Cellular misfolded proteins rescued from degradation by MHC class II molecules are possible targets for autoimmune diseases

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The major function of major histocompatibility complex (MHC) class II molecules is the presentation of peptide antigens to helper T cells. However, when misfolded proteins are associated with MHC class II molecules in the endoplasmic reticulum, they are transported to the cell surface by MHC class II molecules without processing to peptides. Of note, misfolded proteins complexed with MHC class II molecules are specifically recognized by autoantibodies produced in patients with autoimmune diseases such as rheumatoid arthritis and antiphospholipid syndrome. Furthermore, autoantibody binding to misfolded proteins complexed with MHC class II molecules is associated with the susceptibility to autoimmune diseases conferred by each MHC class II allele. Therefore, misfolded proteins rescued from degradation by MHC class II molecules may be recognized as ‘neo-self’ antigens by the immune system and be involved in the pathogenicity of autoimmune diseases.

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General Function of MHC Molecules

MHC is a key molecule in the immune system that presents peptide antigens to T cells (1, 2). MHC molecules are categorized into class I and class II. MHC class I molecules present short peptides that consist of 7–9 amino acids derived from intracellular proteins to cytotoxic T cells. On the other hand, MHC class II molecules present long peptide antigens that consist of 10–15 amino acids derived from extracellular proteins to helper T cells. Both MHC class I and class II genes are highly polymorphic and are different among individuals and populations. Immune responses to exogenous antigens such as viral antigens vary depending on the MHC alleles. On the other hand, it is well-accepted that specific MHC alleles, particularly MHC class II alleles, are associated with susceptibility to many autoimmune diseases (3); a strong association has been confirmed by recent genome-wide association studies (4–7). Therefore, it is important to elucidate the mechanism of this association. Peptide antigens presented on MHC molecules are involved not only in T cell responsiveness but also in T cell differentiation in the thymus. Accordingly, it is predicted that specific MHC alleles are involved in the susceptibility to autoimmune diseases by affecting T cell differentiation and/or responsiveness (3, 8). However, peptide antigens that may explain this susceptibility conferred by each MHC allele have not been identified; thus, it is unclear how MHC molecules control autoimmune disease susceptibility.

MHC Class II Molecules Function as Molecular Chaperones to Transport ER-Misfolded Proteins to the Cell Surface

MHC class I molecules form heterodimers with β2-microglobulin (1, 2). Endogenous peptide antigens generated by the proteasome are transported to endoplasmic reticulum (ER) by the transporter associated with antigen processing (TAP) and are presented on MHC class I molecules. In the absence of peptide antigens, MHC class I molecules are not folded properly, thus are not transported to the cell surface. Similarly, β2-microglobulin, which forms a heterodimer with MHC class I molecules, is required for cell surface expression of MHC class I molecules. For human MHC class I molecules, there are unique monoclonal antibodies (mAbs) such as HC10 and L31 (9–11) that specifically recognize misfolded but not normally folded MHC class I molecules (12, 13). Because misfolded MHC class I molecules that have failed to associate with proper peptide antigens or β2-microglobulin would not be expressed on the cell surface as aforementioned, these mAbs should not bind to the cell surface. However, certain human cell lines (9, 10, 12, 13) or activated human T cells (11) are recognized by these mAbs. Therefore, misfolded MHC class I molecules can be transported to the cell surface in certain situations, although the molecular mechanism of how misfolded MHC class I molecules are transported has not been addressed to date.

A study based on expression cloning of molecules that would permit expression of MHC class I molecules on the cell surface unexpectedly identified MHC class II α and β heterodimers (14). MHC class II molecules do not induce cell surface expression of normally folded but induce cell surface misfolded MHC class I expression that is detected by HC10 and L31 mAbs (9, 10). Analyses of various MHC class II alleles suggested that the peptide binding groove of MHC class II molecules is involved in the
induction of misfolded MHC class I expression (14). Furthermore, MHC class II molecules possessing covalently attached peptide antigens failed to induce misfolded MHC class I expression. This observation is consistent with the notion that misfolded MHC class I molecules were induced on the cell surface by associating with the peptide binding groove of MHC class II molecules. In addition, hen egg lysozyme (HEL) proteins were also transported to the cell surface by MHC class II molecules upon binding to their peptide binding groove. Furthermore, misfolded HEL proteins presented on MHC class II molecules stimulated B cells expressing the HEL-specific B-cell receptor (BCR), indicating that MHC class II molecules possess the ability to present protein antigens to B cells (14). This means that MHC class II molecules function as chaperones to transport ER-misfolded proteins to the cell surface and may be involved in as yet unknown function pertaining to the immune response (14) (Fig. 1).

