Selecting the T Cell Receptor Repertoire

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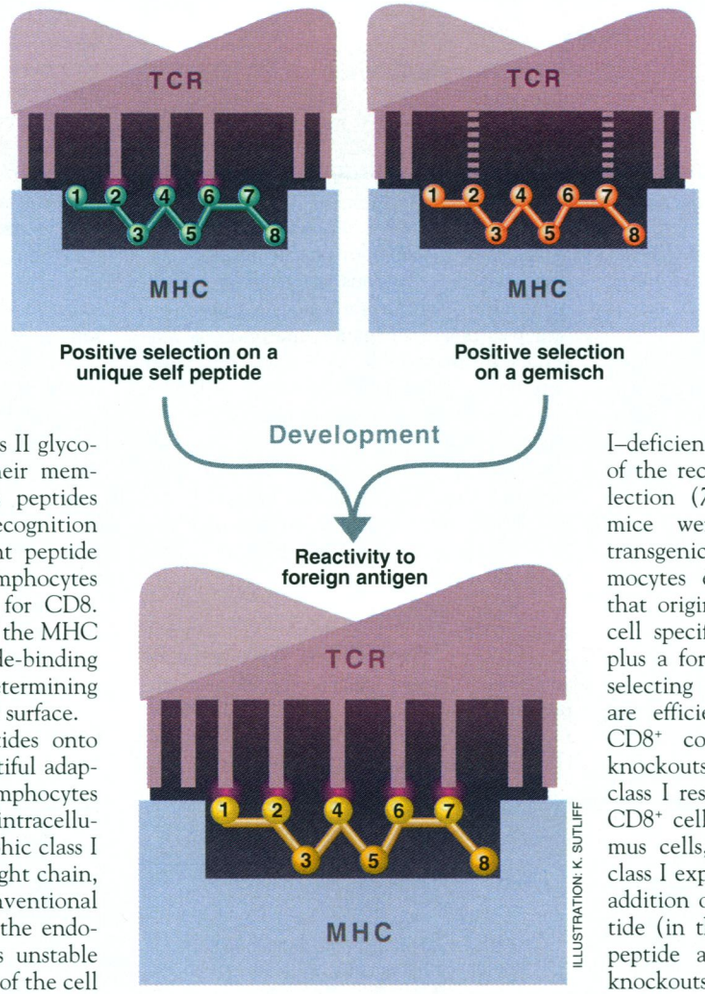
Lymphocytes, circulating cells of the immune system, must be unresponsive to antigens from the organism they are protecting (self antigens) while retaining the ability to respond to foreign pathogens. During their development, immature B and T lymphocytes that react strongly to self antigens are deleted—a process called negative selection. However, recent studies of the maturation of T cells in the thymus suggest that self antigens are also used as mimics or look-alikes of foreign pathogens to positively select the T lymphocyte repertoire.

The major histocompatibility complex (MHC) includes a number of extremely polymorphic genes that encode a set of cell surface glycoproteins whose function is to present foreign, pathogen-derived antigens to T lymphocytes. The class I and class II glycoproteins possess a groove on their membrane distal surface that binds peptides from the cell for T lymphocyte recognition (*1*, *2*). Class I molecules present peptide antigens to $CD8^+$ cytotoxic T lymphocytes and possess an interaction site for CD8. The most polymorphic residues of the MHC glycoproteins affect the peptide-binding specificity of the groove, determining which peptides are brought to the surface.

