing IL-4 and IL-13, which are crucially important for the development of an allergic reaction [4]. Basophils express various surface markers,
some of which are useful as markers of basophil activation. Actually, CD63, which is known as a lysosome-associated membrane glycoprotein (LAMP-3), belongs to the transmembrane-4 superfamily. When basophils are stimulated by antigen via FcεRI, CD63 is expressed on the basophil surface. Recent reports have described that up-regulation of CD63 might reflect an anaphylactic phase [5]. However, CD203c, which is also present in basophils, is a glycosylated type 2 transmembrane family that belongs to the ectonucleotide pyrophosphatase/phosphodiesterase enzyme family [5, 6]. Actually, CD203c is expressed constitutively on basophil surface membranes with lower concentration, which is up-regulated by allergen stimulation. These activation mechanisms suggest that CD203c is related to piecemeal degranulation [7–9]. The reaction of piecemeal degranulation lasts longer than anaphylactic degranulation by slow and persistent mediator release without crosslinking of FcεRI by antigen [10, 11]. These mechanisms might contribute to the pathogenesis of chronic allergic inflammation. Özdemir et al. [12] reported that a CD203c expression pattern clearly discriminated patients with pollen allergy from healthy subjects with high sensitivity and specificity. Complementary components also play crucially important roles in allergic inflammation. Anaphylatoxins C3a and C5a are involved in asthma pathogenesis [13]. It had been reported that the levels of C3a and C5a in bronchoalveolar lavage (BAL) fluid were elevated in asthmatic subjects after allergen challenge [14]. Furthermore, the levels of C3a and C5a in serum are increased in acute exacerbations of asthma [15].

For this study, we asked 3,453 participants whether they experienced any allergic rhinitis (AR) related symptoms and measured six inhalant-aeroallergen-specific IgEs. Skin tests were also conducted for the six aeroallergens. Results show that the levels of CD203c in peripheral basophils by Cryj1 stimulation were significantly higher in the SAR-JCP group than in non-SAR-JCP groups, irrespective of the results of IgE and skin test. Furthermore, the levels of C3a and C5a in serum were significantly higher in SAR-JCP group than in non-sensitized group. Our results suggest that the responsiveness of peripheral basophils, the levels of CD203c by Cryj1 stimulation, might be a more objective and reliable marker reflecting the allergic reaction by SAR-JCP in vivo than specific IgE in serum and skin test.

Methods

Subjects

Between 2003 and 2011, 3,453 hospital workers and university students were invited to participate in an epidemiological survey of AR. Total and specific IgE (Japanese cedar, Dermatophagoides, Dactylis glomerata, Ambrosia artemisiifolia, Candida albicans, and Aspergillus) were measured using the Immuno CAP method (Pharmacia Diagnostics AB, Uppsala, Sweden). They were asked whether they had experienced nasal symptoms related to AR. We also invited 50 randomly selected people to participate in this study during March 2011 and 2012, when subjects were exposed to Japanese cedar pollen. The 50 participants were divided into four groups according to AR symptoms, results of Immuno CAP, and skin test against JCP: Group I, specific IgE negative, skin test negative without SAR-JCP symptoms; Group II, specific IgE positive, skin test negative without SAR-JCP symptoms; Group III, specific IgE positive, skin test positive without SAR-JCP symptoms; Group IV, specific IgE positive, skin test positive with SAR-JCP symptoms. The participants’ physical characteristics are presented in Table 2. No participant had been treated using any medicine such as histamine H1 receptor antagonists, oral corticosteroids, or intranasal spray of corticosteroids. All participants provided written informed consent to participate in the study. The study was approved by the ethical committees of the University of Fukui, Japan.

Skin test

We performed intradermal skin tests against JCP allergen using an allergen extract (Torii Pharmaceutical Co. Ltd., Tokyo, Japan). Subjects were injected with 20 μl of allergen fluid in the forearm. Then the diameter of flare and wheal were measured 15 min after the injections. Subjects exhibiting flare of skin of >20 mm or wheal >9 mm were considered “positive” against JCP.

Nasal symptom scores

The numbers of sneezes, numbers of bouts of nasal blowing, and nasal congestion during JCP season were recorded for all 50 participants. Based on that information, the participants were graded on a scale of Japanese Rhino-conjunctivitis Quality of Life Questionnaire (JRQLQ) followed by Japanese Guideline for allergic rhinitis [16].

