

TCLs from subjects with allergy tended to produce higher levels of IL-5 than those from subjects without allergy, although this did not reach a statistical significance. Interestingly, the highest levels of IL-4 and IL-5 production were observed in the higher-avidity TCLs ($EC_{50} \leq 2 \mu\text{g/mL}$) from subjects with allergy (see this article's Fig E3 in the Online Repository at www.jacionline.org). No differences in the production of IL-10 could be observed between the TCLs from subjects with and without allergy, and only a few TCLs produced IFN- γ or IL-17. Collectively, these results demonstrate that some of the TCLs from subjects with allergy, in particular those with a higher functional TCR avidity, are clearly T_H2 -biased, whereas the TCLs from subjects without allergy exhibit mostly an undifferentiated T_H0 phenotype.

Taken together, we have demonstrated in this study that subtle differences in the frequency, functional TCR avidity, and phenotype of Can f 1-specific memory $CD4^+$ T cells exist between subjects with dog allergy and subjects without allergy. However, the mechanisms leading to these differences remain to be elucidated. Importantly, we found no evidence for differences in either the frequency or function of $CD4^+CD25^+Foxp3^+$ Treg cells or IL-10-producing Tr1 cells between the subject groups with and without allergy. This finding is in line with the emerging evidence that immune tolerance to allergens in healthy individuals without allergy cannot be explained solely by Treg cell function.¹⁰ One intriguing possibility that has not been examined so far in detail is that the nature of the nonallergic response may depend on the type of allergen: for some allergens, active suppression by regulatory T cells may be important for maintaining tolerance, whereas for other allergens, T_H1 deviation or immune ignorance may be the prevalent mechanism. Moreover, it is possible that the mechanisms conferring protection against a particular allergen may be different in truly nonatopic individuals than in those sensitized to other allergens. Obviously, studies comparing $CD4^+$ T-cell responses to different allergens in different subject groups are needed to investigate these hypotheses further.

The skillful technical assistance of Virpi Fisk and Raija Tukiainen is gratefully acknowledged.

Suvi Parviainen, MSc^a
Antti Taivainen, MD, PhD^b
Aino Liukko, BM^a
Anssi Nieminen, MSc^a
Marja Rytönen-Nissinen, PhD^a
Tuure Kinnunen, MD, PhD^{a*}
Tuomas Virtanen, MD, PhD^{a*}

From ^athe Department of Clinical Microbiology, Institute of Clinical Medicine and Biocenter Kuopio, University of Eastern Finland; and ^bthe Department of Pulmonary Diseases, Kuopio University Hospital, Kuopio, Finland. E-mail: Tuure.Kinnunen@uef.fi. Supported by Kuopio University Hospital (project no. 5021605) and the Academy of Finland (contract no. 115160).

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

*These authors contributed equally to this work.

REFERENCES

- Bohle B, Kinaciyan T, Gerstmayr M, Radakovics A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol* 2007;120:707-13.
- Yamanaka K, Yuta A, Kakeda M, Sasaki R, Kitagawa H, Gabazza EC, et al. Induction of IL-10-producing regulatory T cells with TCR diversity by epitope-specific immunotherapy in pollinosis. *J Allergy Clin Immunol* 2009;124:842-5.e7.
- Mobs C, Slotosch C, Löffler H, Jakob T, Hertl M, Pfützer W. Birch pollen immunotherapy leads to differential induction of regulatory T cells and delayed helper T cell immune deviation. *J Immunol* 2010;184:2194-203.
- Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J, et al. Relation of $CD4^+CD25^+$ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 2004;363:608-15.
- Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannis C, Cramer R, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004;199:1567-75.
- Van Overtvelt L, Wambre E, Maillere B, von Hofe E, Louise A, Balazuc AM, et al. Assessment of Bet v 1-specific $CD4^+$ T cell responses in allergic and nonallergic individuals using MHC class II peptide tetramers. *J Immunol* 2008;180:4514-22.
- Macaubas C, Wahlstrom J, Galvao da Silva AP, Forsthuber TG, Sonnerström G, Kwok WW, et al. Allergen-specific MHC class II tetramer+ cells are detectable in allergic, but not in nonallergic, individuals. *J Immunol* 2006;176:5069-77.
- Skrindo I, Farkas L, Kvale EO, Johansen FE, Jahnsen FL. Depletion of $CD4^+CD25^+CD127^lo$ regulatory T cells does not increase allergen-driven T cell activation. *Clin Exp Allergy* 2008;38:1752-9.
- Bateman EA, Ardern-Jones MR, Ogg GS. Persistent central memory phenotype of circulating Fel d 1 peptide/DRB1(*0101) tetramer-binding $CD4^+$ T cells. *J Allergy Clin Immunol* 2006;118:1350-6.
- Romagnani S. Coming back to a missing immune deviation as the main explanatory mechanism for the hygiene hypothesis. *J Allergy Clin Immunol* 2007;119:1511-3.

