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Increased association with detergent-resistant membranes/lipid rafts of apically targeted mutants of the interleukin-6 receptor gp80

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Abstract

Interleukin (IL)-6 is an important cytokine in inflammatory processes, differentiation and growth. The IL-6 receptor complex comprises the specific IL-6 receptor (gp80) and two molecules of the signal tranducing component gp130 which transduces the signal into the nucleus via the Jak-STAT pathway. Both, gp80 and gp130 are sorted preferentially to the basolateral membrane of polarised Madin–Darby canine kidney (MDCK) cells. Previously, we have shown that gp130 partially localises to detergent-resistant membranes (DRMs)/lipid rafts and that lipid raft integrity is crucial for signalling to occur. Here we now demonstrate that wild-type gp80 is associated with DRMs only to a minor extent. However, gp80 mutants which lack parts of the cytoplasmic domain and therefore are more apically expressed than the wild type show an increased affinity for the liquid-ordered membrane domain. Studies with non-polarised MDCK cells suggest that the lipid raft association of the different mutants of gp80 precedes the establishment of cell polarity. Our findings suggest that lipid rafts play a role in the sorting of apically targeted gp80.

Keywords: Lipid rafts; Sorting; Signal transduction; Cell polarity; Cytokines; Interleukin-6

Introduction

Interleukin (IL)-6-type cytokines belong to the class of haematopoietic cytokines (Heinrich et al., 1998). They play an important role in inflammatory processes, differentiation, cell survival, haematopoiesis, and in several other essential processes. The IL-6-type cytokine family comprises IL-6, leukaemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), IL-11, cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC) (also referred to as neurotrophin-1 or B cell-stimulating factor-3), and neuropoietin (NP) (Derouet et al., 2004; Heinrich et al., 1998; Senaldi et al., 1999). Common to these cytokines is that their receptor complexes contain the signal transducer gp130 either as a homodimer (e.g. in the IL-6 signalling complex) or as a heterodimer with the signal transducers LIF-R (e.g. in the CNTF or LIF signalling complex) or OSM-R (alternatively in the OSM signalling complex). IL-6, CNTF, IL-11, CT-1, CLC, and NP first interact with specific ligand-binding receptors, e.g. IL-6 receptor (gp80) or CNTF-R. The exact stoichiometry of the signalling complexes is not completely understood and still under investigation (de Serio et al., 1995;

Abbreviations: cd, Cytoplasmic domain; CNTF, Ciliary neurotrophic factor; DRM, Detergent-resistant membrane; gp, Glycoprotein; GPI, Glycosyl-phosphatidylinositol; GPI-AP, GPI-anchored protein; IL, Interleukin; LIF, Leukaemia inhibitory factor; MDCK, Madin–Darby canine kidney; TM, Transmembrane; wt, Wild-type

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Schroers et al., 2005; Schuster et al., 2003). All these cytokines transduce their signal into the cell via receptor-associated janus kinases and signal transducers and activators of transcription (Jak-STAT pathway).

Recently, it was shown that the two components of the IL-6 receptor complex, gp80 and gp130, are sorted to the basolateral plasma membrane in polarised epithelial Madin–Darby canine kidney (MDCK) cells (Martens et al., 2000). These cells are widely used as a model to investigate sorting and polarised expression of proteins (Misfeldt et al., 1976; Rodriguez-Boulan and Powell, 1992). In contrast, the glycosyl-phosphatidylinositol (GPI)-anchored CNTF-R is sorted to the apical membrane and the LIF-R surprisingly distributed evenly at both membranes (Buk et al., 2004). Martens et al. (2000) further assigned the basolateral localisation of gp80 to two motifs within its 82-amino-acid cytoplasmic domain: a membrane-proximal tyrosine-based motif (YSLG) and a membrane-distal dileucine-type motif (LI). Truncated gp80 mutants were increasingly sorted to the apical side depending on the deletion of one or both sorting motifs.

