

Original article

Characterization of allergen-specific monocyte-derived dendritic cells generated from monocytes by a single-step procedure: effect on naïve and memory T cells

Background: Dendritic cells are one of the most potent antigen-presenting cells and when pulsed with allergen can modulate allergen-specific T-cell responses. We sought to establish a single-step method by which to generate allergen-specific monocyte-derived dendritic cells (MoDCs).

Methods: *Dermatophagoides farinae* (Df)-prepulsed MoDCs were generated from monocytes by culturing with Df in the presence interleukin (IL)-4, granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumour necrosis factor (TNF)- α simultaneously. Df-prepulsed MoDC were incubated with autologous naïve and memory T cells in the absence of recall antigen, then proliferation and cytokine production by T cells was determined.

Results: Generation of allergen-prepulsed MoDCs was confirmed by examining expression of surface molecules. Df-prepulsed MoDC selectively induced proliferation of Df-specific T cells in the absence of recall antigen. Under these conditions, Df-prepulsed MoDCs augmented but did not alter the cytokine production profile. In addition, Df-prepulsed MoDCs activated naïve T cells leading to proliferation and selective production of IFN- γ in allergic patients but not in healthy subjects.

Conclusions: These results suggest that Df-prepulsed MoDC generated from monocytes by a simple single-step manipulation can induce Df-specific cellular responses from both naïve and memory T cells in the absence of recall antigen, and these cells potentially can be utilized as immune adjuvants in allergen-specific immunotherapy.

T. Takishita¹, M. Okano¹,
K. Takahashi³, T. Yoshino², Y. Sugata¹,
H. Hattori¹, S. Ohuchi¹, T. Ogawa¹,
K. Nishizaki¹

¹Departments of Otolaryngology – Head & Neck Surgery and ²Pathology, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan; ³Faculty of Health Sciences, Okayama University Medical School, Okayama, Japan

Key words: allergen; cytokine; dendritic cells; memory; naïve; rhinitis; T cell.

Mitsuhiro Okano, MD
Department of Otolaryngology – Head & Neck Surgery
Okayama University Graduate School of Medicine and Dentistry
2-5-1 Shikatacho
Okayama 700-8558
Japan

Accepted for publication 4 May 2004

Allergic rhinitis is strongly associated with expansion of T-helper (Th)2-type immune responses (1). Differentiation and activation of antigen (Ag)-specific T cells require two signals provided by Ag-presenting cells (APCs) (2). Dendritic cells (DCs) are the most potent APCs, and both myeloid and plasmacytoid DC exist in the human nasal mucosa and increase following allergen exposure (3, 4). DC can lead to the activation of not only memory but also naïve T cells in an Ag-specific manner (5–7). In addition, depending on the type of stimulation they receive, DCs can induce the immune deviation (8, 9).

Monocyte-derived DCs (MoDCs) are considered a useful immunotherapeutic tool (10–16). Vaccination with peptide-pulsed MoDCs augments the delayed type

hypersensitivity (DTH) responses associated with the regression of metastasis in melanoma (13). In addition, immunosuppressive strategies utilizing DCs have been developed in several diseases such as graft-*vs*-host disease (GVHD) and autoimmune diseases (14–16).

Monocyte-derived DCs can take up allergens and induce allergen-specific T-cell responses (6, 7, 17–20), and have been shown to result in greater production of interleukin (IL)-4 and/or IL-5 by autologous T cells from allergic patients when compared with nonallergic subjects. On the contrary, the effects on proliferation and IFN- γ production are still disputed (6, 7, 18–20). In most of the investigations, however, MoDCs were first created by treatment with IL-4 and GM-CSF followed by uptake of Ag.

For future use of DCs as allergen-specific immunotherapy, it would be ideal to produce allergen-specific DCs by simple manipulation. The aim of the present study is to establish a simple single-step process by which to generate allergen-specific MoDCs. We pulsed peripheral blood monocytes with allergen while simultaneously stimulating with IL-4, GM-CSF and tumour necrosis factor (TNF)- α ;

Abbreviations: APC, antigen-presenting cell; CM, culture medium; Df, *Dermatophagoides farinae*; GM-CSF, granulocyte-macrophage colony-stimulating factor; MoDC, monocyte-derived dendritic cell; PBMC, peripheral blood mononuclear cell; PPD, purified protein derivative; TCL, T-cell lines.

no further manipulation is needed to establish allergen-specific MoDCs following this protocol. We investigated the duration of Ag presentation by these allergen-prepulsed MoDCs as well as surface molecule expression and activation of naïve and Ag-specific memory T cells, and compared the ability of Ag presentation by allergen-prepulsed DC with DC generated before Ag stimulation. Our observations indicate that allergen-prepulsed MoDCs generated by our one-step procedure has the potential to be a novel tool for allergen-specific immunotherapy.

