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Dopamine Selectively Induces Migration and Homing of Naive CD8+ T Cells via Dopamine Receptor D3

Yoshiko Watanabe,*† Takashi Nakayama,* Daisuke Nagakubo,* Kunio Hieszima,* Zhe Jin,* Fuminori Katou,† Kenji Hashimoto,† and Osamu Yoshie2*

The nervous systems affect immune functions by releasing neurohormones and neurotransmitters. A neurotransmitter dopamine signals via five different seven-transmembrane G protein-coupled receptors termed D1 to D5. The secondary lymphoid tissues are highly innervated by sympathetic nerve fibers that store dopamine at high contents. Lymphocytes also produce dopamine. In this study, we examined expression and function of dopamine receptors in lymphocytes. We found that D3 was the predominant subtype of dopamine receptors in the secondary lymphoid tissues and selectively expressed by naive CD8+ T cells of both humans and mice. Dopamine induced calcium flux and chemotaxis in mouse L1.2 cells stably expressing human D3. These responses were almost completely inhibited by pertussis toxin, indicating that D3 was coupled with the Gαi class of G proteins. Consistently, dopamine selectively induced chemotactic responses in naive CD8+ T cells of both humans and mice in a manner sensitive to pertussis toxin and D3 antagonists. Dopamine was highly synergistic with CCL19, CCL21, and CXCL12 in induction of chemotaxis in naive CD8+ T cells. Dopamine selectively induced adhesion of naive CD8+ T cells to fibronectin and ICAM-1 through activation of integrins. Intraperitoneal injection of mice with dopamine selectively attracted naive CD8+ T cells into the peritoneal cavity. Treatment of mice with a D3 antagonist U-99194A selectively reduced homing of naive CD8+ T cells into lymph nodes. Collectively, naive CD8+ T cells selectively express D3 in both humans and mice, and dopamine plays a significant role in migration and homing of naive CD8+ T cells via D3.

Dopamine is an important neurotransmitter in the CNS and is involved in the control of locomotion, emotion, cognition, and neuroendocrine secretion (1). In the periphery, dopamine is primarily the precursor of norepinephrine and epinephrine, the major neurotransmitter of the sympathetic nerve system, and the major adrenomedullary hormone, respectively. Dopamine is also released from the sympathetic nerve endings and may function as a neurohormone (2). In particular, dopamine is known to directly control the functions of kidney and vasculature by affecting Na+ homeostasis, hormone secretion, renal blood flow, and cardiovascular function (1, 3). Furthermore, plasma contains a significant level of dopamine, most of which is conjugated to sulfate or glucuronide (2). Five types of dopamine receptors have been identified to date and termed D1, D2, D3, D4, and D5 (1, 4). All of the dopamine receptors belong to the family of seven-transmembrane G protein-coupled receptors. Based on the genomic structure and pharmacological properties, dopamine receptors have been classified into two subgroups. D1 and D5 form the D1-like group that couples with the Gαs class of G proteins, while D2, D3, and D4 form the D2-like group that couples with the Gαi/o class of G proteins (1, 4).

The central and peripheral nervous systems are known to modulate immune functions by releasing soluble factors such as neurohormones and neurotransmitters (5). Furthermore, both the primary and secondary lymphoid organs are highly innervated by the sympathetic nerves that store a large amount of dopamine (6, 7). Lymphocytes are also capable of producing dopamine themselves (8). The existence of dopamine receptors on lymphocytes has been demonstrated by RT-PCR for specific mRNA expression (9–12), specific binding of dopaminergic ligands (13–18), and immunocytochemistry using subtype-specific Abs (19, 20). However, most of these results are still inconclusive and even contradictory. Furthermore, lymphocytes are the mixture of different classes and functional subsets (21, 22). Thus, different lymphocyte classes and subsets may express different dopamine receptor subtypes. As for the biological effects of dopamine on lymphocyte functions, dopamine was shown to inhibit proliferation and cytokine/Ig production of human lymphocytes, and even to induce apoptosis in peripheral mononuclear cells (12, 23), suggesting a predominantly immunosuppressive activity of dopamine. However, the concentrations of dopamine used in these in vitro studies might be too high as a physiological concentration of dopamine in the immune system. Furthermore, immunostimulatory effects of dopamine following in vivo administration were also described (24). Recently, Levite et al. (25) have reported that dopamine induces adhesion of human T cells to fibronectin by triggering activation of the β1 integrins. Thus, dopamine can activate at least one T cell function in vitro, which is closely associated with cell trafficking and tissue microenvironmental localization. However, a potential role of dopamine in the trafficking of lymphocytes has not been explored yet.

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3 Abbreviations used in this paper: D1–5, dopamine receptor 1–5; DPAT, (di-n-propylamino)tetralin; ELC, EBV-induced molecule 1 ligand chemokine; PTX, pertussis toxin; SDF-1, stromal cell-derived factor-1; SLC, secondary lymphoid tissue chemokine.
In this study, we demonstrate that D3 is the predominant dopamine receptor in the secondary lymphoid tissues and expressed highly selectively in naïve CD8\(^+\) T cells of both humans and mice. We also demonstrate that dopamine induces chemotactic responses selectively in human and mouse naïve CD8\(^+\) T cells via D3. Dopamine is also highly synergistic with homoeostatic chemokines in attraction of human naïve CD8\(^+\) T cells. Dopamine also induces adhesion of human naïve CD8\(^+\) T cells to fibronectin and ICAM-1 through activation of integrins via D3. Intraperitoneal injection of mice with dopamine rapidly attracts naïve CD8\(^+\) T cells into the peritoneal cavity via D3. Furthermore, we provide evidence that dopamine promotes homing of mouse naïve CD8\(^+\) T cells into the secondary lymphoid tissues via D3.

