Immunobiology 222 (2017) 797–806

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Comparative analysis of the internalization of the macrophage receptor sialoadhesin in human and mouse primary macrophages and cell lines

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ARTICLE INFO

Article history:
Received 14 June 2016
Received in revised form
18 November 2016
Accepted 20 November 2016
Available online 21 November 2016

Keywords:
Sialoadhesin/Siglec-1/CD169
Internalization
Antibodies

ABSTRACT

Sialoadhesin (Sn) is a surface receptor expressed on resident macrophages with the ability to bind with sialic acids. During inflammation, an upregulation of Sn is observed. Upon binding of monoclonal antibodies to Sn, the receptor becomes internalized and this has been observed in multiple species. The latter characteristic, combined with the strong upregulation of Sn on inflammatory macrophages and the fact that Sn-positive macrophages contribute to certain inflammatory diseases, makes Sn an interesting entry portal for phenotype-modulating or cytotoxic drugs. Such drugs or toxins can be linked to Sn-specific antibodies which should enable their targeted uptake by macrophages. However, the activity of such drugs depends not only on their internalization but also on the intracellular trafficking and final fate in the endolysosomal system. Although information is available for porcine Sn, the detailed mechanisms of human and mouse Sn internalization and subsequent intracellular trafficking are currently unknown. To allow development of Sn-targeted therapies, differences across species and cellular background need to be characterized in more detail. In the current report, we show that internalization of human and mouse Sn is dynamin-dependent and clathrin-mediated, both in primary macrophages and CHO cell lines expressing a recombinant Sn. In primary macrophages, internalized Sn-specific F(ab')$_2$ fragments are located mostly in the early endosomes. With Fc containing Sn-specific antibodies, there is a slight shift towards lysosomal localization in mouse macrophages, possibly because of an interaction with Fc receptors. Surprisingly, in CHO cell lines expressing Sn, there is a predominant lysosomal localization. Our results show that the mechanism of Sn internalization and intracellular trafficking is concurrent in the tested species. The cellular background in which Sn is expressed and the type of antibody used can affect the intracellular fate, which in turn can impact the activity of antibody-based therapeutic interventions via Sn.

1. Introduction

Macrophages are important cell types within the immune system, in steady state conditions and during inflammation (Elhelu, 1983; Robbins and Swirski, 2010). They have different roles in the innate and adaptive immunity such as phagocytosis of pathogens, antigen presentation as well as tissue remodeling and repair (Aderem and Underhill, 1999; Brancato and Albina, 2011; Randolph et al., 2008). These immune cells also play an important role in some inflammatory diseases and infections (Li et al., 2012; Davignon et al., 2013; Jain et al., 2013; Kumar and Herbein, 2014; Noy and Pollard, 2014). During rheumatoid arthritis, macrophages are activated, releasing proinflammatory or regulatory cytokines and chemokines (Kinne et al., 2000). In cancer, macrophages are present in all stages, for example in primary tumors, macrophages are described to enhance tumor cell invasion (Noy and Pollard, 2014). Macrophages are also one of the early targets of HIV-1, which uses macrophages for replication (Kumar and Herbein, 2014). Because of the role of macrophages in different diseases and infections, target-

Abbreviations: Sn, Sialoadhesin.

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http://dx.doi.org/10.1016/j.imbio.2016.11.013
0171-2985/

ing macrophages with phenotype-modulating or cytotoxic drugs is an interesting therapeutic strategy. Several macrophage surface receptors, such as CD64 and CD163 are currently being investigated for treatment of inflammatory diseases (Graversen et al., 2012; Hristodorov et al., 2012; Etzerodt and Moestrup, 2013).

Another macrophage-specific surface receptor with interesting characteristics for antibody-based targeting is sialoadhesin (CD169 or Siglec-1; Sn). Sn is a member of the Ig superfamily of Siglecs (sialic acid-binding immunoglobulin-like lectins) which are expressed on different cells of the immune system (Crocker and Varki, 2001). Like other Siglecs, Sn is able to recognize and bind with sialic acids, a nine carbon sugar found on vertebrates and pathogens (Pillai et al., 2012). Chronic inflammation, such as rheumatoid arthritis and HIV-1, are associated with an upregulation of Sn, suggesting that Sn plays a role in these diseases (van der Kuyl et al., 2007; Hartnell et al., 2001). Sn was first thought to be an adhesion molecule since it has no known signalization motif in the cytoplasmic tail (Pillai et al., 2012; Crocker et al., 2007). However, further research showed that Sn is involved in cell-pathogen interactions and cell signaling (Crocker et al., 2007; Macauley et al., 2014). Moreover, Sn has the ability to internalize after binding with ligands such as monoclonal antibodies (mAbs), suggesting that Sn indeed has signaling functions (Akiyama et al., 2015; Vanderheijden et al., 2003; Chen et al., 2012; Izquierdo-Useros et al., 2012). For HIV-1, it was shown that the interaction with human Sn (hSn) also results in uptake of the virus via Sn. However, detailed analysis showed that this uptake was an invagination of the plasma membrane and not a complete internalization (Akiyama et al., 2015). Whether this peculiar form of uptake is specific for the cell type and hSn is not known and it is not fully clear if mAb binding to hSn also results in plasma membrane invaginations. Even though no known signalization motif has yet been detected in Sn, recent evidence shows that Sn can induce signaling via interaction with DNAX-activating protein of 12 kDa (DAP12). This interaction results in the recruitment of TRIM27 and suppression of antiviral innate immune responses (Zheng et al., 2015). More specifically, this also results in the inhibition of type I IFN production, which seems to be a negative feedback loop since IFN also upregulates Sn expression (Rempel et al., 2008; Delputte et al., 2007a; Puryear et al., 2013).