Methods

Subjects

Twelve Japanese patients (mean 29.2 years old) with perennial allergic rhinitis and seven healthy control subjects (mean 29.1 years old) were examined. All patients showed an elevation of serum IgE specific for *Dermatophagoides farinae* (Df) by CAP-RAST (Pharmacia, Uppsala, Sweden). Informed consent was obtained from each subject. None of the subjects used immunosuppressive drugs or underwent immunotherapy during the study.

Antigen

Crude extract of Df was provided by Torii Co (Tokyo, Japan) (21). Purified protein derivative (PPD) was purchased from Nihon BCG Seizo Co. (Tokyo, Japan). Protein concentration of Ag was determined by bicinchoninic acid assay according to the manufacturer's instructions (Pierce, Rockford, IL, USA).

Generation of Df-specific, MoDCs

Peripheral blood mononuclear cells (PBMCs) in RPMI 1640 (Sigma chemical Co., St Louis, MO, USA) supplemented with 10% FCS (Sigma), 100 U/ml penicillin, 100 µg/ml streptomycin, and 20 mM L-glutamine (Gibco, Grand Island, NY, USA) [culture medium (CM)] were incubated in a tissue culture flask at 37°C for 30 min in a 5% CO₂ atmosphere. After washing, 1×10^6 /ml adherent cells were incubated in CM containing 10 µg/ml Df and 10 ng/ml GM-CSF (Peprotech EC, London, UK), 10 ng/ml IL-4 (Peprotech EC) and 10 ng/ml TNF-α (Promega, Madison, WI, USA). After 7 days of culture, cells corresponding to the MoDC-enriched fraction were harvested, washed and used for subsequent experiments.

Flowcytometric analysis

The method of flowcytometry has been described previously (22). The following monoclonal antibodies were used throughout the study: fluorescein isothiocyanate (FITC)-conjugated anti-CD1a, anti-CD54 or anti-CD80; phycoerythrin (PE)-conjugated anti-CD86, anti-CD40, anti-CD83 or anti-CD14; Cy-chrome-conjugated anti-human leucocyte Ag (HLA)-DR (Becton Dickinson, Mountain View, CA, USA).

Cell preparation

CD45RA+, CD14+, and CD19+ cells were separated from PBMCs using antibody (Ab)-coated magnetic microbeads (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) according to the

manufacturer's instructions. When microbeads coupled to anti-CD45RA, anti-CD14 and anti-CD19 mAb were used, >95, 95 and 85% of the sorted cells were CD45RA+, CD14+ and CD19+ by flowcytometry, respectively. Df and PPD-specific T-cell lines (TCLs) were generated as described previously (23). Flowcytometric analysis revealed that >97% cells in Ag-specific TCLs expressed both CD45RO and CD4.

Ag-specific T-cell responses

Monocyte-derived DC-driven proliferation and cytokine production by naïve and Ag-specific memory T cells was tested as described previously (23). In brief, 1×10^5 of either CD45RA+ cells or Ag-specific TCLs suspended in 200 µl CM were co-cultured with serial dilutions of 30 Gy-irradiated autologous APCs in the presence or absence of recall stimulation with 10 µg/ml Df. CD45RA+ cells and Df-specific TCLs were cultured for 7 and 3 days, respectively.

Cytokine production assays and determination

Levels of IL-4 and IFN-γ in culture supernatants were measured by Opt EIA™ sets (Becton Dickinson) according to the manufacturer's instructions. The detection limits in these assays were 5 pg/ml for IL-4 and 10 pg/ml for IFN-γ.

Statistical analysis

Data were analysed through use of the paired *t*-test or Mann-Whitney's *U*-test. *P* < 0.05 was considered statistically significant. Values were given as mean ± standard deviation (SD).

Results

Effect of Df prepulse on the expression of surface molecules on MoDCs

Peripheral blood mononuclear cell-derived monocytes from allergic patients were cultured with GM-CSF, IL-4 and TNF-α in the presence or absence of Df for 7 days. They expressed CD1a, HLA-DR, CD54, CD80, and CD86, but expression of CD14 was downregulated, suggesting maturation into MoDC (Fig. 1). Although expression of CD1a, HLA-DR, CD54, CD80, and CD86 was similar between Df-prepulsed and non-prepulsed MoDCs, expression of CD40 was significantly upregulated in Df-prepulsed MoDCs (*P* = 0.005) (Fig. 2).

