Microbiology and Tumor Biology Center
Karolinska Institute, Stockholm, Sweden

REGULATION OF T CELL ACTIVATION AND DEATH BY THE AFFINITY OF TCR FOR PEPTIDE/MHC COMPLEXES

Cheng-Hong Wei, M.D.

Stockholm 2002

Published and printed by Karolinska University Press
Box 200, SE-171 77 Stockholm, Sweden

ISBN 91-7349-239-6

To my family

Regulation of T Cell Activation and Death by the Affinity of TCR for peptide/MHC complexes

by Cheng-Hong Wei, Microbiology and Tumor Biology Center, Karolinska Institute, Box 280, S-171 77 Stockholm, Sweden.

ABSTRACT

The aim of this study is to investigate the role of the affinity of peptide:MHC/TCR interaction in the regulation of T cell activation, death and repertoire selection. Three aspects pertinent for our understanding of this issue have been analyzed.

First, we analyzed the effect of partially agonistic peptides on the activation and survival of CTL clones specific for a highly immunogenic HLA A11-restricted peptide epitope derived from the EBV nuclear Ag 4(EBNA 4), IVTDFSVIK (designated IVT). Several analogues with substitutions of TCR contact residues were able to trigger cytotoxic activity without induction of IL-2 mRNA and protein or T cell proliferation. Triggering with these partial agonists in the absence of exogenous IL-2 resulted in down-regulation of the cytotoxic potential of the specific CTLs. One analogue selectively triggered apoptosis as efficiently as the original epitope, subdividing the partial agonists into apoptosis-inducing and non-inducing ligands. Analysis of early T cell activation events did not reveal significant differences between the two types of analogue peptides. These results demonstrate that some partial agonists can dissociate the induction of CTL death from CTL activation. Then, we characterized the apoptotic programs induced by the immunogenic peptide and its partially agonistic analogues in the IVT-specific CTL clones. Our major finding is that CTL triggering with partially agonistic peptide ligands can initiate death receptor dependent and independent apoptotic programs in the effector cells. In contrast to classical AICD, death receptors are not essential for the elimination of CTLs activated with partially agonistic peptides. In addition, death receptor independent apoptosis requires caspases other than caspase 3 and 8. Induction of anti-apoptotic BCl-2 and BCl-X_L expression is associated with resistance to this form of apoptosis. Also, IL-2 enhances classical and inhibits death receptor independent AICD. We concluded that TCR triggering not accompanied by IL-2 production may result in elimination of T-lymphocytes in death receptor independent manner. Our data demonstrated that engagement of TCR by MHC-peptide complexes can trigger diverse apoptotic programs of AICD and that the choice between these programs is determined by the agonistic potency of MHC-peptide ligand.

Second, the molecular basis of different outcomes of CTLs stimulation with immunogenic and partially agonistic peptide ligands was analyzed. The role of MHC:peptide/TCR affinity in the regulation of T cell activation was characterized using tetramer technology. Our results demonstrated that the A11 complexes assembled with the partial agonist dissociated from the surface of IVT-specific CTLs with a faster kinetics as compared with complexes containing the immunogenic peptide. We also showed that the efficiency of CTL recognition correlates with the stability of interaction between the specific TCR and MHC:peptide complex. Tetramer binding and secretion of INFγ were shown to be compatible with T-cell activation by partially agonistic peptides. In conclusion, our results indicate that the affinity of TCR/MHC:peptide interaction determines the strength of TCR signalling, extent of CTL activation as well as the apoptosis pathway that operates in CTLs in the course of AICD.

The third aim of our study was to investigate the influence of the affinity of TCR/MHC interaction on the selection and maintenance of T-cell receptor (TCR) repertoire of peptide specific CTLs. Using tetramer technology, we investigated the restriction of TCR usage among CTL responses against a subdominat EBNA 4 derived peptide referred to as AVF. In agreement with the earlier findings, ex vivo analysis of AVF-specific CTLs using AVF-containing HLA A11 tetramers revealed the same degree of conservation of the AVF-specific response both in healthy virus carriers and in the course of primary EBV infection. Tetramer binding and dissociation experiments performed with AVF-specific CTLs or CTLs expressing a very diverse set of TCRs and specific to another immunodominant A11-restricted EBV-derived peptide epitope did not support a model of affinity driven selection of restricted TCR repertoires. Characterization of individuals that fail to mount responses to the immunodominant A11-restricted CTL epitope but efficiently respond to the AVF-peptide argued against interclonal competition as the reason for the observed TCR conservation. Collectively, our data confirm the existence of naturally induced peptide-specific CTL responses with highly restricted TCR usage. Our data do not support a major role for affinity of MHC:TCR interaction in the selection of structurally conserved TCR-repertoires and suggest that such conservations may be due to structural constrains in MHC:peptide/TCR interactions or endogenous pre-selection of certain clonotypes.

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. **Wei CH,** Beeson C, Masucci MG, Levitsky V. A partially agonistic peptide acts as a selective inducer of apoptosis in CD8+ cytotoxic T lymphocytes. Journal of Immunology, 1999, 163(5): 2601-2609.

II. **Wei CH,** Yagita H, Masucci MG, Levitsky V. Different programs of activation-induced cell death are trigered in mature activated cytotoxic T lymphocytes by immunogenic and partially agonistic peptide ligands**.** Journal of Immunology. 2001, 166(2): 989-995.

III. **Wei CH,** Uhlin M, Masucci MG, Levitsky V. Tetramer binding and secretion of interferon-γ in response to antigenic stimulation are compatible with a range of affinities of MHC:TCR interaction and distinct programs of cytotoxic T lymphocyte activation. Submitted.

