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ORIGINAL ARTICLE

Interleukin-8 produced by T cells is under the control of dopamine signaling

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Abstract

Objective Interleukin (IL)-17 produced by T helper (Th)17 cells is considered to be accepted by the IL-17 receptor expressed by the airway epithelium, thereby influencing the migration of neutrophils by inducing the production of IL-8, a neutrophil migration chemokine. However, it was recently reported that Th17 cells show marked production of IL-8. Our previous studies showed that Th17 cell differentiation is governed by dopamine signals. We therefore analyzed the role of dopamine signals in IL-8 production.

Methods We cocultured dopamine D2-like receptor agonists and an antagonist with peripheral blood mononuclear cells isolated from the peripheral blood of healthy human volunteers, as well as cloned Th cells established from peripheral blood mononuclear cells. We then determined the levels of IL-8.

Results Cloned Th1 and Th17 cells, but not Th2 cells, showed marked production of IL-8. Interferon-γ and IL-17 levels, but not those of IL-5, showed a positive correlation with IL-8 levels. The IL-8 production of activated T cells was augmented by a dopamine D2-like receptor antagonist and suppressed by dopamine D2-like receptor agonists.

Conclusions The dopamine signal is not only a differentiation induction factor of Th17, but is also involved in IL-8 production from activated T cells. It is suppressed by dopamine D2-like receptor agonists, which are known to ameliorate Parkinson's disease.

Introduction

Bronchial asthma is a condition characterized by chronic inflammation of the airway that is mediated by inflammatory cells, such as eosinophils, neutrophils, lymphocytes and mast cells. Cells that determine the airway structure are affected by this inflammation, including airway epithelial cells, fibroblasts and airway smooth muscle cells.[1] Asthma is classified into neutrophil-dominant and eosinophil-dominant types.[2] The neutrophil-dominant type presents with neutrophilic inflammation derived from T helper (Th)1/17 responses,[3,4] whereas the eosinophil-dominant type presents with eosinophilic inflammation derived from Th2 responses to environmental allergens in general.[5]

Recently, interleukin (IL)-33, a cytokine derived from epithelial cells, was newly suggested to be involved in asthma pathology through group 2 innate lymphoid cells.[6,7] Neutrophilic asthma is often quite severe,[8] and this severity has been reported to be correlated with the concentration of tumor necrosis factor and IL-8 contained in the sputum.[9] It might be that such IL-8 is from the neutrophils themselves. However, at the initiation stage of neutrophilic airway inflammation, IL-17 produced by Th17 cells is considered to be accepted by the IL-17 receptor of the airway epithelium, thereby influencing the migration of neutrophils by inducing IL-8 production. However, it was recently reported that Th17 cells show marked production of IL-8.[10] In the present study, we analyzed the

effects of T cells on the direct induction of neutrophilic inflammation. Our previous studies showed that Th17 cell differentiation is governed by dopamine signals.[11] We therefore analyzed the role of dopamine signals in IL-8 production. In our previous study on rheumatoid arthritis, we determined dopamine concentration not only in synovial fluids, but also in peripheral blood mononuclear cell (PBMC) culture supernatant fluids, by using the ethylenediamine condensation method.[12] The latter ranged from 5 to 30 pg/mL (30–200 pmol/L). Because RC50 of dopamine for D2R is 1.3 nmol/L, dopamine produced by antigen-presenting cells in an immunological microenvironment should affect T cells that express D2R, which attests to the effect of a D2R antagonist, haloperidol.[13,14]

The dopamine receptor is divided into two groups based on the nature of coupled G proteins. Dopamine receptors are seven-transmembrane G protein-coupled receptors and consist of five subtypes, from D1 to D5.[15] These subtypes are classified into two subgroups. D1 and D5 are D1-like receptors that couple with Gαs, which increases the levels of intracellular cyclic adenosine monophosphate (cAMP). In contrast, D2, D3 and D4 are D2-like receptors that couple with Gαi, which decreases the levels of intracellular cAMP.[14,16] Dopaminergic signaling through D1-like receptors couples with the increase of cAMP production in T lymphocytes, leading to the production of IL-6 and differentiation to the Th17 phenotype.[13] Dopamine itself has been shown to inhibit the proliferation of human lymphocytes and even to induce apoptosis in peripheral mononuclear cells. We analyzed the effect of dopamine signals on IL-8 production using D2-like receptor antagonists and agonists in the present study.