Materials and Methods

Reagents and animals

Dopamine hydrochloride, 7-hydroxy-(d)-n-propylamino)tetratin (DPAT) hydrobromide (7-OH-DPAT), U-99194 maleate salt, SCH23390, raclopride, haloperidol, and clozapine were all purchased from Sigma-Aldrich. Pertussis toxin (PTX) was purchased from Invitrogen Life Technologies. Human recombinant EBV-induced molecule 1 ligand chemokine (ELC)/CCL19, secondary lymphoid tissue chemokine (SLC)/CCL21, stromal cell-derived factor-1 (SDF-1)/CXCL12, and mouse recombinant ELC/CCL19 were purchased from R&D Systems. Female C57BL/6 mice were purchased from Japan SLC and kept in specific pathogen-free conditions for at least 1 wk before experiments. All animal experiments were conducted following the guidance of the Center of Animal Experiments, Kinki University School of Medicine.

Cells

A murine L1.2 pre-B cell line (26) was provided by E. Butcher (Stanford University School of Medicine, Stanford, CA). Human CD4\(^+\) T cells, CD8\(^+\) T cells, CD45RA\(^+\) CD8\(^+\) T cells, and CD45RO\(^+\) CD8\(^+\) T cells were prepared by positive selection by using MACS system (Miltenyi Biotec). The purity of each fraction was as follows: CD4\(^+\) T cells, >99%; CD8\(^+\) T cells, >97%; CD45RA\(^+\) CD8\(^+\) T cells, >96%; CD45RO\(^+\) CD8\(^+\) T cells, >98%. Mouse CD3\(^+\) T cells were prepared from spleen cells by negative selection using IMagnet system (BD Pharmingen). The purity of each fraction was as follows: CD4\(^+\) T cells, >99%; CD8\(^+\) T cells, >97%; CD45RA\(^+\) CD8\(^+\) T cells, >96%; CD45RO\(^+\) CD8\(^+\) T cells, >98%. Mouse CD3\(^+\) T cells were prepared from spleen cells by negative selection using IMagnet system. After negative selection, the purity of mouse CD3\(^+\) T cells was consistently >99%. For preparation of mRNA, mouse CD4\(^+\) T cells, CD8\(^+\) T cells, and F4/80\(^+\) macrophages were prepared from spleen cells by positive selection using MACS system (Miltenyi Biotec). The purity of each fraction was as follows: CD4\(^+\) T cells, 95%; CD8\(^+\) T cells, 95%; B220\(^+\) B cells, 97%; F4/80\(^+\) macrophages, 82%.

RT-PCR

The first-strand cDNA samples derived from various human leukocyte fractions and organs/tissues were purchased from BD Biosciences. Fractionated mouse cells (see above) were lysed with TRIZol reagent (Invitrogen Life Technologies), and total RNAs were purified by using RNeasy (Qiagen). Total RNA (1 μg) was reverse transcribed using oligo(dT)\(_{12}\) primer and SuperScript II reverse transcriptase (Invitrogen Life Technologies). The first-strand cDNA (equivalent to 20 ng of total RNA) was amplified in a final volume of 20 μl containing 10 pmol of each primer and 1 U of Ex-Taq polymerase (Takara Shuzo). The amplification conditions were denaturation at 94°C for 30 s (5 min for the first cycle), annealing at 60°C for 30 s, and extension at 72°C for 40 s (5 min for the last cycle) for 36 cycles for all the dopamine receptors expressed in human and mouse cells, 37 cycles for all the dopamine receptors expressed in human tissues cDNAs, and 27 cycles for GAPDH expressed in all samples. The amplification products (10 μl each) were separated by electrophoresis on 2% agarose and stained with ethidium bromide. The primers used were as follows: +5'-CTGCGCAAGAATAAGGCTAGA-3' and +5'-TTGT GTGATGGTGTATCTCTC-3' for human D1; +5'-GGTATGGATCTTGAAAGCCGCA-3' and -5'-ACGTCGATGCTGATGATGCTC-3' for human D2; +5'-GGGATGCTGAGGTATTACAGC-3' and -5'-GTTGGTATGAGATGCACTAGCA-3' for human D3; +5'-GATGTAGATGCTGATGATGCT-3' for human D5; +5'-GCTATCTGGCCGAGAATGACTGAC-3' and -5'-CTTGTGCGTCTCTC-3' for mouse D1; +5'-TGATACCGGAGAAGGGCTGC-3' and -5'-TCTGTTGCTGTTGAGATGATGCTC-3' for mouse D2; +5'-GAGGCTGCTGAGCGTCTCACTATCG-3' and -5'-CTCTGTGTTGAGAAGGGCAG-3' for mouse D3; +5'-TACT GAGGAGGTGCTTGGCCTGT-3' and -5'-TCAGCAAAAGAGGCGCA-3' for mouse D4; +5'-CTTTGACTACAGGCTGCTACTG-3' and -5'-CTTTGACTACAGGCTGCTACTG-3' for mouse D5; +5'-GCGAAGGTGCTTCACTGACAATGCG-3' and -5'-GCGTCTTCAACCCCTTGTGATGC-3' for human and mouse GAPDH.

Immunocytochemistry

Fractionated human T cells (see above) were applied into glass chamber slides precoated with poly-L-lysine (Sigma-Aldrich) and kept at 37°C for 1 h. After washing, cells were pretreated with 10% normal human serum and 10% normal rabbit serum. After washing, cells were reacted with anti-human CD45RA FITC (HI100, mouse IgG2b) (BD Biosciences), anti-human CD45RO FITC (UCHL1, mouse IgG2a) (BD Biosciences), or anti-human CD27 FITC (LT27, mouse IgG2a) (Serotec) for 30 min on ice. After washing, cells were fixed with 2% paraformaldehyde in PBS for 5 min at room temperature and then permeabilized with 0.2% saponin in PBS containing 7% rabbit serum for 10 min at room temperature. Fixed and permeabilized cells were reacted with anti-human D3 (5 μg/ml, goat IgG) (Santa Cruz Biotechnology) overnight at 4°C. After washing, cells were successively treated with biotin-labeled anti-goat IgG (5 μg/ml) (Vector Laboratories) for 1 h at room temperature and then with SA-Alexa 546 (Molecular Probes) at 2 μg/ml for 1 h at room temperature. Single- and double-color fluorescence images were taken using a confocal microscope (LSM-5; Carl Zeiss).