Available online July 12, 2010.
doi:10.1016/j.jaci.2010.06.005

Fluctuation of blood and skin plasmacytoid dendritic cells in drug-induced hypersensitivity syndrome

To the Editor:

Drug-induced hypersensitivity syndrome (DIHS), also known as drug reaction with eosinophilia and systemic symptoms, is a severe adverse systemic reaction that usually occurs about several

TABLE I. Clinical and laboratory data in DIHS

Case	Age (y)	Sex	Drugs	Latency period (d)	Fever	LA	WBC (/μL)	Eos (/μL)	Atypical lymph (/μL)	ALT (U/L)	Serum IgG (mg/dL)	Viral reactivation (d)
1	49	F	PB	27	+	+	18,700	5,423	935	338	ND	HHV-6 (T,30)
2	67	F	CBZ	24	+	+	21,500	645	1,505	166	648	HHV-6 (T,32)
3	33	M	CBZ	34	+	+	14,600	146	1,314	147	498	HHV-6 (T,25)
4	62	M	CBZ	28	+	+	22,900	3,893	3,435	159	680	HHV-6 (T,29)
5	46	M	CBZ	24	+	+	10,600	307	106	79	766	HHV-6 (T,21)
6	65	M	CBZ	58	+	+	10,300	ND	ND	39	857	HHV-6 (D,14)
7	48	F	PB, CBZ	449	+	+	17,200	4,128	ND	43	ND	HHV-6 (T,28)(D,14), CMV, EBV
8	39	M	CBZ	37	+	+	31,500	2,205	6,615	263	ND	HHV-6 (T,19)(D,11)

Clinical characteristics of the 8 patients, including age, sex, causative drugs (as determined by a drug-induced lymphocyte stimulation test), latency period, fever, and lymphadenopathy, are shown. Laboratory data were gathered at the peak of illness. Viral reactivation was confirmed serologically, based on increased antibody titers (T) from the baseline or high DNA levels (D), and the date (days after the onset of skin rash) of reactivation is included.

Atypical lymph, Atypical lymphocyte count; ALT, alanine aminotransferase; CBZ, carbamazepine; CMV, cytomegalovirus; Drugs, causative drugs; Eos, eosinophil count; F, female; LA, lymphadenopathy; M, male; ND, not done; PB, phenobarbital; WBC, white blood cell count.

weeks after exposure to certain drugs such as anticonvulsants.¹ It is characterized by multiorgan involvement, high fever, hypogammaglobulinemia,² and sequential reactivation of various latent herpesviruses, such as human herpesvirus (HHV)-6, HHV-7,¹ cytomegalovirus, or EBV. However, the mechanism underlying the reactivation of these herpesviruses remains unknown.

Human plasmacytoid dendritic cells (pDCs) are a subset of leukocytes capable of producing large amounts of IFN- α/β , which enables neighboring cells to differentiate into mature dendritic cells or mature IgG-producing B cells, thereby resisting viral infection.³ pDCs have been identified by using the lineage (CD3, CD14, CD15, CD16, CD19, and CD56)⁻ CD123⁺ phenotype⁴ in the blood and inflamed secondary lymphoid organs.^{3,5} It has been hypothesized that pDCs are specialized for viral recognition and antiviral responses and that activated pDCs are involved in psoriasiform and lichenoid drug reactions.^{6,7} In this study, we sought to determine the distribution of pDCs in the blood and skin of patients with DIHS and to discuss the possibility that pDCs are involved in the reactivation of viruses in DIHS.

The diagnosis of all patients enrolled in this study was confirmed according to the criteria for DIHS proposed by a Japanese consensus group.¹ Clinical characteristics and laboratory data of the 8 patients are shown in Table I. After diagnosis was made, the causative drugs were withdrawn, and treatment with prednisolone (0.5–1 mg/kg daily) was initiated. As controls, age-matched and sex-matched healthy donors and patients with generalized maculopapular drug eruption (MPE) were enrolled.

