Dopamine Induces IL-6–Dependent IL-17 Production via D1-Like Receptor on CD4 Naive T Cells and D1-Like Receptor Antagonist SCH-23390 Inhibits Cartilage Destruction in a Human Rheumatoid Arthritis/SCID Mouse Chimera Model

Kazuhisa Nakano, Kunihiro Yamaoka, Kentaro Hanami, Kazuyoshi Saito, Yasuyuki Sasaguri, Nobuyuki Yanagihara, Shinya Tanaka, Ichiro Katsuki, Sho Matsushita and Yoshiya Tanaka

*J Immunol* 2011; 186:3745-3752; Prepublished online 9 February 2011; doi: 10.4049/jimmunol.1002475

http://www.jimmunol.org/content/186/6/3745

References This article cites 66 articles, 22 of which you can access for free at:

http://www.jimmunol.org/content/186/6/3745.full#ref-list-1

Subscription Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts

*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2011 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Dopamine Induces IL-6–Dependent IL-17 Production via D1-Like Receptor on CD4 Naive T Cells and D1-Like Receptor Antagonist SCH-23390 Inhibits Cartilage Destruction in a Human Rheumatoid Arthritis/SCID Mouse Chimera Model

Kazuhisa Nakano,* Kunihiro Yamaoka,* Kentaro Hanami,* Kazuyoshi Saito,* Yasuyuki Sasaguri,† Nobuyuki Yanagihara,‡ Shinya Tanaka,§ Ichiro Katsuki,¶ Sho Matsushita,‖ and Yoshiya Tanaka*†

A major neurotransmitter dopamine transmits signals via five different seven-transmembrane G protein-coupled receptors termed D1–D5. Several studies have shown that dopamine not only mediates interactions into the nervous system, but can contribute to the modulation of immunity via receptors expressed on immune cells. We have previously shown an autocrine/paracrine release of dopamine by dendritic cells (DCs) during Ag presentation to naive CD4⁺ T cells and found efficacious results of a D1-like receptor antagonist SCH-23390 in the experimental autoimmune encephalomyelitis mouse model of multiple sclerosis and in the NOD mouse model of type I diabetes, with inhibition of Th17 response. This study aimed to assess the role of dopaminergic signaling in Th17-mediated immune responses and in the pathogenesis of rheumatoid arthritis (RA). In human naive CD4⁺ T cells, dopamine increased IL-6–dependent IL-17 production via D1-like receptors, in response to anti-CD3 plus anti-CD28 mAb. Furthermore, dopamine was localized with DCs in the synovial tissue of RA patients and significantly increased in RA synovial fluid. In the RA synovial/SCID mouse chimera model, although a selective D2-like receptor antagonist haloperidol significantly induced accumulation of IL-6* and IL-17* T cells with exacerbated cartilage destruction, SCH-23390 strongly suppressed these responses. Taken together, these findings indicate that dopamine released by DCs induces IL-6–Th17 axis and causes aggravation of synovial inflammation of RA, which is the first time, to our knowledge, that actual evidence has shown the pathological relevance of dopaminergic signaling with RA. The Journal of Immunology, 2011, 186: 3745–3752.
CD4+ T cells, on activation and expansion, develop into different Th cell subsets with different cytokine profiles and distinct effector functions. Th17 cells are the newest member of the effector Th cell family and are characterized by their ability to produce specific cytokines such as IL-17, IL-22, IL-17F, IL-21, and CCL20. TGF-β, IL-6, IL-1β, IL-21, and IL-23 are important for the polarization of Th17 cells from human CD4+ naive T cells (20, 21), and the absence of TGF-β mediates a shift from a Th17 profile to a Th1 profile (21, 22). Recent studies have demonstrated that Th17 cells, rather than Th1 cells, play a pivotal role in the pathogenesis of autoimmune disease models, including experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis (23, 24). Furthermore, a number of observations suggest that Th17 cytokines may be important in rheumatoid arthritis (RA), a representative human autoimmune inflammatory disease. IL-17 is found in RA synovial fluid and in the T cell-rich areas of RA synovial tissue (25–28). In RA patients, in a 2-y prospective study, the cytokine expression of TNF-α, IL-1β, and IL-17 was predictive of joint destruction, whereas IFN-γ was protective (29). Although the direct proinflammatory effects of IL-17 are often small when compared with those of IL-1β and TNF-α, IL-17 may enhance the effects of other cytokines. Using RA synovial tissue fibroblasts, IL-17 enhanced IL-1–mediated IL-6 and CCL20 production (30, 31) and the TNF-α–induced synthesis of IL-1β, IL-6, IL-8, and CCL20 (31, 32), indicating that the effects of IL-17 may be caused by its ability to promote inflammation by inducing cytokines and chemokines (33, 34). Furthermore, direct effector functions of Th17 cells in RA have been demonstrated in that receptor activator for nuclear factor of activated T cells (RANKL) expression on the surface of Th17 cells induces osteoclastogenesis (25, 35, 36), promoting cartilage and bone destruction independently of TNF-α and IL-1β (37, 38). These proinflammatory cytokines induced by IL-17 might even feedback on the generation and expansion of further Th17 cells in this specific microenvironment of the joint. Actually, initial observations from phase I trials show that signs and symptoms of RA are significantly suppressed after treatment with anti–IL-17 Abs, without notable adverse effects, indicating that IL-17 is an important therapeutic target for the treatment of RA (39).