Receptor-specific antibodies can be used to target cells during diseases, either resulting in induction of a signalization cascade or by allowing intracellular delivery of drugs or toxins (Scott et al., 2012). For instance in cancer, antibodies can activate or antagonize immunological pathways after antibody targeting (Scott et al., 2012; Leach et al., 1996). Immunotoxins can also be used as an effective treatment for cancer (Scott et al., 2012). Different toxins or toxin fragments derived from bacteria, plants- and human enzymes can be fused with antibodies (Hristodorov et al., 2012). For these toxins to be active, internalization alone is not sufficient and efficient translocation from endocytic vesicles to the cytoplasm of the cell is usually critical. The efficiency of this translocation depends on the characteristics of the toxin but can also vary depending on the intracellular route of the internalized immunotoxin (Antignani and Fitzgerald, 2013). For instance, antibodies binding transferrin are only internalized into endosomes, while CD163-specific antibodies are delivered to the lysosomes (Graversen et al., 2012; Ritchie et al., 2013). Therefore, an important aspect in the development of immunotoxins is the knowledge on the mechanism of internalization of the receptor, followed by the intracellular route and the final fate.

Some researchers have already evaluated Sn as possible therapeutic target or entry point using liposomes or immunotoxins. Liposomes containing sialic acids on their surface that can bind to mouse Sn (mSn), are selectively internalized after binding and the cargo incorporated in these liposomes was shown to be released for processing (Macauley et al., 2014; Kawasaki et al., 2013). Porcine Sn (pSn) has been used as a model to target vaccines to Sn expressing cells. Fusing peptides to a pSn-specific mAb lead to targeted delivery of the peptide and this resulted in an increased immune response in contrast to non-targeted peptides (Ooms et al., 2013). Furthermore, targeting of immunotoxins to pSn leads to efficient killing of Sn-expressing macrophages (Delputte et al., 2011). These interesting observations demonstrate the potential of Sn as a therapeutic target. However, extrapolation of the findings for pSn to hSn is not straightforward. First, no specific sequences involved in internalization, intracellular trafficking or signalization have been identified in the cytoplasmic tail of Sn in any species, so it is not possible to check for conservation of such motifs in hSn. In addition, although the extracellular part of Sn is well conserved in between species, the cytoplasmic tail is very divergent both in length and in amino acid sequence (Klaas and Crocker, 2012). Since specific amino acid sequences in the cytoplasmic tail of receptors can influence the route of internalization and subsequent intracellular trafficking, the clathrin-dependent endocytosis of pSn and the localization of intracellular pSn-monoclonal antibody (pSn-mAb) complexes in early endosomes, might be different for hSn and mSn (Pillai et al., 2012; Delputte et al., 2011).

In this study, the internalization mechanism of hSn and mSn and the intracellular fate was analyzed in primary macrophages. Since the background of the Sn-expressing cells may also influence internalization characteristics, the internalization of Sn was also studied in CHO cell lines expressing recombinant Sn (Sn^+-CHO). This allows a direct comparison of Sn of different species independent of the cellular background. Our results show that antibody-induced Sn internalization is dynamin- and clathrin-mediated for both hSn and mSn in all cell types evaluated. Surprisingly, different cellular localizations of the endocytosed Sn-mAb complexes are observed in primary macrophages and Sn^+-CHO cell lines. In primary macrophages, the Sn-mAb complexes are observed predominantly in early endosomes. However, Sn^+-CHO cell lines transport the Sn-mAb complexes more to lysosomes.

2. Material and methods

All products were purchased from Thermo Scientific, unless stated otherwise.

2.1. Ethical statement

The experiments with BALB/c mice were authorized by the Ethical Committee for Animals of the University of Antwerp; permit number 2014-17. The use of human monocytes was authorized by the Ethical Committee of the Antwerp University Hospital and the University of Antwerp; permit number 16108.

2.2. Cells

CHO-K1 cells were kindly provided by Dr. J. D. Esko (University of California, San Diego, CA, USA) and were transfected or transduced to obtain stable cell lines expressing hSn (hSn^+-CHO) and mSn (mSn^+-CHO) (De Schryver et al., 2016). These cells were subcultured in RPMI with 10% heat inactivated fetal bovine serum (iFBS). Primary mouse bone marrow macrophages were collected in RPMI medium. After removing red blood cells with an ammonium-chloride-potassium lysis buffer (VWR and Janssen Chimica), macrophages were seeded in RPMI with 10% iFBS, 1% non-essential amino acids, 1% sodium pyruvate, 1% glutamine and with addition of L929 supernatant and 500 U/ml mouse IFN-α (R&D) in the medium. L929 supernatant was collected from cells kindly provided by Dr. C. Uyttenhove (Ludwig Institute for Cancer Research, Brussels, Belgium). Human monocytes were obtained from blood of healthy volunteers from the Belgian Red Cross-Flanders. The