Gold-plating MHC class II molecules

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Square-planar, four-coordinate metal complexes strip peptides from the binding site of MHC class II molecules. Removal of peptides derived from self-antigens could prevent class II–dependent activation of self-reactive T cells in autoimmune diseases such as rheumatoid arthritis.

In 1935, Forestier reported that gold complexes could be used to treat rheumatoid arthritis[1]. Gold therapy is still in use today and is an effective and inexpensive alternative to more modern biologic anti-arthritis or nonsteroidal anti-inflammatory drugs[2]. Yet what is amazing is that this use of gold compounds was originally based on the mistaken view that rheumatoid arthritis was a form of tuberculosis, a disease that was also effectively treated with gold compounds. Fast-forward more than 70 years, and in this issue of *Nature Chemical Biology* De Wall *et al.*[3] propose a molecular mechanism to explain Forestier's finding: gold complexes remove antigenic peptides from major histocompatibility (MHC) class II molecules, thus inhibiting the activation of the self-reactive T lymphocytes that cause rheumatoid arthritis or other autoimmune diseases.

Rheumatoid arthritis is a chronic inflammatory disease that is thought to be caused by the activation of self-reactive $CD4^+$ T cells in the synovial fluid of joints. Self-reactive T cells do not directly recognize irritating self-antigens in the joints; instead they recognize small polypeptide fragments (10–20 amino acids in length) of these antigens[4]. These fragments are not soluble, but are immobilized, or 'presented' to the T cell, by an antigen-presenting cell (APC) that displays the fragments bound to a peptide-binding protein called the major histocompatibility complex (MHC) class II molecule. Because MHC class II molecules bind peptides derived from self-antigens and foreign antigens equally well, potential solutions to fighting autoimmune diseases entail either (i) deleting disease-causing self-reactive T cells or (ii) preventing the activation of self-reactive T cells. One way to do this is by removing the self-antigens from the peptide-binding groove of MHC class II molecules, thereby limiting their ability to activate disease-causing self-reactive T cells.

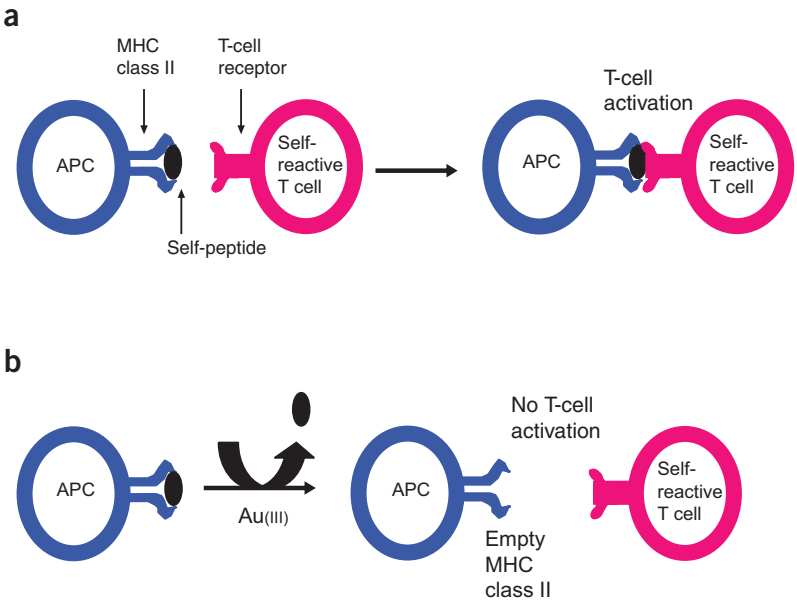

Figure 1 Peptide binding is required for MHC class II-restricted T-cell activation. Antigen-presenting cells (APCs) possess surface MHC class II molecules that bind both self-peptides and foreign peptides. (**a**) When an APC displays a self-peptide to a self-reactive T cell, the T cell is activated and this can lead to autoimmune diseases. (**b**) The ability of square-planar metal complexes to strip peptides from MHC class II molecules will limit activation of self-reactive T cells.

Normally, MHC class II molecules bind peptide fragments of antigenic proteins in lysosome-like antigen-processing compartments in APCs[4]. Class II molecules are escorted into these compartments by a protein called invariant chain. This protein blocks the peptide-binding groove of class II molecules until it reaches antigen-processing compartments, where it is removed by another protein called HLA-DM. HLA-DM not only removes residual invariant-chain peptides from the peptide-binding groove of MHC class II molecules, but also serves to remove weakly bound peptides from class II molecules, thereby acting as a peptide editor that ensures that only high-affinity peptides remain bound to the class II molecules.