After informed consent was obtained, PBMCs were obtained from the subjects at the indicated time points. Frequencies of lineage⁻ CD123⁺ pDCs were evaluated by flow cytometry. Patients with DIHS showed low percentages and numbers of circulating pDCs, especially on viral reactivation at 3 to 4 weeks after the onset of DIHS (Fig 1, A and B). These values were significantly lower than those in healthy subjects and patients with MPE (Fig 1, B). We considered a possibility that pDC counts were affected by treatment with oral corticosteroids, but patients with MPE who were treated with 0.5 to 1 mg/kg prednisolone did not show decreased circulating pDC levels (Fig 1, B).

We obtained biopsy specimens from patients with DIHS or MPE and healthy donors. These frozen specimens were stained with rat anti-human CD123, CD16, and isotype-matched control antibodies (BD Biosciences, San Jose, Calif). Significantly high numbers of CD123⁺CD16⁻ pDCs were detected in the dermis of patients with DIHS (Fig 1, C); the infiltration occurred to a much lesser extent in patients with MPE (Fig 1, D), and only rarely in healthy donors (Fig 1, D).

Recently, pDCs have been revealed to play a defensive role against viruses.⁸ In this study, we focused on the distribution of pDCs in DIHS and found that pDCs accumulate in the skin of DIHS, and that the number of pDCs in circulation decreases significantly around the time point of viral reactivation. These findings suggest that the numerical reduction of circulating pDCs might be caused by their accumulation to the skin. Because we could not define the exact date of viral reactivation, it is difficult for us to state whether the decrease of pDCs precedes or follows viral reactivation. However, we speculate that the paucity of circulating pDCs could depress the antiviral activity in patients with DIHS. This hypothesis is supported by the observation that circulating IgG levels are low in DIHS, because pDCs induce B-cell maturation to produce IgG.² As to pDC dynamics, it still