Dendritic cells (DCs) are the most powerful APCs that determine the balance of differentiation of Th cell subsets. In particular, cytokines produced by DCs are regarded as the key to T cell differentiation. For example, IL-12 produced by DCs induces the differentiation to Th1 (40). In our previous studies, we showed that human monocyte-derived DCs stored dopamine, and that dopamine was released on Ag-specific interaction with naive CD4+ T cells (41). Furthermore, antagonizing dopamine receptor subtypes differentially affected Th17 polarization in vitro (19). D2-like receptor antagonists judged to be Th17 adjuvants in vitro caused a marked deterioration of EAE, whereas D1-like receptor antagonists exhibited a marked improvement in EAE (19). Although the precise mechanism of how D1-like receptor antagonists inhibit IL-17 production remains undetermined, our previous results indicated that DC-derived dopamine might induce the differentiation to Th17.

In this study, we show that dopamine induces IL-6–dependent IL-17 production in vitro, and that antagonizing D1-like receptor inhibits dopamine-mediated IL-6–Th17 axis in vitro and in a human RA/SCID mouse chimera model.

Materials and Methods

**Purification of human naive CD4+ T lymphocytes**

The study using peripheral blood of healthy volunteers was approved by the Human Subjects Research Committee of the University of Occupational and Environmental Health, Japan. Highly purified, untouched CD4+ T cells were prepared from PBMCs of healthy volunteers by exhaustive immunomagnetic negative selection. We routinely used AutoMACS separation columns (Miltenyi Biotec, Germany) and a CD4+ T cell Isolation Kit II (Miltenyi Biotec), and separated into CD45RO+ memory T cells and CD45RA+ naive T cells using CD45RO MicroBeads (Miltenyi Biotec). A FACScalibur flow cytometer (BD Biosciences) showed the purity of these cells to be >99%.

**Synovial tissues and treatments**

Synovial tissues were obtained from patients with active RA, diagnosed according to the criteria of the American College of Rheumatology (formerly the American Rheumatism Association) (42), undergoing joint replacement or synovectomy. This study was approved by the Human Subjects Research Committee of our university. Informed consent was obtained from all subjects enrolled in the study. Synovial membrane samples were perfused with 5% glutaraldehyde, and paraffin sections (3 μm thick) were prepared for immunohistochemical studies. Synovial membrane and cartilage were also dissected under sterile conditions in PBS and immediately prepared for coimplantation to SCID mice.