The loading of cellular peptides onto class I MHC molecules is a beautiful adaptation that allows cytotoxic T lymphocytes to perform a surveillance role for intracellular antigens (*3*, *4*). The polymorphic class I heavy chain and the associated light chain, β_2-microglobulin (β_2M), are conventional membrane proteins produced in the endoplasmic reticulum. The dimer is unstable and does not move to the surface of the cell unless it contains a tightly bound peptide. Remarkably, these peptides originate largely in the cytosol, that is, on the other side of the endoplasmic reticulum membrane. Current wisdom holds that proteasomes fragment denatured proteins: Peptide pumps, which are encoded in the MHC and are referred to as transporters associated with antigen processing (TAP), translocate the peptides into the endoplasmic reticulum lumen. Peptides that bind to MHC class I tightly stabilize the complex, which then moves to the surface. None of these components—proteasomes, TAPs, or MHC chains—can distinguish peptides that originate from within the organism (self) from externally originating peptides (non-self). That ability to distinguish is only achieved by the repertoire of T cell receptors present in the adult animal. Thus, all of the MHC class I molecules on the cell surface are normally stabilized by a diverse set of self peptides. A particular cell type expressing 10^5 copies of one class I molecule may present 10^4 different bound peptides, each represented at 10^0 to 10^3 copies per cell (*5*).



Possible mechanisms of positive selection. Octameric peptides presented by an MHC molecule. Peptide side chains 3, 5, and 8 serve as MHC anchors and peptide side chains 1, 2, 4, 6, and 7 can interact with the T cell receptor. During selection, a unique self peptide (**top**, **left**) could provide a low-affinity mimic of the antigenic peptide or a diverse set of self peptides (a "gemisch") (**top**, **right**) could provide a high ligand density and allow receptor contact with the MHC, with minimal contribution from the peptide side chains. In the adult (**bottom**), all five "up" side chains of foreign antigenic peptides may contact the receptor.

In addition to determining antigen presentation during an immune response, MHC molecules play an equally dramatic role in driving thymocyte development. MHC molecules on thymus epithelial cells select for maturation and export to the peripheral lymphoid organs those immature thymocytes bearing receptors that will best be able to react to a foreign peptide presented by the same MHC molecule (*6*). This requirement for receptor-mediated positive selection means that mice lacking MHC class I expression in the thymus as a result of a targeted disruption of the β_2M or TAP gene do not select $CD8^+$ T cells. A number of recent reports have exploited MHC class I–deficient mice to explore the specificity of the recognition step during positive selection (*7*–*9*). β_2M or TAP "knockout" mice were bred to T cell receptor transgenic mice in which all immature thymocytes expressed a transgenic receptor that originated from a $CD8^+$, cytotoxic T cell specific for a class I MHC molecule plus a foreign peptide. In the appropriate selecting thymus, transgenic thymocytes are efficiently selected into the mature $CD8^+$ compartment. In β_2M or TAP knockouts, the absence of folded MHC class I results in a failure to select mature $CD8^+$ cells. In organ cultures of fetal thymus cells, it is possible to restore MHC class I expression on the cell surface by the addition of exogenous class I binding peptide (in the TAP knockout) or of both a peptide and β_2M (in the case of β_2M knockouts). In this way the enormous complexity of self peptides normally present in the class I groove can be reduced to a single synthetic peptide.

In organ cultures of the thymus from the T cell receptor transgenic mice, variants of the original antigenic peptide were found to cause positive selection, that is, to drive the differentiation of immature thymocytes into $CD8^+$ T cells (*7*, *8*). These peptides could bind to MHC but, because they were slightly different than the original peptide, they presumably bound with a weaker affin-

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ity to the T cell receptor. Indeed, some peptide variants that could mediate positive selection could not activate mature T cells bearing the same receptor, that is, they did not bind well enough to be agonists for mature T cells. However, in some cases these variant peptides were antagonists for mature T cells. When loaded onto target cells bearing a much lower density of agonist peptide, these ligands could disrupt T cell receptor–mediated signaling events, possibly by preventing receptor aggregation. Many other peptides that bind to class I MHC as well as the agonist or antagonist peptides, but which are unrelated to the original peptide, had no effect in this system. Thus, low-affinity ligands of the receptor that are related to the original foreign agonist can promote thymocyte selection.