Expression stable of human D3

This was conducted as described previously. In brief, the coding region of human D3 was amplified from a cDNA library generated from human CD8\(^+\) T cell by PCR using primers: +5'-CATGTCGACAGCGCTTCTCGATGTCAGCT-3' and -5'-CATGTCGACAGCGCTTCTCGATGTCAGCT-3'. The cDNA was cloned into a retroviral vector pMX-HEPES (pMX-HEPES/EGFP). (27) The recombinant retroviruses carrying D3 or vector alone were prepared. Murine pre-B L1.2 cells (26) were infected with the recombinant viruses, and stable transfectants expressing green fluorescence were sorted by flow cytometry using FACS Calibration (BD Biosciences).

Calcium mobilization assay

This was conducted as described previously (28). In brief, cells were suspended at 10\(^5\) cells/ml in HBSS containing 1 mg/ml BSA and 10 mM HEPES (pH 7.4), and loaded with 3 μM Fura 2-AM fluorescence dye (Molecular Probes). After washing, cells were placed in a fluorescence spectrophotometer (F2000; Hitachi) and stimulated with dopamine and other reagents. Emission fluorescence at 510 nm was measured upon excitation at 340 and 380 nm, and the fluorescence intensity ratio (R340/380) was obtained.

Chemosat assay

This was conducted using Transwell plates with 5-μm pore polycarbonate membrane filters (Corning), as described previously (28). Cells migrated into lower wells were lysed with 0.1% Triton X-100 (WAKO) and quantitated using PicoGreen dsDNA quantitation reagent (Molecular Probes).

Flow cytometric analysis

Mononuclear cells were prepared from mouse lymph nodes and suspended in ice-cold PBS containing 2% FBS and 0.1% sodium azide (staining medium). All of the following steps were done on ice. Cells were first treated with anti-mouse CD32/16 (Beckman Coulter) at 1 μg/ml to block the Fc receptors. After washing, cells were incubated with a mixture of FITC-, PE-, PC5-, or allophycocyanin-labeled anti-mouse CD3 (14-2C11, hamster IgG); anti-mouse CD4 (GK1.5, rat IgG2b); anti-mouse CD8a (53-6-7, rat IgG2a); and anti-mouse CD44 (IM7, rat IgG2b) for 30 min. After washing, cells were immediately analyzed on FACSDiCaliber (BD Biosciences). The Abs were all purchased from BD Pharmingen.

Adhesion assays

Human fibroblasts, human ICAM-1 Fc chimeric protein, and control Fc protein were purchased from R&D Systems. We coated 96-well microtiter plates (High-Binding; Corning) with fibronectin at 10 μg/ml, control Fc at 10 μg/ml, or ICAM-1 Fc at 10 μg/ml. After that, microtiter plates were blocked with 1% BSA, T cell subsets were prepared from human PBMC, as described above. Purified T cells were suspended in adhesion medium (RPMI 1640 without phenol red and supplemented with 1% BSA, 20 mM HEPES, and 0.02% sodium azide). Cells were pretreated without or with 1
μM U-99194A (a D3 antagonist) (29), 10 μg/ml anti-integrin α (HP2/1), 10 μg/ml anti-integrin α (SAM1), 10 μg/ml anti-integrin α (25.3.1), or 10 μg/ml control IgG for 20 min. The Abs were all purchased from Beckman Coulter. After that, dopamine, 7-OH-DPAT (a D3-selective agonist) (1), or CCL19 was added to cell suspensions, and cells were immediately added in duplicate to 96-well microtest plates at 1 × 10⁵ cells/well. Plates were centrifuged for 15 s at 1000 rpm and placed at 37°C for 5 min. Unbound cells were removed by gently washing with PBS three times. Bound cells were lysed with 0.1% Triton X-100 and quantitated using PicoGreen dsDNA quantitation reagent.

In vivo migration experiments

Mice received injections i.p. with PBS or PBS containing dopamine or 7-OH-DPAT (a D3 agonist) (1). After various time points, mice were sacrificed by cervical dislocation after diethyl ether anesthesia and cells were recovered from the peritoneal cavity with 3 ml of PBS. After staining for various cell surface markers, differential cell counts were done using FACS Calibur (BD Biosciences).

Mice received injections i.p. with PBS alone or PBS containing U-99194A (a D3 antagonist) (29) at every 12 h. At 24, 48, and 72 h, mice were sacrificed by cervical dislocation after diethyl ether anesthesia and inguinal lymph nodes were obtained. After staining for various cell surface markers, as described above, differential cell counts were performed using FACS Calibur (BD Biosciences).

We also analyzed lymph node homing of fluorescent dye-labeled T cells. Splenic CD8⁺ T cells were purified and incubated without or with 100 nM ELC/CCL19 at 37°C for 30 min. After that, cells were labeled with PKH26 (Sigma-Aldrich), as described previously (30). Mice received i.p. injections with PBS or PBS containing U-99194A (a D3 antagonist) (29). After 1 h, mice received i.v. injections with labeled T cells. At 1, 2, and 4 h after cell inoculation, mice were sacrificed by cervical dislocation after diethyl ether anesthesia and inguinal lymph nodes were removed. After staining various cell surface markers, as described above, differential cell counts were performed for PKH26-labeled cells using FACS Calibur (BD Biosciences).

