reported to contribute to IgG-mediated anaphylaxis[24]. We recently showed that basophils are the most important cells in IgG-mediated but not IgE-mediated systemic anaphylaxis under certain experimental conditions[12] (FIG. 1b). Basophil depletion using the antibody Ba103 before allergen challenge protected mast-cell-deficient mice from death that was due to active systemic anaphylaxis. Basophils in the peripheral blood efficiently captured allergen–IgG immune complexes through IgG-binding Fc receptors, and were then activated to release platelet-activating factor (PAF), which in turn increased vascular permeability with ~10,000 times higher efficacy than histamine[12] (FIG. 1b). So, the small number of basophils in the blood can induce systemic anaphylaxis through the release of the potent vasoamine PAF (instead of histamine) after stimulation with immune complexes. In allergen-sensitized animals, IgE and IgG1 antibodies that are specific for the allergen are both produced. Therefore, both the classical pathway that is mediated by mast cells, IgE and histamine, and the alternative pathway that is mediated by basophils, IgG and PAF can operate. Which pathway is dominant might be determined by the nature of the allergens and the quantities of allergen and specific antibodies that are produced. It remains to be determined whether this alternative pathway also functions in humans. Of note in this regard, the concentration of PAF in human sera has been reported to correlate with the severity of human anaphylaxis[25].

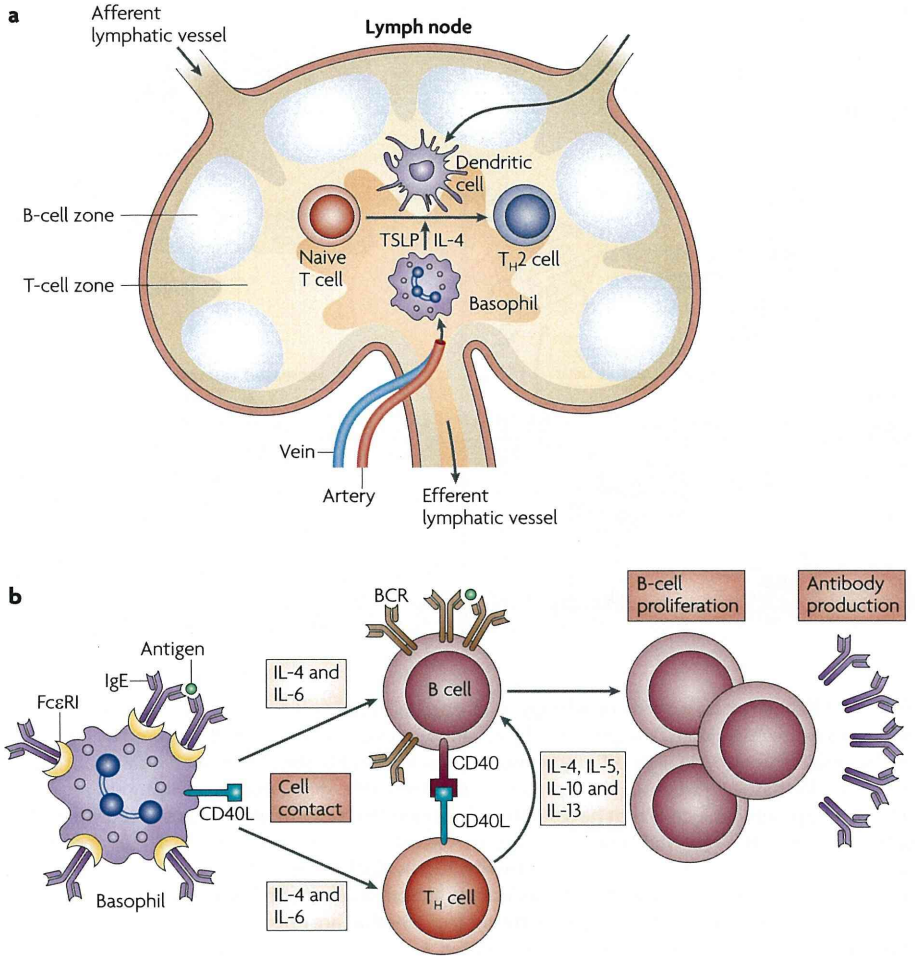

Figure 2 | **Roles for basophils in immune regulation. a** | Basophils drive T helper 2 (T_H2)-cell differentiation during the primary immune response. In response to subcutaneously administered protease antigens, basophils are transiently recruited to the T-cell zone of the draining lymph nodes. Basophils are directly activated by protease antigens, either before or after their entry into the lymph nodes. They then secrete interleukin-4 (IL-4) and thymic stromal lymphopoietin (TSLP), which induce the differentiation of naive T cells into T_H2 cells in cooperation with antigen-presenting dendritic cells in the lymph nodes. **b** | Basophils enhance antibody production in the secondary immune response. In antigen-sensitized animals, antigen-specific IgE antibodies are produced and captured by circulating basophils through the high-affinity Fc receptor for IgE (FcεRI). Following re-exposure to the same antigen, basophils efficiently bind free antigens through IgE–FcεRI complexes, become activated and secrete IL-4 and IL-6. These antigen-stimulated basophils interact with antigen-specific B cells and T_H cells during the secondary immune response, most probably at the interface of the B- and T-cell zones in the lymph nodes. The cytokines that are secreted by activated basophils, including IL-4 and IL-6, promote B-cell proliferation and antibody production in cooperation with the cytokines that are secreted by T_H cells, which are also activated by basophils. BCR, B-cell receptor; CD40L, CD40 ligand.

Basophils in immune regulation

Basophils drive T_H2-cell differentiation. It is well known that IL-4 is fundamental for the differentiation of naive $CD4^+$ T cells into IL-4-producing T_H2 cells, but the cellular source of the initial IL-4 is unknown[26]. Possible sources include T cells, natural killer T cells, basophils, mast cells and eosinophils. As mentioned earlier, basophils readily generate large quantities of T_H2-type cytokines in response to various stimuli, including IL-3 and parasite antigens. The culture of antigen-stimulated naive T cells with basophils promoted robust T_H2-cell differentiation *in vitro*[27,28]. Mice that were deficient for interferon-regulatory factor 2 (IRF2), which is a negative regulator of IL-3-mediated signalling, and wild-type mice that had been treated with IL-3 had an increased number of basophils and showed accelerated T_H2-cell differentiation *in vivo* compared with control mice[27,28]. These results indicated that basophils might be involved in T_H2-cell differentiation.

However, an important outstanding question was whether basophils and naive $CD4^+$ T cells actually encountered each other under physiological conditions in the lymph nodes, which is where T_H2-cell differentiation is thought to occur. Medzhitov and colleagues[13] recently showed that basophils were activated in response to protease allergens, such as papain, and transiently migrated to the draining lymph nodes, albeit in small numbers (~0.3% of lymph-node cells), just before T_H2 cells differentiated there (FIG. 2a). Importantly, the papain-induced T_H2-cell differentiation was abolished in mice that had been treated with the FcεRIα-specific monoclonal antibody MAR-1, which eliminates basophils but not mast cells when administered *in vivo*. By contrast, mice that were deficient in mast cells mounted normal T_H2-cell responses to papain. These results suggest that basophils have an essential and non-redundant role in the development of T_H2-cell responses to a protease allergen.

In the Medzhitov study, basophils that had been stimulated with protease allergens produced IL-4 and thymic stromal lymphopoietin (TSLP), which are both involved in T_H2-cell differentiation *in vivo*[13] (FIG. 2a). Basophils seemed to be directly activated by protease allergens in a Toll-like-receptor-independent manner, probably through an unidentified sensor of proteolytic activity. It is not clear where basophils encounter allergens to become activated (possible locations include the site of allergen injection, the lymph, the blood and the draining lymph nodes) and how basophils are recruited to the lymph nodes. In addition, it will be important to determine whether the entry of basophils into the lymph nodes is indeed required for the development of T_H2-cell responses or whether the production of T_H2-type cytokines by basophils from other parts of the body is sufficient. It will also be important to clarify whether the basophil-driven T_H2-cell differentiation in response to protease allergens is relevant to the T_H2-cell differentiation that is induced by allergens which have no apparent protease activity or by T_H2-cell-inducing adjuvants, such as alum.

Basophils increase humoral memory responses. IgE antibodies in an unbound form have a short half-life in the blood. However, when they are captured by mast cells and basophils through FcεRI binding, they stabilize on the cell surface by forming a complex with FcεRI[29], which in turn transduces signals to increase their survival[30]. One study showed that in the early phase of a secondary immune response, basophils, but not mast cells, secreted large amounts of IL-4 after being stimulated with antigen through the IgE–FcεRI complexes on their surface. By contrast, memory T cells became important producers of IL-4 in the later phase of the response[31]. However, the physiological relevance of basophil-derived IL-4 in this memory response remained uncertain. In a recent report, basophils were shown to increase humoral memory immune responses by producing IL-4 and IL-6 when stimulated by re-exposure to the allergen to which specific IgE had been produced in the primary immune response[14] (FIG. 2b). The same group had previously identified that basophils can function as antigen-capturing cells by trapping soluble antigens when antigen-specific IgE is bound to FcεRI on their surface[32]. In this recent study, they showed that basophils in mice that had been immunized with the fluorescent protein allophycocyanin had antigen-capturing ability both *in vitro* and *in vivo*, even 6 weeks after immunization[14]. Basophils were found to be the main source of IL-4 and IL-6 in the spleen and bone marrow of immunized mice following re-stimulation with allophycocyanin *in vitro* or *in vivo*, a finding that is consistent with the results of an earlier study[33]. Of note, the *in vivo* depletion of basophils using the MAR-1 antibody before the second antigen challenge markedly decreased humoral memory responses, as indicated by decreased serum titres of antigen-specific IgG1 and IgG2a in both mast-cell-sufficient and mast-cell-deficient mice[14]. Consistent with this, the frequency of antigen-specific B cells and plasma cells in the spleen and bone marrow was ~50% lower in the basophil-depleted mice. Conversely, the adoptive transfer of basophils from antigen-sensitized mice increased antigen-specific antibody production in naive mice following stimulation with the allergen[14]. These results clearly showed that basophils have a crucial role in promoting humoral immune responses.

The clinical importance of this role for basophils in humoral immune responses was highlighted by the observation that mice were more susceptible to sepsis following infection with *Streptococcus pneumoniae* if basophils were depleted before a second vaccination with the pneumococcal antigen[14]. Although the precise mechanism underlying the basophil-mediated enhancement of memory responses *in vivo* needs to be clarified, the *in vitro* experiments showed that activated basophils provide support for B-cell proliferation and antibody production in the presence of activated $CD4^+$ T cells through the secretion of IL-4 and IL-6, and through cell–cell contact[14] (FIG. 2b). Basophils also induce phenotypic changes in $CD4^+$ T cells that provide them with T_H2-type characteristics and therefore make them better 'B-cell helpers'. If these phenotypic changes indeed occur *in vivo*, it remains to be shown where the interactions between basophils, B cells and T cells take place, and how basophils that have captured antigen in the blood, spleen or bone marrow are recruited to the site of interaction.

Box 2 | The lineage relationship between basophils and other haematopoietic cells

Our understanding of the developmental origin of basophils remains poor. Do they share a progenitor with mast cells, eosinophils or other haematopoietic cells or do they have a lineage-restricted unique progenitor that is derived directly from a multipotent progenitor? So far, growth factors or transcription factors that are specifically involved in basophil differentiation have not been identified, which is in contrast to other myeloid lineages. In humans, basophils seem to have a closer lineage relationship with eosinophils than with mast cells[36], as determined by *in vitro* colony assays, by two case reports of patients that were deficient in both basophils and eosinophils[37,38] and by the presence of granulocytes that have a basophil–eosinophil hybrid phenotype in some patients with leukaemia. By contrast, a recent study of mouse haematopoiesis identified progenitors that can differentiate into basophils and mast cells but not other lineages[39]. The transcription factor CCAAT/enhancer-binding protein-α had an instructive role in the differentiation of the bipotent progenitor into basophils. The same study also identified basophil-committed monopotent progenitors, which are probably derived from granulocyte/monocyte progenitors[39]. Another study identified mast-cell-committed progenitors that are derived directly from multipotent progenitors instead of common myeloid progenitors or granulocyte/monocyte progenitors[40]. So, the differentiation pathways of mouse basophils and mast cells remain to be clarified. The observed discrepancy in the lineage relationship of basophils and other haematopoietic cells between humans and mice might be due to the species-specific differences in haematopoiesis or it might simply reflect the existence of multiple pathways for the differentiation of basophils in both species. Further investigation, including the identification of growth and/or differentiation factors and transcription factors that govern the differentiation of the basophil lineage, would provide us with a better understanding of this issue and help us to establish the genetic models of basophil deficiency.

New era in basophil research

Since the discovery of basophils by Paul Ehrlich at the end of the nineteenth century, the functional significance of this small population of cells in the blood has remained an enigma, even though basophils are found in most vertebrates. Recent advances in the field, as discussed in this Progress article, have markedly changed our understanding of basophils, and we now appreciate that they are key players in the immune system. We have learned that although a subpopulation of cells might be small, this does not necessarily mean that it does not have an important biological role. Basophils are no longer regarded as redundant because of their similarities to mast cells, and we now recognize that basophils regulate immune and allergic responses at many levels. So, basophil research has entered an exciting new era. Nevertheless, there are still many

unresolved issues, including the pathways of commitment and differentiation of basophils during haematopoiesis (BOX 2), the molecular mechanisms underlying the induction and regulation of chronic allergic inflammation by basophils, the effect of basophil-derived T_H2-type cytokines on dendritic cells and macrophages, and the involvement of basophils in autoimmune diseases and in the protective immunity against pathogens, particularly parasites such as blood-feeding ticks[34].