IV. **Wei CH,** Liu D, Alexander J, Sette A, Masucci MG, Levitsky V. Highly restricted TCR usage in Epstein-Barr virus specific HLA A11-restricted CTL response: analysis of underlying mechanisms with the use of tetramer technology. Submitted.

Contents

Abbreviations

Ag - Antigen
AICD – Activation-induced cell death
APC – Antigen presenting cell
AP-1 – Activation protein-1
APL – Altered peptide ligand
AVF - AVFDRKSDAK
β2m – β2-microglobulin
CAD – Caspase-activated DNAse
CDR – Complementarity determining region
CsA – Cyclosporin A
CTL – Cytotoxic T lymphocytes
EBV – Epstein-Barr virus
EBNA – EBV-encoded nuclear antigen
ER – endoplasmic reticulum
FACS – Fluorescence activated cell sorter
FADD – Fas-associated death domain
FasL – Fas ligand
FLICE – FADD-like interleukin-1 converting enzyme
FLIP – FLICE-inhibitory protein
HC – heavy chain
HLA – Human leukocyte antigen
IAP – Inhibitor of apoptosis
ICAD – Inhibitor of caspase-activated DNAse
IFN - Interferon
Ig – Immunoglobulin
IL-2 – Interleukin-2
IS – Immunological synapse
ITAM - Immunoreceptor tyrosine-based activation motif
IVT - IVTDFSVIK
JNK - Jun N-terminal kinase
LCL – Lymphoblastoid cell line
LCMV – Lymphocytic choriomeningitis virus
MAP kinase – mitogen activated protein kinase
MFI – Mean fluorescence intensity
MKK – MAP kinase kinase
NFAT - nuclear factor of activated T cells
NF-κB – nuclear factor κB
NK – Natural killer
PAMP – Pathogen-associated molecular patterns
PKC – Protein kinase C
PLC – Phospholipase C
pMHC – peptide: MHC molecule complexes
PTK – Protein tyrosine kinase
RT-PCR - reverse transcriptase polymerase chain reaction
SMAC - supramolecular activation clusters
Smac(DIABLO) – Second mitochondria-derived activator of caspase

TAP - transporter-associated with antigen processing
TCR – T cell receptor
TNF - Tumor necrosis factor
TNFR – TNF receptor
TRAIL – TNF-related apoptosis-inducing ligand
Y5 - IVTDYSVIK

I. General introduction

The immune system is a complex, co-ordinated network composed of cells, anatomical structures, substances and procedures which function together to protect the host from infections and malignant cell outgrowth. According to the mode of activation, involvement of unspecific or specific recognition, time kinetics of recruitment and functions, the immune system can be divided into the innate immune system and the adaptive immune system (1-3). Innate immune system consists of skin and mucosal epithelium cells, macrophages, neutrophils, dendritic cells, and natural killer (NK) cells, antimicrobial substances and complement are also important components of the innate immunity. The innate response is initiated through the pattern recognition receptors (PRRs) which recognize frequently encountered structures called pathogen-associated molecular patterns (PAMP) produced by microorganisms. For example, CD14 receptors on macrophages can bind to bacterial endotoxin leading to macrophage activation, and the mannose receptor on phagocytes binds to microbial glycoproteins or glycolipids. Innate immunity acts early in the host's defence against microbes and functions to control or eradicate the infection until the more sophisticated and specific adaptive response has mounted to a sufficient level. In contrast, the adaptive immunity shows diversified, exquisite specificity for distinct macromolecules and an ability to ¨ remember ¨ and respond more strongly to repeated exposure to the same microbe. The components of adaptive immunity are T and B lymphocytes and their products. Adaptive immunity depends on the clonal expansion of T and B cells which can specifically recognize a certain antigen, and the adaptive immunity's extraordinary ability to distinguish among different, even closely related microbes and macromolecules (for example, two proteins differed with only one amino acid) is mainly attributed to the existence of a large pool of specific receptors (sIg and TCR for B and T lymphocytes, respectively) which bear a huge array of diversity (theoretically approximately 10^{11} for Ig repertoire and 10^{16} for TCR $\alpha\beta$ repertoire)(4). However, one should keep in mind that the innate and adaptive immune responses are components of an integrated system of host defence in which numerous cells and molecules function cooperatively. The two systems are bridged through two important links. First, the innate immune response to microbes stimulates

adaptive immune responses and influences the nature of the adaptive responses. Second, adaptive immune responses use many of the effector mechanisms of innate immunity to eliminate microbes, and they often function by enhancing the antimicrobial activities of the defence mechanisms of innate immunity. In addition, processes such as antigen presentation, and chemokines and cytokines incorporate the two systems together to implement the task of defending the host from pathogens.

The adaptive immune response is more precise and efficient in eliminating the pathogen, although the kinetics of the responses to infection is much slower. There are two types of adaptive immune responses, one is humoral immunity and the other is cell-mediated immunity. Humoral immunity is mediated by molecules in the blood, called antibodies, that are produced by B lymphocytes. Humoral immunity is the principal defence mechanism against extracellular microbes and their toxins because secreted antibodies can bind to these microbes and toxins and assist in their elimination. Cellular immunity is mediated by T lymphocytes. Intracellular microbes, such as viruses and some bacteria, survive and proliferate inside phagocytes and other host cells, where they are inaccessible to circulating antibodies. Defence against such infections is a function of cell-mediated immunity, which promotes the destruction of microbes residing in phagocytes or the lysis of infected cells. For example, CD4+ T helper cells activate macrophages to kill phagocytosed microbes, and CD8+ T cells usually directly destroy infected cells. Among T lymphocytes, CD8+ cytotoxic T lymphocytes (CTLs) play an important role in the immune surveillance of various viral infections (5-22) and malignancies (23-29) in vivo. CTLs expressing the TCR $\alpha\beta$ heterodimer and CD8 coreceptor recognize peptides derived from intracellularly processed antigens presented in the context of major histocompatibility complex class I molecules (30-32). Following activation, they become effector cells and can then kill these cells by either lyzing them through the release of pore-forming proteins and caspase-activating granzymes at the target cells (33, 34) (35-37) or by inducing apoptosis in the target cells through the Fas-FasL interaction (38).