Results

Selective expression of D3 in naive CD8⁺ T cells

The dopamine receptors were reported to be expressed not only in the brain, but also in various peripheral tissues and organs (1, 4). We, therefore, first compared expression of the five types of dopamine receptors (D1, D2, D3, D4, and D5) in various human tissues and organs by RT-PCR. The results are shown in Fig. 1A. Most notably, D3 is consistently expressed at relatively high levels in the secondary lymphoid organs/tissues, such as spleen, tonsil, and lymph nodes. This suggested a potential role of D3 in the immune system.

We next examined the expression of the five types of dopamine receptors in fractionated human peripheral blood lymphocytes and monocytes by RT-PCR. As shown in Fig. 1B, resting CD4⁺ T cells faintly expressed D2 and D3. Upon activation of CD4⁺ T cells with Con A, the expression of D2 was slightly up-regulated and that of D3 was completely down-regulated. Resting CD8⁺ T cells strongly expressed D3 and faintly expressed D4. Upon activation of CD8⁺ T cells with PHA, the expression of both D3 and D4 was completely down-regulated. Resting CD19⁺ B cells faintly expressed D4, which was again completely down-regulated upon activation of CD19⁺ B cells with PWM. Resting CD14⁺ monocytes faintly expressed D4. We also confirmed that D3 was dominantly and selectively expressed in mouse splenic CD8⁺ T cells (Fig. 1C). Thus, the selective expression of D3 in CD8⁺ T cells is conserved across the species.

We next examined the protein expression of D3 in purified human CD4⁺ and CD8⁺ T cells. As shown in Fig. 2A, 70–80% of CD8⁺ T cells were stained positive for D3. No such staining was observed with control Ab (data not shown). D3 was hardly detected in CD4⁺ T cells, although CD4⁺ T cells weakly expressed D3 mRNA (Fig. 1B). By using the pattern of expression of CD45RA, CD45RO, and CD27, human CD8⁺ T cells can be further fractionated into four functional subsets: naive (CD45RA⁺CD27⁻), memory (CD45RO⁺CD27⁺), early effector (CD45RO⁺CD27⁻), and terminally differentiated effector (CD45RA⁺CD27⁻) (31, 32). We therefore performed double staining of purified CD8⁺ T cells for D3 and CD45RA or CD45RO, and of purified CD45RA⁺CD8⁺ T cells for D3 and CD27. D3 merged completely with CD45RA (Fig. 2B), but not with CD45RO (Fig. 2C). Furthermore, D3 merged mostly with CD27 in purified CD45RA⁺CD8⁺ T cells (Fig. 2D).

Taken together, CD45RA⁺CD27⁺ naive CD8⁺ T cells are the cells that selectively express D3. Thus, dopamine may play a significant role in the function of resting naive CD8⁺ T cells via D3.

Dopamine induces calcium mobilization and chemotaxis via D3

D3 is known to be coupled with the Gα₁₆ class of G proteins (1, 4). Furthermore, D3 merged completely with CD27 (Fig. 2), but not with CD45RO (Fig. 2C). Furthermore, D3 merged mostly with CD27 in purified CD45RA⁺CD8⁺ T cells (Fig. 2D). Taken together, CD45RA⁺CD27⁺ naive CD8⁺ T cells are the cells that selectively express D3. Thus, dopamine may play a significant role in the function of resting naive CD8⁺ T cells via D3.

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Dopamine induces chemotaxis in human and mouse naive CD8$^+$ T cells. A, Purified CD4$^+$ T cells and CD8$^+$ T cells were stained with anti-D3 (red). Note that only the majority of CD8$^+$ T cells are positive for D3. B, Purified CD8$^+$ T cells were double stained with anti-D3 (red) and anti-CD45RA (green). Note that D3$^+$ cells and CD45RA$^+$ cells mostly overlap. C, Purified human CD8$^+$ T cells were double stained with anti-D3 (red) and anti-CD27 (green). Note that D3$^+$ cells and CD27$^+$ cells mostly overlap. Arrows indicate CD45RA$^+$CD27$^+$ terminally differentiated effector CD8$^+$ T cells (31, 32) that are negative for D3. Representative results from three separate experiments are shown. The scale bars indicate 30 μm (A) and 10 μm (B, C, and D).

We next examined migratory responses of human T cell subsets to dopamine. As shown in Fig. 4A, both dopamine and 7-OH-DPAT induced cell migration in L1.2-D3 with a typical bell-shaped dose-response curve. No such responses were seen with control L1.2-vector. Furthermore, a D3-selective antagonist U-99194A (29) dose dependently suppressed dopamine-induced migratory responses of L1.2-D3 (Fig. 4B). In Fig. 4C, we further confirmed that migratory responses of L1.2-D3 to dopamine were suppressed by raclopride (an antagonist for D2 and D3), U-99194A (an antagonist for D3), and haloperidol (an antagonist for D2, D3, and D4), but not by SCH23390 (an antagonist for D1 and D5) or clozapine (an antagonist for D4) (1, 29). These results clearly demonstrate that D3 mediates dopamine-induced transient calcium mobilization in L1.2 transfectants through coupling with the Gαi class of G proteins.

