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Dynamic reorganization of chemokine receptors, cholesterol, lipid rafts, and adhesion molecules to sites of CD4 engagement

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Abstract

T cell polarization and redistribution of cellular components are critical to processes such as activation, migration, and potentially HIV infection. Here, we investigate the effects of CD4 engagement on the redistribution and localization of chemokine receptors, CXCR4 and CCR5, adhesion molecules, and lipid raft components including cholesterol, GM1, and glycosyl-phosphatidylinositol (GPI)-anchored proteins. We demonstrate that anti-CD4-coated beads (αCD4-B) rapidly induce co-capping of chemokine receptors as well as GPI-anchored proteins and adhesion molecules with membrane cholesterol and lipid rafts on human T cell lines and primary T cells to the area of bead–cell contact. This process was dependent on the presence of cellular cholesterol, cytoskeletal reorganization, and lck signaling. Lck-deficient JCaM 1.6 cells failed to cap CXCR4 or lipid rafts to αCD4-B. Biochemical analysis reveals that CXCR4 and LFA-1 are recruited to lipid rafts upon CD4 but not CD45 engagement. Furthermore, we also demonstrate T cell capping of both lipid rafts and chemokine receptors at sites of contact with HIV-infected cells, despite the binding of an HIV inhibitory mAb to CXCR4. We conclude that cell surface rearrangements in response to CD4 engagement may serve as a means to enhance cell-to-cell signaling at the immunological synapse and modulate chemokine responsiveness, as well as facilitate HIV entry and expansion by synaptic transmission.
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Keywords: Chemokine receptor; Lipid rafts; Cholesterol; CD4; HIV

Introduction

Chemokine receptors belong to the family of seven transmembrane G protein-coupled receptors that are essential for immune cell migration and homing in response to soluble mediators called "chemokines." We have previously demonstrated that chemokine receptor conformation, more specifically CCR5 and CXCR4, is significantly altered upon cholesterol extraction with β-cyclodextrin (BCD) [1,2]. Interestingly, these studies also revealed that SDF-1α (CXCL12) and MIP-1β (CCL4) preferentially bind to chemokine receptors associated with cholesterol- and sphingolipid-enriched membrane microdomains termed "lipid rafts," despite the observation that the majority of cell surface chemokine receptors did not co-localize with lipid rafts on these cells. There is a current debate on chemokine receptor–lipid raft localization where several groups have demonstrated significant levels of chemokine receptors associated with rafts before and/or after stimulation, while others have failed to identify any association [3–6]. This issue is partly confounded by the differences in raft isolation techniques and the varying cell types examined in such studies as well as possible differences in the levels of receptor expression, which may influence receptor–raft localization. Nonetheless, lipid rafts appear to play an important role in the function of many chemokine receptors and the significance of receptor–raft interplay remains to be fully understood.

Polarization and lipid raft recruitment in immune cells have been demonstrated in response to cross-linking of various receptors, including CD3 and CD28, as well as by cytokine and chemokine stimulation [3,7]. For example, formation of the immunological synapse (IS) between an antigen presenting cell and a T cell causes significant

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redistribution of T cell lipids and proteins, such as ganglioside GM1, LFA-1, and TCR, to specific regions of the contact zone [8]. Other distinct immune synapses are also observed with NK cell interactions as well [8]. Signaling through these contact zones is designed to transmit the proper signals from one cell to the other, which can include signals for cell activation, inactivation, or cytolysis. The redistribution of lipid rafts to the IS on T cells is believed to be critical for the transmission of the appropriate signal, especially during T cell activation, by providing stable platforms for the accumulation of intracellular signaling molecules and by providing sites for cytoskeletal assembly [9]. Additionally, polarization of cellular components including chemokine receptors and lipid rafts has been demonstrated in immune cells responding to migratory signals such as chemokine gradients [3,10,11]. Furthermore, chemokine signaling is believed to enhance T cell responsiveness during activation [12–15]. The redistribution of chemokine receptors to the IS in the context of CD4 signaling, which may influence T cell signaling outcomes, has not been determined.

