Antigen presentation

A protease draws first blood

Matthew Bogyo and Hidde L. Ploegh

Biologists used to approach proteolysis with ambivalence and apprehension. Proteolytic cleavages cannot be predicted from a cloned sequence and, because it can destroy or confound one's favourite subjects, proteolysis is ignored at one's peril. During antigen processing, however, all of the proteolytic stops are pulled out because protein breakdown is necessary to provide molecules of the major histocompatibility complex (MHC) with their peptide cargo. On page 695 of this issue, Manoury and co-workers[1] add a new element to the story by showing that a protease, asparaginyl endopeptidase (AEP), is involved in MHC-restricted antigen presentation. This enzyme seems to deliver the initial blow to the tetanus toxin antigen, and leads to further processing of the antigen into epitopes that are recognized by the immune system.

For initiation of an immune response, antigenic fragments are usually presented to antigen-specific T lymphocytes by the products of the MHC. Most antigens that enter the antigen-presenting cell via the endocytic pathway are presented by class II MHC products[2]. Class II molecules are directed to the endocytic pathway by transient association with the invariant chain (Ii), which is the conceptual equivalent of a pro-piece — Ii must be removed for class II molecules to become fully active. This is done by lysosomal proteases, which destroy Ii and, at the same time, attack internalized antigens to convert them to suitably sized peptides. Removal of Ii is necessary not only for the class II MHC to acquire its peptide-binding properties, but also for its proper transport, as shown by experiments using pharmacological[3] or even endogenous inhibitors of lysosomal proteases[4].

Which lysosomal proteases can cleave the antigens and Ii? Attention was initially paid to the best-studied lysosomal cysteinyl and aspartyl proteases, such as cathepsins B and D. *In vitro*, at least, such enzymes can proteolytically remove Ii from the class II–Ii complex[5], and generate T-cell epitopes from intact protein antigens[6]. But, presumably, lysosomal proteases must work in concert to deliver a carefully orchestrated series of cuts if the correct peptide is to be generated. Antigen-presenting cells from knockout mice lacking cathepsins D, B or L show no obvious defects in antigen presentation[7,8], indicating that other proteases are involved in both removing Ii and converting the antigen into peptides.

One might assume, perhaps naively, that many lysosomal proteases are functionally redundant in view of their broad substrate specificities. Indeed — and consistent with such redundancy — deficiencies in lysosomal proteases are quite rare. Toulouse-Lautrec's short stature was attributed to a deficiency in cathepsin K, a minor lysosomal protease involved in bone remodelling[9]. But of the cathepsin knockouts studied so far, only animals lacking cathepsin D are sick, dying at around three weeks of age. Yet the fact that some lysosomal proteases have a restricted tissue distribution (such as cathepsin S, which is involved in proteolytic removal of Ii in professional antigen-presenting cells) raises the ante for the identification of further proteases in antigen presentation[7,8].

Consequently, the search has been on to implicate new proteases, many of them lurking in databases as cathepsin homologues. One of these has now been identified by Manoury *et al.*[1] in B cells as the cysteine protease AEP. A direct relative of the legumains, which were first discovered in plants, AEP is likely to be identical to the recently cloned legumain homologue from mammalian tissue[10]. The authors believe that AEP draws first blood and executes initial digestion of a protein antigen, tetanus toxin. It could help to unfold its target antigen to allow further digestion by the cathepsins.

To work out how a specific protease is involved in antigen processing, we need to be able to modulate its activity selectively. Inhibitors — often modified peptides that mimic an enzyme's substrates — transiently or permanently modify the active-site nucleophile, leading to a loss of activity. Manoury and co-workers used high concentrations of natural peptide substrates to block the activity of AEP *in vitro* and *in vivo*. But although the inhibitors were active against purified AEP, showing little or no effect on other cysteine proteases of the cathepsin family, caution is in order when transposing *in vitro* inhibitor studies to living cells. Many lysosomal cathepsins have similar substrate specificities *in vitro*, and inhibitors that are specific for single proteases are hard to find[11]. So, small-molecule inhibitors that not only block activity, but also allow the molecular targets to be identified, are high on the wish-list. It is anyone's guess how many proteases with functional properties