We next examined migratory responses of CD8$^+$ T cells to dopamine and a D3-selective agonist 7-OH-DPAT (1). As shown in Fig. 4A, both dopamine and 7-OH-DPAT induced cell migration in L1.2-D3 with a typical bell-shaped dose-response curve. No such responses were seen with control L1.2-vector. Furthermore, a D3-selective antagonist U-99194A (29) dose dependently suppressed dopamine-induced migratory responses of L1.2-D3 (Fig. 4B). In Fig. 4C, we further confirmed that migratory responses of L1.2-D3 to dopamine were suppressed by raclopride (an antagonist for D2 and D3), U-99194A (an antagonist for D3), and haloperidol (an antagonist for D2, D3, and D4), but not by SCH23390 (an antagonist for D1 and D5) or clozapine (an antagonist for D4) (1, 29). A checkerboard-type analysis confirmed that dopamine-induced calcium mobilization in L1.2-D3 was suppressed by raclopride (an antagonist for D2 and D3), U-99194A (a D3-selective antagonist), and haloperidol (an antagonist for D2, D3, and D4), but not by SCH23390 (an antagonist for D1 and D5) or clozapine (an antagonist for D4) (1, 29). These results were highly consistent with the selective expression of D3 in CD45RA$^+$ naive CD8$^+$ T cells subset (Fig. 2). Furthermore, a D3-selective antagonist U-99194A (29) dose dependently suppressed migratory responses of CD45RA$^+$CD8$^+$ T cells to dopamine (Fig. 5B). In Fig. 5C, we further confirmed that migratory responses of CD45RA$^+$CD8$^+$ T cells to dopamine were suppressed by raclopride (an antagonist for D2 and D3), U-99194A (an antagonist for D3), and haloperidol (an antagonist for D2, D3, and D4), but not SCH23390 (an antagonist for D1 and D5) or clozapine (an antagonist for D4) (1, 29). These results were highly consistent with the selective expression of D3 in CD45RA$^+$ naive CD8$^+$ T cells.

We also examined chemotactic responses of mouse T cells to dopamine. As shown in Fig. 5E, dopamine as well as 7-OH-DPAT (a D3-selective agonist) (1) selectively induced chemotaxis in CD44$^{low}$ naive CD8$^+$ T cells (34, 35). No such responses to dopamine were seen in CD4$^+$ T cells or CD44$^{high}$ effector/memory CD8$^+$ T cells (34, 35). In Fig. 5F, we confirmed that chemotactic responses of CD44$^{low}$CD8$^+$ T cells to dopamine were suppressed by raclopride (an antagonist for D2 and D3), U-99194A (an antagonist for D3), and haloperidol (an antagonist for D2, D3, and D4), but not SCH23390 (an antagonist for D1 and D5) or clozapine (an antagonist for D4) (1, 29). Thus, in both humans and mice, dopamine selectively induces migratory responses in naive CD8$^+$ T cells via D3.

Synergistic effects of dopamine and homeostatic chemokines in migration of naive CD8$^+$ T cells
Naive T cell are known to continuously recirculate between the secondary lymphoid tissues and blood (21, 36–38). It is also known that CCR7 and CXCR4, via their respective chemokine ligands, play important roles in homing of naive T cells to the
Collectively, dopamine and homeostatic chemokines are indeed highly synergistic in induction of migration in naive CD8^+ T cells.

**Dopamine induces integrin-dependent adhesion of naive CD8^+ T cells to fibronectin and ICAM-1.**

Previously, dopamine was shown to induce adhesion of human T cells to fibronectin via activation of VLA-4 and VLA-5 (25). We, therefore, examined whether dopamine selectively induces adhesion of human naive CD8^+ T cells to fibronectin via D3 through activation of VLA-4 and VLA-5. As shown in Fig. 6A, dopamine as well as 7-OH-DPAT (a D3-selective agonist) (1) indeed selectively induced adhesion of CD45RA^+ naive CD8^+ T cells to fibronectin. No such responses were seen with CD4^+ T cells or CD45RO^+CD8^+ T cells. U-99194A, an antagonist for D3 (29), almost completely inhibited induction of adhesion of CD45RA^+CD8^+ T cells to fibronectin by dopamine. Furthermore,
Dopamine induces migration of human and mouse naive CD8\(^+\) T cells via D3. A. Dose-response experiments. Chemotactic responses of human CD4\(^+\) T cells, CD8\(^+\) T cells, CD45RA\(^+\)CD8\(^+\) T cells, and CD45RO\(^+\)CD8\(^+\) T cells to dopamine at indicated concentrations were determined. B. Inhibition by U-99194A. Chemotactic responses of human CD45RA\(^+\)CD8\(^+\) T cells to 10 nM dopamine were determined in the presence of indicated concentration of U-99194A (a D3 antagonist) (29). C. Effects of various dopamine receptor antagonists. Chemotactic responses of human CD45RA\(^+\)CD8\(^+\) T cells to 10 nM dopamine were determined in the presence of indicated concentration of U-99194A (a D3 antagonist) (29). D. Dose-response experiments. Chemotactic responses of mouse CD4\(^+\) T cells, CD4\(^+\)CD8\(^+\) naive T cells, and CD44\(^{low}\)CD8\(^+\) memory T cells to medium (control), 10 nM dopamine, or 10 nM 7-OH-DPAT (a D3 agonist) (1) were determined. E. Effects of various dopamine receptor antagonists. Chemotactic responses of mouse CD4\(^+\) T cells were pretreated without or with 500 ng/ml PTX for 30 min and determined for chemotactic responses to 10 nM dopamine, as indicated. F. Chemotactic responses of mouse T cell subsets. Chemotactic responses of mouse CD4\(^+\) T cells, CD4\(^+\)CD8\(^+\) naive T cells, and CD44\(^{low}\)CD8\(^+\) memory T cells to medium (control), 10 nM dopamine, or 10 nM 7-OH-DPAT (a D3 agonist) (1) were determined. F. Effects of various dopamine receptor antagonists. Chemotactic responses of mouse CD4\(^+\) CD8\(^+\) naive T cells to 10 nM dopamine were determined in the presence of indicated dopamine receptor antagonists at 100 nM (see above). G. Synergistic effects of dopamine and homeostatic chemokines. Chemotactic responses of human CD45RA\(^+\)CD4\(^+\) T cells (left) or CD45RA\(^+\)CD8\(^+\) T cells (right) to 10 nM ELR/CCL19, 10 nM SLC/CCL21, or 0.1 nM SDF-1/CXCL12 were determined with medium only (control), 1 nM dopamine, or 1 nM dopamine + 100 nM U-99194A (a D3 antagonist) (29). A–F. All assays were done in duplicate, and numbers of cells migrated to lower wells were expressed as percentage of input cells. Results are shown as mean ± SEM from three separate experiments.