Methods

Preparation of PBMC

Peripheral blood was obtained from healthy volunteers under protocols approved by the Saitama Medical University Ethics Committee (#787-II). The blood was centrifuged for 10 min at 450 *g*, and separated into blood cells and plasma. After adding RPMI1640 (Sigma-Aldrich, St. Louis, MO, USA) to the blood cells, the sample was overlayed onto a Ficoll-Paque PLUS (GE Healthcare, Buckinghamshire, England, UK) and centrifuged for another 40 min at 450 *g*. PBMC were recovered from the top-most layer of Ficoll-Paque. Two to seven individuals were used for the analyses.

Preparation of purified protein derivative of tuberculin-specific cloned T cells

PBMC (1.5×10^5 cells/well) were cocultured with purified protein derivative (PPD) antigen (Ag; 1 μg/mL; Japan BCG Laboratory, Tokyo, Japan) in RPMI 1640 (Sigma-Aldrich) medium containing 10% human serum, 1% L-glutamine, 50 IU/mL penicillin and 50 μg/mL streptomycin (R10H medium) at 37°C in a humidified atmosphere at 5% CO_2. After 7 days, the resulting PPD-specific T cells were seeded at three cells/wells by the limiting dilution method in a microculture plate (Nunc#163118; Fisher Scientific, Roskilde, Denmark) and cocultured with irradiated (30 Gy) PBMC (2×10^4 cells/well) as an alternative to antigen-presenting cells (APC) at 37°C in a humidified atmosphere at 5% CO_2. After 7 days, 5–10% of wells were positive for proliferative responses and transferred to 96-well plates. The cells were then cultured to grow sufficiently by adding the irradiated (30 Gy) PBMC (1.5×10^5 cells/well) as an alternative to APC in the presence of PPD Ag.

Culture of PBMC and PPD-specific cloned T cells

PBMC (1.5×10^5 cells/well) were cocultured for 3 days in R10H medium containing an agonistic anti-CD28 antibody (Ab; 1 μg/mL; clone: CD28.2; BD Biosciences, San Diego, CA, USA) in the presence or absence of D2-like receptor antagonist (haloperidol) at 37°C in a humidified atmosphere at 5% CO_2 in a flat-bottomed 96-well plate that had been coated overnight with an anti-CD3 Ab (2 μg/mL in phosphate-buffered saline; clone: HIT3a; BD Biosciences). As described above, PPD-specific T cloned cells (5×10^4 cells/well) were also cocultured. We then determined the IL-8 concentration of the supernatant.

Preparation of allo-reactive Th cloned cells using a mixed lymphocyte reaction

HLA-DR-non-shared PBMC of two donors (3000 cells/individual donor/well of microculture plate; Nunc#163118; Fisher Scientific) were cocultured in R10H medium to induce a two-way mixed lymphocyte reaction (MLR) at 37°C in a humidified atmosphere at 5% CO_2. IL-2 (50 U/mL) and IL-4 (10 U/mL) were added to generate Th1 and Th2 cells, respectively. Transforming growth factor-β (10 ng/mL), tumor necrosis factor-α (10 ng/mL), IL-1β (10 ng/mL) and IL-6 (10 ng/mL) were added to generate Th17. After 10 days, the allo-reactive Th1/2/17 cloned cells that had proliferated (positive rate 5–10%) were transferred to 96-well microplates. These

cells were then cultured to grow sufficiently by adding the irradiated (30 Gy) PBMC (1.5×10^5 cells/well) as an alternative to APC.

