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New T-cell epitope mathematics

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When predicting T-cell epitopes from a protein - a useful technique for cancer and vaccine research - it has been generally thought that theoretically chopping the proteins into eight- to ten-amino acid fragments would give all its possible epitopes. But in the January 15 Nature, Ken-ichi Hanada and colleagues from the National Institutes of Health have discovered that the posttranslational splicing of protein fragments can generate new epitope variants and suggest that there are far more possible epitopes than previously thought (*Nature* 2004, **427:**252-256).

"We started by looking for proteins on kidney cancer cells that would be recognized by the immune system," said James C. Yang, principal investigator of the study, who together with Hanada and colleagues had previously cloned a human T cell (C2 cytotoxic T-lymphocytes, or CTLs) that killed cancer cells overexpressing fibroblast growth factor-5 (FGF-5).

"[We] wanted to proceed to find the specific nine or ten amino acids of FGF-5 that was the recognized epitope," Yang told us. The group therefore set out truncating the FGF-5 gene and determining if these truncations, when expressed in human (HLA)-A3-expressing COS-7 cells, could be recognized by C2 CTLs. "This led to a problem," Yang said, since the stimulatory piece apparently transversed 60 amino acids of FGF-5 and the synthetic peptides corresponding to all the 8-, 9-, and 10-residue peptides (in all three reading frames) contained within these 60 amino acids were not recognized by the CTLs.

The team therefore returned to their genetic approach and attempted internal deletions of the FGF-5 gene, ultimately finding that FGF-5 residues 172-176 and 199-220 together formed the smallest recognized pieces. That surprised Yang, since it indicated the FGF-5 epitope was being fused together from separated sections of the protein. Using a combination of synthetic fusion peptides, natural acid-stripped peptides and high-performance liquid chromatography purification, the authors went on to rule out RNA splicing or ribosome skipping as the cause, leaving a novel form of protein splicing to make the fused peptide.

Two categories of natural protein splicing have been described: one in single-celled organisms regulated by 'inteins and the other involving reverse proteolysis as seen in plants. The researchers suggest that it is a third category. Francine Perler, from New England Biolabs, agreed. "This is clearly not a case of intein-mediated protein splicing," she told us. It is likely an enzymatic process, as Yang's team suggests, said Perler, who was not involved in the study.

The finding suggests that the compliment of human proteins and protein derivatives - the proteome - could be larger than expected, according to Hans-Georg Rammensee from the University of Tübingen and author of a News and Views article accompanying the Yang group's paper. "The new principle of protein splicing in human cells increases the potential number of possible gene products enormously. This is especially important for people trying to design molecularly defined tumor vaccines," Rammensee told us.

For the field of immunology, "the possibility of splicing at the protein level certainly adds to the complexity of the possible repertoire of peptides displayed to T lymphocytes," Stanislav Vukmanovic from New York University Medical Center told us.

"In due time, we will hopefully be able to tell whether this represents a case of peculiar processing of a protein, or a mechanism that contributes to the diversity of peptide presentation to the immune system. It is already clear, however, that all of us thinking about potential peptide presentation to T cells will have to take the possibility of protein splicing into account," said Vukmanovic, who was not involved in the study.

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