Roles of Df-prepulsed MoDCs in the activation of Ag-specific memory T cells in the absence of recall stimulation

Four Df-specific CD4+ TCLs were generated from peripheral blood of two donors (T.T. and S.O.). Df-prepulsed MoDCs could induce the proliferation of all TCLs in the absence of recall Ag (Fig. 3A). However, Df-prepulsed MoDC did not induce the responses of PPD-specific TCLs without recall stimulation (data not shown). This activation occurred even at a MoDC to T-cell ratio of 1 : 25, and the maximal activation was observed when the

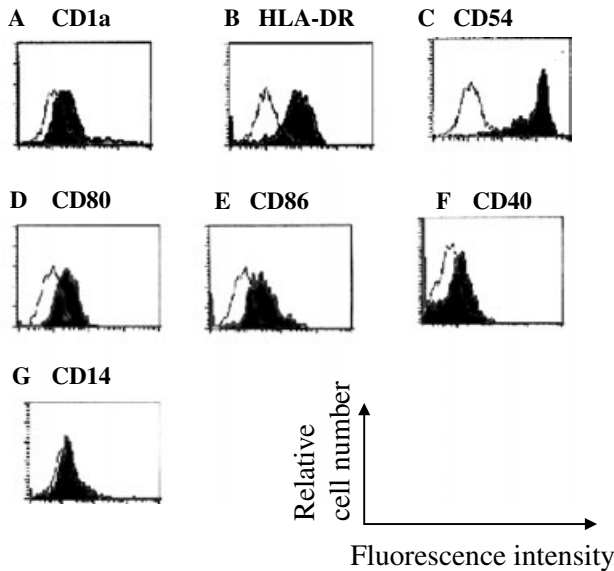


Figure 1. Phenotypic characterization of MoDC. Monocytes from allergic patients were incubated with 10 ng/ml IL-4, 10 ng/ml GM-CSF and 10 ng/ml TNF- α for 7 days. The expression of surface molecules were analysed by flowcytometry. Bold and fine line represent the intensity with and without fluorescence-conjugated mAb, respectively.

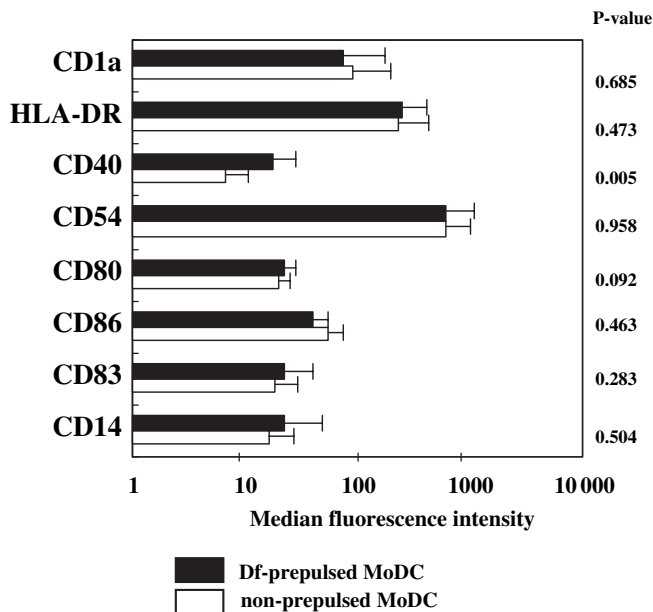


Figure 2. Effect of prepulse with Df on the expression of surface molecules in MoDC. Monocytes from allergic patients ($n = 7$) were incubated with 10 ng/ml IL-4, 10 ng/ml GM-CSF and 10 ng/ml TNF- α in the presence (closed bar) or absence (open bar) of 10 μ g/ml Df for 7 days. Then the generated MoDC were harvested, and the expression of surface molecules were analysed by flowcytometry. *P*-value was obtained through use of paired *t*-test.