It is well recognized that MHC class II molecules present peptide antigens to T cells. To acquire peptide antigens, newly synthesized MHC class II molecules associate with the invariant chain that has an endolysosomal targeting signal (1, 2, 15). MHC class II molecules associated with the invariant chain are transported to endo-lysosomal compartments where they acquire peptide antigens derived from extracellular proteins (1, 2, 15) (Fig. 1). However, considering that invariant chain itself is a Type II membrane protein, MHC class II molecules originally exhibit a capacity to associate with proteins via peptide binding groove. Because the association of MHC class II molecules with the invariant chain is relatively strong, it is thought that MHC class II molecules do not bind to other proteins in ER. However, the affinity between MHC class II molecules and the invariant chain varies depending on the MHC class II alleles (16). Therefore, if the avidity of the association between MHC class II molecules and certain misfolded proteins is higher than the avidity of the association between MHC class II molecules and the invariant chain, the misfolded proteins are associated with newly synthesized MHC class II molecules in ER instead of the invariant chain. MHC class II molecules present long peptides compared to MHC class I molecules (17) because both ends of the peptide binding groove of MHC class II molecules are open (18). Therefore, it is structurally possible that peptide-like structures exposed on misfolded proteins are associated with the peptide binding groove of MHC class II molecules. Direct association between misfolded MHC class I molecules and MHC class II molecules were observed in MHC class II expressing cells (14). Indeed, it has been suggested that MHC class II molecules associate with large protein in certain situations (19–21). However, the immunological function of MHC class II molecules to transport cellular misfolded proteins to the cell surface has not been addressed to date.

**Misfolded Proteins/MHC Class II Complexes in Autoimmune Diseases**

Autoantibodies are produced in most autoimmune diseases such as rheumatoid arthritis (RA) (22). Because the specificities of autoantibodies are different depending on the autoimmune disease, detection of disease-specific autoantibodies is important for their diagnosis (22). However, it is unclear how disease-specific autoantibodies are produced in autoimmune diseases. Antibodies are generally produced against non-self antigens and not against self-antigens. Therefore, exposure of cryptic epitopes on self-antigens in certain situations has been thought to be involved in autoantibody production (23–25).

When proteins are synthesized in the cells, both correctly folded and misfolded proteins are constitutively produced in the cells (26). However, such misfolded proteins are promptly degraded or refolded in the cells and are not transported outside the cells in the steady state (26). Because cellular misfolded proteins are not present outside the cells, immune cells are not exposed to the misfolded proteins in normal situations. Therefore, immune cells may not be tolerant to cellular misfolded proteins.

Although MHC class I molecules are expressed in almost all cell types, MHC class II expression is restricted to certain cell types such as dendritic cells and B cells. Most non-immune cells normally do not express MHC class II molecules. However, in case of human, when cytokines such as interferon (IFN)-γ are produced by inflammation induced by certain infections, MHC class II expression is induced even on non-immune cells that do not express MHC class II molecules in the steady state (27–29). In such a situation, ER-misfolded proteins are associated with MHC class II molecules, thus are transported to the
cell surface without processing to peptides (14). Such abnormal misfolded proteins complexed with MHC class II molecules may expose cryptic autoantibody epitopes that are not present in normally folded protein. Namely, misfolded proteins complexed with MHC class II molecules may be recognized as ‘neo-self’ antigens by immune cells and may induce an antibody response against the complex (Fig. 2).

It has been reported that many autoimmune-diseased tissues aberrantly expressed MHC class II molecules (30–33). For example, thyroid tissues from patients with Hashimoto’s thyroiditis or Graves’ disease aberrantly express MHC class II molecules, although normal thyroid tissues do not express MHC class II molecules (30). Similarly, it has been reported that MHC class II molecules are aberrantly expressed on non-immune cells in other autoimmune diseases (31–33). Although the unusual MHC class II expression was initially considered to be involved in the pathogenicity of autoimmune diseases, these non-immune cells do not express the co-stimulatory molecules such as CD80 or CD86 that are required to initiate T cell responses. Therefore, the aberrant MHC class II expression on non-immune cells has been considered to be simply a result of inflammation and not a cause of autoimmune disease. However, considering that misfolded proteins complexed with MHC class II molecules have a capacity to stimulate antigenspecific B cells (14), there is a possibility that the misfolded proteins complexed with MHC class II molecules are involved in autoimmune diseases as a target for autoantibodies.

Rheumatoid factor is a traditional autoantibody against denatured IgG heavy chain. Because 70–80% of patients with RA are positive for rheumatoid factor, the titers of which have been used for the diagnosis of RA (34). However, it is currently unclear why autoantibodies against denatured IgG are produced in RA, and what are the natural target antigens for rheumatoid factor. IgG is composed of a heavy and a light chain, where the heavy chain alone is not secreted or expressed on the cell surface. However, IgG heavy chain alone is transported to the cell surface in the presence of MHC class II molecules (35). Because IgG heavy chain is expressed even in the absence of light chain, the structure of IgG heavy chain is likely to be different from normal IgG. Furthermore, IgG heavy chain complexed with MHC class II molecules was recognized by autoantibodies from RA (35). On the other hand, the membrane form of IgG BCR was not recognized by the autoantibodies. In addition, autoantibody binding to IgG heavy chain complexed with MHC class II molecules was not observed in rheumatoid factor-positive non-RA individuals (35). Therefore, IgG heavy chain complexed with MHC class II molecules seems to be a specific target for autoantibodies in RA. Indeed, IgG heavy chain/MHC class II complex was detected in the synovial membrane of RA patients.