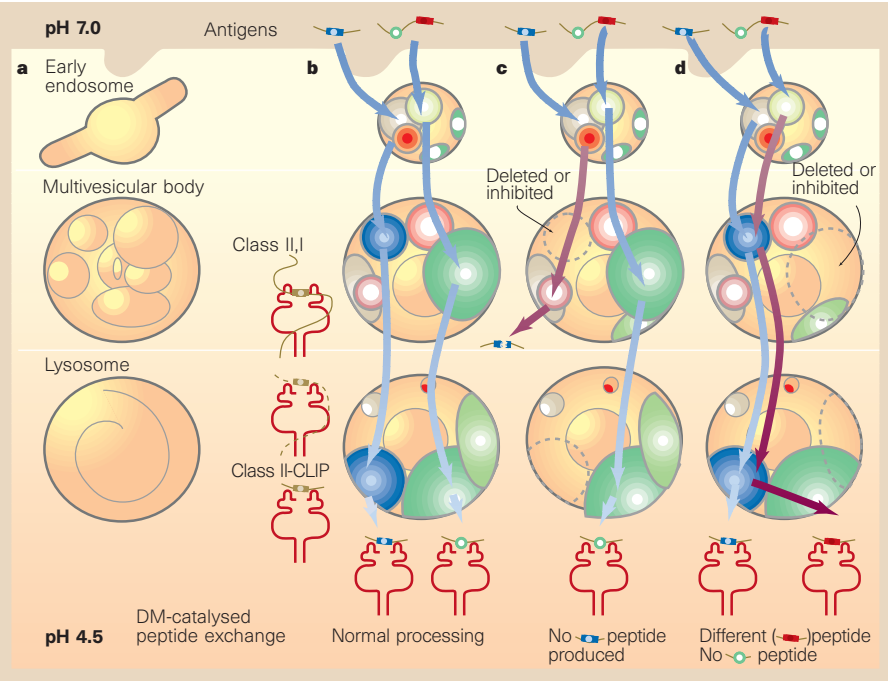

Figure 1 **The endocytic pathway and proteolytic conversion of antigens. Coloured circles represent different specificities and activities of distinct endosomal/lysosomal proteases, and the geometric symbols in the antigen reflect different epitopes.** a, **The endocytic pathway. Newly synthesized class II major histocompatibility complex (MHC) molecules, complexed with the invariant chain (Ii), enter the endocytic pathway. Ii is removed by proteolysis to yield an Ii remnant, CLIP. The class II–CLIP complexes are substrates for peptide exchange catalysed by the class II-like product DM (ref. 2), and the result is formation of class II–peptide complexes.** b, **Under normal conditions (pale blue arrows), each antigen may sample distinct or (partially) overlapping proteases to generate T-cell epitopes.** c, **If a certain protease is ablated or inactivated, other proteolytic pathways may be accessed (burgundy arrows). Some epitopes may no longer be generated (for example, by cleavage within the epitope in the newly accessed pathway), whereas others are produced normally.** d, **Alternatively, enzyme ablation may produce epitopes that are not generated under normal circumstances. (For review see ref. 12.)**

like those of AEP will be discovered. Nonetheless, the fact that antigen presentation is inhibited by competition for substrate at all, favours AEP as the protease that selectively makes the first cut in tetanus toxin.

An exciting prospect is the as-yet-hypothetical involvement of discrete, possibly unique, sets of proteases in converting certain self-proteins into the class II MHC–peptide complexes that trigger an autoimmune response. By identifying such proteases — and AEP could be amongst them — we could selectively inhibit them to blunt the severity of an autoimmune attack, if not prevent it altogether. Given the malleability of protease inhibitors in the medicinal chemist's hand, proteases may turn out to be the preferred targets for development of small-molecule immunomodulators. □

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Superconductivity

An analogue of superfluid ^{3}He

Maurice Rice

Two letters to *Nature*[1,2] published in the past few months, and one in this issue[3] (page 658), report experiments that uniquely specify the unconventional superconductivity in strontium ruthenate (Sr_2RuO_4). They establish that it conforms to long-held views on how superconductivity driven by electron–electron (rather than electron–phonon) interactions should behave. These developments contrast with the heated debates that have swirled for a decade around the high-temperature copper oxide superconductors. Yet there are many similarities in the underlying electronic structures of the ruthenates and the copper oxides.