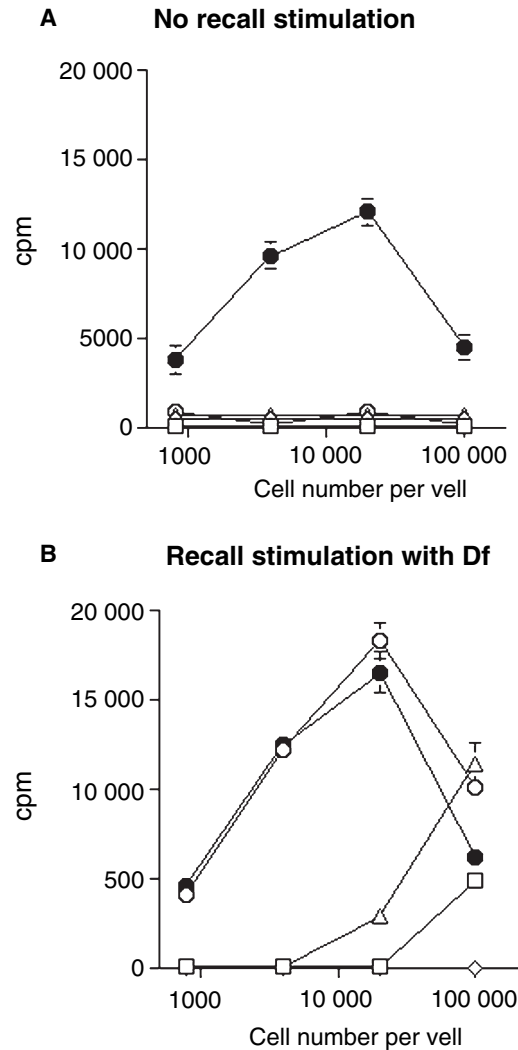


Figure 3. Proliferative responses of Df-specific TCL by APCs with or without recall stimulation; 2×10^4 Df-specific TCL (TF-4) were incubated with serial numbers of 30 Gy-irradiated PBMC (open square), B cell (open diamond), monocyte (open triangle), Df-nonpulsed MoDC (open circle) or Df-prepulsed MoDC (closed circle) in the absence (A) or presence (B) of 10 μ g/ml Df for 72 h. Cell proliferation was estimated by mean uptake of ^{3} H-thymidine (count per minute) in triplicate culture. Bars indicate mean $\pm$ SD. Results were concordant with data from other TCLs such as TF-3, OD-1 and OD-2.

ratio was 1 : 1. Non-prepulsed MoDC, B cells, monocytes and PBMC did not induce the responses (Fig. 3A). With recall Ag stimulation, both Df-prepulsed and non-prepulsed MoDCs exhibited similar abilities to induce Df-specific T-cell proliferation (Fig. 3B).

Effect of Df-prepulsed MoDCs on cytokine production by Ag-specific memory T cells

Two TCLs (TF4 and OD2) were defined as Th0, as they produced detectable amounts of both IL-4 and IFN- γ in

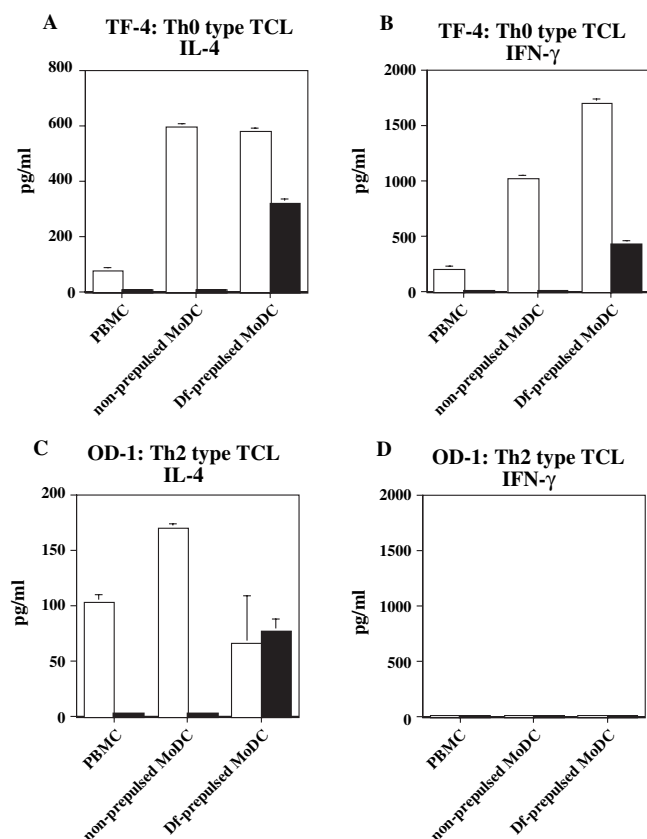


Figure 4. Effect of Df-prepulsed MoDC on cytokine production by Df-specific memory TCL; 2×10^4 of Th0-type (TF-4; A and B) or Th2-type (OD-1; C and D). TCL-specific for Df were incubated with 30 Gy-irradiated PBMC, non-prepulsed MoDC or Df-prepulsed MoDC in the presence (open bar) or absence (closed bar) of 10 μ g/ml Df for 65 h. After incubation, supernatants were collected and IL-4 (A and C) and IFN- γ (B and D) were determined by means of enzyme-linked immunosorbent assay (ELISA). Results indicate mean $\pm$ SD of triplicate wells. Data are representative for two separate experiments, and concordant with data from TF-3 and OD-2 TCLs.

