氏名	蔣（ジャン） 妍（ヤン） (Yan Jiang)
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学位論文名	Transport of misfolded endoplasmic reticulum proteins to the cell surface by MHC class II molecules （ミスフォールド小胞体タンパク質の主要組織適合性遺伝子複合体クラスII分子による細胞表面への輸送）
論文審査委員	（主査） 教　授　荒瀬　尚 （副査） 教　授　熊ノ郷　淳　　教　授　竹田　潔

論文内容の要旨

〔Purpose〕

MHC class I and II molecules play a central role in the immune system by presenting peptide antigens to T cells. In general, peptides derived from extracellular proteins are presented by MHC class II molecules. Structural analyses of MHC class II molecules have revealed that both ends of the MHC class II peptide-binding groove are open and long peptides can be presented. Considering the structure of the MHC class II peptide-binding groove, it is possible that nascent MHC class II products associate with a variety of different proteins other than Ii. Here, we demonstrate that both human and mouse MHC class II molecules bind to host cell-derived misfolded proteins via the peptide-binding groove and transport them to the cell surface.

〔Methods/Results〕

We found that HLA-Cw4 cannot be expressed on the cell surface of human 293T kidney epithelial cells. But when HLA-Cw4 was transfected into cell line 721.221, HLA-Cw4 was readily expressed on the cell surface. We generated a cDNA library from 721.221 cells and transfected the library into 293T cells and found that HLA-Cw4 was expressed on the surface of cells transfected with both HLA-DRα and HLA-DRβ chains.

HLA-Cw4 expressed on 293T cells was recognized by the L31, which is specific for misfolded HLA-C heavy chains that lack peptide and β2-microglobulin. This suggests that 293T cells express only structurally altered, or misfolded, HLA-Cw4 in the presence of HLA-DR proteins on the cell surface.

We analyzed HLA-Cw4 transport by different HLA-DR β chain alleles and found that the HLA-DRA*01:01 / HLA-DRB1*01:03 complex did not enable cell surface expression of HLA-Cw4 whereas HLA-DRA*01:01 / DRB1*01:01, which we cloned from the 721.221 cDNA library, did so efficiently. Three amino acids are different between DRB1*01:01 and DRB1*01:03 at residues 67, 70, and 71. These are the residues that are involved in the formation of pockets 6 and 7 of the HLA-DR peptide-binding groove, suggesting that misfolded HLA-Cw4 binds to the peptide-binding groove and is thus transported to the cell surface. The HLA-DRA*01:01 / TfRpep-HLA-DRB1*01:01 complex did not allow surface HLA-Cw4 expression. Therefore, the HLA-Cw4 heavy chain seems to associate with the peptide-binding groove of certain HLA class II molecules. Co-transfection of Ii together with the HLA-DRA*01:01 and HLA-DRB1*01:01 cDNA resulted in a significant decrease of cell surface HLA-Cw4 expression. However, HLA-Cw4 transported by the HLA-DRA*01:01 / HLA-DRB1*04:04 complex was only slightly affected by Ii. Therefore, the efficiency of transportation of misfolded HLA-Cw4 to the cell surface by HLA-DR molecules is affected by a balance between the strength of association of HLA-Cw4 and Ii to HLA-DR and/or the relative amounts of HLA-DR and Ii proteins present.

When the extracellular domain of HLA-Cw4, lacking the transmembrane region, was co-transfected with HLA-DRA*01:01 and HLA-DRB1*01:01, it was detected on the cell surface in the presence of HLA-DR but not in the absence of HLA-DR. In contrast, HLA-Cw4 lacking the signal sequence, which is required for nascent proteins to be transported to the endoplasmic reticulum (ER), was not transported to the cell surface by HLA-DR, suggesting that HLA-Cw4 associates with HLA-DR in the ER. When HLA-DR protein was immunoprecipitated from lysates of HLA-DR- and HLA-Cw4-co-transfectants, HLA-Cw4 was co-precipitated from HLA-DRA*01:01 and HLA-DRB1*01:01-transfectants but not from cells transfected with HLA-DRA*01:01 and TfRpep-HLA-DRB1*01:01. It is the whole misfolded HLA-Cw4 protein that associates in the ER with the HLA-DR peptide-binding groove, and is then transported to the cell surface.

When wild-type HEL and HEL with mutations at two cysteine residues, Cys30 and Cys64, were transfected, wild-type HEL but not mutant HEL protein was secreted into the culture supernatant. This indicated that the cysteine mutations induced misfolding of HEL. Cysteine-mutant HEL co-transfected with I-Ak was efficiently presented on I-Ak relative to wild-type HEL. HELpep-I-Ak failed to present cysteine-mutant HEL. These data suggest that misfolded intact mutant HEL protein also associates with the peptide-binding groove of MHC class II molecule in the ER and is transported to the cell surface. In co-immunoprecipitation experiments, mutant HEL was co-precipitated with I-Ak, but this was not seen using HELpep-I-Ak. This indicates that the whole HEL protein is present at the peptide-binding site of I-Ak. HyHEL10 is specific for correctly folded HEL protein whereas Gloop4 mAb recognizes HEL protein independently of its structure. Mutant HEL protein presented on I-Ak was recognized by Gloop4 mAb but not HyHEL10 mAb. Notably, wild-type secreted HEL protein was also recognized by Gloop4 but not by HyHEL10 mAb, indicating that misfolded HEL protein was specifically presented on I-Ak even when wild-type HEL protein was also present. These data support the notion that MHC class II molecules associate with misfolded proteins and transport them to the cell surface.

We generated a mouse A20 B-cell line expressing the HEL-specific Gloop4 IgM BCR (G4-A20) and analyzed cell activation by monitoring expression of CD69. Wild-type HEL- or cysteine-mutant HEL-transfectants and soluble HEL protein did not stimulate G4-A20 cells. On the other hand, cysteine-mutant HEL protein presented on MHC class II molecules stimulated G4-A20 cells more efficiently than plate-bound HEL protein or membrane-tethered HEL protein. These results suggest that misfolded proteins presented on MHC class II molecules can efficiently activate antigen-specific B cells.

〔Conclusion〕

We suggest that MHC class II molecules present not only peptides but also intact host cell-derived proteins on the cell surface. These findings provide new insights into the function of MHC class II molecules.

論文審査の結果の要旨

免疫応答の中心的な機能を担うMHCクラスII分子は、いままでペプチド抗原をT細胞に提示すると長年考えられてきた。ところがNK細胞レセプターであるKIRのリガンドとなるMHCクラスIの認識機構について解析をすすめたところ、MHCクラスII分子によってミスフォールドしたMHCクラスIの発現が誘導されることが明らかになった。さらに解析を進めることによって、MHCクラスII分のペプチド結合部位にミスフォールドしたHLAクラスIが結合し細胞表面に輸送されることが判明した。MHCクラスI分子ばかりでなく、小胞体内の様々なミスフォールド蛋白質がMHCクラスII分にペプチドに分解されることなく提示された。また、MHCクラスIIに提示されたミスフォールド蛋白質は抗原特異的なB細胞を直接活性化することも明らかになった。本研究によりMHCクラスII分子の新たな機能が明らかになり、今後MHCクラスII分子の機能を研究する上でも重要な研究成果である。以上より、本研究は、学位の授与に値すると考えられる。