II. Structural and functional aspects of MHC: TCR interaction

II.1 The major histocompatibility complex

The major functions of T cells are defence against intracellular microbes and activation of other cells such as macrophages, dendritic cells and B lymphocytes. The task of presenting antigens to T cells are performed by cell surface molecules which are encoded by genes located within the major histocompatibility complex (MHC). In humans the MHC is also referred to as HLA (human leukocyte antigens) and is located on chromosome 6, and in the mouse it is called H-2 and located on chromosome 17 (39, 40). The human classical MHC class I molecules are called HLA-A,-B, and –C; the corresponding murine MHC class I molecules are called H-2K, -D and –L. HLA-DR, -DP and –DQ are the human classical class II molecules while for mouse they are termed as I-A and I-E. Many different genes are located within the MHC and most of them are related to the immune system and its functions. Two general features of the MHC enable the adaptive immunity to defend the host from diverse pathogens: (i) the MHC is polygenic; each individual has several different genes at specific positions on the chromosomes (loci) encoding the two main types of transplantation antigens MHC class I and class II, (ii) the MHC is polymorphic; at each classical MHC class I and class II locus one of a multitude of different but related genes (alleles) is expressed in a given individual (41, 42).

The two major classes of MHC molecules, class I and II, are expressed differentially on cells. MHC class I molecules are glycoproteins expressed on the plasma membrane of virtually all nucleated cells, while expression of class II molecules is restricted to specialized antigen presenting cells (APCs) such as macrophages, dendritic cells and B lymphocytes. MHC class I molecules bind peptides derived from intracellularly processed viral, bacterial or endogenous proteins. Their main function is to transport and present these peptides to CD8+ T cells (i.e. CTLs). In contrast, MHC class II molecules bind peptide antigens generated within endosomal compartments derived from endocytosed extracellular proteins, they then present these peptides on the surface of professional APCs for interaction with CD4+ T cells (helper T cells)(32).

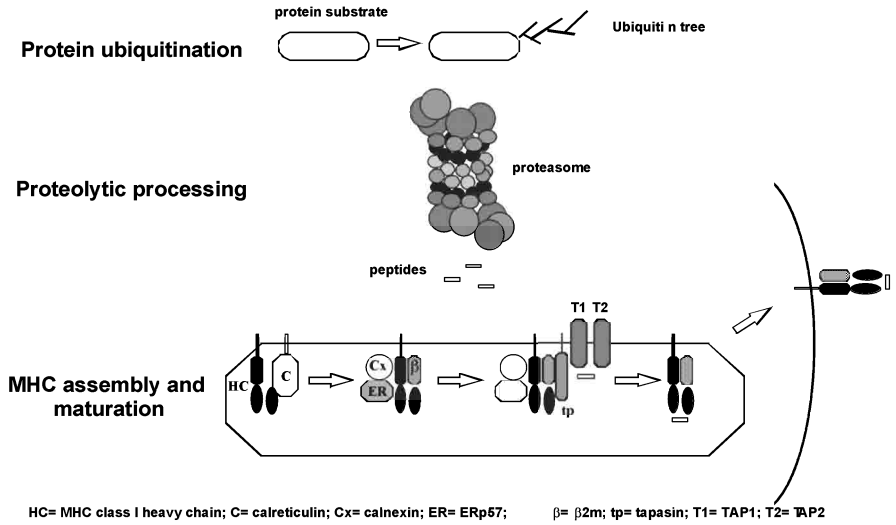
The interaction of peptide-MHC complexes on the surface of APC with specific antigen receptors on the surface of T cells triggers T cell activation and effector functions, e.g. cytolysis, release of cytokines, macrophage activation, B cell activation and secretion of immunoglobulin, etc (43). Professional APCs all express high level of costimulatory molecules (e.g. B7-1, B7-2, CD40) which can be recognized by corresponding receptors (e.g. CD28, CTLA-4) or ligands (e.g. CD154, also called CD40L) on T cells. For priming of naïve T cells, both signal 1 (i.e. TCR-MHC/peptide interaction) and signal 2 (costimulation) are required, TCR triggering in the absence of the second signal will lead to anergy or death of naïve T cells (44, 45).

II.2 MHC class I antigen processing and presentation pathway

In addition to the MHC class I and II genes, several other genes involved in antigen presentation are also encoded within the MHC. The two genes encoding the transporter associated with antigen processing (TAP) lie in the MHC class II region, in close association with the LMP genes that encode components of the proteasome, where the genes for tapasin which binds to both MHC class I and TAP in the endoplasmic reticulum (ER), lies at the centromeric edge of the MHC. The functions of these proteins will be discussed in more details later. In addition to the highly polymorphic MHC class I genes, there are non-classical MHC class I genes (MHC class Ib genes) exhibiting limited polymorphism (46).

The generation of mature peptide-loaded class I molecules requires the co-ordination of three essential processes: degradation of antigenic proteins to peptides in the cytosol, translocation of peptides across the ER membrane, and assembly of MHC class I heavy chain:β2m:peptide complexes and their transport to the cell surface(47, 48). **Figure 1** summarises the main steps involved in MHC class I restricted antigen presentation.

