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“A Cell-Free Recapitulation of Antigen Processing for MHC Class II: the Role of HLA-DM in the Selection of Immunodominant Epitopes”

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Abstract:

The mechanisms responsible for the phenomenon of immunodominance in antigen presentation to T cells are not well understood. In antigen presentation by class II major histocompatibility complex (MHC) molecules, HLA-DM, a “peptide editor” that allows certain peptides to remain associated with class II MHC molecules while causing others to dissociate, may be needed for the selection of the immunodominant epitope from the pool of peptides generated from a protein antigen. To test this hypothesis, we have established a cell-free system, composed of purified proteins, that mimics the class II MHC antigen presentation process: recombinant soluble forms of HLA-DR1 and HLA-DM, together with purified endosomal proteases, are incubated with an intact antigen protein. We used the influenza hemagglutinin (HA) protein as our model antigen because its immunodominant epitope with respect to HLA-DR1 is already known. We show that, in the presence of HLA-DM, HLA-DR1 and the HA protein associate with each other through HLA-DR1's peptide-binding groove even before any proteolytic degradation of the HA protein takes place. We further demonstrate that, upon the addition of endosomal proteases, a proteolytic fragment of the HA protein containing the immunodominant epitope remains bound to HLA-DR1. This fragment can be detected as a prominent species in the mass spectrometric analysis of peptides eluted from the peptide/HLA-DR1 complexes formed in this reaction. Our approach demonstrated here may prove to be useful in the *de novo* identification of immunodominant epitopes in antigens whose immunodominant epitopes are not yet known. We further demonstrate that the presence of HLA-DM reduces the number of HA protein-derived peptide species captured by HLA-DR1, thereby providing evidence for HLA-DM's role as an editor of peptide-class II MHC complex formation.