Why do our bodies keep the number of basophils so low under physiological conditions? Basophils readily respond to various stimuli and release immune modulators, so it is possible that a high number of basophils could disturb the homeostasis of the immune system and potentially lead to systemic anaphylaxis. The establishment of genetically engineered mice that are deficient only for basophils would further our understanding of the *in vivo* functions of basophils (BOX 1). Accumulating evidence indicates that basophils have pivotal roles in the regulation of the immune system in spite of their small numbers. Therefore, basophils and their products might be promising therapeutic targets for immunological disorders.

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1. Galli, S. J. Mast cells and basophils. *Curr. Opin. Hematol.* **7**, 32–39 (2000).
2. Prussin, C. & Metcalfe, D. D. IgE, mast cells, basophils, and eosinophils. *J. Allergy Clin. Immunol.* **111**, S486–S494 (2003).
3. Gibbs, B. F. Human basophils as effectors and immunomodulators of allergic inflammation and innate immunity. *Clin. Exp. Med.* **5**, 43–49 (2005).
4. Dvorak, A. M. The mouse basophil, a rare and rarely recognized granulocyte. *Blood* **96**, 1616–1617 (2000).
5. Urbina, C., Ortiz, C. & Hurtado, I. A new look at basophils in mice. *Int. Arch. Allergy Appl. Immunol.* **66**, 158–160 (1981).
6. Seder, R. A. *et al.* Mouse splenic and bone marrow cell populations that express high-affinity Fcε receptors and produce interleukin 4 are highly enriched in basophils. *Proc. Natl Acad. Sci. USA* **88**, 2835–2839 (1991).
7. Schroeder, J. T., MacGlashan, D. W. Jr. & Lichtenstein, L. M. Human basophils: mediator release and cytokine production. *Adv. Immunol.* **77**, 93–122 (2001).
8. Voehringer, D., Shinkai, K. & Locksley, R. M. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity* **20**, 267–277 (2004).
9. Min, B. *et al.* Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. *J. Exp. Med.* **200**, 507–517 (2004).
10. Mukai, K. *et al.* Basophils play a critical role in the development of IgE-mediated chronic allergic inflammation independently of T cells and mast cells. *Immunity* **23**, 191–202 (2005).
11. Obata, K. *et al.* Basophils are essential initiators of a novel type of chronic allergic inflammation. *Blood* **110**, 913–920 (2007).
12. Tsujimura, Y. *et al.* Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis. *Immunity* **28**, 581–589 (2008).
13. Sokol, C. L., Barton, G. M., Farr, A. G. & Medzhitov, R. A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nature Immunol.* **9**, 310–318 (2008).
14. Denzel, A. *et al.* Basophils enhance immunological memory responses. *Nature Immunol.* **9**, 733–742 (2008).
15. Kojima, T. *et al.* Mast cells and basophils are selectively activated *in vitro* and *in vivo* through CD200R3 in an IgE-independent manner. *J. Immunol.* **179**, 7093–7100 (2007).
16. Macfarlane, A. J. *et al.* Basophils, eosinophils, and mast cells in atopic and nonatopic asthma and in late-phase allergic reactions in the lung and skin. *J. Allergy Clin. Immunol.* **105**, 99–107 (2000).
17. Gauvreau, G. M. *et al.* Increased numbers of both airway basophils and mast cells in sputum after allergen inhalation challenge of atopic asthmatics. *Am. J. Respir. Crit. Care Med.* **161**, 1473–1478 (2000).
18. Sampson, H. A. *et al.* Second symposium on the definition and management of anaphylaxis: summary report — second national institute of allergy and infectious disease/food allergy and anaphylaxis network symposium. *J. Allergy Clin. Immunol.* **117**, 391–397 (2006).
19. Simons, F. E. *et al.* Risk assessment in anaphylaxis: current and future approaches. *J. Allergy Clin. Immunol.* **120**, S2–S24 (2007).
20. Galli, S. J. Pathogenesis and management of anaphylaxis: current status and future challenges. *J. Allergy Clin. Immunol.* **115**, 571–574 (2005).
21. Finkelman, F. D. Anaphylaxis: lessons from mouse models. *J. Allergy Clin. Immunol.* **120**, 506–515 (2007).
22. Miyajima, I. *et al.* Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and FcγRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. *J. Clin. Invest.* **99**, 901–914 (1997).
23. Dombrowicz, D. *et al.* Absence of FcεRIα chain results in upregulation of FcγRIII-dependent mast cell degranulation and anaphylaxis. Evidence of competition between FcεRI and FcγRIII for limiting amounts of FcRβ and γ chains. *J. Clin. Invest.* **99**, 915–925 (1997).
24. Strait, R. T., Morris, S. C., Yang, M., Qu, X. W. & Finkelman, F. D. Pathways of anaphylaxis in the mouse. *J. Allergy Clin. Immunol.* **109**, 658–668 (2002).
25. Vadas, P. *et al.* Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. *N. Engl. J. Med.* **358**, 28–35 (2008).
26. Min, B. & Paul, W. E. Basophils and type 2 immunity. *Curr. Opin. Hematol.* **15**, 59–63 (2008).
27. Hida, S., Tadachi, M., Saito, T. & Taki, S. Negative control of basophil expansion by IRF-2 critical for the regulation of Th1/Th2 balance. *Blood* **106**, 2011–2017 (2005).
28. Oh, K., Shen, T., Le Gros, G. & Min, B. Induction of Th2 type immunity in a mouse system reveals a novel immunoregulatory role of basophils. *Blood* **109**, 2921–2927 (2007).
29. Kubo, S., Nakayama, T., Matsuoka, K., Yonekawa, H. & Karasuyama, H. Long term maintenance of IgE-mediated memory in mast cells in the absence of detectable serum IgE. *J. Immunol.* **170**, 775–780 (2003).
30. Kawakami, T. & Galli, S. J. Regulation of mast-cell and basophil function and survival by IgE. *Nature Rev. Immunol.* **2**, 773–786 (2002).
31. Khodoun, M. V., Orekhova, T., Potter, C., Morris, S. & Finkelman, F. D. Basophils initiate IL-4 production during a memory T-dependent response. *J. Exp. Med.* **200**, 857–870 (2004).
32. Mack, M. *et al.* Identification of antigen-capturing cells as basophils. *J. Immunol.* **174**, 735–741 (2005).
33. Aoki, I., Kinzer, C., Shirai, A., Paul, W. E. & Klinman, D. M. IgE receptor-positive non-B/non-T cells dominate the production of interleukin 4 and interleukin 6 in immunized mice. *Proc. Natl Acad. Sci. USA* **92**, 2534–2538 (1995).
34. Falcone, F. H., Pritchard, D. I. & Gibbs, B. F. Do basophils play a role in immunity against parasites? *Trends Parasitol.* **17**, 126–129 (2001).
35. Galli, S. J., Grimbaldeston, M. & Tsai, M. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. *Nature Rev. Immunol.* **8**, 478–486 (2008).
36. Arock, M., Schneider, E., Boissan, M., Tricottet, V. & Dy, M. Differentiation of human basophils: an overview of recent advances and pending questions. *J. Leukoc. Biol.* **71**, 557–564 (2002).
37. Juhlin, L. & Michaelsson, G. A new syndrome characterised by absence of eosinophils and basophils. *Lancet* **1**, 1233–1235 (1977).
38. Mitchell, E. B., Platts-Mills, T. A., Pereira, R. S., Malkovska, V. & Webster, A. D. Acquired basophil and eosinophil deficiency in a patient with hypogammaglobulinaemia associated with thymoma. *Clin. Lab. Haematol.* **5**, 253–257 (1983).
39. Arinobu, Y. *et al.* Developmental checkpoints of the basophil/mast cell lineages in adult murine hematopoiesis. *Proc. Natl Acad. Sci. USA* **102**, 18105–18110 (2005).
40. Chen, C. C., Grimbaldeston, M. A., Tsai, M., Weissman, I. L. & Galli, S. J. Identification of mast cell progenitors in adult mice. *Proc. Natl Acad. Sci. USA* **102**, 11408–11413 (2005).

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
CD200R3 | FcεRI | IL-3 | IL-4 | IRF2 | PAF | TSLP

FURTHER INFORMATION
Hajime Karasuyama's homepage: http://www.tmd.ac.jp/med/mbch/top_English.htm

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Molecular Pathogenesis of Genetic and Inherited Diseases

Flaky Tail Mouse Denotes Human Atopic Dermatitis in the Steady State and by Topical Application with *Dermatophagoides pteronyssinus* Extract

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The barrier abnormality, a loss-of-function mutation in the gene encoding filaggrin (*FLG*), which is linked to the incidence of atopic dermatitis (AD), is a recently discovered but important factor in the pathogenesis of AD. Flaky tail (*Flg^{ft}*) mice, essentially deficient in filaggrin, have been used to investigate the role of filaggrin on AD. However, the relevancy of *Flg^{ft}* mice to human AD needs to be determined further. In this study, we observed the clinical manifestations of *Flg^{ft}* mice in the steady state and their cutaneous immune responses against external stimuli, favoring human AD. Under specific pathogen-free conditions, the majority of *Flg^{ft}* mice developed clinical and histological eczematous skin lesions similar to human AD with outside-to-inside skin barrier dysfunction evaluated by newly devised methods. In addition, cutaneous hapten-induced contact hypersensitivity as a model of acquired immune response and a mite extract-induced dermatitis model physiologically relevant to a human AD were enhanced in *Flg^{ft}* mice. These results suggest that the *Flg^{ft}* mouse genotype has potential as an animal model of AD corresponding with filaggrin mutation in human AD. *(Am J Pathol 2010, 176:2385–2393; DOI: 10.2353/ajpath.2010.090957)*

Atopic dermatitis (AD), which affects at least 15% of children in developed countries, is characterized by eczematous skin lesions, dry skin, and pruritus.[1–3] Although the precise pathogenic mechanism of AD is as yet unknown, several accumulated lines of evidence suggest that a defective skin barrier to environmental stimuli may contribute to its pathogenesis. It has long been thought that the barrier abnormality in AD is not merely an epiphenomenon but rather is the "driver" of disease activity.[4] The evidence for a primary structural abnormality of the stratum corneum in AD is derived from a recently discovered link between the incidence of AD and loss-of-function mutations in the gene encoding filaggrin (*FLG*). Individuals carrying the *FLG* null allele variants tend to develop AD.[5–7]

Filaggrin protein is localized in the granular layers of the epidermis. Profilaggrin, a 400-kDa polyprotein, is the main component of keratohyalin granules.[8–10] In the differentiation of keratinocytes, profilaggrin is dephosphorylated and cleaved into 10 to 12 essentially identical 27-kDa filaggrin molecules, which aggregate in the keratin cytoskeleton system to form a dense protein-lipid matrix.[10] This structure is thought to prevent epidermal water loss and impede the entry of external stimuli, such as allergens, toxic chemicals, and infectious organisms. Therefore, filaggrin is a key protein in the terminal differentiation of the epidermis and in skin barrier function.[11]

Because AD is a common disease for which satisfactory therapies have not yet been established, understanding the mechanism of AD through animal models is an essential issue.[1,12] Flaky tail (*Flg^{ft}*) mice, first introduced in 1958, are spontaneously mutated mice with

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abnormally small ears, tail constriction, and a flaky appearance of the tail skin, which is most evident between 5 and 14 days of age.[13] Mice of the *Flg*^{ft} genotype express an abnormal profilaggrin polypeptide that does not form normal keratohyalin F granules and is not proteolytically processed to filaggrin. Therefore, filaggrin is absent from the cornified layers in the epidermis of the *Flg*^{ft} mouse.[14–16]

Recently, it has been revealed that the gene responsible for the characteristic phenotype of *Flg*^{ft} mice is a nonsense mutation of 1-bp deletion analogous to a common human *FLG* mutation.[15] These mice developed eczematous skin lesions after age 28 weeks under specific pathogen-free (SPF) conditions[17] and enhanced penetration of tracer perfusion determined by ultrastructural visualization,[16] and were predisposed to develop an allergen-specific immune response after epicutaneous sensitization with the foreign allergen ovalbumin (OVA).[15,17] On the other hand, general immunity through intraperitoneal sensitization with OVA was comparable between *Flg*^{ft} mice and control mice.[15,17]

Despite these recent advances, there still remain several issues with *Flg*^{ft} mice to be addressed. For example, serial close observation of clinical manifestations in reference to human AD will be informative. It is of value to evaluate the responses to external stimuli relevant to human AD, such as mite extracts, instead of OVA that has been used previously. A comparative study on the skin-mediated contact hypersensitivity (CHS) response and non-skin-mediated delayed-type hypersensitivity response is important to evaluate the impact of barrier dysfunction on immune responses *in vivo*. In addition, although it has now been determined that the barrier dysfunction is a key element in the establishment of AD, there is no established method to evaluate the outside-to-inside barrier function quantitatively.