response to Df when irradiated PBMCs were used as APC. The other TCLs (TF3 and OD1) were defined as Th2 as they produced IL-4 but not IFN- γ in the same stimulation. In Th0 TCLs, Df-prepulsed but not non-prepulsed MoDCs induced production of both IL-4 and IFN- γ in the absence of recall Ag (Fig. 4A,B). In Th2 TCLs, only Df-prepulsed MoDCs induced IL-4 but not IFN- γ production in the absence of recall Ag (Fig. 4C,D). Furthermore, we compared cytokine production by Ag-specific CTLs in response to Df-prepulsed MoDCs and non-prepulsed MoDCs in presence of recall Ag. We found no differences in stimulatory ability between Df-prepulsed MoDCs and non-prepulsed MoDCs (data not shown).

Duration of Ag presentation by Df-prepulsed MoDCs

Monocytes were incubated with Df in the presence of GM-CSF, IL-4 and TNF- α for 1, 2 and 4 weeks,

thereafter cells were harvested and co-cultured with TCLs. MoDCs harvested in the first week displayed the most potent Ag presentation. This ability was reduced when the cells were incubated for longer periods, however, cells harvested at 4 weeks could still induce proliferation and cytokine production by TCLs. However, non-prepulsed MoDC did not display the ability during the periods observed without recall Ag (Fig. 5).

Role of Df-prepulsed MoDCs in the activation of naïve T cells in the absence of recall stimulation

We co-cultured Df-prepulsed MoDCs with CD45RA + T cells from either allergic patients or healthy subjects (Fig. 6). The proliferation of naïve T cells to Df-prepulsed MoDCs was significantly higher in allergic patients than healthy subjects (Fig. 6A). IL-4 production was not present in either group (Fig. 6B). However, Df-prepulsed MoDCs induced IFN- γ production only in allergic patients (Fig. 6C).

Discussion

In the present study, we characterized the ability of Ag-prepulsed MoDCs to present Ag to both naïve and Ag-specific T cells. The most novel aspect of our method to generate Ag-specific MoDCs was to pulse monocytes with Ag and cytokine cocktails simultaneously at the beginning of culture. Although several methods by which to pulse DCs with Ag have been reported, these systems require multiple manipulations (19, 20). Our method may be beneficial for the future use of immunotherapy because of its simplicity.

Several reports have demonstrated the phenotypic differences in DCs between allergic patients and healthy donors in the presence or absence of Ag (7, 19, 20). By our method, Df-prepulsed DCs expressed higher levels of CD40 when compared with non-prepulsed DC. Engagement via CD40 is known to induce IgE isotype switching, eosinophil survival and cytokine release by memory T cells (24–26). Thus our results suggest that Df-prepulsed MoDC may augment Df-specific T-cell responses via enhanced expression of CD40.

Monocyte-derived DCs prepulsed with Df at the beginning of their generation could activate Ag-specific memory T cells in the absence of recall Ag. This capacity was seen at MoDC to T-cell ratios ranging from 1 : 25 to 5 : 1, thus falling in line with previous reports of strong responses at a DC to T-cell ratio of $\leq 1 : 10$ (6, 7, 19, 20). Stimulator/responder ratio affects Th1 or Th2 differentiation from naïve T cell (9). In addition, DCs have been shown to induce immune deviation (27, 28). Df-prepulsed MoDC elicited similar patterns of IL-4 and IFN- γ production by Df-specific TCLs at several DC to T cell ratios ranging from 1 : 625 to 5 : 1. Immune deviation to a Th1 response may be useful for prevention of or