Measuring levels of CD203c in peripheral basophils

Whole blood was collected with heparin during the JCP season. Reportedly, basophils are distinguishable from other cells using three-color flow cytometry to detect cells that are CD3–, CRTH2+, and CD203c+ [17]. Actually, CRTH2, also called CD294, is expressed on basophils, eosinophils, and Th2 cells [18]. The levels of CD203c in basophils were measured by Allergenicity Kit (Beckman Coulter, CA). We used Cryj1 as a major allergen of JCP. Whole blood with heparin was incubated at two Cryj1 concentrations (0.5 ng/
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ml, 50 ng/ml) for 15 min. Anti-IgE antibody at 8 μg/ml as a positive control and PBS as a negative control were used for stimulation. The levels of CD203c on basophils were determined using the fluorescence of negative control and positive control. PC7-conjugated anti-CD3, FITC-conjugated anti-CRTH2, and PE-conjugated anti-CD203c antibodies were added and analyzed by BD FACSCalibur (BD Bioscience, San Diego). At least 500 basophils were detected at each assay.

Measuring anaphylatoxins in serum

Venous blood was collected during the JCP season. Samples were then centrifuged at 4°C. Supernatants were stored at −80°C until use. Subsequently, C3a and C5a enzyme-linked immunosassays (ELISAs) were performed according to the manufacturer’s instructions (BD Biosciences, San Diego).

Statistical methods

All data are reported as the median. Differences between groups were analyzed using unpaired t-tests. A p-value of less than 0.05 was considered statistically significant.

Results

For this epidemiological survey of AR, 3,453 volunteers were recruited. Subject characteristics are presented in Table 1. In this study, subjects showing Immuno CAP score ≥2 were defined as sensitized. The most common airborne allergen is JCP, and sensitized ratios of the six inhalant aeroallergens are the following: JCP (57.5%), dust mites (39.6%), D. glomerata (24.4%), A. artemisiifolia (9.9%), C. albicans (5.6%), and Aspergillus (2.1%) (Table 1). These results are in line with those reported from an earlier study [2]. Among the 1,987 subjects of the JCP sensitized group, 1,435 (72.2%) had experienced seasonal allergic symptoms during the JCP season. SAR-JCP subjects (group IV) revealed high nasal symptom scores (sneezing 2.20 ± 0.86, nasal congestion 1.60 ± 1.06, nasal blowing 2.47 ± 1.13, nasal itchiness 2.2 ± 1.15) that were higher than those of the other three groups (Fig. 1).

To assess the responsiveness of peripheral basophil for JCP stimulation, we conducted basophil activation tests using JCP extract (Cryj1). The levels of CD203c in peripheral basophils by Cryj1 stimulation were significantly higher in group IV (p < 0.05) than in any of the three other groups (Fig. 2). Although, clinically, skin testing is the most reliable examination to distinguish SAR-JCP to date [16], group III subjects showed no SAR-JCP symptoms in spite of positive skin tests. This result suggests that a positive result on a JCP skin test does not invariably distinguish patients as SAR-JCP. The levels of CD203c in peripheral basophils by Cryj1 stimulation in group IV were significantly higher than in group III subjects (Cryj1 0.5 ng/ml: 38.8 ± 15.7% vs. 60.2 ± 27.4%, p < 0.05, Cryj1 50 ng/ml: 46.8 ± 23.3% vs. 68.0 ± 21.2%, p < 0.05) (Fig. 2). Therefore, measuring CD203c in peripheral basophils by Cryj1 stimulation might be a more reliable examination than skin testing for SAR-JCP diagnosis. Most importantly, group II (specific IgE positive, skin test negative without SAR-JCP symptoms) showed quite low levels of CD203c in peripheral basophils by Cryj1 stimulation than group IV (SAR-JCP subjects) did, indicating that not all people with JCP-specific IgE will present symptoms of SAR-JCP. Another component that was not JCP specific IgE in serum might be involved in representing symptoms of SAR-JCP.