**Cell cultures**

Synovial tissue samples were dissected under sterile conditions in PBS and immediately prepared for culture of fibroblast-like synovial cells. In brief, the tissue sample was minced into small pieces and digested with collagenase dispase (Sigma Aldrich, Tokyo, Japan) in serum-free DMEM (Life Technologies, NY). After filtering through a nylon mesh, the cells were extensively washed and suspended in DMEM, supplemented with 10% FCS (Bio-Pro, Karlsruhe, Germany). Finally, isolated cells were seeded in 25-cm2 culture flasks (Falcon, Lincoln Park, NJ) and cultured in a humidified 5% carbon dioxide atmosphere. After overnight culture, nonadherent cells were removed and incubation of adherent cells was continued in a fresh medium. At confluence, the cells were trypsinized, passaged at a 1:3 split ratio, and recultured. The medium was changed twice each week, and the cells were used after three to five passages.

**Quantitative analysis of IFN-γ, IL-3, IL-17, IL-1β, TGF-β1, and IL-6**

Cytokine concentrations in culture supernatant were measured by ELISA kit (R&D Systems).

**Quantitative real-time PCR**

Total RNA was extracted using the RNaseasy mini kit (Qiagen, Tokyo, Japan), according to the manufacturer’s instructions. cDNA was reverse transcribed using the high-capacity RNA-to-cDNA Master Mix (Applied Biosystems, Tokyo, Japan). Primers and probes were all predesigned TaqMan Gene Expression Assays from Applied Biosystems. Real-time PCR was performed on the StepOnePlus Real-Time PCR System (Applied Biosystems) machine. Predesigned specific primers were used to detect rorc (Hs01076112) and 18s (Hs99999901). Target gene expression was normalized using 18s expression level and expressed in arbitrary units.

**Cell viability assay**

Cell viability was assessed using a TetraColor One kit including WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium] and electron carrier mixture (Seikagaku, Tokyo, Japan). The WST-8 assay is based on the conversion of tetrazolium salt WST-8 to the highly water-soluble formazan. Cells (1 × 105) were seeded and incubated on a 96-well flat-bottom plate (Iwaki) in DMEM containing 10% FCS in a final volume of 0.1 ml for 24 h at 37°C. They were treated with different agents for various time intervals. Ten milliliters of a solution containing 5 mM WST-8, 0.2 mM 1-methoxy-5-methylphenazium methosulfate, and 150 mM NaCl was added to each well. After incubation for 2 h at 37°C, the OD of each well was measured on a microplate reader at 450 nm.

**Flow cytometric detection of cell-surface dopamine receptors**

Staining and flow cytometric analysis of synovial fibroblasts were carried out by standard procedures as already described using a FACScalibur (BD Biosciences). Anti-dopamine D1–D5 receptor rabbit polyclonal Abs were obtained from Calbiochem. In brief, synovial fibroblasts (1 × 105 cells/sample) were incubated with anti-dopamine receptor polyclonal Ab for 20 min at 4°C, followed by FITC-conjugated anti-rabbit IgG (Sigma) at saturating concentrations in FACS medium consisting of HBSS (Nissui, Tokyo, Japan), 0.5% human serum albumin (Mitsubishi Pharma, Osaka, Japan), 0.5% BSA, 0.1% sodium azide, and 20% FCS. After each incubation step, cells were washed with FACS medium, fixed with 3% paraformaldehyde, and washed again before analysis by FACSCalibur flow cytometry (BD Biosciences). This study was approved by the Human Subjects Research Committee of the University of Occupational and Environmental Health, Japan.
Japan), and 0.2% Na2HPO4 (Sigma) for 20 min at 4°C. After three washes in FACS medium, the cells were analyzed with the FACSCalibur.