It is now generally accepted that specialised membrane microdomains – so-called lipid rafts – exist in the plasma membrane of eukaryotic cells. Lipid rafts are highly enriched in cholesterol and sphingolipids and form distinct liquid-ordered domains in the plane of the liquid-disordered plasma membrane (Brown and London, 1998; Simons and Ikonen, 1997). Many proteins were reported to associate with rafts via different mechanisms like posttranslational modifications, e.g. palmitoylation, double acylation, N-glycosylation, or addition of a GPI anchor. Several transmembrane (TM) proteins (e.g. G protein-coupled receptors or receptor tyrosine kinases) also show affinities for these domains; however, in this case the targeting motifs are as yet not fully understood (Macdonald and Pike, 2005). Caveolins, structural proteins found in caveolae, are also enriched in low-density plasma membrane fractions. Thus, caveolin-1 or other constitutively raft-/caveolae-associated proteins serve as markers for these membrane domains. Due to their physicochemical properties lipid rafts can be isolated with non-ionic detergents at 4 °C as low-density, detergent-resistant membranes (DRMs; containing all kinds of raft domains: lipid rafts, caveolae) on sucrose density gradients (Brown and Rose, 1992). However, it is currently still under hot debate in how far DRMs do indeed represent lipid rafts or related structures in vivo (Helms and Zurzolo, 2004; Pike, 2004; Zurzolo et al., 2003). In this paper, for simplification we do not distinguish between the terms 'DRMs' and 'rafts'.

Lipid rafts are important entities in intracellular transport, endocytosis, cholesterol trafficking, and recently a role of lipid rafts in the sorting of membrane proteins, especially of GPI-anchored and/or N-glycosylated proteins, from the trans-Golgi network to the plasma membrane has been shown (Brown and Rose, 1992; Füllekrug and Simons, 2004; Helms and Zurzolo, 2004; Muniz and Riezman, 2000; Weimbs et al., 1997). Moreover, lipid rafts play a crucial role in many different signal transduction pathways like EGF-, GDNF-, IgE-, IL-2- and IL-6-type cytokine signalling (Buk et al., 2004; Pike, 2003; Simons and Toomre, 2000; Smart et al., 1999). Receptor subunits are either recruited into raft domains after ligand binding and signalling occurs within rafts (e.g. FcεRI (Field et al., 1995)) or receptor components move out of rafts to form functional signalling complexes (e.g. IL-2 receptor subunits (Marmor and Julius, 2001)). Other receptors constitutively associate with lipid rafts. We could demonstrate that the CNTF-R is constitutively associated with lipid rafts whereas only a portion of gp130 and the LIF-R were found in DRMs (Buk et al., 2004). The detection of differential raft association was strongly dependent on the choice of the detergent. Signalling of IL-6, CNTF, and LIF was shown to be dependent on intact lipid rafts as it can be reversibly disrupted with the cholesterol-sequestering drug methyl-β-cyclodextrin (MCD).

In this study we analysed the lipid raft association of gp80, the ligand-binding subunit of the IL-6 receptor complex. Furthermore, we employed two deletion mutants lacking either 57 amino acids (aa) in the cytoplasmic domain (Δ412, dileucine-type motif missing) or the complete cytoplasmic domain (82 aa, tyrosine-based and LI-motif missing) (Δcd) (Gerhartz et al., 1994; Martens et al., 2000) to investigate whether lipid rafts play a role in the sorting of gp80 mutants to the apical membrane. For comparison, we also analysed the raft association of an apically targeted gp130 mutant lacking the complete cytoplasmic domain (gp130Δcd) (Martens et al., 2000). We find a direct correlation between apical sorting and association with lipid rafts for the gp80 variants but not for those of gp130. The increased association with rafts of apically sorted gp80 mutants was already seen in non-polarised cells indicating that this distribution is independent of the development of cell polarity.

Material and methods

Materials

Normal donkey serum was supplied by DAKO (Hamburg, Germany) and normal goat serum by Sigma-Aldrich (Taufkirchen, Germany). Protein A Sepharose™ CL-4B and the ECL Western blotting detection system were purchased from Amersham Pharmacia Biotech (Freiburg, Germany). Protease inhibitor cocktail, aprotinin, leupeptin, and pepstatin