Evaluation of the effect of cAMP inhibitors on IL-8 production

PBMC (1.5×10^5 cells/well) were cocultured for 3 days in R10H medium containing an anti-CD28 Ab (1 μg/mL) in the presence or absence of D2-like receptor antagonist (haloperidol; Sigma-Aldrich) and an adenylyl cyclase inhibitor (MDL-12,330A; BIOMOL International, Plymouth Meeting, PA, USA) at 37°C in a humidified atmosphere at 5% CO_2 in a flat-bottomed 96-well plate that had been coated overnight with an anti-CD3 Ab (2 μg/mL in phosphate-buffered saline). We then determined the IL-8 concentration of the supernatant.

Evaluation of the effect of dopamine D2-like receptor agonists (pramipexole and ropinirole) on IL-8 production

PBMC (1.5×10^5 cells/well) were cocultured for 7 days in medium (RPMI1640, 10% bovine serum, 1% L-glutamine, 50 IU/mL penicillin, 50 μg/mL streptomycin) containing a *Candida* Ag (15 μg/mL; Torii Pharmaceutical, Tokyo, Japan) in the presence of D2-like receptor agonists (pramipexole and ropinirole; Sigma-Aldrich) separately at 37°C in a humidified atmosphere at 5% CO_2 in a flat-bottomed 96-well plate.

Determining the cytokine concentration by an enzyme-linked immunosorbent assay

The cytokine concentration of the culture supernatant was determined using an interferon-γ, IL-5, IL-8 or IL-17 enzyme-linked immunosorbent assay kit (R&D Systems, Guthrie, MN, USA).

Statistical analysis

A two-tailed Student's *t*-test was used to assess the significance of differences between groups in an enzyme-linked immunosorbent assay. $P < 0.05$ was considered significant.

Results

Effects of stimulation by anti-CD3/28 Ab and haloperidol on the IL-8 production of PBMC

PBMC were stimulated by agonistic anti-CD3/28 Ab and cultured. After 3 days, the IL-8 levels in the supernatant were measured by an enzyme-linked immunosorbent assay. PBMC stimulated by anti-CD3/28 Ab showed marked production of IL-8 (Fig. 1; haloperidol 0 μmol/L). However, in the presence of haloperidol, the IL-8 production of PBMC stimulated by anti-CD3/28 Ab was significantly augmented (Fig. 1; haloperidol 10 μmol/L: $P < 0.001$).

Effects of haloperidol on PPD-specific cloned T cells in the absence of APC

PBMC contain not only T cells, but also APC, which are also a source of dopamine. To eliminate the effect of APC, we prepared PPD-specific cloned T cells as described earlier. These T cells, mainly consisting of Th1 cells, in the absence of APC stimulated by anti-CD3/28 Ab were cultured in the presence or absence of haloperidol. The IL-8 production of the PPD-specific cloned T cells stimulated by anti-CD3/28 Ab was again significantly augmented by haloperidol (Fig. 2; 10 μmol/L: $P < 0.05$ and 1 μmol/L: $P < 0.01$).

Positive correlation of Th1/17 cytokines with the IL-8 production

We prepared allo-reactive cloned Th1/2/17 cells as described earlier. These Th1/2/17 cells and irradiated allogeneic PBMC as an alternative to APC were cocultured. After 7 days, the supernatant was collected. The correlation between IL-8 and interferon-γ (Th1 cytokine) of the Th1 cells, IL-5 (Th2 cytokine) of the Th2 cells and IL-17 (Th17 cytokine) of the Th17 cells was analyzed. The IL-8 levels in the Th1/17 cells were approximately 10-fold higher than those in the Th2 cells ($P < 0.0001$). Furthermore, there was a significant positive correlation between interferon-γ and IL-8 (Fig. 3a; $r = 0.3172$, $P < 0.01$),