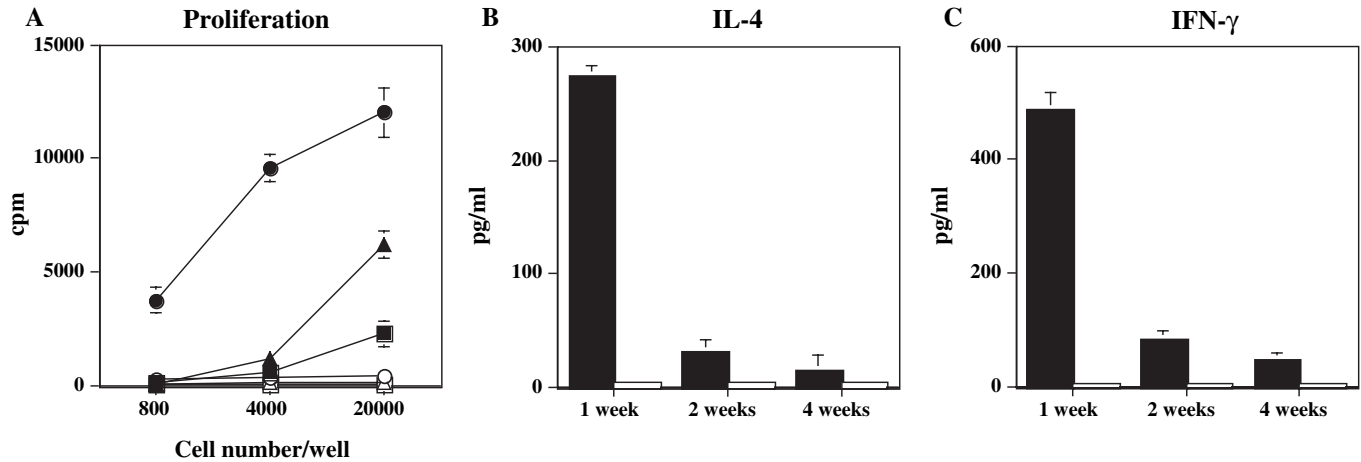


Figure 5. Duration of Ag-presenting capacity of Df-prepulsed Mo-DC. Monocytes from allergic patient (TT) were cultured with (closed symbols and bars) or without (open symbols and bars) 10 μ g/ml Df together with 10 ng/ml IL-4, 10 ng/ml GM-SCF and 10 ng/ml TNF- α . 1 (circle), 2 (triangle) and 4 (square) weeks after the beginning of culture, the generated MoDC were harvested, washed and irradiated for 30 Gy. Then serial numbers of MoDC were cultured with autologous 2×10^4 Df-specific TCLs (TF-4) without recall stimulation for 72 h. Cell proliferation of TCLs was measured by incorporation of ^3H -thymidine (A). Results indicate mean cpm $\pm$ SD of triplicate cultures. And after incubation for 65 h, supernatants were collected, and IL-4 (B) and IFN- γ (C) were measured by means of ELISA. Results indicate mean cpm $\pm$ SD of triplicate wells. Data are representative for two separate experiments, and concordant with data from OD-2 TCL.

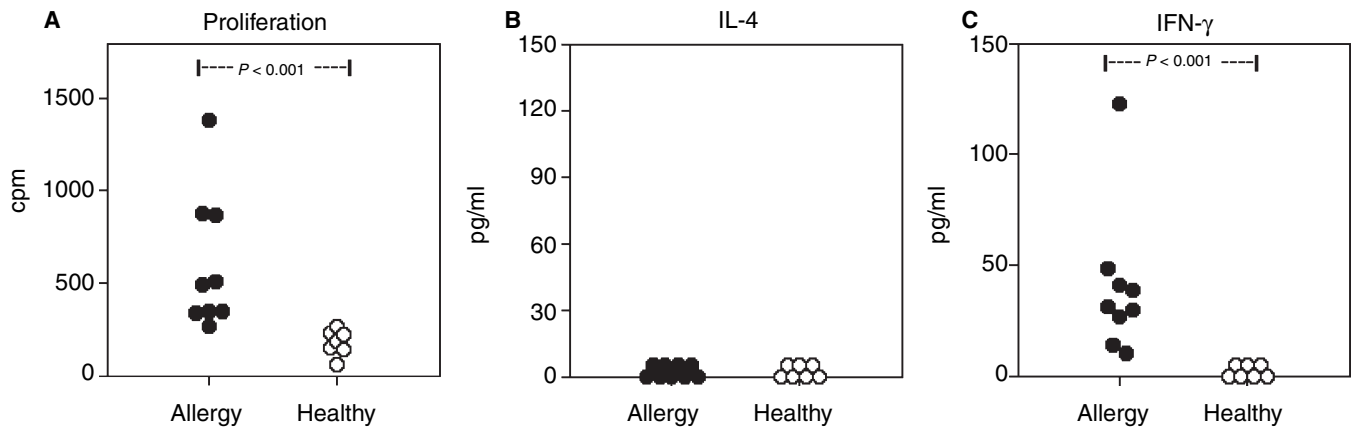


Figure 6. Proliferation and cytokine production of naïve T cells induced by Df-prepulsed MoDC without recall stimulation. Df-prepulsed MoDC from nine allergic patients with perennial rhinitis (closed circle) and seven healthy subjects (open square) were generated as described above; 30 Gy-irradiated 2×10^4 Df-prepulsed MoDC were co-cultured with the same number of autologous CD45RA+ cells for 7 days. ^3H -thymidine was added, and incorporation of ^3H -thymidine was measured as described above. After 3 days incubation, supernatants were collected, and IL-4 (B) and IFN- γ (C) were determined by means of ELISA. P-value was obtained through use of Mann-Whitney's U-test.

immunotherapy for type I allergy (27, 28). However, our results suggest that Df-prepulsed MoDCs produced by our method have no potential to induce immune deviation of Df-specific memory T cells.