Numerous lines of evidence suggest that ubiquitinylation and protein degradation by the multicatalytic protease complex, defined also as proteasome, are involved in antigen processing. Michalek et al. have shown that ubiquitinylation is required for



HC= MHC class I heavy chain; C= calreticulin; Cx= calnexin; ER= ERp57; β= β2m; tp= tapasin; T1= TAP1; T2= TAP2

Figure 1. The MHC class I antigen presentation pathway

(Adapted from van Endert, P.M. (1999). Curr. Op. Immunol . *11*, 82-88)

the generation of antigenic peptides (49), and presentation of an OVA-derived epitope was prevented by treatment of the APCs with proteasome inhibitors (50). The proteasome is composed of a 20S cylindrical proteolytic core that binds at each end activator complexes designated ¨ 19S regulator ¨ and ¨ 11S regulator ¨ (or PA28). The 19S regulator associates with the 20S proteasome to form the 26S protease, which is involved in the targeted degradation of ubiquitin-conjugated proteins (51). Substrate proteins are first ubiquitinylated and then unfolded. Within the proteasome, these proteins are cleaved into peptides. The proteasome possesses five distinct peptidase activities, including: chymotrypsin-like activity (cleavage after hydrophobic residues), trypsin-like activity (cleavage after basic residues), peptidylglutamyl peptide-hydrolyzing activity (PGPH, cleavage after acidic residues), a small neutral amino acid-preferring (SNAAP) activity (cleavage of peptide bonds between the small neutral amino acid Ala or Gly) and a branched chain amino acid-preferring (BrAAP) activity (cleavage of peptide bonds on the carboxyl side of branched residues, such as Leu, Ile and Val) (52). Incorporation of Lmp2 and Lmp7 in the 20S proteasome core following IFN-γ treatment may alter the peptidase activity, favouring cleavage at the carboxyl side of hydrophobic, basic and branched chain amino acids (53, 54). This type of proteasome complex is defined as ¨ immunoproteasome ¨. Consistent with the

proteasome peptidase activity, sequence analysis of peptides eluted from HLA class I molecules highlights a limited variation at the carboxyl termini: Ile, Leu, Val, Arg, Lys, Tyr or Phe are found at the C-terminus in 95% of all class I restricted epitopes (55).

Following proteasome degradation, the produced peptides are then actively transported by the TAP-1/TAP-2 heterodimer from the cytosol into the ER, where they associate with heterodimers of MHC class I heavy chain and β2-microglobulin (β2m). It has been shown that TAPs preferentially translocate peptides of approximately the size usually found associated with MHC class I molecules (56-58). The efficiency of TAP translocation drops significantly for peptide substrates shorter than 8 and longer than 14 amino acids. The ability of TAP to transport peptides with different C-termini varies from species to species. Human TAP is permissive for peptides with hydrophobic and basic C-terminal amino acids (59, 60), whereas mouse TAP transports peptides with hydrophobic C-termini (57, 59).

ER resident proteins like calnexin, clareticulin and Erp57 are important in retaining class I-β2m dimers in the ER in anticipation of peptide binding. Peptide binding of the class I-β2m dimer is dependent on interaction with TAP, which is mediated by tapasin (61). When stably assembled, the trimeric complexes are transported to the cell surface through the ER and Golgi network. The peptide binding cleft of MHC class I molecules has a strict peptide length requirement of 8-11 amino acids, and displays an allele-specific motif for preferential peptide binding (62). Once the peptide is bound deep within the cleft, further processing is not likely to occur. Despite example of peptide trimming in the ER, it has been established that the cytosol is the main site of processing of class I presented epitopes. This antigen processing and presentation pathway is operating in almost all types of cells, enabling the CTLs to detect intracellular antigens and destroy infected cells.

It should be noted that although intracellular antigens are the main sources of MHC class I peptide ligands, processing of exogenous antigens for presentation on class I molecules has been observed in different model systems. In fact, MHC class I-mediated presentation of exogenous antigens by the specialized APCs, termed cross-

priming or cross-presentation, appears to be critical for CTL immune surveillance against microorganisms that do not infect the specialized APCs themselves (63-65).

II.3 Structure of the MHC class I molecules

Early studies on the crystal structures of MHC class I molecules have revealed that they are composed of a highly polymorphic membrane-anchored heavy chain (~45 KD) and a lighter invariant soluble non-covalently attached β2m (~12 KD) (66, 67). The heavy chain consists of three extracellular domains α1, α2 and α3, a transmembrane region and a cytoplasmic tail. The α1 domain does not contain any cystein residues capable of forming disulphide bonds. The α2, α3 domains and β2m each contain two cystein residues involved in the disulphide-bond formation. Only the class I heavy chain spans the membrane. β2m as well as the α3 domain of the heavy chain have a folded structure that closely resembles that of the immunoglobulin. In contrast, the α1 and α2 domains of the heavy chain form two α-helices topping a sheet of eight β-strands (**Figure 2**). This part of the structure, corresponding to the most polymorphic region of the heavy chain, forms a cleft where peptide antigens bind (68, 69). The structural integrity of the complex depends on the presence of all three components. β2m interacts extensively with the α1/α2 peptide-binding domain and its position relative to the peptide-binding region is rather well conserved in all the known structures of class I molecules. The α3 domain does not seem to have any major impact on peptide binding.

The peptide-binding clefts of class I molecules are closed at both ends, restricting the size of the bound peptides to 8-11 amino acids, and the peptides are held in a largely extended conformation and run the length of the cleft.