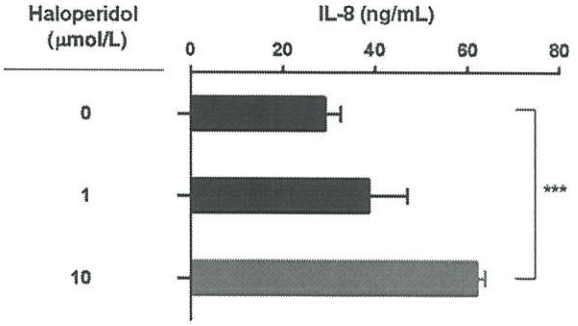

Figure 1 The interleukin (IL)-8 production of peripheral blood mononuclear cells stimulated by anti-CD3/28 antibody in the presence or absence of haloperidol (1 and 10 μmol/L). The results are representative of four experiments. ***$P < 0.001$.

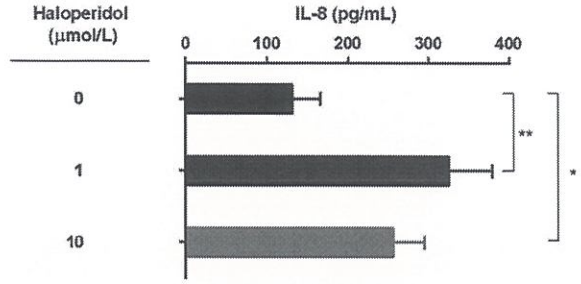

Figure 2 The interleukin (IL)-8 production of purified protein derivative-specific cloned T cells stimulated by anti-CD3/28 antibody in the presence or absence of haloperidol (1 and 10 μmol/L). The results are representative of three experiments. *$P < 0.05$, **$P < 0.01$.

whereas no such correlation was observed between IL-5 and IL-8 (Fig. 3b; $r = -0.0522$, not significant). There was also a significant positive correlation between IL-17 and IL-8, as shown in Figure 3c ($r = 0.5485$, $P = 0.01$).

Effects of an adenylyl cyclase inhibitor on the IL-8 production of anti-CD3/28 Ab-stimulated PBMC

PBMC were stimulated by agonistic anti-CD3/28 Ab, and cultured in the presence or absence of haloperidol and MDL-12,330A, an adenylyl cyclase inhibitor. The IL-8 production was significantly augmented by haloperidol (10 μmol/L), as described earlier ($P < 0.05$). In contrast, the IL-8 production was significantly suppressed in the presence of MDL-12,330A (Fig. 4; $P < 0.01$).

Effects of the dopamine D2-like receptor agonists, pramipexole and ropinirole, on the IL-8 production of PBMC stimulated by *Candida albicans* Ag

Human PBMC react to exposure to *Candida albicans* Ag through Th1/17 responses. Therefore, IL-8 production might be high under such conditions, as speculated based on the findings in Figure 3. PBMC were stimulated by *C. albicans* Ag and then cultured in the presence or absence of the D2-like receptor agonists, pramipexole and ropinirole. After 7 days, the levels of IL-8 in the supernatant were measured by an enzyme-linked immunosorbent assay. The IL-8 production was significantly suppressed at all concentrations of D2-like receptor agonists tested compared with the control group (Fig. 5).

Discussion

High levels of IL-8 were produced by PBMC stimulated by anti-CD3/28 Abs, as well as by PPD-specific cloned T cells. We confirmed in the present study the previous finding that Th17 cells produce high levels of IL-8 to induce the migration of neutrophils.

The IL-8 production of PBMC stimulated by anti-CD3/28 Ab was augmented by haloperidol, and the same finding was noted with PPD-specific cloned T cells in the absence of APC, thereby eliminating the influence of APC. Signaling through D2-like receptor expressed on Th cells might be inhibited by haloperidol, a D2-like receptor antagonist. The cAMP levels in Th cells would then be increased by the dopamine these cells produced, thereby augmenting the IL-8 production. It is therefore likely that the dopamine signaling through the D1-like receptor positively influences the IL-8 production from T cells, whereas that through D2-like receptor negatively influences it.