DeWall *et al.* developed a high-throughput assay designed to identify small molecules that could disrupt MHC class II–peptide interactions. In a screen of 28,000 compounds, they found that square-planar d^8 metal complexes of Pt(II) were able to very efficiently strip peptides from human class II molecules. Most importantly, they found that these compounds, as well as related Pd(II) and Au(III) complexes, blocked the ability of peptide-loaded APCs to stimulate antigen-specific $CD4^+$ T cells, thus leading the way to understanding the mechanism by which noble metal complexes could inhibit rheumatoid arthritis by preventing the activation of autoreactive $CD4^+$ T cells by APCs.

As in the mechanism of peptide removal by HLA-DM[5], it is likely that these metal complexes do not interact with the MHC class II peptide-binding groove itself. Although the

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details of the mechanism of action of these square-planar metals on class II–peptide complexes remains unclear, circular dichroism and monoclonal antibody reactivity studies revealed that Pt(II)- and Au(III)-treated class II molecules seem to be in a conformation that resembles that of empty class II molecules, demonstrating that these compounds remove peptides without fully denaturing the class II molecule itself. Furthermore, these compounds render the empty class II molecules unable to subsequently bind peptides, so in this respect they work in a way that is very different from that of HLA-DM. What is most important is that these square-planar metal compounds enhance the peptide dissociation kinetics of even high-affinity peptides by up to 60-fold. Because even modest changes in peptide dissociation rate can profoundly influence immune responses *in vivo*[6], efficient self-peptide release mediated by metal complexes could assist in limiting the activation of self-reactive $CD4^+$ T cells in individuals with rheumatoid arthritis.

This study is a first step in the design of additional square-planar metal complexes that could specifically target MHC class II–self-peptide complexes. Although this work is exciting, there are still challenges to be addressed. For instance, class II molecules also bind peptides derived from pathogens, and these 'good' class II-peptide complexes are required to initiate robust immune responses against these pathogens; thus, the nonselectivity of metal complexes in stripping peptides from class II molecules could compromise the immune system of patients. However, this is a general problem that must be addressed with all forms of immunosuppressive therapies. Most significantly, what started in the 1930s as a treatment for the 'infectious agent' that caused rheumatoid arthritis now may finally have a clear molecular mechanism. Modern medicinal chemists will certainly use the findings of DeWall *et al.* in their attempts to tailor even more specific compounds to fight rheumatoid arthritis and a variety of autoimmune diseases.

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How to evolve a silk purse from a sow's ear

David E Cane

The enzyme γ-humulene synthase normally generates a mixture of more than 50 sesquiterpene products from a single substrate. Targeted evolution allowed the design of new mutants with greatly enhanced product specificity. The results provide a model for the divergent evolution of enzyme specificity from ancestral proteins of promiscuous function.

Understanding the relationship between protein structure and biochemical function, as well as how these properties coevolve, represents one of the greatest challenges at the frontier of structural biology and proteomics. A team of researchers from the University of California at Berkeley and the University of California at San Francisco have now developed an ingenious and powerful method to study the evolution of enzyme specificity as a function of random but focused changes in protein sequence. In the March 16 issue of *Nature*, Jay D. Keasling and colleagues report that controlled alteration of a small number of active-site amino acids can dramatically alter the product specificity pattern of a naturally occurring enzyme that normally makes a plethora of terpene natural products[1]. Their results provide unexpected insights into the relationship of protein structure to enzyme specificity, while adding a promising new tool to the repertoire that can be used for rational enzyme design and engineering.

The team chose to examine an enzyme called γ-humulene synthase[2], a sesquiterpene synthase belonging to a large class of proteins that have been isolated from a variety of plant and microbial sources and that convert the 15-carbon acyclic precursor, farnesyl diphosphate (FPP), to any of more than 300 distinct sesquiterpene hydrocarbons or alcohols[3]. Each such cyclase uses a variation of a common reaction mechanism in which the FPP substrate is ionized to a carbocationic intermediate that then undergoes a cascade of cyclization and rearrangement reactions before generation of the final, characteristic monocyclic, bicyclic or tricyclic products. The structure and stereochemistry of these eventual products are believed to be a direct consequence of the precise folding of the acyclic substrate in the enzyme active site. The enzyme then triggers the ionization that launches the cyclization, stabilizes the successive reaction intermediates, and quenches the cationic product by specific deprotonation or hydration, all the while protecting the highly reactive carbocation intermediates from premature quenching by bulk water. Whereas most terpene synthases of microbial origin seem to generate exclusively or predominantly a single cyclization product, plant terpene synthases frequently generate complex mixtures of products. The enzyme γ-humulene synthase, first cloned from grand fir (*Abies grandis*) by Rodney Croteau and his collaborators, represents an extreme example of such product promiscuity, generating 52 different sesquiterpenes from the single substrate FPP[2] (**Fig. 1**). This impressive catalytic sloppiness is presumably a reflection of the loose binding of the farnesyl portion of FPP in the active site of γ-humulene synthase[4].

Many previous investigations of terpene synthase specificity have focused on the use of site-directed mutagenesis to alter the metal-binding residues that have been implicated in the key ionization step[5]. Other studies have involved attempts to morph

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