One of the unique properties of DCs is that they can activate naïve T cells (5, 6). The present study showed that Df-prepulsed MoDCs activate naïve T cells which leads to not only to proliferation but also to IFN- γ production in allergic patients but not in healthy subjects (Fig. 6). However, Bellinghausen et al. (6) reported that a

Th2 cytokine profile was induced in naïve T cells from atopic but not from nonatopic donors after stimulation with autologous allergen-pulsed DCs. Recent investigations revealed that DCs may not have an intrinsic capacity to direct either Th1 or Th2 cell development, but rather might be modified by external factors such as dose of Ag and a range of stimuli including pathogen-derived products and inflammatory mediators (29, 30). Thus the difference between these studies may arise from the methods used to generate allergen-pulsed DCs such as

the use of TNF- α at the beginning of culture and the concentration of cytokines loaded. Our method appears to have a beneficial advantage for the future use as allergen-specific immunotherapy as Df-prepulsed MoDCs generated by our method can promote the immune deviation into Th1 responses in naïve T cells from allergic but not healthy subjects (27, 28).

In conclusion, we have provided *in vitro* evidence that MoDCs prepulsed with Df at the beginning of their generation display an ability to stimulate both memory and naïve T cells in the absence of recall Ag. This capacity can last up to 4 weeks after loading with Df. In addition, Df-prepulsed MoDC can promote allergen-specific Th1 responses from naïve T cells only in allergic patients.

References

- Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage colony stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J Immunol* 1992;**148**:2390–2394.
- Liu Y, Linsley Y. T cell costimulation. *Curr Opin Immunol* 1992;**4**:265–270.
- Jahnsen FL, Lund-Johansen F, Dunne JF, Farkas L, Haye R, Brandtzaeg P. Experimentally induced recruitment of plasmacytoid (CD123high) dendritic cells in human nasal allergy. *J Immunol* 2000;**165**:4062–4068.
- Till SJ, Jacobson MR, O'Brien F, Durham SR, KleinJan A, Fokkens WJ et al. Recruitment of CD1a+ Langerhans cells to the nasal mucosa in seasonal allergic rhinitis and effects of topical corticosteroid therapy. *Allergy* 2001;**56**:126–131.
- Lanzavecchia A, Sallusto F. Regulation of T cell immunity by dendritic cells. *Cell* 2001;263–266.
- Bellinghausen I, Brand U, Knop J, Saloga J. Comparison of allergen-stimulated dendritic cells from atopic and nonatopic donors dissecting their effect on autologous naïve and memory T helper cells of such donors. *J Allergy Clin Immunol* 2000;**105**:988–996.
- Bellinghausen I, Brand U, Steinbrink K, Enk AH, Knop U, Saloga J. Inhibition of human allergic T-cell responses by IL-10-treated dendritic cells: differences from hydrocortisone-treated dendritic cells. *J Allergy Clin Immunol* 2001;**108**:242–249.
- Rissoan MC, Soumelis V, Kadowaki N, Grouard G, Briere F, Malefy R et al. Reciprocal control of T helper cell and dendritic cell differentiation. *Science* 1999;**283**:1183–1186.
- Tanaka H, Demeure CE, Rubio M, Delespesse G, Sarfati M. Human monocyte-derived dendritic cells induce naïve T cell differentiation into T helper 2 (Th2) or Th1/Th2 effectors: role of stimulator/responder ratio. *J Exp Med* 2000;**192**:405–411.
- Akagawa KS, Takasuka N, Nozaki Y, Komuro I, Azuma M, Ueda M et al. Generation of CD1+ RelB+ dendritic cells and tartrate-resistant acid phosphatase-positive osteoclast-like multinucleated giant cells from human monocytes. *Blood* 1996;**88**:4029–4039.
- Tsai V, Southwood S, Sidney J, Sakaguchi K, Kawakami Y, Appella E et al. Identification of subdominant CTL epitopes of the GP100 melanoma-associated tumor antigen by primary *in vitro* immunization with peptide-pulsed dendritic cells. *J Immunol* 1997;**158**:1796–1802.
- Chapuis F, Rosenzweig M, Yagello M, Ekman M, Biberfeld P, Gluckman JC. Differentiation of human dendritic cells from monocytes *in vitro*. *Eur J Immunol* 1997;**27**:431–441.
- Nestle FO, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R et al. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat Med* 1998;**4**:328–332.
- O'Rourke RW, Kang SM, Lower JA, Feng S, Ascher NL, Baekkeskov S et al. A dendritic cell line genetically modified to express CTLA4-IG as a means to prolong islet allograft survival. *Transplantation* 2000;**69**:1440–1446.
- Kim SH, Kim S, Evans CH, Ghivizzani SC, Oligino T, Robbins PD. Effective treatment of established murine collagen-induced arthritis by systemic administration of dendritic cells genetically modified to express IL-4. *J Immunol* 2001;**166**:3499–3505.
- Morita Y, Yang J, Gupta R, Shimizu K, Shelden EA, Endres J et al. Dendritic cells genetically engineered to express IL-4 inhibit murine collagen-induced arthritis. *J Clin Invest* 2001;**107**:1275–1284.
- van den Heuvel MM, Vanhee DD, Postmus PE, Hoefsmit EC, Beelen RH. Functional and phenotypic differences of monocyte-derived dendritic cells from allergic and nonallergic patients. *J Allergy Clin Immunol* 1998;**101**:90–95.
- Sung SJ, Taketomi EA, Smith AM, Platts-Mills TA, Fu SM. Efficient presentation of house dust mite allergen Der p 2 by monocyte-derived dendritic cells and the role of beta 2 integrins. *Scand J Immunol* 1999;**49**:96–105.
- De Wit D, Amraoui Z, Vincart B, Michel O, Michils A, Van Overvelt L et al. Helper T-cell responses elicited by Der p 1-pulsed dendritic cells and recombinant IL-12 in atopic and healthy subjects. *J Allergy Clin Immunol* 2000;**105**:346–352.
- Hammad H, Charbonnier AS, Duez C, Jacquet A, Stewart GA, Tonnel AB et al. Th2 polarization by Der p 1-pulsed monocyte-derived dendritic cells is due to the allergic status of the donors. *Blood* 2001;**98**:1135–1141.
- Dandeu JP, Le Mao J, Lux M, Rabillon J, David B. Antigens and allergens in *Dermatophagoides farinae* mite. II. Purification of AG f1, a major allergen in *Dermatophagoides farinae*. *Immunology* 1982;**46**:679–687.