Several groups have recently demonstrated that CD4, chemokine receptors, LFA-1, and cytoskeletal proteins on target cells are recruited to sites of contact with an infected dendritic cell or T cell, dubbing this union the "HIV synapse," supporting the models for cell-to-cell transmission of HIV and other retroviruses [16–22]. This process bears amazing resemblance to the formation of the IS where T cell interactions with APCs results in the recruitment of lipid rafts, adhesion molecules, and the T cell receptor (TCR) complex to the point of cell-to-cell contact [23]. The similarity between these immune-associated synapses suggests a commonality between the processes mediating these raft recruitment. CD4 signaling and raft association in the IS has also been demonstrated to be required for TCR/PKCγ raft association and clustering [24]. Here, we sought to examine if solid-phase engagement of the CD4 molecule alone is sufficient to induce human T cells redistribution of chemokine receptors, lipid rafts, and adhesion molecules to the point of cell contact. Our studies revealed that engagement of CD4 alone on human T cells does induce chemokine receptor, cholesterol, GM1, adhesion molecules, and GPI-anchored proteins, but not CD45, recruitment to contact sites. This process requires lck signaling, F-actin polymerization, and the presence of bioactive cholesterol. Moreover, we also demonstrate that both lipid rafts and CXCR4 on target T cells become polarized to sites of contact with HIV-infected cells. The implications of these findings on T cell activation and HIV infectivity will be discussed.

Materials and methods

Cells and reagents

Jurkat T cells (clone E6.1), Molt-4, Sup-T1, and CEM-NKR-CCR5 (referred to as CEM-R5) cells were obtained through the AIDS Research Reagents and Reference Program (Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health) from Dr. A. Weiss and Dr. A. Trkola. The JCaM 1.6 variant of E6.1 Jurkat cells was obtained from Dr. Ron Wange at the National Institute on Aging. Cells were grown in RPMI-1640 (Mediatech; Cellgro, Herndon, VA) supplemented with 10% heat-inactivated FBS (Biosource International, Rockville, MD), 10 mM HEPES, and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) [cRPMI]. Activated T cells were derived from human donors, purified on human T cell enrichment columns (R&D Systems), stimulated with immobilized anti-CD3, and subsequently grown in medium supplemented with IL-2. Monoclonal antibodies to CXCR4 (12G5), CD3 (UCHT1), CD45 (HI30), CD59 (p282[H19]), CD48 (cTU145), CD11a (HI111), CD29 (MAR4), and CD49d (9F10) were purchased from BD Pharmingen (San Diego, CA). Alexa Fluor 488-labeled phalloidin, Alexa Fluor 594-labeled cholera toxin B subunit (CTB-AF594), Alexa Fluor 488-labeled goat anti-mouse IgG (GAM-AF488), and marina blue-labeled goat anti-mouse IgG (GAM-MB) were purchased from Molecular Probes (Eugene, OR). Filipin and cytochalasin D were purchased from Sigma-Aldrich (St. Louis, MO). PP2 (4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo(3,4-d]pyrimidine) was purchased from Calbiochem (San Diego, CA). Hydroxypropyl-β-cyclodextrin (Trappsol) was purchased from Cyclodextrin Technologies Development (Gainesville, FL).

β-cyclodextrin treatment

Cells (1×10^6/ml) were incubated with 10 mM hydroxypropyl-β-cyclodextrin (BCD) in PBS for 30 min at 37°C. Cells were washed with PBS to remove BCD and resuspended in cRPMI for experiments. Low toxicity was confirmed by exclusion of trypan blue dye in treated and untreated cells.

Immunofluorescence microscopy and bead–cell capping

Cells (2×10^6) were labeled with 1 μg of mAb at 4°C for 30 min, followed by GAM-AF488 (1 μg) and CTB-AF594 (1 μg) at 4°C for 30 min. Labeled cells were resuspended in 500-μl cRPMI along with 10^6 αCD4-coated beads (αCD4-B) or αCD45-coated beads (Dynal Biotech Inc., Lake Success, NY) at a T cell to bead ratio of 2:1 and then incubated at 37°C for 30 min to allow capping. The cells were then washed with cold PBS, fixed with 1% paraformaldehyde in PBS for 20 min, and subsequently resuspended in PBS containing 5% FBS (PBS-F). For filipin staining, 4 μl of filipin at 5 mg/ml in ethanol was added to the bead-attached fixed cells in 100 μl of PBS-F, incubated at room temperature for 1 h, washed with PBS, and then resuspended in PBS-F. Stained cell–bead conjugates were then spun onto glass slides using a Shandon cytospin at 1000 rpm for 3 min (Thermo Shandon, Pittsburgh, PA).