FIGURE 6. Dopamine induces adhesion of naive CD8\(^+\) T cells to fibronectin and ICAM-1 via D3 through activation of integrins. Adhesion to fibronectin (A) and ICAM-1 (B). Indicated T cell fractions were pretreated without or with 1 μM U-99194A (a D3 antagonist) (29), 10 μg/ml anti-α\(_5\) integrin, 10 μg/ml anti-α\(_4\) integrin, or 10 μg/ml control IgG for 20 min. After that, dopamine, 7-OH-DPAT (a D3 agonist) (1), or CCL19 was added to cell suspensions at a final concentration of 100 nM. Then, cells were immediately added in duplicate to microtiter plates precoated with control BSA or fibronectin (A) or control IgG or ICAM-1-Fc (B). Plates were centrifuged for 15 s at 1000 rpm and placed at 37°C for 5 min. After washing with PBS, bound cells were lysed with 0.1% Triton X-100 and quantitated using PicoGreen dsDNA quantitation reagent. All assays were done in duplicate, and numbers of bound cells were expressed as percentage of input cells. Results are shown as mean ± SEM from three separate experiments.

anti-α\(_5\) integrin and anti-α\(_4\) integrin, but not control IgG, significantly inhibited dopamine-induced adhesion of CD45RA\(^+\)CD8\(^+\) T cells to fibronectin (25). Thus, dopamine selectively induces adhesion of CD45RA\(^+\) naive CD8\(^+\) T cells to fibronectin via D3 through activation of VLA-4 and VLA-5.

We next examined whether dopamine also induces adhesion of human naive CD8\(^+\) T cells to ICAM-1 through activation of LFA-1, the process known to be critical in transendothelial migration of lymphocytes (36). As shown in Fig. 6B, dopamine as well as 7-OH-DPAT (a D3-selective agonist) (1) indeed selectively induced adhesion of CD45RA\(^+\)CD8\(^+\) T cells to ICAM-1. Again, no such responses were seen with CD4\(^+\) T cells or CD45RO\(^+\)CD8\(^+\) T cells. Induction of adhesion of CD45RA\(^+\)CD8\(^+\) T cells to ICAM-1 was completely blocked by U-99194A (an antagonist for D3) (29) and by anti-α\(_5\) integrin, but not by control IgG. CCL19 is a chemokine known to act via CCR7 that is expressed by naive
T cells and the majority of memory T cells (21, 22). Therefore, we also examined CCL19-induced adhesion of T cell subsets to ICAM-1. CCL19 induced adhesion of not only CD45RA+/CD8+ T cells, but also CD4+ T cells and CD45RO+/CD8+ T cells to ICAM-1. CCL19-induced adhesion of these subsets to ICAM-1 was also completely blocked by anti-α4 integrin, but not by control IgG. It was also notable that, as far as CD45RA+/CD8+ T cells were concerned, dopamine induced their adhesion to ICAM-1 as efficiently as CCL19. Collectively, dopamine selectively induces adhesion of naive CD8+ T cells to ICAM-1 via D3 through activation of LFA-1.

### In vivo mobilization of naive CD8+ T cells by dopamine

To test a chemotactic activity of dopamine in vivo, we injected dopamine or 7-OH-DPAT (a D3-specific agonist) (1) into the peritoneal cavity of mice. As shown in Fig. 7A, dopamine as well as 7-OH-DPAT selectively induced a rapid accumulation of CD44low naive CD8+ T cells (34, 35) in the peritoneal cavity with a peak at 30 min. No such changes in cell number were seen with CD4+ T cells or CD44high memory/effectector CD8+ T cells (34, 35). As shown in Fig. 7B, U-99194A (a D3-specific antagonist) (29) effectively suppressed dopamine-induced mobilization of CD44low/CD8+ T cells into the peritoneal cavity without affecting the numbers of CD4+ T cells or CD44high/CD8+ T cells. Collectively, dopamine is indeed capable of inducing a selective mobilization of naive CD8+ T cells via D3 in vivo.

### D3 is involved in homing of mouse naive CD8+ T cells to lymph node

We further tested whether dopamine plays a role in homing of naive CD8+ T cells to the secondary lymphoid tissues via D3. We treated mice with a D3-selective antagonist U-99194A (29) at every 12 h and enumerated various T cell subsets in inguinal lymph nodes at 24, 48, and 72 h. Fig. 8A shows the results at 24 h. U-99194A, but not vehicle alone, significantly and selectively reduced the numbers of naive CD44low/CD8+ T cells (34, 35) in the inguinal lymph nodes. Similar results were obtained at 48 and 72 h (data not shown). We also examined effects of U-99194A on lymph node homing of CD3+ T cells that had been labeled with a fluorescent dye PKH26 (30). A fraction of CD3+ T cells was also pretreated with ELC/CCL19 to desensitize CCR7 (39, 40). Mice were pretreated with U-99194A for 1 h and i.v. inoculated with PKH26-labeled CD3+ T cells. At 1, 2, and 4 h after cell inoculation, inguinal lymph nodes were removed. Cells were isolated and stained for appropriate surface markers. Differential cell counts

![FIGURE 7](http://www.jimmunol.org/)  
**FIGURE 7.** In vivo mobilization of naive CD8+ T cells by dopamine. A. Selective mobilization of naive CD8+ T cells by dopamine. Mice received injections i.p. with 200 μl of PBS, 200 μl of PBS containing 0.1 nmol dopamine, or 200 μl of PBS containing 0.1 nmol 7-OH-DPAT (a D3 agonist) (1). At indicated time points, cells were recovered from the peritoneal cavity and stained for indicated cell surface markers. Differential cell counts were made on FACSCalibur. Each point represents mean ± SEM from five separate experiments.  