As the clonal frequency of allo-reactive T cells in the HLA-DR-non-shared combination was as high as 10^{-4}, we were able to prepare cloned T cells easily. Furthermore, T cells activated by MLR are mainly naïve CD4 T cells.[17] Therefore, it is possible to induce Th1/2/17 cells by changing the cytokine environment in the induction phase. IL-8 production

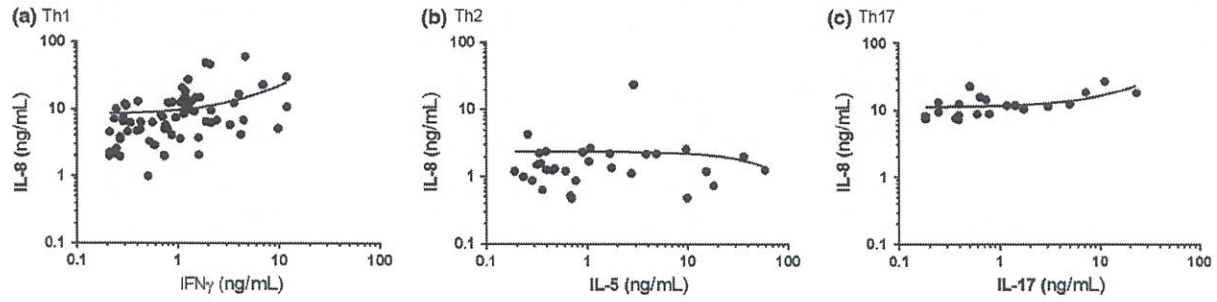

Figure 3 (a) The correlation between interleukin (IL)-8 and interferon-γ (IFNγ) produced from allo-reactive T helper (Th)1 cloned cells ($r = 0.3172$, $P < 0.01$). (b) The correlation between IL-8 and IL-5 produced from allo-reactive Th2 cloned cells ($r = -0.0522$, not significant). (c) The correlation between IL-8 and IL-17 produced from allo-reactive Th17 cloned cells ($r = 0.5485$, $P = 0.01$).

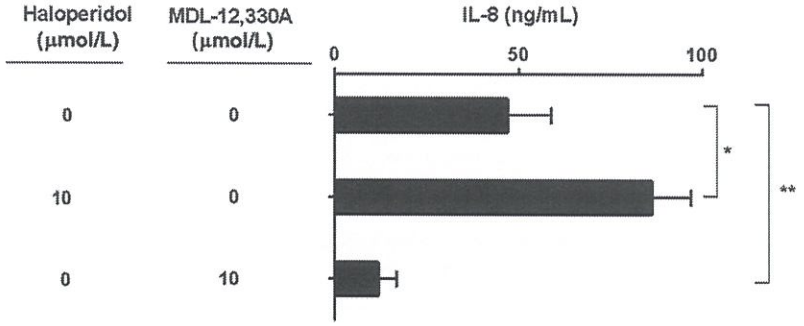

Figure 4 The interleukin (IL)-8 production of peripheral blood mononuclear cells stimulated by anti-CD3/28 antibody in the presence or absence of haloperidol (10 μmol/L) and MDL-12,330A (10 μmol/L). The results are representative of three experiments. *$P < 0.05$, **$P < 0.01$.