The first two domains (α1 and α2) of the MHC class I proteins form an extremely polymorphic peptide binding groove, approximately 30Å long and 12Å in the middle. Each α-helical domain contributes half of the eight-stranded β-sheet floor of the groove and α-helix wall. The most variable residues point into this groove and up from the tops of both helices, conferring unique peptide-binding and TCR-binding

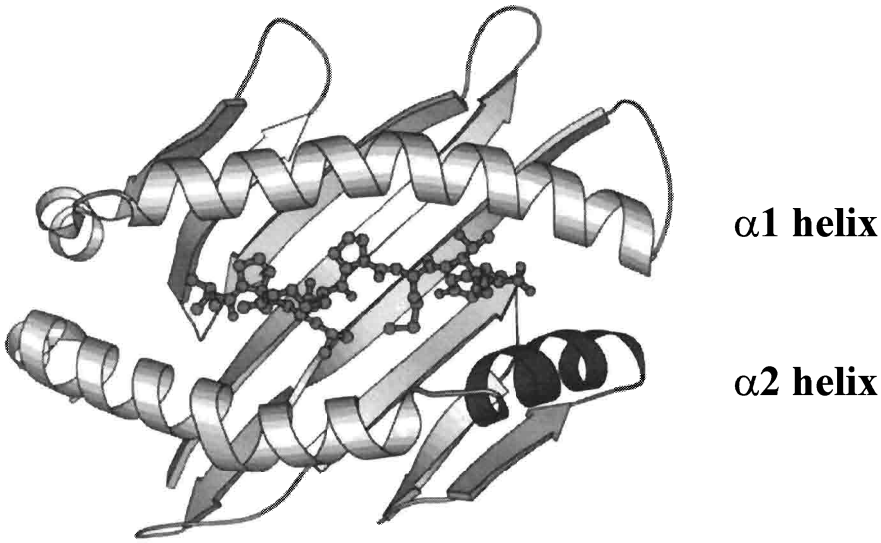


Figure 2. MHC class I–peptide complex. A schematic diagram of the binding groove and peptide for the HLA B35*01 HIV Nef peptide complex. The 8-mer peptide is shown in ball-and-stick representation and MHC residues 138–149 in the □2 helix are highlighted by darker shading. This portion of the MHC framework appears to shift in response to the precise positioning of the peptide carboxy-terminus. Adapted from Jones E. Y., Curent Opinion in Immunology 1997, 9(1): 75-79.

specificity of each MHC molecule. The majority of these variable residues are located in the central portion of the cleft, whereas clusters of highly conserved residues that stabilize/anchor the peptide termini occur at both ends of the groove. Some of these conserved, primarily aromatic residues block the ends of the binding groove, while others create a network of hydrogen bonds with associated peptides. The relative arrangement of the polymorphic residues that line the peptide-bonding cleft creates pockets which accommodate amino acid side chains of the peptide, thereby anchoring the peptide onto the class I molecule. In the analysis of the structure of the first determined MHC class I molecule, HLA-A2, six major pockets were identified and labeled as A through F (70). Residues that compromise each region are approximately the same in all defined MHC class I structures, so that each section of the primary sequence is located at the same place in the binding cleft in any MHC class I

molecule. All the structures have two distinct and fairly conserved pockets A and F at each end of the peptide-binding groove. Pocket B, C, D, E have distinct sizes and characters in different allelic variants of MHC class I molecules, thereby imposing different sequence constraints on the peptide bound. A consequence of this is that class I binding peptides contain allele-specific sequence motifs, defined by the position and identity of at least a couple of anchoring residues, one of which is the C-terminus. In addition, there are secondary pockets that can enhance and further tune the affinity of a particular peptide.

II.4 Structure of the MHC class I: peptide complexes (pMHC)

In most class I crystals the peptide is deeply buried in the binding groove, allowing for multiple interactions between the groove and side chains of the bound peptide. These contacts increase the overall binding affinity, and align the peptide in the site so that part of its sequence can be recognized by the TCR. The side chain contacts differ between different alleles, and determine the peptide sequence specificity. Important contacts occur between A and F pockets of the MHC groove and the peptide. In all class I:peptide complexes the N-terminal residue is accommodated with the A pocket and the C-terminal residue is accommodated with the F pocket.

The binding of peptides to MHC molecules is a noncovalent interaction mediated by residues both in the peptides and in the clefts of the MHC molecules. These peptides bind to the cleft in an extended conformation. Once bound, the the peptide and their associated water molecules fill the clefts, making extensive contacts with the amino acid residues that form the β-strands of the floor and the α-helices of the side of the cleft. In most MHC molecules, the β-strands in the floor of the cleft contain "pockets". The amino acid residues of a peptide may contain side chains that fit into these pockets and bind to complementary amino acids in the MHC molecule, often via hydrophobic interactions. Such residues of the peptide are called anchor residue, because they contain most of the favorable interactions of the binding (i.e., they anchor peptide in the cleft of the MHC molecule). The anchor residues of peptides may be located in the middle or at the ends of the peptide. Each MHC-binding peptide usually contains only one or two anchor residues, and this presumably allows

greater variability in other residues of the peptide, which are the residues that are recognized by specific T cells. Specific interactions of peptides with the α-helical side chain of the cleft also contribute to peptide binding by forming hydrogen bonds or charge interactions (salt bridges). Class I-binding peptides usually contain hydrophobic or basic amino acids at their carboxy termini that also contribute to the interaction.

Because many of the residues in and around the peptide-binding cleft of MHC molecules are polymorphic (i.e., they differ among various MHC alleles), different alleles favor the binding of different peptides. This is the structural basis of the function of MHC genes as "immune response genes"; animals that are responders to a particular peptide express MHC alleles that can bind and display it to T cells.

A portion of the bound peptide is exposed from the open top of the cleft of the MHC molecule, and the amino acid side chains of this portion of the peptide are recognized by the antigen receptors of specific T cells. The same T cell receptor also interact with polymorphic residues of the α-helices of the MHC molecule itself. Thus, amino acids from both the antigenic peptide and the MHC molecules contribute to T cell antigen recognition, with the peptide being responsible for the fine specificity of antigen recognition and the MHC residues accounting for the MHC restriction of the T cells. Predicably, variations either in the peptide antigen or in the peptide-binding cleft of the MHC molecule will alter presentation of that peptide and/or its recognition by T cells. By introducing mutations in an immunogenic peptide, it is possible to identify residues involved in binding to MHC molecules and those that are critical for T cell recognition. Also, one can enhance the immunogenicity of a peptide by incorporating into it a residue that strengthen its binding to commonly inherited MHC molecules in a population. This approach is being tried for the synthesis of custom-designed vaccines.