Fractional catecholamine analysis

Ten patients with RA and 10 control patients with osteoarthritis (OA) were diagnosed according to the American College of Rheumatology criteria (42). Synovial fluid specimens were collected during either diagnostic or therapeutic arthrocentesis of the knee (10 knees in 10 cases with RA and 10 knees in 10 cases with OA; Table I). All synovial samples were collected under sterile conditions, and the cellular components were removed immediately after centrifugation. The supernatants were stored at −30°C. The supernatants were transferred immediately to a test tube containing perchloric acid (final concentration, 0.4 M). Catecholamines (dopamine, noradrenaline, and adrenaline) were adsorbed onto aluminum hydroxide and estimated by the ethylenediamine condensation method using a fluorescence spectrophotometer (F-4010; Hitachi, Tokyo, Japan) with excitation and emission wavelengths of 420 and 540 nm, respectively (43).

Preparation of human RA/SCID chimera mice and treatment with dopamine receptor antagonists

Human RA/SCID chimera mice were evaluated as models for the treatment study. A total of 18 male SCID mice (CR.17/lcr; CLEA Japan, Tokyo, Japan), 6- to 7-wk-old, which had been bred under specific pathogen-free conditions at our university animal center, were used for establishment of the human RA/SCID chimera mouse model. Pannus tissue from synovial membrane, cartilage, and bone, collected as a mass from RA patients at the time of surgery, was used for implantation. The size of the removed specimen was adjusted to a block almost 4–8 mm in diameter before implantation. The mice were anesthetized with diethyl ether, according to the guidelines established by the animal ethics committee of the University of Occupational and Environmental Health, Japan. The tissue implants were grafted s.c. on the backs of the mice. All surgical procedures were performed under sterile conditions. The mice were randomly assigned to three groups at 1 wk after the implantation. The mice in the test groups were s.c. administered 0.3 mg/kg haloperidol (a D2-like receptor antagonist; Sigma) twice a week for 3 wk. The mice in the control group received PBS served as a negative control. Thirty days after implantation, the mice were anesthetized and their implanted tissues were removed. The animal experiments were approved by and performed in compliance with the guidelines of the Institutional Animal Care and Use Committee.

Fractional catecholamine analysis

The 3-μm-thick sections prepared from RA synovial tissues were incubated with rabbit anti-dopamine Ab (Chemicon) or S-100 and then incubated with secondary Ab (EnVision+; Dako) (44, 45). The implanted tissues were removed and histologically observed after H&E staining. To examine biological activities of the implanted tissues, we performed immunohistochemical staining as previously described (44, 45). The sections were stained with the following anti-human monoclonal Abs and an immunostaining kit: IFN-γ, IL-17, IL-6, and EnVision+. These were purchased from Dako Japan.

Statistical analysis

Parametric testing among three or more groups was performed by ANOVA. Nonparametric testing was performed using the Mann–Whitney rank sum test.

Results

Dopamine increases IL-6-dependent IL-17 secretion from human T cells

We have previously reported that only D1 was specifically expressed at a high level in CD4+ naive T cells, and that dopamine increased the cAMP concentrations in CD4+ naive T cells via D1-like receptors, and subsequently induced the secretion of IL-4 and IL-5 (19, 41). In this study, we first examined the pattern of cytokine secretion including IL-17 from CD4+ naive T cells, which were stimulated by anti-CD3/CD28 Abs and simultaneously added dopamine. Dopamine slightly increased IL-5 secretion without affecting IFN-γ production, whereas dopamine markedly increased IL-17 secretion in a dose-dependent manner, indicating dopamine as an important factor for Th17 differentiation rather than Th2 differentiation (Fig. 1A). We also measured the concentration of Th17-inducible cytokines such as IL-6, IL-1β, and TGF-β. Interestingly, IL-6 secretion also increased in a dose-dependent manner (Fig. 1B), whereas minimum IL-1β and no TGF-β secretion were observed (data not shown). At the same time, we investigated the response of naive human T cells to