In this study, we found that *Flg*^{ft} mice showed spontaneous dermatitis with skin lesions mimicking human AD in a steady state under SPF conditions: serial occurrence of manifestations as scaling, erythema, pruritus, and erosion followed by edema in this order. We also successfully evaluated outside-to-inside barrier dysfunction in *Flg*^{ft} mice quantitatively using a newly developed method. In addition, we determined that the Th1/Tc1-mediated immune response was enhanced by immunization through skin but not through non-skin immunization. Last, we induced severe AD-like skin lesions in *Flg*^{ft} mice by application of mites as a physiologically relevant antigen for human AD, which will be an applicable animal model of AD.

Materials and Methods

Mice

C57BL/6NCrSlc (B6) mice were purchased from SLC (Shizuoka, Japan). Flaky tail (STOCK *a/a ma ft/ma ft*/J; *Flg*^{ft} mice) mice have double-homozygous filaggrin (*Flg*) and matted (*ma*) mutations.[13,14] We used B6 mice as a control of *Flg*^{ft} mice because *Flg*^{ft} mice were described to be outcrossed onto B6 mice at The Jackson Laboratory (Bar Harbor, ME)[13,14] (of note, although the strain was crossed with B6, it is not a B6 congenic strain but rather a hybrid stock that is probably semi-inbred). Female mice were used in all experiments unless otherwise stated; they were maintained on a 12-hour light/dark cycle at a temperature of 24°C and at a humidity of 50 + 10% under SPF conditions at Kyoto University Graduate School of Medicine. Routine colony surveillance and diagnostic workup verified that mice were free of Ectromelia virus, lymphocytic choriomeningitis virus, mouse hepatitis virus, Sendai virus, *Mycoplasma pulmonis*, cilia-associated respiratory bacillus, *Citrobacter rodentium* [*Escherichia coli* O115a,c:K(B)], *Clostridium piliforme* (Tyzzer's organism), *Corynebacterium kutscheri*, *Helicobacter hepaticus*, *Pasteurella pneumotropica*, *Salmonella* spp., parasites, intestinal protozoans, *Enterobius*, and ectoparasites. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Kyoto University Graduate School of Medicine.

Clinical Observation and Histology

The clinical severity of skin lesions was scored according to the macroscopic diagnostic criteria that were used for the NC/Nga mouse.[18] In brief, the total clinical score for skin lesions was designated as the sum of individual scores, graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe), for the symptoms of pruritus, erythema, edema, erosion, and scaling. Pruritus was observed clinically for more than 2 minutes.

For the histological portion of the study, the dorsal skin of mice was stained with H&E. Toluidine blue staining was used to detect mast cells, and the number of mast cells was calculated as the average from five different fields of each sample ×40 magnification).

Flow Cytometric Analysis and Quantitative RT-PCR

Cells from the skin-draining axillary and inguinal lymph nodes (LNs) and from the spleen were analyzed with flow cytometry. Fluorescent-labeled anti-CD4 and anti-CD8 antibodies were obtained from eBioscience (San Diego, CA) and used to stain cells. The total number of cells per organ and the number of cells in each subset were calculated through flow cytometry using the FACSCanto II system (Becton Dickinson, San Diego, CA). Quantitative RT-PCR was performed as described previously, using the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as a control.[19]

Total and Mite-Specific Serum IgE

Total serum IgE levels were measured with a mouse IgE ELISA Kit (Bethyl Laboratories, Montgomery, TX) according to the manufacturer's protocols. For the measurement of mite-specific IgE levels, the same type of mouse IgE ELISA Kit was used with slightly modifications. Specifi-

cally, plates were coated and incubated with 10 μg/ml *Dermatophagoides pteronyssinus* (Dp) (Biostir, Kobe, Japan) diluted with coating buffer for 60 minutes. After a blocking period of 30 minutes, 100 μl of 5× diluted serum was added into each well and incubated for 2 hours. Anti-mouse IgE-horseradish peroxidase conjugate (1:15,000; 100 μL) was used to conjugate the antigen-antibody complex for 60 minutes at room temperature; from this point on the ELISA Kit was used according to the manufacturer's protocol. Absorbance was measured at 450 nm. The difference between the sample absorbance and the mean of negative control absorbance was taken as the result.

Skin Barrier Function

The dorsal regions of the skin were shaved in all mice before measurement. To evaluate inside-to-outside barrier function, transepidermal water loss (TEWL) was measured with a Tewameter Vapo Scan (Asahi Biomed, Tokyo, Japan) at 24°C and 46% relative humidity.

Outside-to-inside barrier function was assessed by means of fluorescein isothiocyanate isomer I (FITC) (Sigma-Aldrich, St. Louis, MO). The shaved dorsal skin of mice was treated with 100 μl of 1% FITC diluted in acetone and dibutyl phthalate (1:4); 3 hours later, this area was tape-stripped (Scotch tape, 3M, St. Paul, MN) nine times to remove the stratum corneum containing the remnant of FITC. The painted area (1.2 cm × 1.2 cm) was removed, and FITC concentration was measured. Each skin sample was soaked in PBS at 60°C for 10 seconds, after which the dermis and epidermis were separated. The epidermis was soaked in 500 μl of PBS, homogenized, and spun down at 2200 × *g*. The supernatant was collected, and fluorescence was measured at an excitation wavelength of 535 nm and an emission wavelength of 460 nm using an Arvo SX 1420 counter (Wallac, PerkinElmer, Waltham, MA). The fluorescence value was compared with a standard curve using FITC serial dilutions.

For the evaluation of fluorescence intensities of FITC penetrated into the epidermis, a 1 × 1 cm skin sample was taken after tape stripping, and a 10-μm Tissue-Tek (Sakura Finetek, Tokyo, Japan)-embedded section was analyzed using a BZ-9000 Biorevo digital microscope (Keyence, Osaka, Japan) at the same time exposure.

An *in situ* dye permeability assay with toluidine blue was performed using embryos at 18 days (littermates). Unfixed, untreated embryos were dehydrated by a 1-minute incubation in an ascending series of methanol (25, 50, 74, and 100%) and rehydrated with the descending same methanol series, washed in PBS, and stained with 0.01% toluidine blue.

Scratching Behavior

Scratching behavior was measured in detail using the Sclaba Real system (Noveltec, Kobe, Japan). Mice were put into the machine 20 minutes before measurement to allow them to adapt to the new environment. Ointment was then applied, and the number and duration of scratching sessions were counted according to the manufacturer's protocol for 15 minutes.[20]

Dermatitis Models

For the assessment of irritant contact dermatitis, 20 μl of 0.2 mg/ml phorbol myristate acetate (PMA) (Sigma-Aldrich) was applied to both sides of the ears. Ear thickness change was measured at 1, 3, 12, and 24 hours as well as 5 days after application.

To induce a CHS response, 25 μl of 0.5% 1-fluoro-2.4-dinitrobenzene (DNFB) (Nacalai Tesque, Kyoto, Japan) was painted on the shaved abdomens of mice for sensitization. Five days later, the ears were challenged with 20 μl of 0.2% DNFB, and ear thickness change was measured at 24 and 48 hours after application. Nonsensitized mice were used as a control. A delayed-type hypersensitivity response model was established using OVA (Sigma-Aldrich). Mice were sensitized with 200 μl of 0.5 mg/ml of OVA in complete Freund's adjuvant (Difco Laboratories, Detroit, MI) intraperitoneally and challenged 5 days later with an injection of 20 μl of 1 mg/ml of OVA in incomplete Freund's adjuvant (Difco Laboratories) into the hind footpads. Footpad thickness was measured before and 24 hours after challenge. Nonsensitized mice were used as a control. Footpad swelling was calculated by (footpad thickness change of sensitized mice) − (footpad thickness change of nonsensitized mice). To induce murine AD-like skin lesions, 40 mg of 0.5% Dp in white petrolatum was topically applied to the ears and upper back twice a week for 8 weeks. Petrolatum without Dp was used as a control. One gram of Dp body product (Biostir) contained 1.78 mg of total protein with 2.47 μg of Dp protein (Der p1). Ear thickness and clinical scores were measured every week. Mite-specific IgE levels, TEWL, and histological appearance of eczematous skin were observed 12 hours after the final application.

Statistical Analysis

Data were analyzed using an unpaired two-tailed *t*-test. $P < 0.05$ was considered to be significant.

Results

Spontaneous Dermatitis of Flg^{ft} *Mice in the Steady State under SPF Conditions*

As described previously,[14,15] the expression of the filaggrin monomer was barely detectable by Western blotting in the dorsal skin of *Flg^{ft}* mice compared with that of B6 mice (data not shown). Here, we investigated the clinical manifestations seen in the skin of *Flg^{ft}* mice raised in a steady state under SPF conditions and found that *Flg^{ft}* mice developed spontaneous dermatitis (Figure 1A). The clinical severities of skin lesions, including pruritic activity, erythema, edema, erosion, and scaling, were scored. The total clinical scores of *Flg^{ft}* mice increased with age

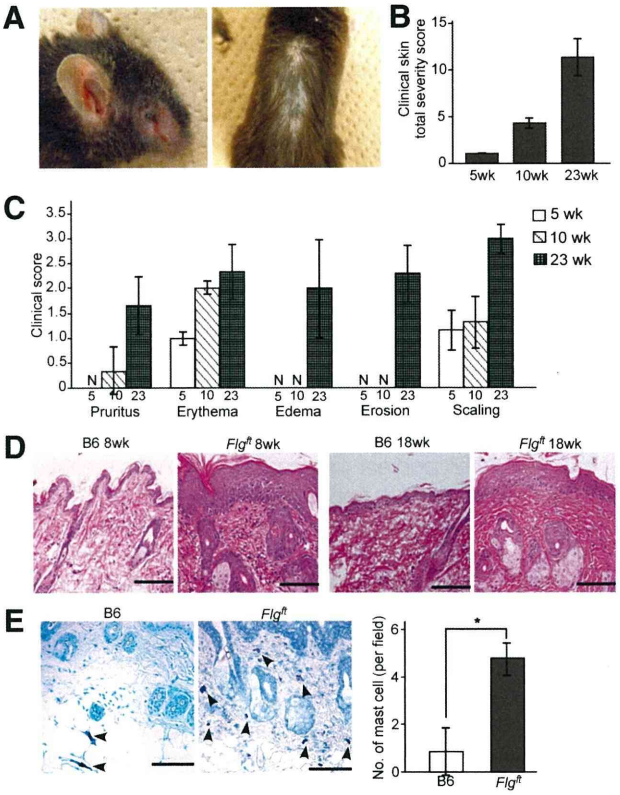

Figure 1. Spontaneous dermatitis in *Flg*$^{ft/ft}$ mice in SPF. **A:** Clinical photographs of 20-week-old *Flg*$^{ft/ft}$ mice. Total clinical severity scores (**B**) for each particular item (**C**) in 5-, 10- and 23-week-old *Flg*$^{ft/ft}$ mice. N, none. **D:** H&E-stained sections in 8- and 18-week-old mice. Scale bar = 100 μm. **E:** Toluidine blue staining of the skin from 8-week-old B6 and *Flg*$^{ft/ft}$ mice and the numbers of mast cells (**arrowheads**) per field are shown. $^*P < 0.05$.

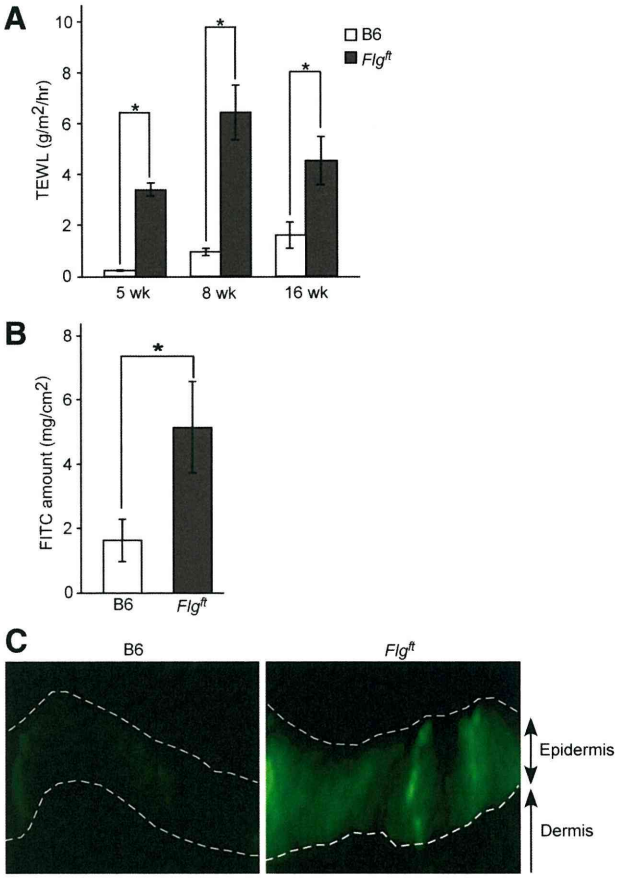

Figure 2. Skin barrier dysfunction in *Flg*$^{ft/ft}$ mice. **A:** TEWL through dorsal skin of 5-, 8-, and 16-week-old B6 and *Flg*$^{ft/ft}$ mice. **B:** Amount of FITC in the skin of B6 and *Flg*$^{ft/ft}$ mice after topical application. **C:** Fluorescence intensities of FITC of the skin after topical application. **Dashed white lines** indicate the border between the epidermis and the dermis, and the top of the epidermis. $^*P < 0.05$.