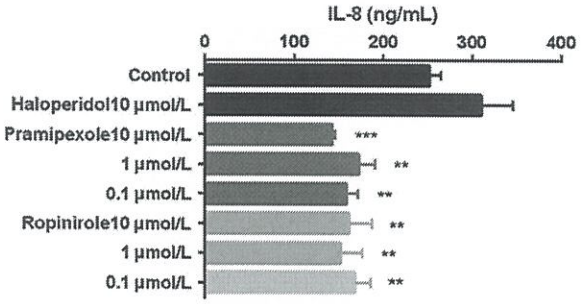

Figure 5 The interleukin (IL-8) production of peripheral blood mononuclear cells stimulated by *Candida* antigen in the presence or absence of haloperidol (10 μmol/L) and pramipexole and ropinirole (0.1, 1 and 10 μmol/L). The results are representative of four experiments. **$P < 0.01$, ***$P < 0.001$.

was detected in the supernatant of allo-reactive Th1/2/17 cells induced by MLR. There was a positive correlation between the Th1/17 cytokines and the IL-8 production. The levels of IL-8 produced by Th2 cells were approximately 90% lower than those produced by Th1/17. Furthermore, no correlation was observed between Th2 cytokine and IL-8 in Th2 cells. The capacity of Th2 cells to augment neutrophilic inflammation seems to be rather weak, and IL-8 derived from Th1/17 responses is likely to mainly augment neutrophilic inflammation.

The IL-8 production was significantly suppressed in the coculture of PBMC and MDL-12,330A, an adenylyl cyclase inhibitor. It is therefore conceivable that a decrease in the levels of intracellular cAMP in T cells was induced, leading to decreased IL-8 production. Taken together, these findings show that the dopamine signaling is involved in the direct production of IL-8 by T cells, which is responsible for the increase and decrease in the intracellular cAMP levels.

PBMC stimulated by *C. albicans* Ag produced high levels of IL-8, which were subsequently significantly suppressed by the dopamine D2-like receptor agonists, pramipexole and ropinirole. D2-like receptor on T cells is stimulated by these agonists, which should lead to decreased intracellular cAMP levels. This likely then leads to a decrease in the IL-8 produced directly by T cells. The IL-8 production had no significant difference at 1 nmol/L of D2-like receptor agonists, pramipexole and ropinirole, compared with the control group.

Severe asthma is characterized by not only eosinophilic inflammation, but also by neutrophilic inflammation.[18] Both Th1/17 and group 3 innate lymphoid cells are reportedly involved in neutrophilic airway inflammation.[19] Pepe *et al.* reported that IL-8 is the most important factor influencing the severity of human airway neutrophilic inflammation.[20] We herein reported that dopamine signaling affects the direct production of IL-8 by T cells, especially Th1 and Th17 cells.

Dopamine receptor antagonists and agonists are widely used for the treatment of Parkinson's disease and schizophrenia, both of which originate from aberrant dopamine signals. In the present study, the production of IL-8 by T cells was found to be augmented by the dopamine D2-like receptor antagonist used to treat schizophrenia, and suppressed by the dopamine D2-like receptor agonists used to treat Parkinson's disease.

Rheumatoid arthritis is a typical neutrophilic inflammation, with a prevalence in untreated schizophrenia patients and only one-tenth of that in healthy individuals, according to an epidemiological study.[12] Therefore, the association between the dopamine balance and neutrophilic inflammation has also been suggested in epidemiological surveys.

In the present study, we showed that IL-8 is directly produced by activated T cells, which are under the control of dopamine signaling. Neutrophilic inflammation in chronic inflammatory diseases, such as asthma, rheumatoid arthritis, ulcerative colitis and multiple sclerosis,[21–24] might be suppressed by dopamine D2-like receptor agonists, which are therapeutic agents for Parkinson's disease. An evaluation of the *in vivo* effects of dopamine D2-like receptor agonists is eagerly awaited.

Acknowledgments

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Disclosure of ethical statements

The protocol for this research project has been approved by ethics committee of Saitama Medical University (#787-II), and it conforms to the provision of Declaration of Helsinki. All informed consent was obtained from the participants.

Conflict of interest

S.M. is an employee of iMmno, Inc. The other authors declare no conflict of interest.

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