FIGURE 1. Dopamine-mediated cytokine secretion from CD4+ T cells. Purified human CD4+ naive T cells were stimulated with anti-CD3 and anti-CD28 plate-bound Abs, and with the indicated concentrations of dopamine. A, ELISA for IFN-γ, IL-5, and IL-17 was performed on the supernatants after a 3-d culture period. B, Cytokine beads array for IL-1β and IL-6 was performed on the supernatants after a 3-d culture period. C, Real-time PCR analysis of the expression of nroc after stimulation. mRNA values were normalized to 18s. Purified human CD4+ naive T cells were pretreated with 200 μg/ml tocolizumab or γ-globulin (as a control Ab) for 30 min and stimulated with 50 ng/ml PMA and 1 μg/ml ionomycin (D). PBS served as a negative control. Data are mean ± SD of five experiments in triplicate. Statistically significant differences are indicated by asterisks. **p < 0.01, *p < 0.05 versus control. Figures represent three independent experiments.
Dopamine without anti-CD3/CD28 Abs. However, all cytokines that we tested were undetectable or very low level (data not shown). Furthermore, we investigated the rorc mRNA expression in these cells after stimulation with dopamine by quantitative PCR (Fig. 1C). Stimulation with anti-CD3/CD28 Abs induced the rorc mRNA expression, whereas dopamine markedly increased the expression level at 72 h. The induction of IL-17 secretion, which was dopamine dependent, was almost completely inhibited when human CD4⁺ naive T cells were pretreated with tocilizumab, an anti-human IL-6R Ab (Fig. 1D). These results suggested that dopamine increases IL-17 production from T cells via IL-6 production.

D1-like receptor antagonists inhibit dopamine-mediated IL-6 and IL-17 secretion from human T cells

The IL-6 gene promoter contains a cAMP response element (46). In our previous studies, we showed that dopamine-mediated cAMP elevation in human CD4⁺ naive T cells was completely inhibited by treatment with SCH-23390, a selective D1-like antagonist (41). To evaluate whether antagonizing D1-like receptors decreases dopamine-mediated IL-6 and IL-17 production, we treated CD4⁺ naive T cells with or without SCH-23390, and then IL-6 and IL-17 secretion was determined by ELISA. As shown in Fig. 2, SCH-23390 completely inhibited dopamine-mediated IL-6 and IL-17 secretion from T cells. This inhibitory effect by antagonizing D1-like receptors was demonstrated not only by SCH-23390, but by LE300, another D1-like receptor antagonist (data not shown), indicating that antagonizing D1-like receptor inhibited dopamine-mediated IL-6–Th17 axis.

Dopamine is detected in inflamed synovial tissue in RA

To investigate the roles of dopamine in the pathogenesis of synovitis in RA, we measured catecholamine concentrations in synovial fluid of RA and OA patients (Table I). Although it is necessary to compare RA synovial tissue with synovial tissue from healthy individuals, we could not obtain samples from healthy individuals. Concentrations of noradrenalin and adrenaline did not differ between RA and OA patients, but dopamine was significantly higher in RA patients (Fig. 3A). Presumably, dopamine-producing cells were increased at the site of synovitis in RA. In the synovial tissues from RA patients, S100-positive DCs were also stained with dopamine, and an accumulation of lymphocytes was prominently observed in the surroundings of DCs (Fig. 3B, 3C). Thus, it seems likely that there are DCs that synthesized and stored dopamine in RA synovial tissue.