(Figure 1B). The first manifestations to appear when mice were young were erythema and fine scaling; pruritic activity, erosion, and edema followed later (Figure 1C). In contrast, no cutaneous manifestation was observed in either B6 mice, studied as a control, or heterozygous mice intercrossed with *Flg*^{ft} and B6 mice kept under SPF conditions throughout the experimental period (data not shown). In addition, there was no apparent difference in terms of clinical manifestations between the genders of *Flg*^{ft} mice throughout the period (data not shown).

Histological examination of skin from *Flg*^{ft} mice revealed epidermal acanthosis, increased lymphocyte infiltration, and dense fibrous bundles in the dermis in both younger (8-week-old) and older (18-week-old) *Flg*^{ft} mice; none of these were observed in B6 mice (Figure 1D). In addition, toluidine blue staining to detect mast cells showed an increased number of mast cells, especially degranulated mast cells in the upper dermis, in *Flg*^{ft} mice (Figure 1E). No mouse or human mite bodies were detected in the sections. These data support the diagnosis of spontaneous clinical dermatitis in *Flg*^{ft} mice in the steady state under SPF conditions.

Defect of Skin Barrier Function in Flg^{ft} *Mice*

Because barrier dysfunction is a common characteristic of AD,[4–7,21] we measured TEWL, an established indicator of barrier function.[21] TEWL was significantly higher in *Flg*^{ft} mice than in B6 mice from an early age (4 weeks) to an older age (16 weeks) (Figure 2A). Because TEWL is only a measure of water transportation through the skin from the inside to the outside of the body, another experimental method was necessary to evaluate outside-to-inside barrier function from the perspective of invasion of external stimuli. To address this issue, we measured FITC penetration through the skin from the outside. FITC solution was applied to the shaved dorsal skin of 8-week-old female mice; 3 hours later, the epidermis was separated and homogenized so that the FITC content could be measured with a fluorometer. The epidermis of *Flg*^{ft} mice contained a higher amount of FITC than that of B6 mice (Figure 2B). Neither group had FITC in the dermis after this procedure, however (data not shown). In addition, observation of fluorescence intensities in the epidermis of both mice showed stronger fluorescence in *Flg*^{ft} mice (Figure 2C). To further analyze the skin permeability, we examined the mouse embryos by toluidine blue solution and showed that the *Flg*^{ft} embryo was entirely dye-permeable compared with the control littermate (Supplemental Figure S1, see *http://ajp.amjpathol.org*). These data strongly indicate a defect in the skin barrier of *Flg*^{ft}

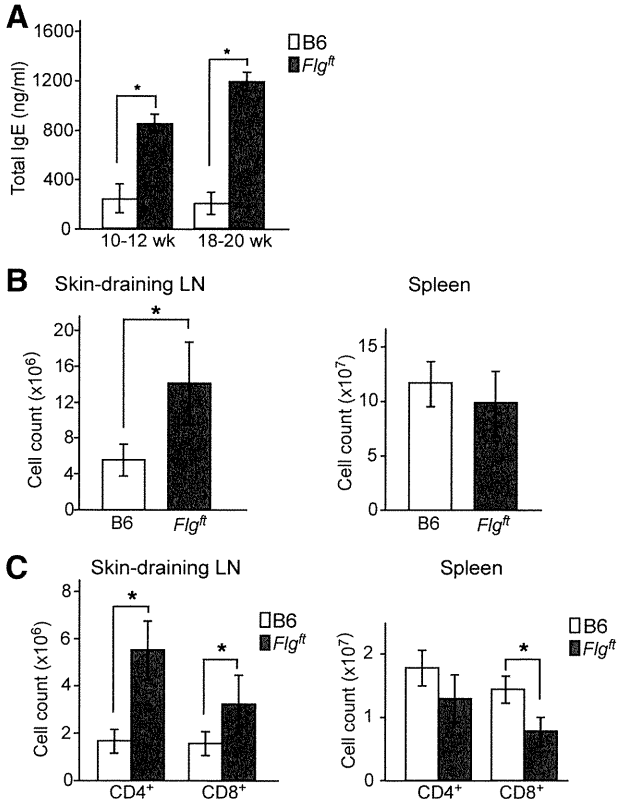

Figure 3. The immune status of *Flg*^{ft} mice in a steady state. **A:** Total serum IgE levels of B6 and *Flg*^{ft} mice as measured by enzyme-linked immunosorbent assay. **B** and **C:** Numbers of total cells (**B**), $CD4^+$ cells, and $CD8^+$ cells in the skin-draining LN and spleen (**C**). $^*P < 0.05$.

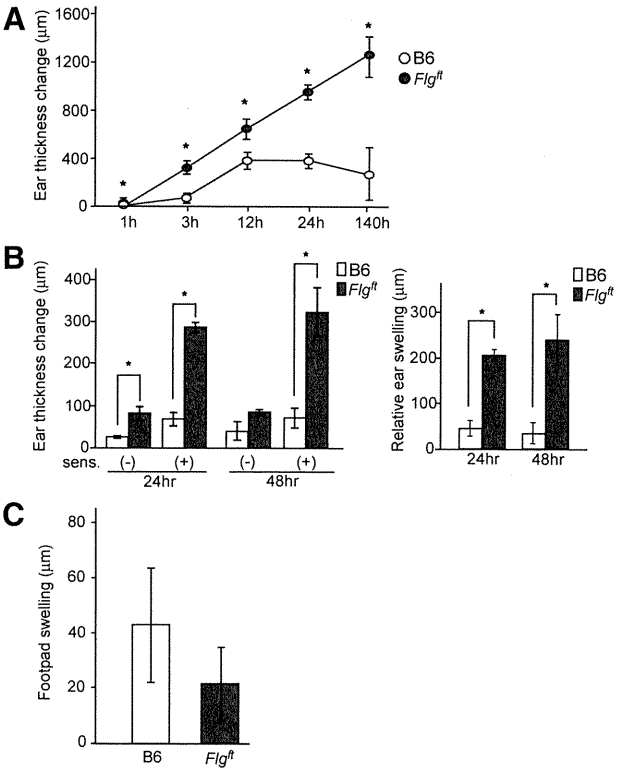

Figure 4. Enhanced cutaneous immune responses in *Flg*^{ft} mice. **A** and **B:** Ear thickness change in B6 and *Flg*^{ft} mice after topical application of PMA as a model of irritant contact dermatitis (**A**), after DNFB challenge on the ears with or without sensitization (**B**, **left panel**) and the relative ear swelling (**B**, **right panel**) as a model of CHS. **C:** Delayed-type hypersensitivity response. B6 and *Flg*^{ft} mice were intraperitoneally sensitized with OVA, and challenged through subcutaneous injection to the footpad. Twenty-four hours later, footpad swelling change was measured. $^*P < 0.05$.

mice, both from inside to outside and from outside to inside.

Immune Status in the Steady State

To further elucidate the immune status of *Flg*^{ft} mice in the steady state under SPF conditions, we measured the levels of total serum IgE, because increased severity of AD is known to be correlated with elevated serum IgE levels.[22] IgE levels were significantly higher in *Flg*^{ft} mice than in age-matched B6 mice in the steady state under SPF conditions (Figure 3A). To investigate this matter in greater detail, single cell suspensions from the skin-draining inguinal and axillary LNs and from the spleen were analyzed. The total mononuclear cell number of the LNs was significantly higher in *Flg*^{ft} mice than in B6 mice, but that of the spleen was comparable (Figure 3B). In addition, *Flg*^{ft} mice exhibited significantly higher numbers of $CD4^+$ and $CD8^+$ cells in the skin-draining LNs, but not in the spleen (Figure 3C). Thus, an enhanced immune reaction seems to be induced in *Flg*^{ft} mice by the condition of their skin.

To further analyze the immune condition of the skin, we measured the Th1 (interferon-γ [IFN-γ]), Th2 (interleukin [IL]-4 and IL-13), and Th17 (IL-17) cytokine mRNA levels of dorsal skin of 9-week-old mice in the steady state. The mRNA expression levels of IFN-γ, IL-4, and IL-13 were similar between *Flg*^{ft} and B6 mice, but there was an enhancement in the IL-17 mRNA expression (data not shown) as reported previously.[17]

Enhanced Dermatitis in Flg^{ft} *Mice under External Stimuli*

To characterize the likelihood of various cutaneous immune responses, mice were exposed to various external stimuli. First, we studied the irritant contact dermatitis response to PMA as an irritant agent. When we applied PMA to the ears of B6 and *Flg*^{ft} mice, *Flg*^{ft} mice exhibited an enhanced ear swelling response compared with age-matched B6 mice throughout the experimental period (Figure 4A). Next, we measured the CHS response to DNFB. DNFB was applied to the abdominal skin for sensitization; 5 days later, the ears were challenged with the same hapten. The ear thickness change was more prominent in *Flg*^{ft} mice than in B6 mice (Figure 4B, left panel). On the other hand, the ear thickness change of mice without sensitization was higher for *Flg*^{ft} mice than B6 mice, suggesting that irritation contact dermatitis was enhanced in *Flg*^{ft} mice as expected. To avoid the involvement of this irritation in CHS, we next analyzed the relative ear swelling by subtracting the ear thickness change without sensitization from the ear thickness change with sensitization. The relative ear swelling was more exten-

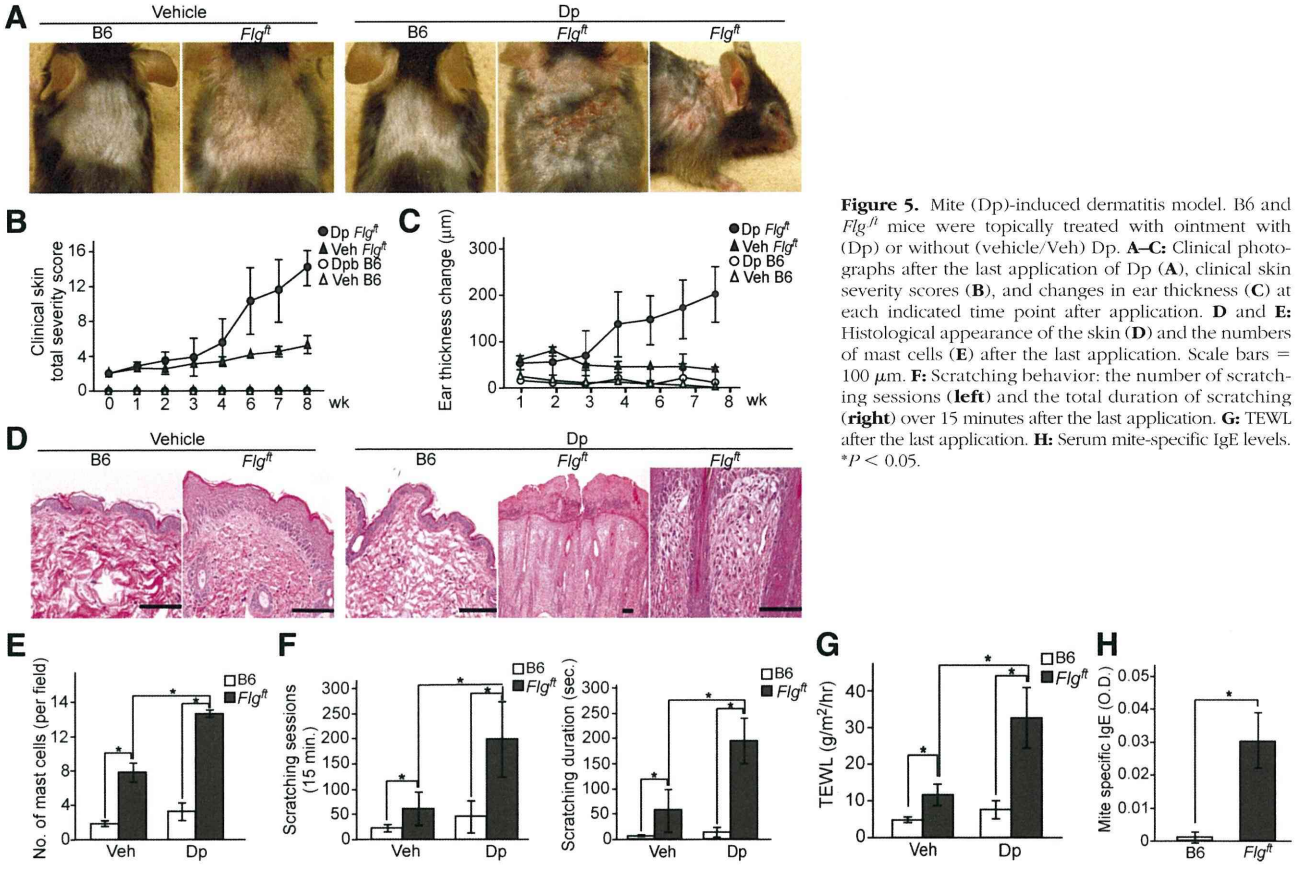

Figure 5. Mite (Dp)-induced dermatitis model. B6 and *Flg*^{ft} mice were topically treated with ointment with (Dp) or without (vehicle/Veh) Dp. **A–C:** Clinical photographs after the last application of Dp (**A**), clinical skin severity scores (**B**), and changes in ear thickness (**C**) at each indicated time point after application. **D** and **E:** Histological appearance of the skin (**D**) and the numbers of mast cells (**E**) after the last application. Scale bars = 100 μm. **F:** Scratching behavior: the number of scratching sessions (**left**) and the total duration of scratching (**right**) over 15 minutes after the last application. **G:** TEWL after the last application. **H:** Serum mite-specific IgE levels. $^{*}P < 0.05$.

sive in *Flg*^{ft} mice than in B6 mice (Figure 4B, right panel). We then measured the relative amount of mRNA for IFN-γ, as a representative Th1 cytokine, to GAPDH as an endogenous control. The relative amount of IFN-γ was higher in the ears of *Flg*^{ft} mice than in those of B6 mice 12 hours after the challenge (0.27 ± 0.13 versus 0.019 ± 0.013, $n = 3$). To further assess the immune responses of *Flg*^{ft} mice, we elicited a delayed-type hypersensitivity response through nonepicutaneous sensitization and challenge. Mice were immunized intraperitoneally with OVA and challenged with a subcutaneous injection of OVA into the footpad. In contrast to the CHS response induced via the skin, the resulting footpad swelling in *Flg*^{ft} mice was lower rather than higher than that in B6 mice (Figure 4C). We also examined the production of mRNA levels of the spleen 3 days after intraperitoneal OVA injection, and it showed a similar level of IFN-γ between *Flg*^{ft} mice and B6 mice (relative mRNA amount to GAPDH: 0.011 ± 0.005 versus 0.016 ± 0.006, $n = 5$). Thus, Th1/Tc1 immune responses were enhanced in *Flg*^{ft} mice only when the stimuli operated via the skin, suggesting that the enhanced immune responses seen in *Flg*^{ft} mice depend on skin barrier dysfunction.

