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論 文 内 容 の 要 旨
Synopsis of Thesis

氏 名 Name	OMAHDI ZAKARIA
論文題名 Title	Structural insight into phosphoglycolipid recognition by C-type lectin receptor DCAR (結核菌受容体DCARによる糖リン脂質認識機構の構造解析)

論文内容の要旨

〔目 的(Purpose)〕

DCAR (dendritic cell immunoactivating receptor 2, *Clec4b1*) is a C-type lectin receptor (CLR) expressed by myeloid cells which was recently reported to function as a receptor for mycobacteria through the recognition of unique phosphoglycolipids abundantly found in mycobacterial envelopes, called acylated phosphatidyl-myo-inositol mannosides (AcPIMs). As AcPIMs induce the expression of inflammation-related genes in human dendritic cells, it suggests the presence of an unidentified human AcPIM receptor and the determination of murine DCAR structure may provide valuable information for the identification of novel bacterial receptor(s).

〔方法ならびに成績(Methods/Results)〕

The carbohydrate recognition domain (CRD) of DCAR was expressed as inclusion bodies, refolded and purified for crystallization and structural analysis which revealed that DCAR, though having a typical C-type lectin fold, also has specific features which mediate the interaction with AcPIMs. By mutagenesis, we demonstrated that the non-canonical carbohydrate binding motif (EPS; Glu-Pro-Ser) of DCAR is necessary for optimal receptor activation and is not only a variant of the typical mannose-binding EPN (Glu-Pro-Asn) motif. Several residues, such as Ala136 and Gln198, present in the vicinity of the calcium coordination site and which were different than those of the similar CLR Mincle, were shown to be required for ligand binding and receptor activation. Mutagenesis analysis suggested that these residues are implicated in the accommodation of the negatively charged phosphate moiety and the formation of a secondary sugar binding site for the interaction with whole AcPIMs. To confirm the direct interaction of DCAR with the phosphosugar moiety of AcPIMs, we generated a water-soluble analog (inositol-monophosphate di-mannose; IPM2). By biolayer interferometry as well as co-crystallization, we demonstrated that DCAR can indeed directly bind one mannose residue of the phosphosugar moiety of AcPIMs IPM2 (dissociation constant of 140 μM ± 7.9 μM). However, we also observed rapid dissociation of the interaction by biolayer interferometry which suggested the presence of another determinant involved in the stable interaction between DCAR and whole AcPIMs. The crystal structure of DCAR in complex with IPM2 revealed a hydrophobic surface extending from the ligand binding site and in a suitable position for the accommodation of the acyl chain(s) of whole AcPIMs. Mutagenesis of the most hydrophobic residue in this area (Ile133) strongly impaired reporter activity in cells stimulated with AcPIMs.

〔総 括(Conclusion)〕

These results show that DCAR binds carbohydrates in a similar fashion to other CLRs through its overall typical C-type lectin fold and structural and mutagenesis analysis also revealed characteristics for the suitable recognition of unique mycobacterial phosphoglycolipids. The present study suggests that the mannose-binding ability and hydrophobic groove of DCAR mediate its specific binding to pathogen-derived phosphoglycolipids, such as mycobacterial AcPIMs.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

結核菌は感染時に宿主に多様な免疫応答を惹起する。中でも結核菌の細胞壁糖鎖はパターン認識受容体の一種であるC型レクチン受容体を介して宿主の免疫応答に関与する。結核菌の糖脂質成分であるPhosphatidyl-inositol mannoside (PIM)はマウスのミエロイド細胞に発現したDendritic Cell Activating Receptor (DCAR)によって認識され、免疫応答を誘発することが先行研究によって明らかとなった。しかしDCARとPIMの相互作用に関する原子レベルのリガンド認識機構は未だ不明であった。本研究は、DCARのC型レクチンドメインを用いて生化学的解析を通してリガンド結合に関与する重要な残基を同定した。さらにC型レクチンドメイン単独および可溶性PIM糖鎖アナログとの複合体のX線結晶構造解析を行い、新奇な糖鎖認識機構を明らかにした。

本研究は結核感染機構の解明に重要な知見を与えるものであり、学位を授与するに充分であると判断される。