The complement components C3a and C5a play crucially important roles in the development of allergic inflammation [13]. We next examined the concentrations of serum anaphylatoxins C3a and C5a. Concentrations of both anaphylatoxins were significantly higher in group IV (SAR-JCP) (64785.1 ± 20136.7 ng/ml, 124.1 ± 46.0 ng/ml, respectively) than in group I (38917.6 ± 9393.6 ng/ml, 63.4 ± 18.4 ng/ml, respectively) (p < 0.05). Although not significantly, the concentrations of both anaphylatoxins were higher in group IV (SAR-JCP) than in group II (specific IgE positive, skin test negative without SAR-JCP symptoms) and group III (specific IgE positive, skin test positive without SAR-JCP symptoms) (Fig. 3) (group II vs. group IV, C3a: p = 0.0670, C5a: p = 0.0832, group III vs. group IV, C3a: p = 0.090, C5a: p = 0.131, respectively). These results suggest that the concentrations of anaphylatoxins influence the SAR-JCP symptoms exactly.
**Table 2.** Characteristics of CD203c study subjects.

|                          | Control (group I) | Sensitized (group II) | Sensitized (group III) | SAR-JCP (group IV) |
|--------------------------|-------------------|-----------------------|------------------------|-------------------|
| Sex (male:female)        | 8:8               | 2:8                   | 5:4                    | 7:8               |
| Age (y), mean ± SD       | 26.1 ± 3.9        | 23.9 ± 5.4            | 28.1 ± 7.8             | 27.9 ± 5.1        |
| Skin test                | (−)               | (−)                   | (+)                    | (+)               |
| Total IgE (IU/ml) mean (range) | 34.2 (5–460)    | 60.3 (26–200)         | 94.1 (8–340)          | 173.8 (29–8700)  |
| JC specific IgE (IU/ml)  | <0.3              | 1.4                   | 6.7                    | 18.3              |
| Dermatophagoides specific IgE (IU/ml) | <0.3              | 0.9                   | 1.4                    | 1.2               |
| Dactylis glomerata specific IgE (IU/ml) | <0.3              | 0.4                   | 0.9                    | 4.9               |
| Ambrosia artemissifolia specific IgE (IU/ml) | <0.3              | 0.3                   | 0.5                    | 0.6               |
| Candida albicans specific IgE (IU/ml) | <0.3              | <0.3                  | <0.3                   | <0.3              |
| Aspergillus spp specific IgE (IU/ml) | <0.3              | <0.3                  | <0.3                   | <0.3              |

**Figure 1.** Symptom scores. Nasal symptom scores (nasal blowing, sneezing, nasal congestion, nasal itchiness) in SAR-JCP subjects (group IV) were significantly higher than both sensitized (groups II and III) and control subjects (group I). **p < 0.01.

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Discussion

The current study revealed that the most common aeroallergen is JCP (1,987 of 3,453 participants (52.7%) were sensitized by JCP); however, 27.8% of sensitized subjects had not experienced any SAP-JCP symptoms, even during the JCP season. We also showed that the levels of CD203c in peripheral basophils by JCP stimulation yielded more objective and more specific data about allergic reactions by comparing skin tests and specific IgE in serum. Both mast cells and basophils possess FcεRI on the cell surface. Both cause allergic inflammation after crosslinking of surface-bound IgE by antigen. Different from mast cells, basophils are obtainable from whole peripheral blood cells. Recent reports in the relevant literature describe that basophil activation reflects pathological and clinical conditions in some allergic diseases [9, 19].

Often, CD63 is used as a basophil activation marker because CD63 expression is associated more closely with degranulation. However, several studies have dissociated the appearance of CD63 from histamine release. Actually, CD203c is expressed on resting basophils at low levels.
concentrations; its expression is rapidly up-regulated following activation. Measuring CD203c expression in basophils might be more consistent with the AR symptoms and pathology than measuring CD63. Reportedly, the level of CD203c expression is correlated significantly with nasal symptoms among SAR patients [20]. We also demonstrated the correlations between the levels of CD203c and nasal symptoms and found the significant correlations: (Cryj1 0.5 ng/ml sneezing: $R^2 = 0.343$, $p < 0.0001$, nasal blowing: $R^2 = 0.344$, $p < 0.0001$, nasal congestion: $R^2 = 0.220$, $p < 0.001$, nasal itchiness: $R^2 = 0.316$, $p < 0.0001$) (Cryj1 50 ng/ml sneezing: $R^2 = 0.313$, $p < 0.0001$, nasal blowing: $R^2 = 0.330$, $p < 0.0001$, nasal congestion: $R^2 = 0.176$, $p < 0.001$, nasal itchiness: $R^2 = 0.330$, $p < 0.0001$). Our current data are consistent with those presented in an earlier report (Fig. 4).