Dopamine receptor antagonists on rheumatoid synovitis in SCID mice engrafted with human RA synovium mice

Next, to evaluate the relevance of these findings in vivo, we studied the effect of dopamine receptor antagonists in a model of the SCID mouse in which active RA synovial tissue and cartilage have been engrafted (SCID mice engrafted with human RA synovium [SCID-HuRAg]) (47). SCID-HuRAg mice were divided into three groups (n = 6) 1 wk after the transplantation, and an antagonist of D1-like receptors (SCH-23390), an antagonist of D2-like receptors (haloperidol), or vehicle (control) was administered twice a week for 3 wk for each group. The grafts were collected 30 d after implantation and pathological evaluation was performed. Macroscopically, remarkable retraction of synovial tissue was observed in the group that received D1-like receptor antagonist. In contrast, vascular proliferation and enlargement of tissue were observed in the group administered with D2-like receptor antagonist (Fig. 4A). The histopathological findings showed only slight

**FIGURE 2.** Suppression of dopamine-mediated upregulation of IL-6 and IL-17 production by a D1-like dopamine receptor antagonist. Purified human CD4⁺ naïve T cells were stimulated with anti-CD3 and anti-CD28 plate-bound Abs, 1 μM dopamine, and with or without 10 μM SCH-23390. White and gray bars represent without and with SCH-23390 exposure, respectively. Results are shown as relative fold changes compared with control cytokine production (not exposed to dopamine and SCH-23390). Data are mean ± SD of five experiments in triplicate. Statistically significant differences are indicated by asterisks. **p < 0.01 versus control.

**FIGURE 3.** Presence of catecholamine in synovial fluids and the storage of dopamine in synovial tissue. A. Synovial fluids obtained from 10 patients with RA and 10 patients with OA were analyzed by HPLC electrochemical detection for dopamine, noradrenaline, and adrenaline. The composite results are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th to 90th percentiles. B and C, Immunohistochemical staining of S-100 protein (B) and dopamine (C) in RA synovial tissue (brown staining). Arrowheads indicate dopamine-stored cells (original magnification ×20). Sections are representative for three patients.

---

**Table I.** Clinical features of the participants who contributed synovial fluid

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RA</th>
<th>OA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>1/9</td>
<td>2/8</td>
</tr>
<tr>
<td>Mean age, y (range)</td>
<td>65.2 (39–85)</td>
<td>69.5 (57–78)</td>
</tr>
<tr>
<td>Mean CRP, mg/dl (range)</td>
<td>2.8 (1.5–4.4)</td>
<td>NA</td>
</tr>
<tr>
<td>Mean MMP3, ng/ml (range)</td>
<td>441.6 (135–738)</td>
<td>NA</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein; MMP3, matrix metalloproteinase-3; NA, not applicable.
cartilage destruction, shrinkage of synovial fibroblasts, and prominent IFN-γ-producing cells in the group administered with D1-like receptor antagonist (Fig. 4B, 4C). In contrast, the group administered with D2-like receptor antagonist had marked cartilage destruction, synovial hyperplasia with angiogenesis, and prominent IL-6+ and IL-17+ cells (Fig. 4B, 4C). The effect of antagonizing D1-like receptor antagonist was demonstrated not only by SCH-23390, but by LE300, which is a selective D1-like receptor antagonist (data not shown).

D1-like receptor antagonists do not influence the viability of RA synovial fibroblasts

To clarify whether the retraction of synovial tissue in SCID-HuRAG mice treated with D1-like receptor antagonists is due to direct effect on synovial fibroblasts, we evaluated cell viability of SCH-23390–treated synovial fibroblasts using a WST-8 assay.

No significant differences were observed between antagonist-treated and untreated groups (Fig. 5A). Furthermore, we investigated the expression of dopamine receptors on synovial fibroblasts derived from RA patients. However, no D1–D5 subtype was detected on synovial fibroblasts (Fig. 5B). These results indicated that antagonizing D1-like receptors did not reduce synovial tissue in SCID-HuRAG mice directly.