II.5 Differentiation of CTL and expression of TCR genes

Lymphocytes are the only cells in the body that express highly diverse antigen receptors that can recognize a wide variety of foreign substances. This diversity is

generated during the development of mature B and T lymphocytes from precursor cells that do not express antigen receptors and cannot recognize and respond to antigens. The process by which bone marrow-derived lymphocyte progenitors are converted to mature lymphocytes that populate the peripheral lymphoid tissues is called lymphocyte maturation. The collection of antigen receptors, and therefore specificities, expressed in B and T lymphocytes composes the lymphocyte repertoire. During maturation, a tightly regulated program of sequential gene expression leads to changes in the phenotype of the developing cells, generation of a diverse repertoire, acquisition of functional competence, and selection events that ensure that most of the lymphocytes that enter peripheral tissues are useful, in that they respond to foreign antigens but not to many self antigens. The expression of antigen receptor genes is the central feature of all these maturation events (71).

The T cell receptor is a heterodimer composed of two polypeptide chains, α and β or γ and δ, which are divided into a variable domain involved in antigen recognition and a constant domain which is important for membrane attachment and T cell activation. The variable domain is encoded by multiple variable (V), diversity (D) and joining (J) gene segments. The constant domain is encoded by constant (C) gene segments. Somatic rearrangement during lymphocyte development in the thymus juxtapose V-J or V-D-J segments that code for the α/γ and β/δ chains respectively. This combinatory potential creates an array of unique TCRs capable of recognizing a large variety of epitopes. The diversity is further increased by several possible α/β or γ/δ pairings and by nucleotide additions and/or trimming at the V(D)J junctions (72-75). **Figure 3** depicts a simplified scheme of rearrangement of TCR α and β gene segments.

Amino acid sequence comparison suggested that T cell receptors are structurally immunoglobulin-like (76). Crystal structure analysis confirmed that the extracellular portion of the αβ TCR heterodimer is structrually similar to the antigen-binding fragment (Fab) of Ig molecule. The V regions of the TCR α and β chains contain short streches of sequences where the variability between different TCRs is concentrated, and these form the hypervariable or complementarity-determining regions (CDRs). Three CDRs in the α chain are juxtaposed to three similar regions in

the β chain to form the part of the TCR that specifically recognizes peptide-MHC complexes. In the α and β chains of the TCR, the third hypervariable regions (which form CDR3) are composed of sequences encoded by V and J gene segments (in the α chain) or V, D and J gene segments (in the β chain). The CDR3 regions also contain sequences that are not present in the genome but are encoded by random nucleotide additions, so-called N regions. Therefore, most of the sequence variability in TCRs is concentrated in CDR3 (4).

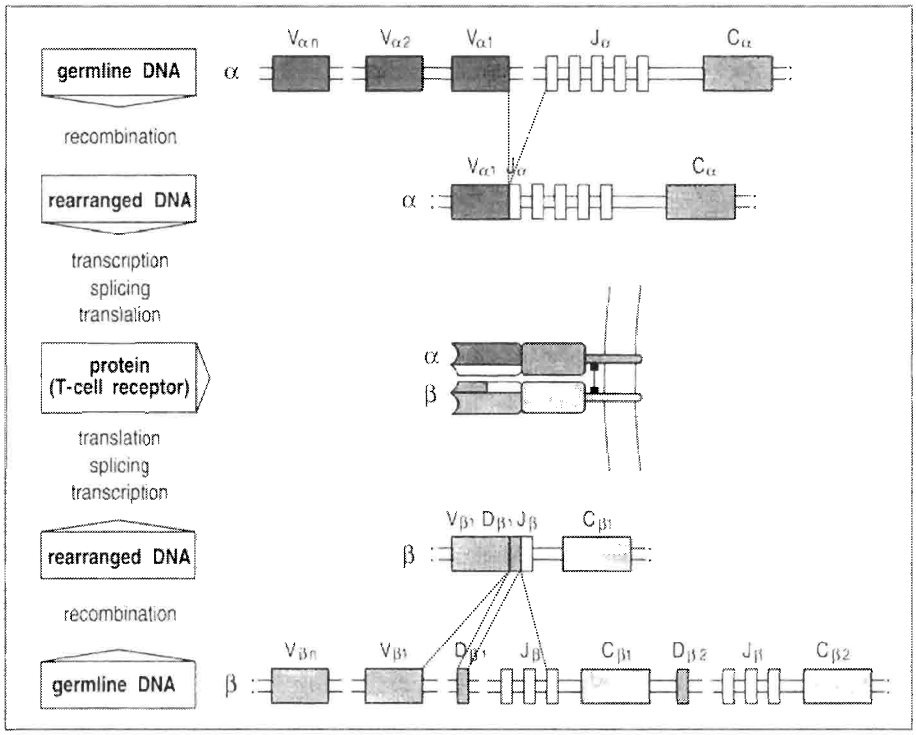


Figure 3. T cell receptor α and β chain rearrangement and expression. The T cell receptor α and β chain genes are composed of discrete segments that are joined by somatic recombination during development of the T cell. For the TCR α chain, a Vα gene segments rearranges to a Jα gene segment to create a functional exon. Transcription and splicing of the VJα exon to Cα generates the mRNA that is translated to yield the the TCR α chain protein. For the TCR β chain, the variable domain is encoded in three gene segments, Vβ, Dβ, and Jβ. Rearrangement of these gene segments generates a functional VDJβ exon that is transcribed and spliced to join to Cβ; the resulting mRNA is translated to yield the TCR β chain. The α and β chains pair soon after their biosynthesis to yield the α:β TCR heterodimer. Not all J gene segments are shown. Adapted from Janeway C. A. et al., *Immunobiology*, 1999, 4th edition.

