

# Functional Reconstitution of the Human Chemokine Receptor CXCR4 with G<sub>i</sub>/G<sub>o</sub>-Proteins in Sf9 Insect Cells

PATRICK KLEEMANN, DAN PAPA, SANDY VIGIL-CRUZ, AND ROLAND SEIFERT

## ABSTRACT

The chemokine stromal cell-derived factor-1 $\alpha$  (SDF-1 $\alpha$ ) binds to the chemokine receptor CXCR4 that couples to pertussis toxin-sensitive G-proteins of the G<sub>i</sub>/G<sub>o</sub>-family. CXCR4 plays a role in the pathogenesis of autoimmune diseases, human immunodeficiency virus infection and various tumors, fetal development as well as endothelial progenitor and T-cell recruitment. To this end, most CXCR4 studies have focused on the cellular level. The aim of this study was to establish a reconstitution system for the human CXCR4 that allows for the analysis of receptor/G-protein coupling at the membrane level. We wished to study specifically constitutive CXCR4 activity and the G-protein-specificity of CXCR4. We co-expressed N- and C-terminally epitope-tagged human CXCR4 with various G<sub>i</sub>/G<sub>o</sub>-proteins and Regulator of G-Protein Signalling (RGS)-proteins in Sf9 insect cells. Expression of CXCR4, G-proteins and RGS-proteins was verified by immunoblotting. CXCR4 coupled more effectively to G $\alpha_{i1}$  and G $\alpha_{i2}$  than to G $\alpha_{i3}$  and G $\alpha_o$  and insect cell G-proteins as assessed by SDF-1 $\alpha$ -stimulated high-affinity steady-state GTP hydrolysis. The RGS-proteins RGS4 and GAIP enhanced SDF-1 $\alpha$ -stimulated GTP hydrolysis. SDF-1 $\alpha$  stimulated [<sup>35</sup>S]guanosine 5'-[ $\gamma$ -thio]triphosphate (GTP $\gamma$ S) binding to G $\alpha_{i2}$ . RGS4 did not enhance GTP $\gamma$ S binding. Na<sup>+</sup> salts of halides did not reduce basal GTPase activity. The bicyclam, 1-[[1,4,8,11-tetrazacyclotetradec-1-ylmethyl]phenyl]methyl]-1,4,8,11-tetrazacyclotetradecane (AMD3100), acted as CXCR4 antagonist but was devoid of inverse agonistic activity. Halides reduced the maximum SDF-1 $\alpha$ -stimulated GTP hydrolysis in the order of efficacy I<sup>-</sup> > Br<sup>-</sup> > Cl<sup>-</sup>. In addition, salts reduced the potency of SDF-1 $\alpha$  at activating GTP hydrolysis. From our data we conclude the following. (i) Sf9 cells are a suitable system for expression of functionally intact human CXCR4. (ii) Human CXCR4 couples effectively to G $\alpha_{i1}$  and G $\alpha_{i2}$ . (iii) There is no evidence for constitutive activity of CXCR4. (iv) RGS-proteins enhance agonist-stimulated GTP hydrolysis, showing that GTP hydrolysis becomes rate-limiting in the presence of SDF-1 $\alpha$ . (v) By analogy to previous observations made for the  $\beta_2$ -adrenoceptor coupled to G<sub>s</sub>, the inhibitory effects of halides on agonist-stimulated GTP hydrolysis may be due to increased GDP-affinity of G<sub>i</sub>-proteins, reducing the efficacy of CXCR4 at stimulating nucleotide exchange.