Although the pathogenicity of autoantibodies in RA is unclear, it has been reported that IgG from RA patients induces arthritis in FcγR1IB-deficient mice that lack an inhibitory Fc receptor (36). In addition, the adaptive transfer of autoantibodies from RA-model mice such as K/BxN mice and Type II collagen-immunized mice into normal mice also induces arthritis (37–39). Therefore, autoantibodies directed against IgG heavy chain complexed with MHC class II molecules might be involved in the pathogenesis of RA by a mechanism similar to recent reports (40, 41).

Antiphospholipid syndrome (APS) is an autoimmune disease that causes thrombosis and pregnancy complications (42). The major target for the antiphospholipid antibody is thought to be β2-glycoprotein I (β2GP1) (43–45). Free β2GP1 forms a circular structure, which is converted to a linear structure when β2GP1 is associated with phospholipid (46, 47). However, it is unclear whether β2GP1 complexed with phospholipid is a natural target for the antiphospholipid antibody. When the association of β2GP1 with MHC class II molecules was studied, β2GP1 was found to be expressed on the cell surface in the presence of MHC class II molecules (48). In addition, β2GP1 complexed with MHC class II molecules was recognized by the antiphospholipid antibody. Particularly, APS patients with a negative anti-β2GP1 autoantibody titer according to a general laboratory test possessed autoantibodies against β2GP1 complexed with MHC class II molecules. Because more than 80% of patients possessed autoantibodies against β2GP1/MHC class II complex, β2GP1 complexed with MHC class II molecules seems to be the major autoantibody target in APS (48). In this way, misfolded proteins complexed with MHC class II molecules may serve as the major target for

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**Rescue cellular misfolded proteins by MHC class II molecules**
autoantibodies produced in various autoimmune diseases.

**Autoimmune Disease Susceptibility and Misfolded Proteins/MHC Class II Complexes**

Specific MHC class II alleles affect susceptibility to many autoimmune diseases (3). MHC class II is the gene most strongly associated with susceptibility to RA (5, 8). For example, individuals who have human leukocyte antigen (HLA)-DR4, a human MHC class II allele, are 10 times more susceptible to RA than those who have HLA-DR3 (8). When autoantibody binding to IgG heavy chain complexed with MHC class II molecules was compared among various HLA-DR alleles, autoantibody binding to IgG heavy chain complexed with each HLA-DR allele was strongly correlated with susceptibility to RA conferred by each HLA-DR allele (35). This suggests that individuals who possess RA-susceptible MHC class II alleles are likely to generate autoantibody targets. Because peptide antigens that are related to RA-susceptibility determined by MHC class II alleles have not been identified (8), the IgG heavy chain is the first molecule that is associated with RA-susceptibility conferred by each HLA-DR allele. Because measuring autoantibodies against IgG heavy chain complexed with HLA-DR is more specific to RA than the traditional rheumatoid factor titer (35), there is a possibility that the IgG heavy chain/HLA-DR complex is directly involved in the pathogenicity of RA. Similar to RA, β2GPI associated with APS-susceptible HLA-DR allele is well recognized by the antiphospholipid antibody (48). In this way, misfolded proteins complexed with MHC class II molecules may be directly involved in the pathogenicity of various autoimmune diseases as a target for autoantibodies.

**Concluding Remarks**

Our studies on misfolded proteins complexed with MHC class II molecules suggested that the complex is involved in the pathogenicity of autoimmune diseases as a specific target for autoantibodies. Therefore, it is important to elucidate the fine mechanisms of how the misfolded proteins/MHC class II complex is formed, how antibodies against misfolded proteins/MHC class II complex are produced and how antibodies against misfolded proteins/MHC class II complex cause autoimmune diseases. In addition, role of T cells in IgG autoantibody production against misfolded protein/MHC class II complex needs to be elucidated in future study. Transport of cellular misfolded proteins to the cell surface would not be the primary function of MHC class II molecules. However, because MHC class II molecules can present long peptide antigens, they may mistakenly transport cellular misfolded proteins to the cell surface when their expression is induced or increased by inflammation or infection. Most studies on misfolded proteins have been restricted to the molecular mechanisms of how misfolded proteins are degraded or refolded. Therefore, studies on the active transport mechanism of misfolded proteins to the cell surface will be important to understand the pathogenicity of autoimmune diseases.

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**References**


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