T cell maturation in the thymus progresses in stages distinguished by expression of antigen receptor genes, CD4 and CD8 coreceptor molecules, and location in the thymus. The earliest T lineage immigrants to the thymus do not express TCRs or CD4 or CD8 molecules. The developing T cells within the thymus, called thymocytes, initially populate the outer cortex, where they undergo proliferation, rearrangement of TCR genes, and surface expression of CD3, TCR, CD4 and CD8 molecules. As the cells mature, they migrate from the cortex to the medulla. The least mature thymocytes, called pro-T cells, are $CD4^-$ $CD8^-$ (double negative, DN), and the TCR genes are in the germline configuration. In the pre-T stage, thymocytes remain double negative, but V-D-J recombination occurs in the TCR β chain locus. Primary β chain transcripts are expressed and processed to bring the Cβ segment adjacent to the VDJ complex, and β chain polypeptides are produced. The β chain associates with the invariant pre-T α chain to form a pre-T receptor. The β:pre-T α receptor transduces signals that inhibit rearrangement on the other β chain allele (allelic exclusion) and promote CD4 and CD8 expression and further proliferation of immature thymocytes. In the $CD4^+$ $CD8^+$ (double positive, DP) stage of T cell development, V-J recombinations occur in the α locus, α chain polypeptides are produced, and low levels of TCRs are expressed on the cell surface.

In order to eliminate TCRs which recognize self antigen or self MHC with too high affinity and lead to autoimmunity, also to eliminate useless TCRs which will not function in antigen recognition, selection processes are very critical after the random somatic rearrangement of TCR germline V, D, and J gene segments. These selection processes drive maturation of TCR-expressing, DP thymocytes and shape the T cell repertoire toward self-MHC restriction and self-tolerance. Positive selection of $CD4^+$ $CD8^+$ TCRαβ double positive thymocytes require low-avidity recognition of peptide-MHC complexes on thymic epithelial cells, leading to a rescue of the cells from programmed death. Negative selection of $CD4^+$ $CD8^+$ TCRαβ double positive thymocytes occurs when these cells recognize, with high avidity, antigens that are present in the thymus. This process is responsible for tolerance to many self antigens. Most of the cortical thymocytes do not survive these selection processes. As the surviving TCRαβ thymocytes mature, they move into the medulla and become single

positive (SP), i.e. either $CD4^+$ $CD8^-$ or $CD4^-$ $CD8^+$. Medullary thymocytes acquire the ability to differentiate into either helper or cytolytic effector cells and finally emigrate to peripheral lymphoid tissues (77, 78).

II.6 The TCR complex

The C regions of both α and β chain continue into short hinge regions, which contain cystein residues that contribute to disulfite bond that links the two chains. The hinge is followed by the hydrophobic transmembrane portions, an unusual feature of which is the presence of positively charged amino acid residues, including a lysine residue (in the β chain) or a lysine and arginine residue (in the α chain). These residues interact with negatively charged residues present in the transmembrane portion of other polypeptides (CD3 and ζ) that form the TCR complex. Both α and β chains have carboxy terminal cytoplasmic regions that are 5 to 12 amino acids long. These cytoplasmic regions are too small to transduce signals, and the CD3 and ζ molecules physically associated with the TCR serve the signal-transduction functions (79).

CD3-specific antibodies recognize the complex which consists of three proteins that are designated CD3 γ, δ, and ε (80). The TCR complexes also contain a disulfite-linked homodimer of the ζ chain. The CD3 and the ζ chain are identical in all T cells regardless of the specificity, which is consistent with their role in signal transduction and not in antigen recognition. The CD3 γ, δ, and ε are homologous to each other. The N-terminal extracellular regions of γ, δ, and ε each contain a single Ig-like domain, therefore these three proteins are members of the Ig superfamily. The transmembrane segments of all three CD3 chains contain a negatively charged aspartic acid residue, which binds to positively charged residues in the transmembrane domains of the TCR α and β chains, thus keeping the complex intact. The cytoplasmic domains of the CD3 γ, δ, and ε proteins range from 44 to 81 amino acid rresidues long, and each of these domains contains one copy of a conserved sequence motif important for signaling functions that is called the immunoreceptor tyrosine-based activation motif (ITAM). The ζ chain has a short extracellular region of nine amino acids, a transmembrane region containing a negatively charged aspartic acid

residue (similar to the CD3 chains), and a long cytoplasmic region (113 amino acids) that contains three ITAMs. It is normally expressed as a homodimer. It has been established that the expression of the TCR complex requires synthesis of all its components. The function of CD3 and ζ chain is to initiate the biochemical events that lead to T cell activation following recognition of antigen by the specific TCR (81).