It has been reported that *Flg*^{ft} mice show an enhanced immune response to OVA.[15,17] Their reaction to clinically relevant allergens such as mites has not been evaluated, however. It has also been reported that BALB/c or NC/Nga mice develop an allergic cutaneous immune response to mite antigens when they are applied to the skin after vigorous barrier disruption by means of tape-stripping or SDS treatment.[23,24] Accordingly, we sought to determine whether skin lesions could be induced in *Flg*^{ft} mice through the application of Dp ointment without any skin barrier disruption procedures to evaluate the physiological significance of filaggrin.

The application of Dp ointment to shaved backs and ears induced no cutaneous manifestation in B6 mice throughout the experimental period (Figure 5, A and B), but the same treatment induced dermatitis in *Flg*^{ft} mice, especially on the ears, face, and dorsal skin. Petrolatum alone, used instead of Dp ointment as a control, induced no skin manifestation (Figure 5, A–C). The clinical severity of Dp-induced dermatitis was scored; after 16 applications of Dp ointment over 8 weeks, *Flg*^{ft} mice had developed a very severe skin condition in contrast with the control groups. Consistently, ear swelling in response to Dp ointment was most prominent in *Flg*^{ft} mice (Figure 5C). Histological examination of H&E-stained sections of involved *Flg*^{ft} skin after 16 applications showed acanthosis, elongation of rete ridges, and dense lymphocyte and neutrophil infiltration in the dermis (Figure 5D), accompanied by an increased number of mast cells in the dermis (Figure 5E). We also measured the scratching behavior of *Flg*^{ft} mice treated with Dp using the Sclaba Real system. The number of scratching sessions and the total duration of scratching were significantly higher in *Flg*^{ft} mice than in B6 mice, even among those mice that had not been treated with Dp ointment (Figure 5F); treatment of *Flg*^{ft} mice with Dp ointment raised the number of scratching sessions and the total duration of scratching even higher.

We further evaluated barrier function by measuring TEWL in Dp-treated and untreated mice of each genotype; TEWL was higher in untreated *Flg*^{ft} mice than in B6 mice, and Dp treatment of *Flg*^{ft} mice raised TEWL even higher (Figure 5G). Finally, we examined mite-specific serum IgE levels after the last application and found that *Flg*^{ft} mice had higher levels of Dp-specific IgE than B6 mice had (Figure 5H). Thus, the treatment of *Flg*^{ft} mice with Dp ointment, even without prior barrier disruption, remarkably enhanced both the clinical manifestations and the laboratory findings that correspond to indicators of human AD.

Discussion

Here, we demonstrated that *Flg*^{ft} mice exhibit spontaneous dermatitis with lymphadenopathy, elevated IgE levels, and skin barrier disruption in a steady state under SPF conditions. These outcomes are compatible with the features of human AD, which include chronic eczema, pruritus, and dry skin with elevated TEWL and serum IgE levels.[1–4,25,26] In addition, *Flg*^{ft} mice exhibit enhanced susceptibility to irritant contact dermatitis, CHS, and mite-induced dermatitis compared with B6 mice; these characteristics are also reminiscent of human AD. These results suggest that the barrier defect in this strain of mice leads to spontaneous dermatitis and enhances cutaneous immune responses and inflammation.

Since the first introduction of *Flg*^{ft} mice in 1972,[13] there have been only a few reports of these mice. The first report demonstrated that *Flg*^{ft} mice without the *ma* mutation showed flaky skin as early as postnatal day 2 but became normal in appearance by 3 to 4 weeks of age without spontaneous dermatitis except for their slightly smaller ears.[13] Later, the lack of filaggrin in the epidermis was proposed in the commercially available strain of *Flg*^{ft} mice used in this study, which has both *Flg* and *ma* mutations, as a model of ichthyosis vulgaris, and therefore the cutaneous inflammatory conditions from the perspective of AD was not discussed.[14] There have been three recent studies using *Flg*^{ft} mice as a model of filaggrin deficiency: Fallon et al[15] used *Flg*^{ft} mice from which the *ma* mutation had been eliminated with four additional backcrosses to B6 mice, and others used the commercially available *Flg*^{ft} mice.[16,17] The first report showed only a histological abnormality without clinical manifestations,[15] the second report demonstrated spontaneous eczematous skin lesions after 28 weeks of age,[17] and the third report did not indicate any spontaneous dermatitis in *Flg*^{ft} mice.[16] In our experiment, we observed a spontaneous dermatitis as early as 5 weeks of age with mild erythema and fine scales. These symptoms gradually exacerbated, accompanied by scratching, erosion, and edema, respectively, and became prominent at the age of 23 weeks. The discrepancies among these results seem to be related to the presence or absence of the *ma* mutation and/or variation in the genetic backgrounds of the different strains used and to environmental factors. It has been reported that Japan has higher morbidity for AD than other countries,[27,28] possibly attributable to environmental factors such as pollen.

It has been reported that TEWL, an indicator of inside-to-outside barrier function, is high in both AD patients with the *FLG* mutation[29] and *Flg*^{ft} mice.[15] In consideration of the immunological defense by the skin, however, it is more important to assess outside-to-inside barrier function rather than inside-to-outside barrier function. In fact, outside-to-inside barrier dysfunction has recently been proposed as the most important aspect in the pathogenesis of AD.[9,26] Scharschmidt et al[16] reported increased bidirectional paracellular permeability of water-soluble xenobiotes by ultrastructural visualization in *Flg*^{ft} mice, suggesting a defect of the outside-to-inside barrier. However, the quantitative measurement of this parameter has not been addressed. Here, we propose a new method for evaluating outside-to-inside barrier function quantitatively by measuring the penetrance of FITC through the skin. This method has a parallel correlation with the qualitative measurement of FITC penetrated in epidermis and an established method for skin permeability assay, the *in situ* dye staining method. Therefore, by using this new method, we were able to detect outside-to-inside barrier dysfunction in *Flg*^{ft} mice quantitatively.

The skin abnormality associated with AD is well known to be a predisposing factor to sensitive skin[30,31] and allergic contact dermatitis,[32,33] but patients with AD produce a tuberculin response similar to that of healthy control subjects.[34,35] In humans, sensitive skin is defined as reduced tolerance to cutaneous stimulation, with symptoms ranging from visible signs of irritation to subjective neurosensory discomfort.[30,31] The question of whether human AD patients are more prone to allergic contact dermatitis than nonatopic individuals is still controversial.[33] To address this question, we evaluated skin responsiveness to PMA as an irritant and found that irritant contact dermatitis was enhanced in *Flg*^{ft} mice. In addition, *Flg*^{ft} mice showed an increased skin-sensitized CHS reaction, a form of classic Th1- and Tc1-mediated delayed-type hypersensitivity to haptens, emphasized by increased IFN-γ production. In contrast, when mice were sensitized intraperitoneally, no difference was observed between *Flg*^{ft} and B6 mice *in vivo* or *in vitro*. This finding is consistent with the observation that humans with and without AD respond comparably to tuberculin tests[34,35] and suggests that skin barrier function regulates cutaneous immune conditions, which hints at a possible mechanism involved in human AD.

Clinical studies have provided evidence that a house dust mite allergen plays a causative or exacerbating role in human AD[36] and that a strong correlation exists between patients with *FLG* null alleles and house dust mite-specific IgE.[37] AD-like skin lesions can be induced by repeated topical application of a mite allergen in NC/Nga mice but not in BALB/c mice.[23] In the present study, we induced skin lesions that were clinically and histologically similar to AD, along with increased TEWL, increased scratch behavior, and increased levels of mite-specific IgE, in *Flg*^{ft} mice through the application of Dp. Dp is a common aeroallergen that is frequently involved in induction of human AD. It has protease activities, spe-

cifically from Der p1, Der p3, and Der p9, which may activate protease-activated receptor-2 in human keratinocytes.[38,39] A recent report has shown that activation of protease-activated receptor-2 through Dp application significantly delays barrier recovery rate in barrier function-perturbed skin or compromised skin.[39] Therefore, Dp may play a dual role in the onset of AD, both as an allergen and proteolytic signal and as a perturbation factor of the barrier function, leading to the persistence of eczematous skin lesions in AD.[39,40]

To address the issue of variable genetic background, we observed immune responses in mice of other genotypes, such as BALB/c and C3H, as controls, but both of these lines exhibited much less severe CHS responses compared with those in *Flg^{ft}* mice (data not shown), suggesting that the enhanced immune responses seen in *Flg^{ft}* mice were not solely due to their genetic background. The effect of the *ma* mutation in relation to the *ft* mutation in commercially available *Flg^{ft}* mice in the development of AD-like skin lesions needs to be clarified in future studies. Furthermore, our study showed that heterozygous mice intercrossed with *Flg^{ft}* mice and B6 mice did not develop spontaneous dermatitis. In this way they are unlike human AD patients, most of whom are heterozygous for the *FLG* mutation. Not only human studies but also additional mouse studies are required to clarify these relationships.

In this study, we have shown that *Flg^{ft}* mice exhibit spontaneous dermatitis resembling human AD, enhanced irritation dermatitis and a contact hypersensitivity response, and mite-induced AD-like skin lesions, which provide hints for possible mechanisms in the human disease. These results suggest that *Flg^{ft}* mice have the potential to serve as an animal model of human AD and further accentuate the important role of filaggrin in skin barrier function in the pathogenesis of AD.

