Karolinska Institutet

Institutionen för Medicinsk Biokemi och Biofysik

Structural and functional studies of class A scavenger receptors MARCO (SCARA2) and SCARA5

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Samuelssonsalen, Tomtebodavägen 6, Karolinska Institutet, Solna

Fredagen den 15 mars, 2013, klo 9.30

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Stockholm 2013

ABSTRACT

Our bodies are constantly exposed to various microorganisms and there is a constant battle between their survival and ours. Fortunately we are equipped with our immune system that takes remarkably good care of us, but when it fails we all know the consequences. This thesis work concentrates on innate immunity and the molecular mechanisms set in place to fight infections and to keep our bodies functional. More specifically, the focus is on germline-encoded receptors MARCO and SCARA5 capable of recognizing and handling pathogen- or damage-associated molecular patterns (PAMPs and DAMPs).

Macrophage receptor with a collagenous structure (MARCO) is a pattern recognition receptor (PRR) expressed by professional phagocytes, macrophages and dendritic cells, and participates in the clearance of bacteria and pollution particles. It is a trimeric molecule with a short N-terminal cytosolic domain, a single pass transmembrane domain followed by a large extracellular region with a spacer domain, a long collagenous domain, and C-terminal scavenger receptor cysteine-rich (SRCR) domain. In this study it was established that the C-terminal SRCR domain of MARCO is the main functional unit mediating both ligand-binding and adhesion.

We could show that a soluble form of MARCO binds bacteria cell wall components lipopolysaccharide and lipoteichoid acid. Utilizing the soluble protein we identified several hydrophobic peptides that bound to the SRCR domain of the receptor. The peptide sequences were identified as part of complement component C4b that functions as an opsonin once bound to the surface of bacteria. We could detect some binding of C4b to MARCO, but the peptide sequence was not involved in the binding. Another ligand, acetylated low-density lipoprotein (AcLDL), was also found to bind to the SRCR domain. With the help of mutational analysis and by solving the crystal structure of a monomeric and dimeric form of the SRCR domain to 1.78 and 1.77 Å, respectively, we could identify in more detail that a β-sheets structure with several positively charged arginines and a negative cluster residing in a long loop area of the structure were important for the ligand-binding functions. These areas affected also MARCO mediated adhesion to various surfaces. Based on the ion-binding site found in the long loop region, we were able to show that Ca^{2+} is needed for ligand binding.

Other half of the thesis work focused on the physiological function of SCARA5, a MARCO related scavenger receptor having an additional α-helical coiled coil domain between the spacer domain and the collagenous domain. Similar to MARCO, SCARA5 was found to promote cell adhesion and bind and internalize ligands such as bacteria and LPS. In contrast, we could only see limited binding of AcLDL, the knowledge of which we used in the mutational analysis made to map the ligand-binding region in the MARCO SRCR domain. Further, we utilized the strong cell adhesion for selection of stable cell lines without using antibiotics and were able to improve cell viability and handling of the cells in serum-free culture conditions during protein production. Our *in vivo* functional studies of SCARA5 revealed that in contrast to MARCO, the receptor is expressed by a vimentin and platelet-derived growth factor receptor α (PDGFRα) positive subpopulation of fibroblasts participating in immune related homeostasis. The homeostatic function was evident on the aging SCARA5 deficient mice that developed antinuclear antibodies and connective tissue related autoimmune disease-like symptoms with lymphoid cell accumulations in several organs, especially lung.

ISBN 978-91-7549-045-8