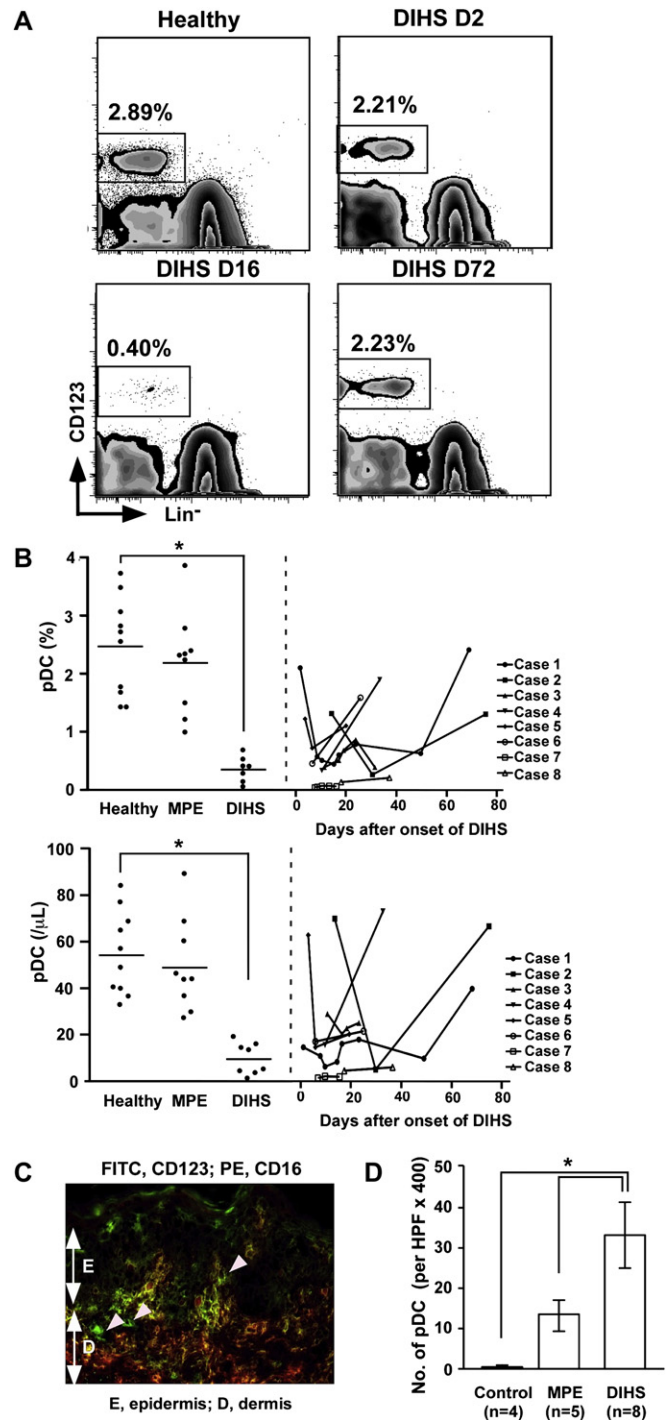


FIG 1. pDC distribution in the blood and in the skin. **A and B,** Fluorescence-activated cell sorting plots of PBMCs in a healthy donor and a patient (case 1) with DIHS 2, 16, and 72 days after the onset (A). The percentages (upper panel) and the numbers (lower panel) of lineage⁻ (Lin⁻) CD123⁺ pDCs in healthy donors, patients with MPE, and patients with DIHS at viral reactivation and at other indicated time points. **C,** Immunohistochemistry of the skin from a patient with DIHS. Arrowheads indicate pDCs. Skin materials were obtained around the peak of illness. **D,** Numbers of pDCs in the skin of healthy donors, patients with MPE, and patients with DIHS per high power fields (HPF). Data are presented as means $\pm$ SDs. * $P < .05$ (Student *t* test). PE, Phycoerythrin.

remains unknown how pDCs accumulate in the skin preferentially in patients with DIHS. Because HHV-6 transcripts were detected in the skin of patients with DIHS,⁹ pDCs may accumulate to attack the herpesviruses. In addition, the causative drugs such as anticonvulsants might stimulate fibroblasts and/or endothelial cells to release chemoattractants for pDCs in the skin.

pDCs are known to induce autoimmune diseases.¹⁰ The rapid increase in the frequency of pDCs in the blood after treatment for DIHS (Fig 1, B) may be associated with the occurrence of certain autoimmune diseases, such as type 1 diabetes mellitus, which are more likely to occur after DIHS.¹¹ It has been reported that the reduced number of circulating pDCs in patients with SLE can be caused by their accelerated migration to the sites of inflammation including skin lesions.¹² It would be of interest to examine whether administration of type I IFN prevents viral reactivation of DIHS and whether blockade of type I IFN suppresses the occurrence of autoimmune diseases after DIHS. Although we still have much to learn, our studies on pDCs may shed light on the mechanisms underlying DIHS.

Kazunari Sugita, MD, PhD^{a*}
Mikiko Tohyama, MD, PhD^{b*}
Hideaki Watanabe, MD, PhD^c
Atsushi Otsuka, MD, PhD^d
Saeko Nakajima, MD^d
Masafumi Iijima, MD, PhD^c
Koji Hashimoto, MD, PhD^b
Yoshiki Tokura, MD, PhD^a
Yoshiki Miyachi, MD, PhD^d
Kenji Kabashima, MD, PhD^d

From ^athe Department of Dermatology, University of Occupational and Environmental Health, Kitakyushu; ^bthe Department of Dermatology, Ehime University School of Medicine, Toon; ^cthe Department of Dermatology, Showa University School of Medicine, Tokyo; and ^dthe Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan. E-mail: kaba@kuhp.kyoto-u.ac.jp.

Supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labor, and Welfare of Japan.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

*These authors contributed equally to this work.