References

1. Jin H, He R, Oyoshi M, Geha RS: Animal models of atopic dermatitis. J Invest Dermatol 2009, 129:31–40
2. Wollenberg A, Bieber T: Atopic dermatitis: from the genes to skin lesions. Allergy 2000, 55:205–213
3. Novak N, Bieber T, Leung DY: Immune mechanism leading to atopic dermatitis. J Allergy Clin Immunol 2003, 112:S128–S139
4. Elias PM, Hatano Y, Williams ML: Basis for the barrier abnormality in atopic dermatitis: outside-inside-outside pathogenic mechanism. J Allergy Clin Immunol 2008, 121:1337–1343
5. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, Goudie DR, Sandilands A, Campbell LE, Smith FJ, O'Regan GM, Watson RM, Cecil JE, Bale SJ, Compton JG, DiGiovanna JJ, Fleckman P, Lewis-Jones S, Arseculeratne G, Sergeant A, Munro CS, El Houate B, McElreavey K, Halkjaer LB, Bisgaard H, Mukhopadhyay S, McLean WH: Common loss-of function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 2006, 38:441–446
6. Nomura T, Sandilands A, Akiyama M, Liao H, Evans AT, Sakai K, Ota M, Sugiura H, Yamamoto K, Sato H, Palmer CN, Smith FJ, McLean WH, Shimizu H: Unique mutations in the filaggrin gene in Japanese patients with ichthyosis vulgaris and atopic dermatitis. J Allergy Clin Immunol 2007, 119:434–440
7. Morar N, Cookson WO, Harper JI, Moffat MF: Filaggrin mutations in children with severe atopic dermatitis. J Invest Dermatol 2007, 127:1667–1672
8. Dale BA: Filaggrin, the matrix protein of keratin. Am J Dermatopathol 1985, 7:65–68
9. Listwan P, Rothnagel JA: Keratin bundling proteins. Methods Cell Biol 2004, 78:817–827
10. Candi E, Schmidt R, Melino G: The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 2005, 6:328–340
11. Gan SQ, McBride OW, Idler WW, Markova N, Steinert RM: Organization, structure, and polymorphisms of the human profilaggrin gene. Biochemistry 1990, 29:9432–9440
12. Shiohara T, Hayakawa J, Mizukawa Y: Animal models for atopic dermatitis: are they relevant to human disease? J Dermatol Sci 2004, 36:1–9
13. Lane P: Two new mutations in linkage group XVI of the house mouse. J Hered 1972, 63:135–140
14. Presland RB, Boggess D, Lewis SP, Hull C, Fleckman P, Sundberg JP: Loss of normal profilaggrin and filaggrin in flaky tail (ft/ft) mice: an animal model for the filaggrin-deficient skin disease ichthyosis vulgaris. J Invest Dermatol 2000, 115:1072–1081
15. Fallon PG, Sasaki T, Sandilands A, Campbell LE, Saunders SP, Mangan NE, Callanan JJ, Kawasaki H, Shiohama A, Kubo A, Sundberg JP, Presland RB, Fleckman P, Shimizu N, Kudoh J, Irvine AD, Amagai M, McLean WH: A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. Nat Genet 2009, 41:602–608
16. Scharschmidt TC, Man MQ, Hatano Y, Crumrine D, Gunathilake R, Sundberg JP, Silva KA, Mauro TM, Hupe M, Cho S, Wu Y, Celli A, Schmuth M, Feingold KR, Elias PM: Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens. J Allergy Clin Immunol 2009, 124:496–506, 506.e1–e6
17. Oyoshi MK, Murphy GF, Geha RS: Filaggrin-deficient mice exhibit Th17-dominated skin inflammation and permissiveness to epicutaneous sensitization with protein antigen. J Allergy Clin Immunol 2009, 124:475–493, 493.e1
18. Leung DY, Hirsch RL, Schneider L, Moody C, Takaoka R, Li SH, Meyerson LA, Mariam SG, Goldstein G, Hanifin JM: Thymopentin therapy reduces the clinical severity of atopic dermatitis. J Allergy Clin Immunol 1990, 85:927–933
19. Mori T, Kabashima K, Yoshiki R, Sugita K, Shiraishi N, Onoue A, Kuroda E, Kobayashi M, Yamashita U, Tokura Y: Cutaneous hypersensitivities to hapten are controlled by IFN-γ-upregulated keratinocyte Th1 chemokines and IFN-γ-downregulated Langerhans cell Th2 chemokines. J Invest Dermatol 2008, 128:1719–1727
20. Orito K, Chida Y, Fujisawa C, Arkwright PD, Matsuda H: A new analytical system for quantification scratching behavior in mice. Br J Dermatol 2004, 150:33–38
21. Gupta J, Grube E, Ericksen MB, Stevenson MD, Lucky AW, Sheth AP, Assa'ad AH, Khurana Hershey GK: Intrinsically defective skin barrier function in children with atopic dermatitis correlates with disease severity. J Allergy Clin Immunol 2008, 121:725–730
22. Novak N: New insights into the mechanism and management of allergic diseases: atopic dermatitis. Allergy 2009, 64:265–275
23. Kang JS, Lee K, Han SB, Ahn JM, Lee H, Han MH, Yoon YD, Yoon WK, Park SK, Kim HM: Induction of atopic eczema/dermatitis syndrome-like skin lesions by repeated topical application of a crude extract of Dermatophagoides pteronyssinus in NC/Nga mice. Int Immunopharmacol 2006, 6:1616–1622
24. Yamamoto M, Haruna T, Yasui K, Takahashi H, Iduhara M, Takaki S, Deguchi M, Arimura A: A novel atopic dermatitis model induced by topical application with Dermatophagoides farinae extract in NC/Nga mice. Allergol Int 2007, 56:139–148
25. Matsumoto M, Ra C, Kawamoto K, Sato H, Itakura A, Sawada J, Ushio H, Suto H, Mitsuishi K, Hikasa Y, Matsuda H: IgE hyperproduction through enhanced tyrosine phosphorylation of Janus kinase 3 in NC/Nga mice, a model for human atopic dermatitis. J Immunol 1999, 162:1056–1063
26. Cooper D, Hales J, Camp R: IgE-dependent activation of T cells by allergen in atopic dermatitis: pathophysiologic relevance. J Invest Dermatol 2004, 123:1086–1091
27. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee: Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet 1998, 351:1225–1232
28. Williams H, Robertson C, Stewart A, Aït-Khaled N, Anabwani G,

Anderson R, Asher I, Beasley R, Björkstén B, Burr M, Clayton T, Crane J, Ellwood P, Keil U, Lai C, Mallol J, Martinez F, Mitchell E, Montefort S, Pearce N, Shah J, Sibbald B, Strachan D, von Mutius E, Weiland S: Worldwide variations in the prevalence of symptoms of atopic eczema in the international study of asthma and allergies in childhood. J Allergy Clin Immunol 1999, 103:125–138

29. Nemoto-Hasebe I, Akiyama M, Nomura T, Sandilands A, McLean WHI, Shimizu H: Clinical severity correlates with impaired barrier in filaggrin-related eczema. J Invest Dermatol 2009, 129:682–689
30. Willis CM, Shaw S, De Lacharrière O, Baverel M, Reiche L, Jourdain R, Bastien P, Wilkinson JD: Sensitive skin: an epidemiological study. Br J Dermatol 2001, 145:258–263
31. Farage MA, Katsarou A, Maibach HI: Sensory, clinical and physiological factors in sensitive skin: a review. Contact Dermatitis 2006, 55:1–14
32. Clayton TH, Wilkinson SM, Rawcliffe C, Pollock B, Clark SM: Allergic contact dermatitis in children: should pattern of dermatitis determine referral? A retrospective study of 500 children tested between 1995 and 2004 in one UK centre. Br J Dermatol 2006, 154:114–117
33. Mailhol C, Lauwers-Cances V, Rance F, Paul C, Giordano-Labadie F: Prevalence and risk factors for allergic contact dermatitis to topical treatment in atopic dermatitis: a study in 641 children. Allergy 2009, 64:801–806
34. Yilmaz M, Bingöl G, Altintas D, Kendirli SG: Correlation between atopic diseases and tuberculin responses. Allergy 2000, 55:664–667
35. Grüber C, Kulig M, Bergmann R, Guggenmoos-Holzmann I, Wahn U, MAS-90 Study Group: Delayed hypersensitivity to tuberculin, total immunoglobulin E, specific sensitization, and atopic manifestation in longitudinally followed early Bacille Calmette-Guérin-vaccinated and nonvaccinated children. Pediatrics 2001, 107:e36
36. Kimura M, Tsuruta S, Yoshida T: Correlation of house dust mite-specific lymphocyte proliferation with IL-5 production, eosinophilia, and the severity of symptoms in infants with atopic dermatitis. J Allergy Clin Immunol 1998, 101:85–89
37. Henderson J, Northstone K, Lee SP, Liao H, Zhao Y, Pembrey M, Mukhopadhyay S, Smith GD, Palmer CN, McLean WH, Irvine AD: The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study. J Allergy Clin Immunol 2008, 121:872–877
38. Vasilopoulos Y, Cork MJ, Teare D, Marinou I, Ward SJ, Duff GW, Tazi-Ahnini R: A nonsynonymous substitution of cystatin A, a cysteine protease inhibitor of house dust mite protease, leads to decreased mRNA stability and shows a significant association with atopic dermatitis. Allergy 2007, 62:514–519
39. Jeong SK, Kim HJ, Youm JK, Ahn SK, Choi EH, Sohn MH, Kim KE, Hong JH, Shin DM, Lee SH: Mite and cockroach allergens activate protease-activated receptor 2 and delay epidermal permeability barrier recovery. J Invest Dermatol 2008, 128:1930–1939
40. Roelandt T, Heughebaert C, Hachem JP: Proteolytically active allergens cause barrier breakdown. J Invest Dermatol 2008, 128:1878–1880

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ORIGINAL ARTICLE

Impact of Sedative and Non-Sedative Antihistamines on the Impaired Productivity and Quality of Life in Patients with Pruritic Skin Diseases

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ABSTRACT

Background: The impairment that pruritic skin diseases have on patient productivity at work, in the classroom, and in daily activities is substantial and needs to be characterized. The objective of this study was to determine how pruritic skin diseases impact patient productivity and quality of life (QOL), in order to improve the measurement of these endpoints to allow the influence of treatment options including sedative and non-sedative antihistamines to be analyzed.

Methods: The impact of pruritic skin diseases and the effect of antihistamine therapy on work, classroom, and daily productivity were evaluated using the Work Productivity Assessment Index-Allergy Specific Questionnaire. The intensity of itch and patient QOL were assessed using a visual analogue scale and Skindex-16, respectively.

Results: Pruritic skin diseases resulted in significant impairment of work, classroom, and daily productivity. The severity of overall work impairment in atopic dermatitis (AD), urticaria, and prurigo was higher than for other diseases analyzed. However, classroom activity was more adversely affected in patients with urticaria relative to other diseases. All pruritic diseases in this study negatively impacted daily activity to a similar degree. Impaired productivity was significantly improved in patients taking non-sedative antihistamines for 1 month, and the improvements correlated with the alleviation of itch and improved QOL.

Conclusions: These results indicate that pruritic skin diseases reduce patient productivity at work, in the classroom, and during daily activities, and that non-sedative antihistamines may offer an advantage over sedative antihistamines for alleviating certain negative consequences of these skin diseases.

KEY WORDS

antihistamine, productivity, pruritic, quality-of-life, skin diseases, WPAI-AS

INTRODUCTION

The impaired quality of life (QOL) and diminished work and classroom productivity of individuals with pruritic skin diseases is a matter of public concern.[1,2] Furthermore, estimates of the impact of pruritic skin diseases on the economic loss in businesses and school performance records have attracted a great deal of interest worldwide.[3,4] Similar unfavorable impacts were identified for certain skin diseases, such as chronic idiopathic urticaria, psoriasis, and chronic hand dermatitis.[5-8] The Work Productivity Assessment Index (WPAI) is commonly used to determine the impact of health and disease on certain parameters related to patient productivity. According to the WPAI, the estimated percent of overall work impairment due to psoriasis, urticaria, and chronic hand dermatitis is 15%, 25%, and 29%, respectively.[5,6,8]

Itching is a key characteristic of allergic skin diseases that dramatically affects a patient's quality of

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life.[9,10] Thus, it is possible that itching alone would affect patient performance in the work place. The allergy specific WPAI (WPAI-AS) can be used to more effectively assess productivity in these patients as itching is a common symptom of allergy-related skin diseases. Recently, we reported the effect of antihistamines on productivity of patients with pruritic skin diseases using the WPAI-AS assessment questionnaire.[11,12] On average, pruritic skin diseases impaired overall workplace productivity, classroom productivity, and daily activity by 39%, 45%, and 42% at baseline, respectively.[12] Furthermore, non-sedative antihistamines (mainly fexofenadine) reduced the intensity of itch and improved work productivity. In contrast, sedative antihistamines failed to improve work productivity, but significantly decreased itch intensity.[12] However, the relative impact of different pruritic diseases on work productivity has not been assessed. In this report, the WPAI-AS evaluation system was applied to each subgroup of patients with different diagnoses of pruritic skin diseases, and the degree of impairment for each disease at baseline was compared using a linear least-squares method. Furthermore, itch severity and patient QOL were assessed using a visual analogue scale (VAS) and Skindex-16, respectively. Finally, after validating the relationships between these parameters, we propose a method to approach the treatment of pruritic skin disease that will improve overall productivity in the workplace, in the classroom, and in daily activities.

METHODS

PATIENTS AND STUDY DESIGN

This study was conducted between April, 2008 and March, 2009. After obtaining approval from the Institutional Review Board (IRB), patients with pruritic skin diseases (n = 216) from Osaka University Hospital or its affiliated hospitals, gave informed consent to participate in this study. The final number of valid responses was n = 206 (male : female=93 : 113; mean age ± SD: 52 ± 20 years). Patients with skin diseases associated with underlying systemic diseases (e.g., serious liver disease, renal dysfunction, and blood diseases), history of epilepsy, history of a previous drug allergy, or women who were pregnant or lactating were excluded from this study. Participants received no medical attention during the week before study initiation. The selection of therapy for each patient, such as oral antihistamines versus external medicine (e.g., steroid ointments, tacrolimus ointments, or certain moisturizers), was left to the physician's discretion (open-label trial). Fexofenadine (n = 72) and loratadine (n = 2), anti-histamines for which the package insert contained no cautionary statement regarding sedative actions, were categorized as "non-sedative". All other antihistamines were classified as "sedative".

STUDY INSTRUMENTS

The Skindex-16 quality-of-life instrument[13] was used to measure the effect of pruritic skin diseases on QOL. The magnitude of the itch sensation was assessed using a VAS (0-100, "0" indicates no-symptom, and "100" indicates most severe symptom). Work and classroom productivity were assessed with the WPAI-AS instrument (score range, 0-100%; higher percentages indicate higher productivity).[11] Work productivity, classroom productivity, and daily activity impairment (%I) were calculated by the effects of the pruritic skin diseases on productivity while working/attending class or other daily activities during the past 7 days. The percentage of work/classroom time missed (%TM = TM/TW) was calculated by the number of work/classroom hours missed due to allergy (TM) and the usual number of hours worked/attending class (TW). Finally, the percentage overall impairment was calculated as follows: %TM + ([100 - %TM] × I%) = % overall impairment.[11] These instruments were patient-administered before (baseline) and 1 month after treatment initiation.

STATISTICAL ANALYSIS

The one-sample t-test was used for analysis of differences between two groups. Pearson's product-moment correlation coefficient was used to determine the significance of correlations between two parameters (Table 1, 2). To examine the significance of the contingency between the certain categorical data, Fisher's exact test (for evaluating the significance between the two kinds of classifications) and Cochran-Mantel-Haenszel general association statistics (for evaluating more than 3 kinds of classifications) were performed (Table 3). The bias of evaluative consequences to one variable was analyzed using univariate analysis (Table 4). A linear least-squares method was used to evaluate the degree of impairment in each disease at baseline. Because heterogeneity of starting values was inevitable, the effect measures illustrated in Figure 1 were evaluated using linear models. The results and confidence intervals for the improvement variations were compared visually for each parameter using a forest plot. Improvement variations (change ratios) were calculated as follows: change ratio = (evaluated value 1 month after the initiation of treatment-baseline value)/(baseline value). In all tests, values of $P < 0.05$ were considered statistically significant.