B. Effect of U-99194A on dopamine-induced mobilization of naive CD8+ T cells. Mice received injections i.p. with 200 μl of PBS, 200 μl of PBS containing 0.1 nmol dopamine, or 200 μl of PBS containing 0.1 nmol dopamine and 1 nmol U-99194A (a D3 antagonist) (29). After 30 min, cells in the peritoneal cavity were recovered and stained for indicated cell surface markers. Differential cell counts were made on FACSCalibur. Each point represents mean ± SEM from five separate experiments.  

![FIGURE 8](http://www.jimmunol.org/)  
**FIGURE 8.** Role of dopamine in homing of naive CD8+ T cells to lymph nodes. A. In vivo effects of U-99194A. Mice received i.p. injections twice at 0 and 12 h with 200 μl of PBS or 200 μl of PBS containing U-99194A (a D3 antagonist; 20 mg/kg; each, n = 7) (29). At 24 h, inguinal lymph node lymphocytes were obtained, and cell numbers of CD4+ T cells, CD44low/CD8+ naive T cells, and CD44high/CD8+ memory T cells were determined by flow cytometry. Percentage of changes from untreated mice were calculated. Results are shown as mean ± SEM from three separate experiments. Statistical significance was determined by Student’s t test, *p < 0.01. B. Effects of U-99194A and CCR7 desensitization on homing of labeled T cells to inguinal lymph nodes. Mice received i.p. injections with 200 μl of PBS or 200 μl of PBS containing U-99194A (a D3 antagonist; 20 mg/kg; each, n = 8) (29). After 1 h, mice were i.v. inoculated with PKH26-labeled CD3+ T cells. For CCR7 desensitization, purified CD3+ T cells were pretreated with 100 nM ELC/CCL19 at 37°C for 30 min before PKH26 labeling. After 2 h, inguinal lymph node lymphocytes were obtained, and cell numbers of PKH26-labeled CD4+ T cells, CD44low/CD8+ T cells, and CD44high/CD8+ T cells were determined by flow cytometry. Percentage of changes from untreated mice were calculated. Results are shown as mean ± SEM from three separate experiments. Statistical significance was determined by Student’s t test, *p < 0.01.

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according to the various surface markers were performed for PKH26-labeled CD3\(^+\) T cells. Fig. 8B shows the results at 2 h after cell inoculation. Treatment of mice with U-99194A significantly and selectively reduced homing of naive CD44\(^{low}\)CD8\(^+\) T cells (34, 35) into inguinal lymph nodes. In contrast, desensitization of CCR7 of injected T cells universally reduced homing of not only naive CD44\(^{low}\)CD8\(^+\) T cells, but also CD4\(^+\) T cells and memory CD44\(^{high}\)CD8\(^+\) T cells into inguinal lymph node (21, 22). When CCR7-desensitized T cells were injected into mice pretreated with U-99194A, this further reduced homing of CD44\(^{low}\)CD8\(^+\) naive T cells in inguinal lymph nodes. Similar results were obtained for other time points (data not shown). Collectively, these results indicate that dopamine indeed plays a significant role in homing of naive CD8\(^+\) T cells into the secondary lymphoid tissues via D3.

**Discussion**

Dopamine is an important neurotransmitter that signals via five different seven-transmembrane G protein-coupled receptors termed D1 to D5 (1, 4). A large number of studies have provided evidence that lymphocytes express dopamine receptors (9–17, 19, 20). According to these reports, lymphocytes appear to express essentially all subtypes of dopamine receptors, with a possible exception of D1. However, most of these studies were conducted using either highly sensitive RT-PCR or various dopamine receptor agonists/antagonists with possible cross-reactivities for specific binding assays. Furthermore, lymphocytes are composed of multiple classes and functional subsets (21, 22). Thus, the exact expression profiles of the dopamine receptor subtypes in various lymphocyte classes and subsets need to be defined. As for potential functions of dopamine receptors in lymphocytes, dopamine has been shown to modulate immune functions (12, 23, 24). In the present study, we have first shown that D3 is the predominant dopamine receptor subtype in the secondary lymphoid tissues (Fig. 1A). Next, we have demonstrated that D3 is mainly expressed in resting CD8\(^+\) T cells in both humans and mice (Fig. 1, B and C). Furthermore, we have shown that D3 is highly selectively expressed in naive CD8\(^+\) T cells of both humans (Fig. 2) and mice (Fig. 5D). Thus, the selective expression of D3 in resting naive CD8\(^+\) T cells is highly conserved across the species. In contrast, expression of other subtypes of dopamine receptors in lymphocytes appears to be less significant (Fig. 1).

D3 belongs to the D2-like dopamine receptor group that couples with the Ga\(\alpha\)i class of G proteins (1, 4). Seven-transmembrane receptors coupled with the Ga\(\alpha\)i class of G proteins are known to be frequently involved in chemotactic responses (33). We have indeed shown that dopamine as well as a D3-selective agonist 7-OH-DPAT (1) induces calcium flux and chemotactic responses in cells of a mouse L1.2 pre-B line (26) stably expressing human D3 in a manner highly sensitive to a D3-selective antagonist U-99194A (29) and PTX (33) (Figs. 3 and 4). Furthermore, we have shown that dopamine as well as a D3-selective agonist 7-OH-DPAT (1) selectively induce cell migration in naive CD8\(^+\) T cells of both humans and mice in a manner highly sensitive to a D3-selective antagonist U-99194A (29) and PTX (33) (Fig. 5). Moreover, i.p. injection of mice with dopamine as well as a D3-selective agonist 7-OH-DPAT (1) selectively attracted naive CD8\(^+\) T cells into the peritoneal cavity (Fig. 7). Collectively, for the first time it is demonstrated that naive CD8\(^+\) T cells selectively express D3 across the species, and dopamine selectively induces chemotaxis in naive CD8\(^+\) T cells by signaling via D3.