Shortly after the Bardeen, Cooper and Schrieffer (BCS) theory of conventional electron–phonon-driven superconductivity appeared in 1957, Kohn and Luttinger[4] proposed a generalization to electron–electron interactions. The BCS theory starts from a normal metallic state with its 'Fermi liquid' of mobile electrons, with their interactions well described by the perturbative Landau theory. As the temperature is lowered an effective attraction between the electrons near the Fermi level arises from the exchange of phonons, or lattice vibrations, which grows in strength and overcomes the Coulomb repulsion. This net attraction binds two electrons to form an isotropic spin-singlet Cooper pair in a state of zero angular momentum (that is, *s*-wave). The origin of superconductivity is the condensation of the centre of mass motion of these pairs to form a coherent quantum state.

Kohn and Luttinger[4] showed that even when the electron–electron interaction was predominantly repulsive it could still cause a subtle attraction, forming Cooper pairs in a higher angular momentum state (such as *p*-wave or *d*-wave). This attraction was estimated to be very weak in simple metals. As a result, any such unconventional superconducting state would be difficult to observe due to a very low transition temperature (T_c), and then only in highly perfect crystals. But the attraction should be higher in transition metals and other materials, where the electronic states are concentrated close to the ions, thereby strengthening the interactions between electrons. In particular, if these interactions are strong enough to drive the metal to the verge of magnetic order, then the attractions would be further heightened by the exchange of magnetic fluctuations. The discovery in the 1970s of just such *p*-wave Cooper pairing in the neutral Fermi-liquid, ^{3}He, stimulated the search for an electronic counterpart.

In the 1980s, superconductivity was discovered in a class of rare earth and actinide compounds known as heavy fermion metals. Here the relevant electronic states (4*f* and 5*f*) are even more compact and the proximity to magnetic order favours superconductivity (see ref. 5 for example). Surprisingly, the large class of transition metal oxides rarely shows superconductivity, although the variety of their spin and charge ordering attests to the presence of strong interactions.

Four years ago, Maeno and collaborators[6] succeeded in preparing high-quality Sr_2RuO_4 and found a metallic state with hallmarks of a Landau–Fermi liquid, and a superconducting transition at $T_c \sim 0.7$ K. The crystal structure is that of the $La_{2-x}Sr_xCuO_4$ copper oxide family. It consists of two-dimensional RuO_2 planes, and quantum coherence between the electrons in different planes occurs at $T < 50$ K. The most definitive

100 YEARS AGO

It is naturally difficult to obtain direct evidence as to how birds rid themselves of the indigestible parts of the fruit they eat. It is a question to which I have given some attention from its bearing on the dispersal of seeds. I have found large quantities of the seeds of hawthorn, dog-rose, mistletoe and ivy evidently voided by birds, as I incline to think generally as faeces, especially in the case of the hawthorn and ivy. Some large bird, I suppose the rook, consumes ivy berries largely in the spring, and gets rid of the seeds in what appears to be a mass of excrementitious matter. Many of these have not lost their vitality, and germinate readily in the same season. I have some thriving ivy plants obtained from such seed sown in 1896 and numerous seedlings this year of similar origin, the seed being sown on April 28, and coming up on June 7. I do not think much stress need be laid on the fact that much of the fruit swallowed is voided undigested, though the mistle-seeds I found were in a mass something like a lump of frog-spawn, with much of the pulp of the berry still adhering to each seed. I fancy birds and beasts, like many human beings, frequently swallow greedily far more than is good for them, especially when they light upon an abundant supply after enforced abstinence.

From *Nature* 15 December 1898.

50 YEARS AGO

In recent discussions on blinking, it has been taken for granted that, in a physical experiment, an object cannot be kept under continuous observation. The difficulty, however, is easily overcome. It is easy to acquire the habit of blinking the eyes alternately and, provided the period is made slightly shorter than that associated with normal blinking, no discomfort results. I have used this technique in the laboratory for more than twenty years, and have always assumed that it was generally known; but this, apparently, is not the case.

From *Nature* 18 December 1948.

Many more abstracts like these can be found in *A Bedside Nature: Genius and Eccentricity in Science, 1869–1953*, a 266-page book edited by Walter Gratzer. Contact Lisa O'Rourke. e-mail: l.orourke@nature.com