Perhaps the most interesting finding from this study is that basophil activation test is a more specific examination than measuring specific IgE in serum or skin tests (Fig. 2). This result indicates that merely possessing antigen specific

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**Figure 4.** Correlations between the levels of CD203c and nasal symptom scores. There are significant correlations between the levels of CD203c and each nasal symptom scores. Cryj1 0.5 ng/ml sneezing: $R^2 = 0.343$, $p < 0.0001$, nasal blowing: $R^2 = 0.344$, $p < 0.0001$, nasal congestion: $R^2 = 0.220$, $p < 0.001$, nasal itchiness: $R^2 = 0.316$, $p < 0.0001$. Cryj1 50 ng/ml sneezing: $R^2 = 0.313$, $p < 0.0001$, nasal blowing: $R^2 = 0.330$, $p < 0.0001$, nasal congestion: $R^2 = 0.176$, $p < 0.001$, nasal itchiness: $R^2 = 0.330$, $p < 0.0001$. 

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IgE alone is insufficient to develop allergic symptoms or the mechanism by which allergic inflammation between skin and nasal mucosa might be different. Regarding venom hypersensitivity, Korosec et al. [21] reported that basophil activation test had significantly higher diagnostic sensitivity than skin tests. Another component might be involved in representing SAR-JCP symptoms.

Complementary components have an important role of innate immune system against bacteria and other pathogens. They form immune complex with surface antigen by “pattern recognition molecules.” Then they start attacking foreign bodies by mediating the variety of inflammatory responses [22, 23]. C3a and C5a are generated not only via the classic IgG/antigen immune-complex pathway; β-trypptase in human mast cells can also generate bioactive C3a and C5a [24]. Basophils possess receptors for C3a and C5a. The binding of anaphylatoxins to these receptors can engender the release of chemical mediators such as histamines, LTC4, IL-4, and IL-13 [25, 26]. The present study showed that although not significant, concentrations of both anaphylatoxins, C3a and C5a, were higher in group IV (SAR-JCP) than those in group II (JCP sensitized, skin test negative without SAR-JCP symptoms) or group III (JCP sensitized, skin test positive without SAR-JCP symptoms) (Fig. 3). These results suggest that anaphylatoxins are involved in representing of SAR-JCP symptoms through basophil activation. Our most recent data showed significantly lower concentrations of both anaphylatoxins in patients with sublingual immunotherapy against JCP (submitted data). Serum anaphylatoxins might play a crucially important role in SAR-JCP development through basophil activation. Additional studies must be conducted to ascertain the precise contribution of anaphylatoxins to basophil activation inducing SAR-JCP.

A member of interleukin-1 family, IL-33, is a ligand for ST2 (IL-33Rx) [27]. IL-33 is expressed constitutively in epithelial and endothelial cells. It can be released by necrotic cells and injured cells, which act as “alarmin” in vivo [28, 29]. According to a great deal of recent reports, IL-33 plays a crucially important role for developing allergic diseases [30, 31]. Basophils express ST2, which is up-regulated by IL-3 [32], and which can be stimulated by IL-33 to produce Th2 cytokines without cross-linking of FcεRI [33, 34]. However, IL-33 also prompts basophils to secrete IL-4, IL-13, and IL-8 with IL-3 and/or FcεRI activation, which accelerate FcεRI-induced mediator release [32]. We reported previously that serum concentrations of IL-33 were significantly higher in patients with SAR-JCP than in controls [35]. These results support the inference that the concentration of IL-33 affects basophil reactivity or symptoms of SAR-JCP. In the present study, we did not analyze the levels of IL-3 and ST2. To emphasize the effects on the up-regulation of CD203c, we consider that more investigations are required.

Among sensitized subjects with positive skin test reaction in our study, four subjects exhibited AR related nasal symptom and diagnosed as SAR-JCP during the next pollen season. They showed moderate to high CD203c expression as well as SAR-JCP subjects (Cryj1 0.5 ng/ml: 48.7–61.8%, Cryj1 50 ng/ml: 25.6–89.9%) (Table 3). These results may indicate that up-regulation of CD203c reflect the onset of AR. To verify the prediction of the onset of AR, we consider that further investigations are needed about sensitized subjects.

In conclusion, results show that in SAR-JCP group, responsiveness of peripheral basophils, the levels of CD203c by Cryj1 stimulation are significantly higher than those of non-SAR-JCP groups (irrespective of possessing JCP-specific IgE in serum or skin test positive). Furthermore, although not statistically significant, concentrations of both anaphylatoxins were increased in one group (SAR-JCP) compared with the other three groups (non-SAR-JCP groups). Our results imply that peripheral basophil reactivity, CD203c expression by Cryj1 stimulation can be a useful examination tool for the diagnosis of SAR-JCP and that serum anaphylatoxins might be involved in the development of SAR-JCP through basophil activation.