Previously, Levite et al. (25) reported that dopamine induced adhesion of human T cells to fibronectin, a major extracellular matrix component, by triggering activation of the \(\beta_1\) integrins VLA-4 and VLA-5. Furthermore, they also demonstrated that a D3-selective agonist 7-OH-DPAT (1) mimicked and a D3-selective antagonist U-99194A (29) blocked the observed effects of dopamine (25). We have extended their observations and shown that dopamine as well as a D3-selective agonist 7-OH-DPAT (1) selectively induce adhesion of human naive CD8\(^+\) T cells to fibronectin via D3 through activation of VLA-4 and VLA-5 integrins (Fig. 6A). Furthermore, we have shown that dopamine as well as a D3-selective agonist 7-OH-DPAT (1) selectively induce adhesion of human naive CD8\(^+\) T cells to ICAM-1 via D3 through activation of LFA-1 integrin (Fig. 6B). The latter observation is highly relevant to the potential role of dopamine in transendothelial migration of naive CD8\(^+\) T cells in vivo (36).

The secondary lymphoid tissues are abundantly innervated by the sympathetic nerves that store a large amount of dopamine, and nerve fibers are particularly concentrated around vascular endothelial cells (7). The concentration of dopamine in the synapse is estimated to be 100–300 \(\mu\)M (24). Lymphocytes are also capable of producing dopamine and contain dopamine at 1.6–8.6 \(\times\) 10\(^{-18}\) mol/cell, suggesting a potential autocrine-paracrine effect of dopamine on lymphocytes (8). Thus, lymphocytes may be exposed to a relatively high concentration of dopamine in the secondary lymphoid tissues, especially in the vicinity of blood vessels, including high endothelial venules, the gateway for lymphocyte homing (36, 39–41). This may suggest that dopamine plays a significant homeostatic role in the homing of naive CD8\(^+\) T cells into the secondary lymphoid tissues by activation of LFA-1 and induction of chemotaxis (Figs. 5 and 6B). Dopamine may also play a role in the microenvironmental localization of naive CD8\(^+\) T cells within the secondary lymphoid tissues by triggering activation of \(\beta_1\) integrins (Fig. 6A) (25). Migration of lymphocytes into the secondary lymphoid tissues is now known to be controlled by the homeostatic chemokines SLC/CCL21 and ELC/CCL19 that are presented on high endothelial venules of the secondary lymphoid tissues (21, 37–41). In addition, SDF-1/CXCL12 and its receptor CXCR4 are reported to participate in these steps (37, 38). We have shown that 1 nM dopamine is highly synergistic with suboptimal ELC/CCL19, SLC/CCL21, and SDF-1/CXCL12 in induction of cell migration in naive CD8\(^+\) T cells (Fig. 5G). This concentration of dopamine is comparable to that of dopamine in the plasma (42). Furthermore, we have demonstrated that treatment of mice with a D3-selective antagonist U-99194A (29) selectively and significantly decreases the numbers of naive CD8\(^+\) T cells in lymph nodes, suggesting that endogenous dopamine indeed plays a significant role in homing of naive CD8\(^+\) T cells via D3 (Fig. 8A). Furthermore, we have shown that treatment of mice with U-99194A before injection of fluorescent dye-labeled T cells selectively blocks homing of naive CD8\(^+\) T cells to lymph nodes, while CCR7 desensitization of labeled T cells universally reduces homing of all T cell subsets, as expected (Fig. 8B). Thus, dopamine indeed plays a significant role in the homing of naive CD8\(^+\) T cells to the secondary lymphoid tissues in cooperation with the chemokines that are expressed in the secondary lymphoid tissues.

Previous in vitro studies testing dopamine at relatively high concentrations demonstrated that dopamine was immunosuppressive (12, 23). In contrast, in vivo administration of pharmacological doses of dopamine was reported to be mostly immunostimulatory (24). Thus, dopamine could be either immunostimulatory or immunosuppressive depending on the experimental conditions used. We have shown that the chemotactic activity of dopamine in naive CD8\(^+\) T cells is bell shaped with the optimal concentrations within the range of 10 to 100 nM (Fig. 5). Similarly, Levite et al. (25) reported that the activation of \(\beta_1\) integrin by dopamine was bell shaped with the optimal concentrations in the range of 10 to 100 nM. Thus, the biphasic type of dose responses may be in part
responsible for such dichotomous activities. Furthermore, dopamine added to cell cultures at high concentrations might be taken up by immune cells (43) and might be metabolized to norepinephrine and epinephrine, which also exert potent immunomodulatory effects via various adrenergic receptors (5, 6).

A notable finding is that the expression of D3 by naive CD8⁺ T cells is almost completely down-regulated upon cell activation (Fig. 1). This is in sharp contrast to a highly elevated expression of chemokine receptors and responsiveness to chemokines of acti-vated T cells (21, 22). Therefore, upon activation, CD8⁺ T cells may no longer need D3-mediated attraction to the secondary lymphoid tissues. Even though the physiological reason for such a restricted use of D3 by resting naive CD8⁺ T cells is not known, the state of the central and peripheral nervous systems may selectively affect the function of naive CD8⁺ T cells via D3. In future studies, mice with target disruption of D3, which were reported to respectively affect the function of naive CD8⁺ T cells, may no longer need D3-mediated attraction to the secondary lymphoid tissues (20).

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Disclosures

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References