RESULTS

STUDY POPULATION CHARACTERISTICS

A total of 216 patients with pruritic skin disease entered the study, and data from 206 patients (average age of 52 ± 20 years) who completed the study were used for analysis. Company employees and part-time workers represented 48% of the patients (n = 99), and retired seniors and unemployed individuals ac-

Table 1 Correlations between baseline parameters and patient outcomes

	Correlations to baseline patient parameters [*P*-value, Pearson's coefficient of correlation (r), *n*]		
Allergic pruritic skin diseases (AD and urticaria)	Itch VAS	Skindex-16 score	Activity impairment
Overall work productivity impairment	NS ($r = 0.2443$, $n = 52$)	$P < 0.001$ ($r = 0.5674$, $n = 51$)	$P < 0.001$ ($r = 0.6712$, $n = 52$)
Overall classroom productivity impairment	NS ($r = 0.1948$, $n = 14$)	NS ($r = 0.0915$, $n = 13$)	NS ($r = 0.1833$, $n = 14$)
Activity impairment	$P = 0.006$ ($r = 0.2893$, $n = 89$)	$P < 0.001$ ($r = 0.7051$, $n = 84$)	- -
Non-allergic skin diseases (All other excluding AD and urticaria)			
Overall work productivity impairment	NS ($r = 0.2904$, $n = 44$)	$P < 0.001$ ($r = 0.4813$, $n = 46$)	$P < 0.001$ ($r = 0.8584$, $n = 47$)
Overall classroom productivity impairment	NS ($r = 0.2604$, $n = 4$)	NS ($r = 0.7963$, $n = 4$)	$P = 0.0014$ ($r = 0.9986$, $n = 4$)
Activity impairment	$P < 0.001$ ($r = 0.3332$, $n = 107$)	$P < 0.001$ ($r = 0.5170$, $n = 109$)	- -

NS, not statistically significant; vs., versus.

Table 2 Correlative relationships between antihistamine treatment groups and the improvement ratio of itch VAS scores to Skindex-16, overall work productivity impairment, and activity impairment

	Correlations to baseline patient improvement ratios by treatment group [*P*-value, Pearson's coefficient of correlation (r), *n*]	
	Non-sedative AH	Sedative AH
Skindex-16 score vs. itch VAS	$P < 0.001$ ($r = 0.5769$, $n = 69$)	NS ($r = 0.2360$, $n = 99$)
Overall work productivity impairment vs. itch VAS	$P = 0.0042$ ($r = 0.4539$, $n = 38$)	NS ($r = 0.2462$, $n = 46$)
Activity impairment vs. itch VAS	$P = 0.0046$ ($r = 0.3448$, $n = 66$)	NS ($r = 0.1203$, $n = 92$)

NS, not statistically significant; AH, anti-histamines; VAS, visual analogue scale.

counted for 43% ($n = 89$). Students made up a relatively small fraction of the study group ($n = 18$, 9%). Patients diagnosed with eczema/dermatitis had the highest representation (36%) among participants, followed in decreasing order by patients with urticaria, atopic dermatitis (AD), pruritus, prurigo, and psoriasis (Table 5).

ASSESSMENT OF WORK, CLASSROOM, AND ACTIVITY IMPAIRMENT

Table 6 shows the baseline work, classroom, and daily activity WPAI-AS productivity scores. Due to the relatively small sample size of each disease group, statistically significant differences in impairment between disease groups were not detected (Fig. 2). However, the results indicate that the overall impairment of work, classroom, and daily activity productivity tended to be larger in the atopic dermatitis, eczema/dermatitis, and urticaria disease groups (Fig. 2). There were also some interesting group-specific observations. Prurigo showed higher overall impairment of work productivity and daily activity. Individuals with urticaria had relatively higher percentages of impairment of overall classroom productivity than that observed in other skin diseases. Daily activity was impaired at high percentages for individuals with AD.

CORRELATION BETWEEN PRODUCTIVITY IMPAIRMENT AND SKINDEX-16, OR LOSS OF DAILY LIFE PRODUCTIVITY

To check the validity of the assessment procedures in this study, we looked for correlations between impaired productivity at work, in the classroom, and in daily activities. In addition, correlations between overall activity impairment, the magnitude of itch sensation as assessed by VAS, and QOL measures as assessed by Skindex-16 were analyzed (Table 1). As shown in Table 1, correlation analyses were divided between allergic (atopic dermatitis and urticaria) and non-allergic skin diseases (all other diagnosis groups). Results specific for allergic skin diseases indicated that impairment in overall work productivity showed a positive correlation with the itch VAS, Skindex-16, and the impairment in daily activity. A correlation between impairment in overall classroom

Table 3 Distribution of patient characteristics in the sedative and non-sedative antihistamine treatment groups

Background factors		Non-sedative AH		Sedative AH		*P*-value†
		n	%	*n*	%	
Age	<50	42	56.8	55	45.8	0.183
	≥50	32	43.2	65	54.2	
Gender	Male	37	50.0	48	40.0	0.183
	Female	37	50.0	72	60.0	
Disease	AD	20	27.0	22	18.3	0.515
	Ec/der	26	35.1	45	37.5	
	Urticaria	16	21.6	33	27.5	
	Other	12	16.2	20	16.7	
Occupation	Worker	45	60.8	51	42.5	0.017
	Student	8	10.8	10	8.3	
	Other	21	28.4	59	49.2	
Duration of disease	<5 years	46	62.2	74	61.7	1.000
	≥5 years	21	28.4	34	28.3	

†Differences in the distribution of patients between sedative and non-sedative antihistame groups was determined by the Fisher's exact test for age, gender, and duration of disease and by the Cochran-Mantel-Haenszel general association statistic for disease diagnostic group and occupation. AH, antihistamines; AD, atopic dermatitis; Ec/der, eczema/dermatitis.

Table 4 Impact of background factors on the improvement of WPAI-AS score

Patient characteristics	Impact of patient characteristics on overal productivity impairment (*P*-value)		
	Overall work impairment	Overall classroom impairment	Daily activity impairment
Age	0.345	0.2986	0.3556
Gender	0.4454	0.5464	0.2615
Disease	0.0646	0.5349	0.4118
Duration of disease: <5 years, ≥5 years	0.0053	0.4793	0.2528
Occupation: worker, student, other	N/A	N/A	0.5097

N/A, not applicable.

productivity and itch VAS, Skindex-16 score, and activity impairment was not observed for the allergic skin diseases (Table 1). However, in the allergic skin disease subgroup there was a positive correlation between the impairment in daily activity and the magnitude of itch and Skindex-16 scores.

Similar analyses were performed on the subgroup of patients with all other skin disease diagnoses except atopic dermatitis and urticaria. This group was designated the non-allergic skin disease group even though varying causative conditions including allergic and non-allergic mechanisms could be responsible for symptoms related to eczema/dermatitis. As shown in Table 1, the correlation profile of this subgroup was very similar to that of the allergic skin disease subgroup with one major difference. There was a significant correlation between overall classroom productivity and activity impairment in the non-allergic skin disease subgroup (Table 1).

IMPACT OF ANTIHISTAMINES ON PATIENT OUTCOMES

Patients were treated with non-sedative antihistamines (n = 74), sedative antihistamines (n = 121), or external medication (n = 11) for a duration of 1 month (Table 7). The patient characteristics in the physician-assigned treatment groups of sedative and non-sedative antihistamines were all well-matched with the exception of occupation (Table 3). We previously reported that the impaired productivity in pruritic skin diseases was significantly improved in patients taking non-sedative antihistamines.[12] Interestingly, for patients taking non-sedative antihistamines in this study, the improvement ratio as assessed using the VAS score showed a significant correlation with improvements in the Skindex-16 score, the reduction in overall work productivity impairment, and the reduction in daily activity impairment. No significant correlations were found among patients taking sedative antihistamines (Table 2).

To eliminate the bias for starting value dispersion, the effects of non-sedative and sedative antihistamines on overall work productivity, daily activity, and overall classroom productivity were corrected by grouping according to background factors or baseline value using the linear least-squares methods (Fig. 1A). Results indicated that non-sedative antihistamines produced greater overall improvements in pro-

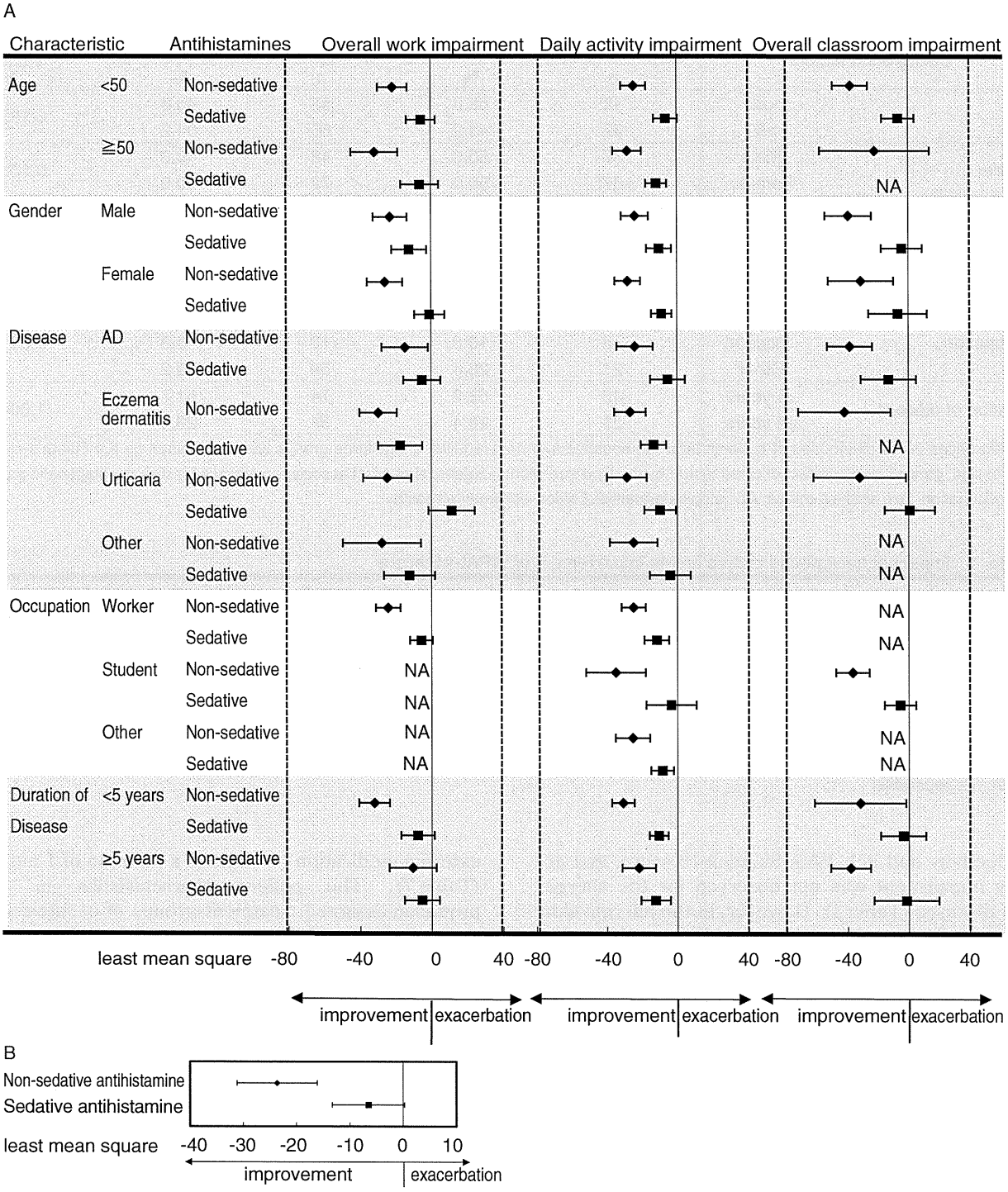

Fig. 1 **A.** The impact of antihistamines on overall work productivity impairment, activity productivity impairment, and overall classroom productivity impairment per-certain parameters of pruritic skin diseases. Changes in the evaluated value of certain parameters from baseline were adjusted with background factors and the initial value (a linear model). Results are shown in a forest plot. Horizontal lines indicate 95% confidence intervals. The rhomboid or square dot on center of the horizontal line indicates the point estimate. Significance is indicated by horizontal lines that do not overlap with the vertical line of least mean square = 0. NA, not applicable. **B.** Comparison of overall work impairment (amount of change) adjusted by background factor (disease duration).

Table 5 Characteristics of patient population by pruritic skin disease diagnostic group

Disease	(*n*)	Male	Female	Average age (yrs ± SD)	Average duration of disease (yrs ± SD)
Atopic dermatitis	43	21	22	33.7 ± 10.1	17.1 ± 13.2
Eczema/dermatitis	75	33	42	61.9 ± 17.8	3.1 ± 8.1
Urticaria	50	17	33	47.3 ± 16.3	5.4 ± 10.1
Pruritus	14	9	5	64.3 ± 18.1	3.4 ± 3.6
Prurigo	8	6	2	59.8 ± 16.6	2.1 ± 1.5
Psoriasis	7	4	3	49.3 ± 19.6	1.1 ± 1.4
Others†	9	3	6	54.7 ± 18.2	10.8 ± 14.9

†Includes patients with systemic lupus erythematodes, tinea pedis, toxicoderma, polymorphic light eruption, von Recklinghausen disease, tuberous sclerosis, scabies, bullous pemphigoid, and lupus erythematodes.