Discussion

Dopamine has been shown to act on receptors present on immune cells, with all subtypes of dopamine receptors found on leukocytes (7, 8, 10). Interestingly, disorders such as schizophrenia and Parkinson’s disease, in which there are changes in dopamine receptors and dopamine signaling pathways not only in brain, but in lymphocytes, are also associated with altered immune functioning (48). For example, in patients with schizophrenia thought to be...
dependent on excessive response of D2-like dopamine receptors (49), the median incidence rate of RA is ~0.09% (50). This incidence is only one tenth that of RA in the general population, suggesting the involvement of dopamine signaling pathway in the pathogenesis of RA. However, there was no clear explanation for this epidemiological phenomenon.

The secondary lymphoid tissues are highly innervated by sympathetic nerve fibers that store high levels of dopamine (51), and lymphocytes also produce dopamine (52, 53). However, although RA synovium has been known to have the characteristics of lymphoid organs with regard to cellular composition and organization, a drastic loss of sympathetic nerve fibers in the RA synovium has been demonstrated (54). In our previous study, we demonstrated that monocyte-derived DC released vesicles containing dopamine toward T cells during DC–T cell interaction (41). Furthermore, we could detect dopamine only in DCs in RA synovium in our current study. These findings suggested that DC-derived dopamine during naive T cell–DC interaction may contribute to the development of RA.

During the development of RA, CD45RA+ T cells may be attracted by chemokines secreted by tissue macrophages/DCs. Naive CD45RA+ T cells expressing ICAM-3, which is a specific ligand of DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN), are abundantly present within the RA synovium (55), suggesting that naive T cell–DC interaction is involved in cell activation and increased release of cytokines and enzymes. Although we had tried to characterize the phenotype of the putative DCs storing significant amounts of dopamine using several Abs to DC markers such as CD1a, CD123, and BDCA-2, these DC markers were not detectable by immunohistochemical staining, which is probably due to reduced antigenicity by fixation synovial membrane samples by 5% glutaraldehyde.

Although the concentration of dopamine in the RA synovial fluid was 0.1–10 nM, calculated dopamine concentration within 1 ms after unitary synaptic release was 100–250 nM within 1 μm in dopaminergic neurons (1). Because the space at DC–T cell synapses is within 1 μm (41), the concentration of dopamine in the synapse is estimated to be 100–250 nM, thus indicating that CD4+ naive T cells might be exposed to a relatively high concentration of dopamine during DC–T cell interaction. The concentration of the dopamine we used was high compared with previous reports. However, when naive CD4 T cells were stimulated with both anti-CD3/CD28 mAb and dopamine, cell viability was not affected by dopamine up to 10 μM (data not shown). Previous in vitro studies testing dopamine at relatively high concentrations demonstrated that dopamine was immunosuppressive (14, 15). In contrast, in vivo administration of pharmacological doses of dopamine was reported to be mostly immunostimulatory (56). Thus, dopamine could be either immunostimulatory or immunosuppressive depending on the experimental condition used. However, it is highly possible that these findings are dependent on the expression pattern and the level of dopamine receptors.

Studies carried out on human and murine T cells have demonstrated that these cells express all subtypes of dopamine receptors, each of which has diverse modulatory effects on the T cell physiology. D1- and D2-like dopamine receptors are coupled to stimulation and inhibition of intracellular cAMP production, respectively (2, 13). Stimulation of the D1-like receptor impairs T cell function by increasing intracellular cAMP levels. Stimulation of D1-like receptor not only inhibits cytotoxic function of CD8+ T cells (57), but impairs function and differentiation of regulatory T cells (Tregs) (53, 58). Stimulation of dopamine D2-like receptors in normal resting peripheral human T lymphocytes induces integrin-mediated adhesion to fibronectin (4) and increases the secretion of TNF-α (primarily via D3 receptors) and the secretion of IL-10 (primarily via D2 receptors) without affecting the secretion of IFN-γ and IL-4 (7). However, because these normal resting peripheral human T lymphocytes were not purified, they were different in quality from CD4+CD45RA+ naive T cells, which we used in this study. We have already reported that there were differences in the expression pattern of dopamine receptor subtypes between naive CD4 T cells and memory CD4 T cells (19), and that dopamine increases intracellular cAMP levels in naive CD4 T cells but decreases them in memory CD4 T cells (41). Therefore, high purity of CD4+CD45RA+ naive T cells was needed to reproduce the series of our results.

