IMMUNE EVASION

Dodging defences

In 1997, an outbreak of deadly influenza virus hit the live poultry markets of Hong Kong. Having jumped straight from chickens to humans, this virus strain, known as H5N1/97, killed six of the 18 people known to be infected. A report in *Nature Medicine* now reveals the secret weapon of the virus.

Postmortems showed that the victims had massive levels of virus in their lungs and that they had probably died as a result of the overproduction of cytokines, such as interferons (IFNs) and tumour-necrosis factor (TNF). These cytokines have potent antiviral effects, which led Heui Seo and colleagues to wonder if the lethal virus strain might somehow be resistant to antiviral cytokines. Remarkably, they found that the replication of H5N1/97 in pig cells was completely unaffected by the addition of IFN- α , IFN- γ or TNF, at any dose, whereas the replication of common influenza subtypes was inhibited.

So, how does H5N1/97 dodge the antiviral effects of cytokines? Several previous studies have reported that influenza-virus non-structural protein 1 (NS1) dampens the host response to IFN- α/β , which makes it a promising candidate resistance gene. The authors inserted the NS1 gene of H5N1/97 into another non-lethal influenza virus, H1N1. They found that replication of the wild-type H1N1 virus was inhibited completely by antiviral cytokines, whereas replication of the recombinant virus was unabated, which confirmed the essential role of NS1 in immune evasion.

A comparison of the amino-acid sequences of NS1 from various influenza viruses showed that the H5N1/97 NS1 is unique in that it has a glutamic acid at position 92. All of the other known influenza-virus strains have an aspartic acid at this position. To test the importance of this residue, the Glu92 of H5N1/97 NS1 was changed to an aspartic acid

T-CELL DEVELOPMENT

Seeking the selectors

The nature of the peptides that mediate thymic positive selection has been much debated. Reporting in the September issue of *Immunity*, Santori and colleagues have characterized peptides that induce positive selection using bioassay- and bioinformatics-based approaches. Reassuringly, both lines of investigation picked out the same two peptide ligands.

Positive selection is the process by which CD4⁺CD8⁺ double-positive thymocytes that interact with self-peptide-MHC complexes are selected to survive and mature. This developmental checkpoint is thought to ensure that all mature T cells can interact with self-MHC molecules. But, what makes a peptide a positive selector and how common are they?

The authors addressed these issues by characterizing naturally occurring peptides that mediate positive selection in a T-cell receptor (TCR)-transgenic model. OT-1 mice express a TCR that is specific for an ovalbumin (OVA) peptide presented by the MHC class I molecule H2-K^b.

In the first approach, bound peptides were extracted from H2-K^b molecules that

had been purified from various cell lines and tissues. Peptide fractions were then tested for activity in an assay for positive selection — the ‘co-receptor-dulling assay’. This measures the ability of a peptide ligand to induce the downregulation of expression of the CD4 or CD8 co-receptors on thymocytes, which is an *in vitro* correlate of positive selection. Candidate peptides in positive fractions were sequenced, then synthesized and tested for their ability to induce positive selection in fetal thymic organ culture. This process identified two positively selecting peptides: one from β -catenin (Catnb_{329–336}) and the other from F-actin capping protein (Cappa1_{92–99}).

The Catnb_{329–336} and Cappa1_{92–99} peptides have some structural homology to the OVA peptide that is the OT-1 TCR agonist. So, can positively selecting ligands be predicted on the basis of sequence homology? The second bioinformatics-based approach tested this theory. A database of predicted mouse MHC ligands was assembled, then algorithms were devised to select ligands that were

similar to the OVA peptide at key TCR contact sites. Only ligand peptides for which the parent protein is expressed in the thymus were considered. Seven candidate peptides were identified which were then tested for their ability to bind to H2-K^b and for activity in the co-receptor-dulling assay. Ruling out peptides that are not actually presented by H2-K^b in the thymus gave an end result of two peptides — Catnb_{329–336} and Cappa1_{92–99} — which, remarkably, are the same peptides that were identified by the bioassay-based approach.

So, it seems that the peptides that induce positive selection are rare and have key TCR contact residues in common. Moreover, this study sets an important precedent for the general use of bioinformatics to predict the ligands that mediate positive selection.

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References and links

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- FURTHER READING** Hogquist, K. A. Signal strength in thymic selection and lineage commitment. *Curr. Opin. Immunol.* **13**, 225–231 (2001)
- WEB SITES**
 Stephen Jameson's lab:
<http://www.micab.med.umn.edu/faculty/Jameson.html>
 Kristin Hogquist's lab:
<http://www.micab.med.umn.edu/faculty/Hogquist.html>
 Stanislav Vukmanovic's lab:
<http://www.med.nyu.edu/research/vukmas01.htm>