Table 6 Baseline WPAI-AS productivity scores (Mean ± SD)

	AD	Ec/Der	Urticaria	Pruritus	Prurigo	Psoriasis	Others
Work	(*n* = 31)	(*n* = 31)	(*n* = 21)	(*n* = 2)	(*n* = 5)	(*n* = 3)	(*n* = 6)
Work productivity impairment	38.7 ± 26.3	41.0 ± 24.8	33.8 ± 25.8	20.0 ± 0	36.0 ± 18.2	26.7 ± 25.2	23.3 ± 29.4
Work time missed	4.9 ± 11.4	2.6 ± 10.3	10.6 ± 26.8	0	12.2 ± 21.7	2.2 ± 3.8	0
Overall work productivity impairment	40.4 ± 26.8	41.3 ± 25.2	41.8 ± 29.5	20.0 ± 0	42.9 ± 24.8	28.9 ± 21.7	23.3 ± 29.4
Classroom	(*n* = 8)	(*n* = 1)	(*n* = 6)	(*n* = 1)	(*n* = 0)	(*n* = 1)	(*n* = 1)
Classroom productivity impairment	41.3 ± 25.3	50.0	63.3 ± 15.1	0	-	0	10
Classroom time missed	0	0	14.5 ± 17.8	0	-	0	0
Overall classroom productivity	41.3 ± 25.3	50.0	70.1 ± 10.5	0	-	0	10
Activity	(*n* = 43)	(*n* = 72)	(*n* = 46)	(*n* = 14)	(*n* = 8)	(*n* = 7)	(*n* = 9)
Activity impairment	50.2 ± 26.9	41.8 ± 23.0	37.6 ± 26.4	37.9 ± 20.1	46.3 ± 22.0	44.3 ± 28.8	34.4 ± 29.2

AD, atopic dermatitis; Ec/Der, Eczema/Dermatitis; SD, Standard deviation.

ductivity in patients with skin diseases than sedative antihistamines (Fig. 1A). Non-sedative antihistamines significantly improved work productivity under almost all background conditions with the exception of disease duration. Sedative antihistamines only had a significant impact on the subpopulation of patients that were male or those that had a diagnosis of eczema/dermatitis (Fig. 1A).

The duration of disease was the only baseline patient characteristic that could significantly influence or bias the outcomes seen from administration of antihistamines (Table 4). Therefore, we compared the amount of change in the overall work impairment in the sedative and non-sedative antihistamine treatment groups after adjusting for the baseline duration of disease (Fig. 1B). These results confirmed that non-sedative antihistamines significantly improved the overall work impairment, while sedative antihistamines did not (Fig. 1B). Evaluation of impact of antihistamines on daily activity impairment and overall classroom impairment also demonstrate the superiority of non-sedative antihistamines over sedative antihistamines (Fig. 1A). Interestingly, sedative antihistamines failed to improve overall classroom productivity in all the patient population groups analyzed (Fig. 1A).

THE EFFECT OF ANTIHISTAMINES ON ATOPIC DERMATITIS

The effect of antihistamines on atopic dermatitis is still controversial.[14,15] Therefore, the treatment effects specifically for patients with atopic dermatitis (n = 43) were analyzed independently from other diagnostic groups (Fig. 3). As expected, treatment with antihistamines significantly reduced itch intensity in atopic dermatitis, while external medicines were ineffective (Fig. 3A). No differences were found between patients taking non-sedative versus sedative antihistamines (Fig. 3A). The impact of all treatments on the Skindex-16 QOL measure was similar to that for the itch VAS, with a significant effect for all antihistamines, but not for topical medications (Fig. 3B). Both non-sedative, and sedative antihistamines improved overall work impairment without statistical significance (Fig. 3C). Alternatively, the non-sedative antihistamine significantly reduced activity productivity impairment, whereas the trend towards improvement seen with sedative antihistamines did not reach statistical significance (Fig. 3D). These patients were prescribed concomitant external medications, but there were no remarkable differences between the non-sedative and sedative antihistamines treatment groups (Fig. 3E).

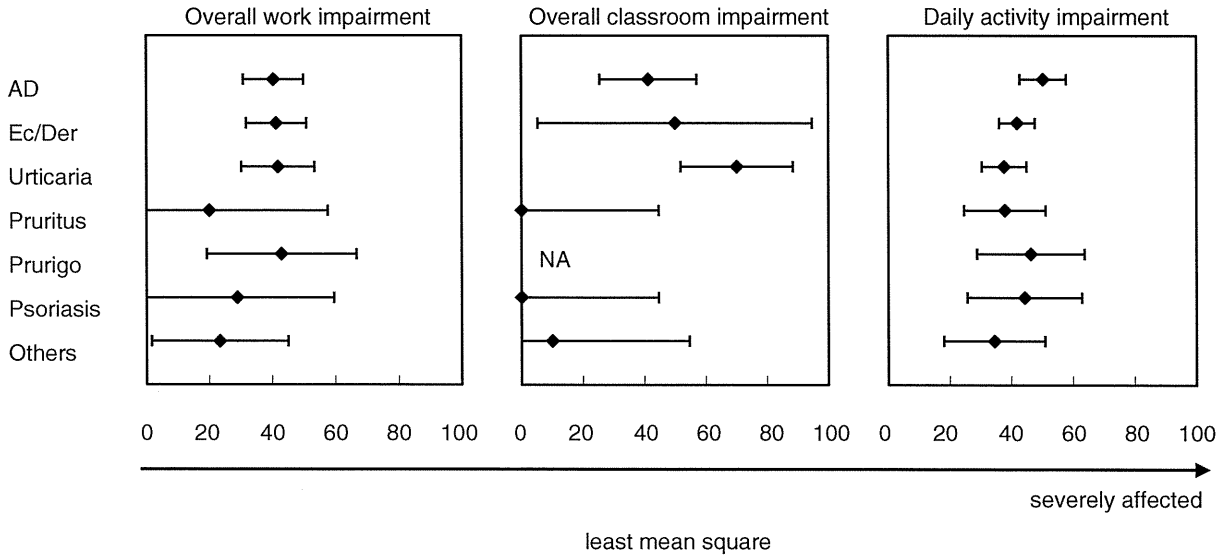

Fig. 2 Forest plots demonstrating the degree of impairment in each disease at baseline was evaluated using a linear least-squares method. Horizontal lines represent 95% confidence intervals. The rhomboid dot on the center of horizontal line indicates the point estimate. NA, not applicable.

Table 7 Number of patients from each skin disease diagnostic group assigned to indicated treatments

	Sedation	*n*	AD	Ec/Der	Uriticaria	Pruritus	Prurigo	Psoriasis	Others
Fexofenadine	NS	72	20	26	14	5	1	1	5
Loratadine	NS	2	0	0	2	0	0	0	0
Olopatadine	S	53	8	18	18	2	3	3	1
Epinastine	S	8	3	3	0	0	0	2	0
Cetirizine	S	9	2	6	0	0	1	0	0
Ebastine	S	11	1	7	1	1	0	0	1
Other 2nd generation	S	19	5	5	7	1	0	0	1
1st generation	S	21	3	6	7	2	2	1	0
External medicine	-	11	1	4	1	3	1	0	1
Total	-	206	43	75	50	14	8	7	9

AH, anti-histamines; NS, non-sedative; S, sedative; AD, atopic dermatitis; Ec/Der, Eczema/Dermatitis.

DISCUSSION

This study demonstrates that allergic skin diseases may have detrimental effects on productivity at work, in the classroom, and during daily activity. Previous reports demonstrated that allergic rhinitis impaired mean overall productivity at work, in the classroom, and in daily activity by ratios of 27-48%, 33-47%, and 42-51%, respectively.[16-19] In the present study, work performance and daily activities were highly and similarly impaired in patients with allergic skin diseases. However, WPAI-AS baseline scores in our study were slightly high relative to previous reports of WPAI (unidentified version) baseline scores for chronic idiopathic urticaria, psoriasis, and chronic hand dermatitis.[5,6,8] It is not currently clear why the present study generated different WPAI baseline scores, but further investigation is warranted.

According to the WPAI-AS values for the various pruritic skin diseases, the impairments in classroom productivity and overall classroom productivity were higher for patients with urticaria (Fig. 2). To clarify the reason why urticaria affected classroom productivity, cases of students with urticaria were analyzed independently for correlations with certain parameters (data not shown). Only the Skindex-16 was significantly associated with classroom impairment in this group ($P = 0.0075$, $r = 0.9282$, $n = 6$). Presumably, urticaria may impair a student's classroom productivity by negatively impacting their QOL.

In previous reports, WAPI scores of overall work impairment in patients with psoriasis were lower than those for patients with chronic idiopathic urticaria and chronic hand dermatitis.[5,6,8] Pearce and colleagues[6] discussed the observation that QOL measures did not exhibit the same trend as WPAI score in

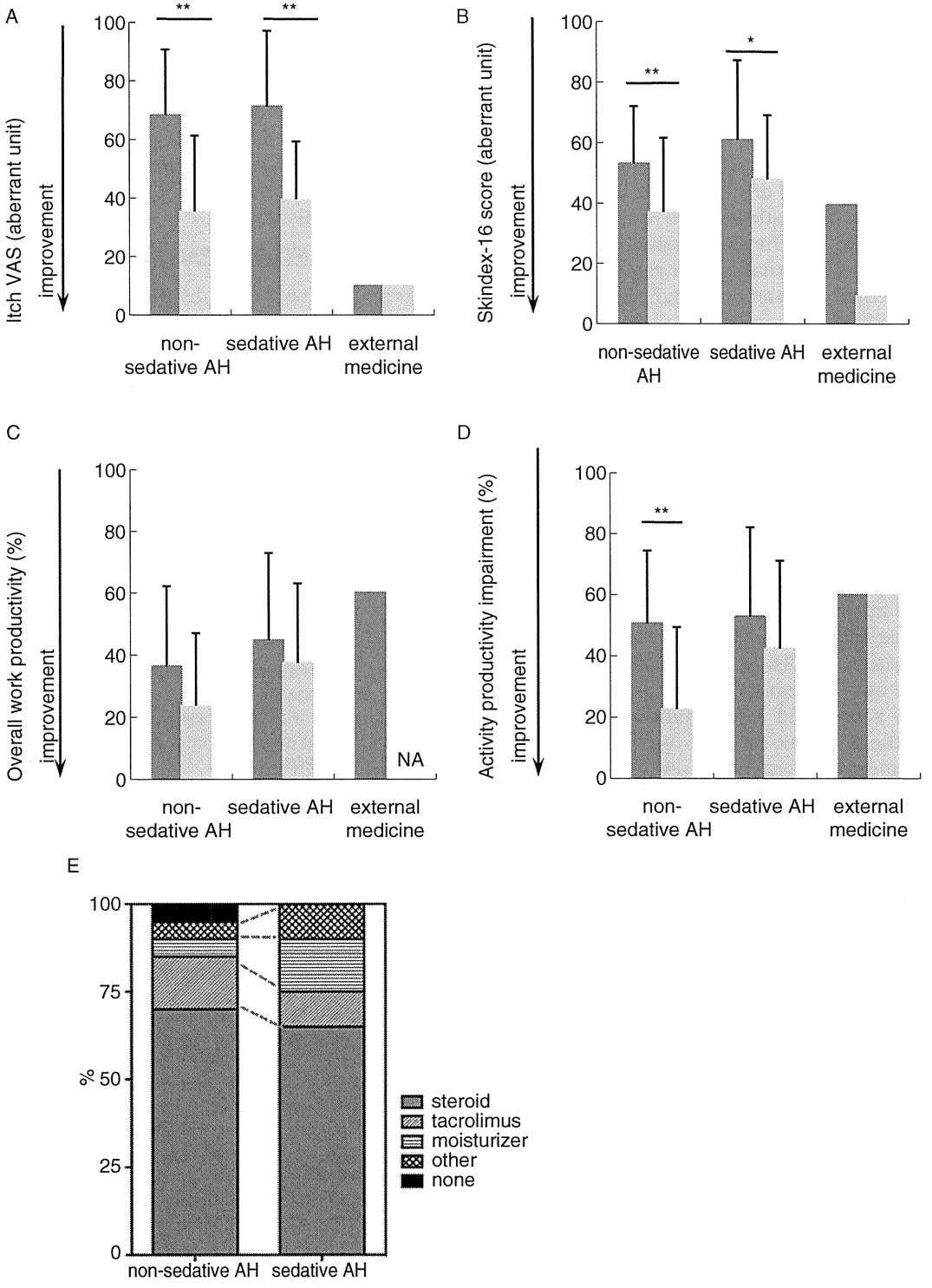

Fig. 3 The impact of antihistamines on (**A**) itch VAS, (**B**) skindex-16 score, (**C**) overall work productivity impairment, and (**D**) daily activity productivity impairment in atopic dermatitis. The data of baseline assessment (dark gray bar) and post treatment assessment (light gray bar) are shown as mean ± SD. **Statistically significant improvement compared with the data of baseline assessment ($P < 0.001$), *$P < 0.01$. NA, not applicable; AH, antihistamines. (**E**) Concomitant external medicine for cases with atopic dermatitis. "Other" includes vitamin D3 or non-steroidal anti-inflammatory ointment.