Acknowledgments

The authors thank Megumi Maeda and Tazuko Fujiwara for excellent assistance, and Yuko Okano for editorial assistance. This work was supported in part by the grants from Ministry of Education, Culture, Sports, Science and Technology, Japan (no. 14704043) and Research on Allergic disease and Immunology of Ministry of Health, Labor and Welfare (no. 14210301).

22. Nakada M, Nishizaki K, Yoshino T, Okano M, Masuda Y, Ohta N et al. CD86 (B7-2) antigen on B cells from atopic patients shows selective, antigen-specific upregulation. *Allergy* 1998;**53**:527–531.
23. Okano M, Kino K, Takishita T, Hattori H, Ogawa T, Yoshino T et al. Roles of carbohydrates on Cry j1, the major allergen of Japanese cedar pollen, in specific T cell responses. *J Allergy Clin Immunol* 2001;**108**:101–108.
24. Oettgen HC. Regulation of the IgE isotype switch: new insights on cytokine signals and the function of epsilon germline transcripts. *Curr Opin Immunol* 2000;**12**:618–623.
25. Bureau F, Seumois G, Jasper F, Vanderplasschen A, Derty B, Pastoert PP et al. CD40 engagement enhances eosinophil survival through induction of cellular inhibitor of apoptosis protein 2 expression: possible involvement in allergic inflammation. *J Allergy Clin Immunol* 2002;**110**:443–449.
26. Rogers NJ, Jackson IM, Jordan WJ, Lombardi G, Delikouras A, Lechler RI. CD40 can costimulate human memory T cells and favors IL-10 secretion. *Eur J Immunol* 2003;**33**:1094–1104.
27. Brand U, Bellinghausen I, Enk AH, Jonuleit H, Becker D, Knop J et al. Allergen-specific immune deviation from a TH2 to a TH1 response induced by dendritic cells and collagen type I. *J Allergy Clin Immunol* 1999;**104**:1052–1059.
28. Hattori H, Okano M, Yamamoto T, Yoshino T, Yamashita Y, Watanabe T et al. Intranasal application of purified protein derivative suppresses the initiation but not the exacerbation of allergic rhinitis in mice. *Clin Exp Allergy* 2002;**32**:951–959.
29. de Jong EC, Vieira PL, Kalinski P, Schuitemaker JH, Tanaka Y, Wierenga EA et al. Microbial compounds selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells *in vitro* and diverse Th cell-polarizing signals. *J Immunol* 2002;**168**:1704–1709.
30. Boonstra A, Asselin-Paturel C, Gilliet M, Crain C, Trinchieri G, Liu Y-J et al. Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: dependency on antigen dose and differential toll-like receptor ligation. *J Exp Med* 2003;**197**:101–109.