REFERENCES

- Shiohara T, Kano Y. A complex interaction between drug allergy and viral infection. *Clin Rev Allergy Immunol* 2007;33:124-33.
- Kano Y, Inaoka M, Shiohara T. Association between anticonvulsant hypersensitivity syndrome and human herpesvirus 6 reactivation and hypogammaglobulinemia. *Arch Dermatol* 2004;140:183-8.
- Siegal FP, Kadowaki N, Shodell M, Fitzgerald-Bocarsly PA, Shah K, Ho S, et al. The nature of the principal type 1 interferon-producing cells in human blood. *Science* 1999;284:1835-7.
- Olweus J, BitMansour A, Warnke R, Thompson PA, Carballido J, Picker LJ, et al. Dendritic cell ontogeny: a human dendritic cell lineage of myeloid origin. *Proc Natl Acad Sci U S A* 1997;94:12551-6.
- Facchetti F, De Wolf-Peters C, Van den Oord JJ, Desmet VJ. Plasmacytoid T cells in a case of lymphocytic infiltration of skin: a component of the skin-associated lymphoid tissue? *J Pathol* 1988;155:295-300.
- Park J, Choi Y, Lee J, Kim S, Lee S, Won Y, et al. Psoriasiform drug eruption induced by anti-tuberculosis medication: potential role of plasma-cytoid dendritic cells. *Acta Derm Venereol* 2010;90:305-6.
- Sontheimer RD. Lichenoid tissue reaction/interface dermatitis: clinical and histological perspectives. *J Invest Dermatol* 2009;129:1088-99.
- Colonna M, Trinchieri G, Liu YJ. Plasmacytoid dendritic cells in immunity. *Nat Immunol* 2004;5:1219-26.
- Watanabe T, Nakashima H, Ohmatsu H, Sakurai N, Takekoshi T, Tamaki K. Detection of human herpesvirus-6 transcripts in carbamazepine-induced hypersensitivity syndrome by in situ hybridization. *J Dermatol Sci* 2009;54:134-6.
- Banchereau J, Pascual V. Type I interferon in systemic lupus erythematosus and other autoimmune diseases. *Immunity* 2006;25:383-92.
- Sekine N, Motokura T, Oki T, Umeda Y, Sasaki N, Hayashi M, et al. Rapid loss of insulin secretion in a patient with fulminant type 1 diabetes mellitus and carbamazepine hypersensitivity syndrome. *JAMA* 2001;285:1153-4.
- Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL. Plasmacytoid dendritic cells (natural interferon-alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions. *Am J Pathol* 2001;159:237-43.

Available online July 12, 2010.

doi:10.1016/j.jaci.2010.06.004

Man's best friend? The effect of pet ownership on house dust microbial communities

To the Editor:

The prevalence of asthma among children has been steadily increasing in westernized nations. Although host genotype likely plays a role in predisposition to allergic disease, the rate at which asthma has increased and the geographically distinct location of this phenomenon implicate environmental factors as being important. Epidemiologic studies have suggested that contact with animals provides protection against allergic disease development¹; childhood farm exposure, specifically to livestock, is associated with a significant decrease in the risk of atopic sensitization, a protective effect that persists into early adulthood. More recently, in our own birth cohort, maternal prenatal exposure to household pets, particularly dogs, has been suggested to affect fetal immune response development.² Higher concentrations of cord blood IgE were associated with mothers unexposed to pets,² a notable finding given that previous studies have demonstrated a link between increased cord blood IgE levels and the risk for subsequent development of allergic disorders.³

In this study we examined whether the presence of dogs or cats or the absence of a furred pet affected the microbial composition of house dust. Using a high-density phylogenetic microarray, the 16S rRNA PhyloChip,⁴ and fungal automated rRNA intergenic spacer analysis, we examined 16 dust samples collected from households with 1 or more dogs (D households; n = 6), 1 or more cats (C households; n = 5), or no furred pets (NP households; n = 5). Five (83.3%), 5 (100%), and 3 (60%) samples from D, C, and NP households, respectively, had sufficient material for microbial analysis. Bacterial community richness (number of bacterial taxa detected), evenness (relative distribution of taxa in communities), and diversity (calculated by using richness and evenness indices) were notably increased in all dog-owning and a subset of cat-owning households. Dust from households with dogs was significantly richer ($P = .04$) and more diverse ($P = .04$) compared with dust from households without pets (Fig 1, A and B); community evenness was not significantly different between these 2 groups ($P = .14$; Fig 1, C, and see Table E1 in this article's Online Repository at www.jacionline.org). These data suggest that dog ownership increases house dust diversity, driven largely by introduction of additional types of bacteria.

Significant differences in taxon relative abundance between the D and NP groups identified 337 taxa significantly increased in abundance in dog-owning houses (see Table E2 and the Methods section in this article's Online Repository at www.jacionline.org). They primarily belonged to the Proteobacteria (112 taxa), Actinobacteria (63 taxa), Firmicutes (47 taxa), Verrucomicrobia (7 taxa),