We have previously demonstrated that D1-like receptors were functionally dominant, and that dopamine increased cAMP levels via D1-like receptors and induced the production of Th2 cytokine such as IL-4 and IL-5, in response to anti-CD3/CD28 mAb (41). Regarding regulation of TCR-triggered signaling by cAMP in T cells, protein kinase A (a protein kinase activated by cAMP) and cAMP induce inhibition of ERK phosphorylation (59) and of JNK activation (60), activate C-terminal Src kinase (61), and block NF-κB activation (62, 63). All of these intracellular biochemical events induce a marked impairment on T cell activation with inhibition of T cell proliferation and of cytokine production (64). In this study, we showed that dopamine markedly increased IL-17 production from T cells via IL-6 production, in response to anti-CD3/CD28 mAb. Because the IL-6 gene promoter contains a cAMP response element (46), it is likely that increased intracellular cAMP via D1-like receptors of T cells induces IL-6 production, which act as autocrine or paracrine stimulus for IL-17 production in short-term culture. Although murine Th17 cells originate from CD4+ naive T cells in the presence of IL-6 and TGF-β, the precise conditions for human Th17 differentiation remain controversial (65). Therefore, further studies are needed to elucidate the potential role of dopamine in human Th17 differentiation by long-term culture in the presence of TGF-β. However, in a human RA/SCID mouse chimera model, antagonizing dopamine receptor subtypes differentially affected cytokine expression, indicating that dopamine produced by DCs that accumulated in synovial tissue of RA established a Th17-predominant immune system via IL-6 and IL-17 production from T cells, leading to aggravation of synovitis and cartilage destruction.

Interestingly, it has been reported that dopamine reduces the suppressive and trafficking activities of naturally occurring Tregs through D1-like receptors in both human and mouse (53, 58), and that dopamine-mediated downregulation of naturally occurring Tregs was selectively reversed by treatment with SCH-23390 (53, 58), indicating that DC-derived dopamine causes prolongation of RA synovitis via inhibition of Treg, as well as IL-6–Th17 bias. In other words, application of a D1-like receptor antagonist can be expected to control autoimmune disorders such as RA via two mechanisms: inhibition of IL-6–Th17 bias and increase in Treg activity. Actually, D1-like receptor antagonists exhibited preventive and therapeutic effects on model mice of autoimmune diseases such as EAE in mice, diabetes mellitus that occurs naturally in NOD mice, and crescent formation in nephrotic serum nephritis mice (19, 66, 67).

In this study, the specific importance of neuroimmune cross talk during pathological processes of various immune-inflammatory diseases was indicated, to our knowledge, for the first time by the discovery of dopamine production in DCs, which are the immunological sentinels at the frontline of tissue defense and subsequent induction of Th17-predominant immune disease. In addition, approach from a different perspective could lead to new treatment applications of these findings.
Acknowledgments
We thank N. Sakaguchi, K. Noda, T. Adachi, and S. Shinohara for technical assistance.

Disclosures
Y.T. has received consulting fees, speaking fees, and/or honoraria from Mitsubishi Tanabe Pharma; Chugai Pharmaceutical Co., Ltd.; Eisai Co., Ltd.; Takeda Pharmaceutical Co., Ltd.; and Abbott Japan and has received research grant support from Mitsubishi Tanabe Pharma; Takeda Pharmaceutical Co., Ltd.; MSD; Pfizer; Astellas Pharma; Chugai Pharmaceutical Co., Ltd.; Abbott Japan; and Eisai Co., Ltd.

References