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**Conflicts of Interest**

None declared.

**References**

1. Yamada T., H. Saito, S. Fujieda. 2014 [cited 2014 Jun 8]. Present state of Japanese cedar pollinosis: the national affliction. J. Allergy Clin. Immunol. 133(3):632–639. e5. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24361081
2. Sakashita M., T. Hirota, M. Harada, R. Nakamichi, T. Tsunoda, Y. Osawa, A. Kojima, M. Okamoto, D. Suzuki, S.
Peripheral basophil reactivity

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10. Dvorak A. M., D. W. MacGlashan, E. S. Morgan, L. M. Lichtenstein. 1996 [cited 2014 Nov 6]. Vesicular transport of histamine in stimulated human basophils. Blood 88(11):4090–4101. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8943842

11. Crivellato E., B. Nico, F. Mallardi, C. A. Beltrami, D. Ribatti. 2003 [cited 2014 Nov 6]. Piecemeal degranulation as a general secretory mechanism? Anat. Rec. A Discov. Mol. Cell Evol. Biol. 274(1):778–784. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12923888

12. Özdemir S. K., D. Gülüşoğlu, B. A. Sin, A. H. Elhan, A. İlkicioğullar, Z. Msrlgil. 2011 [cited 2014 Jun 8]. Reliability of basophil activation test using CD203c expression in diagnosis of pollen allergy. Am. J. Rhinol. Allergy 25(6):e225–e231. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22185730

13. Gerard N. P., C. Gerard. 2002 [cited 2014 Jun 8]. Complement in allergy and asthma. Curr. Opin. Immunol. 14(6):705–708. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12413519

14. Humbles A. A., B. Lu, C. A. Nilsson, C. Lilly, E. Israel, Y. Fujiwara, N. P. Gerard, C. Gerard. 2000 [cited 2014 Jun 8]. A role for the C3a anaphylatoxin receptor in the effector phase of asthma. Nature 406(6799):998–1001. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10984054

15. Nakano Y., S. Morita, A. Kawamoto, T. Suda, K. Chida, H. Nakamura. 2003 [cited 2014 Jun 8]. Elevated complement C3a in plasma from patients with severe acute asthma. J. Allergy Clin. Immunol. 112(3):525–530. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/13679811

16. Okubo K., Y. Kurono, S. Fujieda, S. Ogino, E. Uchio, H. Odajima, H. Takenaka, K. Baba. 2011 [cited 2014 Jun 8]. Japanese guideline for allergic rhinitis. Allergol. Int. 60(2):171–189. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21636965

17. Boumiza R., A.-L. Debard, G. Monneret. 2005 [cited 2014 Jun 8]. The basophil activation test by flow cytometry: recent developments in clinical studies, standardization and emerging perspectives. Clin. Mol. Allergy 3:9. [Internet]. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1190199&tool=pmcentrez&rendertype=abstract

18. Nagata K., H. Hirai, K. Tanaka, K. Ogawa, T. Aso, K. Sugamura, K. Sugamura, M. Nakamura, S. Takano. 1999 [cited 2014 Jun 8]. CRTH2, an orphan receptor of T-helper-2-cells, is expressed on basophils and eosinophils and responds to mast cell-derived factor(s). FEBS Lett. 459(2):195–199. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10518017

19. Ono E., M. Taniguchi, N. Higashi, H. Mita, K. Kajiwara, H. Yamaguchi, S. Tatsuno, Y. Fukutomi, H. Tanimoto, K. Sekiya, et al. 2010 [cited 2014 Jun 8]. CD203c expression on human basophils is associated with asthma exacerbation. J. Allergy Clin. Immunol. 125(2):483–489. e3. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20159259
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20. Nagao M., Y. Hiraguchi, K. Hosoki, R. Tokuda, T. Usui, S. Masuda, M. Yamaguchi, T. Fujisawa. 2008 [cited 2014 Jun 8]. Allergen-induced basophil CD203c expression as a biomarker for rush immunotherapy in patients with Japanese cedar pollinosis. Int. Arch. Allergy. Immunol. 146(Suppl):47–53. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18504407

21. Korosec P., R. Erzen, M. Silar, N. Bajrovic, P. Kopac, M. Kosnik. 2009 [cited 2014 Jun 8]. Basophil responsiveness in patients with insect sting allergies and negative venom-specific immunoglobulin E and skin prick test results. Clin. Exp. Allergy 39(11):1730–1737. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19689457