In two reports, a strong agonist peptide could apparently stimulate positive selection of $CD8^+$ T cells (8, 9). Increasing the dose of the same peptide drove the immature thymocytes to deletion (negative selection). Interesting comparisons can be made here because both groups used the same T cell receptor [the receptor was derived from a cytotoxic T cell clone specific for a glycoprotein peptide from the lymphocytic choriomeningitis virus (LCMV) plus the D^b molecule] and the same agonist peptide (a cysteine residue at a presumed MHC anchor site was replaced by methionine). In one case, in which TAP knockout mice were used, 3×10^{-5} M peptide added to organ culture caused positive selection, whereas 3×10^{-4} M peptide resulted in negative selection (8). In the other case, in β_2M knockout mice, 10^{-12} M peptide gave positive selection and 10^{-6} M peptide gave negative selection (9). This difference in the two systems is difficult to understand: It is actually the reverse of what one would predict, because TAP-deficient cells are easier to load with exogenous peptide than are β_2M-deficient cells.

Another interesting feature of positive selection induced by the strongly antigenic LCMV peptide is that it seems to result in the production of $CD8^{low}$ cells—T cells with less CD8 on their surface than on those produced by positive selection in control cultures (8, 9). In experiments with the other T cell receptor transgenic mouse (specific for a hen ovalbumin peptide plus K^b) and β_2M knockouts, the antigenic peptide did not cause positive selection, only deletion (7). However, in this case a single residue variant that serves as a weak agonist could cause either positive selection at low concentrations or the production of $CD8^{low}$ cells at higher ligand concentrations.

What are the natural peptide ligands for positive selection? The class I molecules on thymus epithelial cells are stable trimers composed of class I heavy chain, β_2M, and a complex assortment of about 10^4 different self peptides derived from ribosomal proteins, histones, and other cytosolic proteins. How is this complexity perceived and utilized in positive selection? The recent experiments show that peptide side chains can contribute to the affinity of the interaction with the T cell receptor and drive positive selection in a highly specific way. Does this mean that each of the approximately 10^4 natural self peptides presented by class I individually selects a subset of the T cell repertoire, or does a mixture of peptides, a "gemisch," select each T cell? If one self peptide–MHC complex positively selects each T cell, then simplistically this implies that 10^4 self peptide ligands select reactivity for the potential universe of pathogen peptides that may be presented by the MHC. If each MHC-bound peptide has five T cell receptor contact positions ("up" residues), then the potential universe of foreign antigens is 20^5, or 3×10^6, ligands. Given that the low affinity of the interaction required for positive selection will capture more receptors, this does not seem to be an unreasonable relation. Previous work with conventional thymocytes expressing endogenously rearranged receptor genes has shown that a single peptide can select a detectable population of mature $CD8^+$ T cells (*10*, *11*). If self peptides serving as strong agonists contribute to positive selection, they would necessarily be more closely related to the antigenic peptide. In fact, there is experimental evidence that natural self peptides do not include strong agonists for peripheral T cells. Fractionation of natural peptides from antigen-expressing cells can reveal discrete peaks of targeting activity for the appropriate T cell when the peptides are loaded onto empty MHC molecules. This activity can be observed at a 1000-fold dilution (*12*, *13*). Significantly, natural peptides from antigen-minus cells yield no targeting activity in this assay, implying that strong agonists do not exist for positive selection.

The number of refolded class I trimers achieved in thymus organ culture is only a fraction of that for wild-type values. For β_2M knockouts it is less than 1%, and for TAP knockouts it is up to 30%. Although no single self peptide occupies a high fraction of MHC molecules, it is conceivable that some T cell receptors could be positively selected by a gemisch, a group of cooperating self peptides—each one of extremely low affinity but adding up to high ligand density—providing sufficient avidity to drive selection. In this model, the selection of any one receptor would not be dependent on one self peptide, and none of the cooperating, selecting ligands would be detected as agonists or antagonists for peripheral T cells. Gemisch selection might actually take two forms. In the first, many different self peptides could contribute some affinity to the T cell receptor by virtue of their exposed side chains. In the second, many different self peptides may allow the T cell receptor to contact the surface of the MHC molecule itself by keeping out of the way.

In a normal thymus, both forms of selection—on a single peptide or a gemisch —may occur for immature T cells of different specificity. But the outcome is the same. Imprinting on self produces a circulating T cell population that is poised to respond to pathogen-modified self.

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