22. Kildsgaard J., T. J. Hollmann, K. W. Matthews, K. Bian, F. Murad, R. A. Wetsel. 2000 [cited 2014 Jun 8]. Cutting edge: targeted disruption of the C3a receptor gene demonstrates a novel protective anti-inflammatory role for C3a in endotoxin-shock. J. Immunol. 165(10):5406–5409. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11067891

23. Boos L., A. J. Szalai, S. R. Barnum. 2005 [cited 2014 Jun 8]. C3a expressed in the central nervous system protects against LPS-induced shock. Neurosci. Lett. 387(2):68–71. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16085360

24. Fukuoka Y., H.-Z. Xia, L. B. Sanchez-Muñoz, A. L. Dellinger, L. Escribano, L. B. Schwartz. 2008 [cited 2014 Jun 8]. Generation of anaphylatoxins by human beta-tryptase from C3, C4, and C5. J. Immunol. 180(9):6307–6316. [Internet]. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2645414&tool=pmcentrez&rendertype=abstract

25. Eglite S., K. Plüss, C. A. Dahinden. 2000 [cited 2014 Jun 8]. Requirements for C5a receptor-mediated IL-4 and IL-13 production and leukotriene C4 generation in human basophils. J. Immunol. 165(4):2183–2189. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10925305

26. Ali H. 2010 [cited 2014 Jun 8]. Regulation of human mast cell and basophil function by anaphylatoxins C3a and C5a. Immunol. Lett. 128(1):36–45. [Internet]. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2815128&tool=pmcentrez&rendertype=abstract

27. Schmitz J., A. Owyang, E. Oldham, Y. Song, E. Murphy, T. K. McClanahan, G. Zurawski, M. Moshrefi, J. Qin, X. Li, et al. 2005 [cited 2014 May 28]. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 23(5):479–490. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16286016

28. Mousson C., N. Ortega, J.-P. Girard. 2008 [cited 2014 Jun 8]. The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel “alarmin”? PLoS ONE 3(10):e3331. [Internet]. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2556082&tool=pmcentrez&rendertype=abstract

29. Liew F. Y., N. I. Pitman, I. B. McInnes. 2010 [cited 2014 Jun 8]. Disease-associated functions of IL-33: the new kid in the IL-1 family. Nat. Rev. Immunol. 10(2):103–110. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20081870

30. Haenuki Y., K. Matsushita, S. Futatsugi-Yumikura, K. J. Ishii, T. Kawagoe, Y. Imoto, S. Fujieda, M. Yasuda, Y. HISA, S. Akira, et al. 2012. A critical role of IL-33 in experimental allergic rhinitis. J. Allergy Clin. Immunol. 130(1):184–94. e11.

31. Imai Y., K. Yasuda, Y. Sakaguchi, T. Haneda, H. Mizutani, T. Yoshimoto, K. Nakanishi, K. Yamanishi. 2013 [cited 2014 Jun 8]. Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. Proc. Natl. Acad. Sci. USA 110(34):13921–13926. [Internet]. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3752227&tool=pmcentrez&rendertype=abstract

32. Pecaric-Petkovic T., S. A. Didichenko, S. Kaempfer, N. Spieg, C. A. Dahinden. 2009 [cited 2014 Jun 8]. Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. Blood 113(7):1526–1534. [Internet]. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2644080&tool=pmcentrez&rendertype=abstract

33. Kondo Y., T. Yoshimoto, K. Yasuda, S. Futatsugi-Yumikura, M. Morimoto, N. Hayashi, T. Hoshino, J. Fujimoto, K. Nakanishi. 2008 [cited 2014 Jun 8]. Administration of IL-33 induces airway hyperresponsiveness and goblet cell hyperplasia in the lungs in the absence of adaptive immune system. Int. Immunol. 20(6):791–800. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18448455

34. Suzukiwa M., M. Ikura, R. Koketsu, H. Nagase, C. Tamura, A. Komiya, S. Nakae, K. Matsuhashi, K. Ohta, K. Yamamoto, et al. 2008 [cited 2014 Jul 24]. An IL-1 cytokine member, IL-33, induces human basophil activation via its ST2 receptor. J. Immunol. 181(9):5981–5989. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18941187

35. Sakashita M., T. Yoshimoto, T. Hirotta, M. Harada, K. Okubo, Y. Osawa, S. Fujieda, Y. Nakamura, K. Yasuda, K. Nakanishi, et al. 2008 [cited 2014 Jun 8]. Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis. Clin. Exp. Allergy 38(12):1875–1881. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19037964