II.7 the pMHC: TCR interactions (structure)

The antigen-binding site of the TCR is a flat surface formed by the CDRs of the α and β chains. This resembles the antigen-binding surface of antibody molecules, which is formed by the V regions of the heavy and light chains. In the few TCR structures that have been studied in details (82-85), though there are some differences among the solved structures, certain common features of the interaction between a TCR and its ligand has been revealed. **Figure 4** shows a representative structure of complex formed between a TCR and its ligand, a peptide/MHC molecule complex. The TCR interfaces the MHC/peptide complex in a diagonal orientation, parallel to the β sheets which form the pedestal of the MHC peptide-binding cleft. The TCR α chain is oriented over the N-terminal region of the peptide, and the TCR β chain is over the C-terminal region of the peptide. CDR2α loop positions over the α2 helix of the MHC class I molecule, and CDR2β appears over the α1 helix. The CDR3 loops are positioned over the center of the MHC-associated peptide. In some TCRs, parts of all six CDR loops make contact with the MHC molecule (84), whereas in others recognition of the MHC molecule is mainly the function of CDR1 and CDR2, while CDR3 contacts the peptide. The dominant role of CDR3 in recognizing the most variable component of the ligands, i.e. the peptide antigens, is consistent with the fact that the greatest variability of the TCR is concentrated in CDR3(86). Many more TCRs that are bound to their peptide-MHC ligands will need to be analysed before generalization can be drawn. However, one surprising result of the analyses already completed is that the side chains of only one or two amino acid residues of the MHC-bound peptide make contact with the TCR. This is structure basis for the remarkable

ability of T cells to distinguish among diverse antigens on the basis of very few amino acid differences.

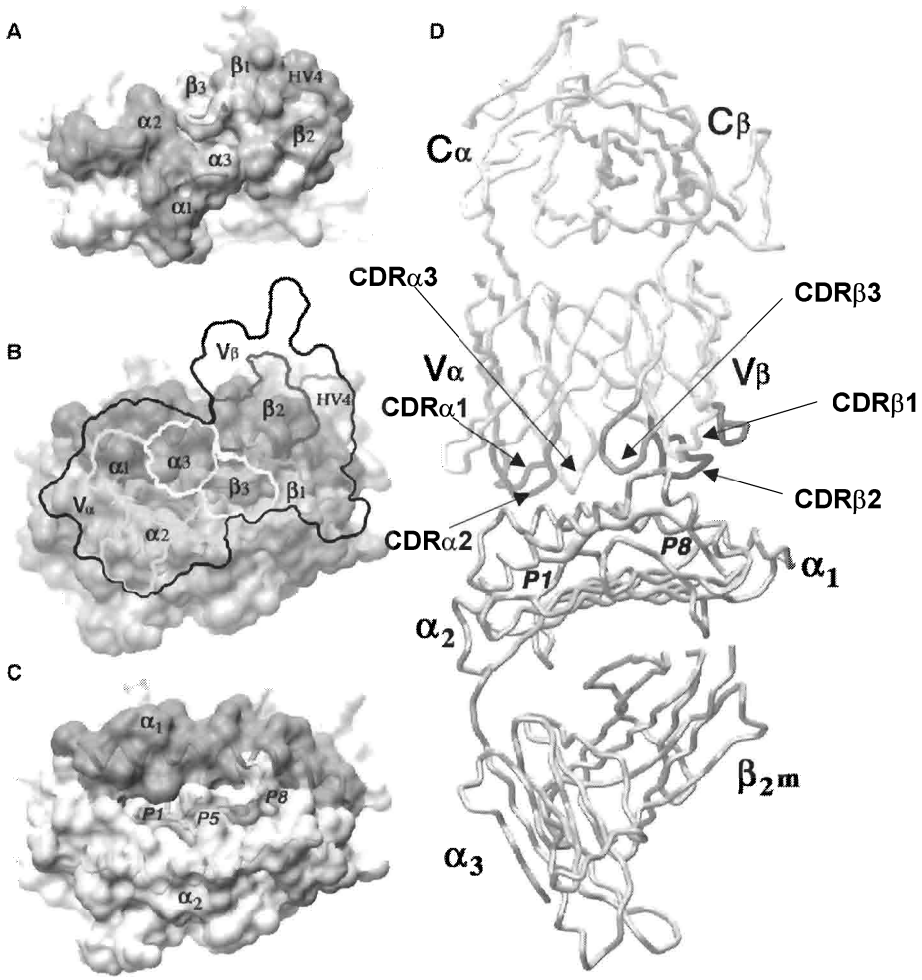


Figure 4. Structure of the complex formed between a T cell receptor, peptide and a MHC class I molecule,as revealed by X-ray crystallography. (A) Molecular surface of the ligand-binding face of the TCR viewed from above the CDR loops of the of the TCRα and TCRβ chains. (B) Footprint of the TCR on the peptide-MHC complex. (C) MHC class I molecule (H-2K^b) with bound peptide. (D) Backbon tube representation of the TCR-pMHC complex. The TCR is on top, with the MHC class I molecule on the bottom. The octamer peptideis ahown as a large tube. Note that all CDRs 1-3 of the α and β chains of the TCR contact both peptide and MHC residues, with CDR3s straddling the middle of the peptide. Adapted from Garcia K. C. et al., Science 1996, 274(5285): 209-219.

II.8 pMHC: TCR interactions (affinity)

The function of TCRs in immunity depend on both their specificity and affinity. Specificity is the potential to bind one unique chemical structure more strongly than a number of similar alternatives, it is established by TCR gene arrangements early in a T cell's ontogeny. Affinity refers to the strength of the binding between a certain antibody or TCR for its antigen or ligand. During the dynamic interaction of TCR and its ligand (pMHC), two opposing reactions are operating at the same time, which are the association reaction TCR + pMHC $\rightarrow$ TCR:pMHC and the dissociation reaction TCR:pMHC $\rightarrow$ TCR + pMHC. The reaction will eventually reach an equilibrium, at which the net velocity is zero because the absolute velocity in the forward reaction (association reaction) equals the absolute velocity in the reverse direction (dissociation reaction). The forward velocity,V*a*, is proportional to the concentration of TCR and pMHC: $Va=K_a\{TCR\}\{pMHC\}$, where K_a is know as the association rate constant (also called K_{on}, its unit is $M^{-1}\times S^{-1}$). The reverse velocity, *Vd*, is proportional to the concentration of TCR:pMHC complex: $Vd=K_d\{TCR:pMHC\}$, where Kd is know as the dissociation rate constant (also known as K_{off}, its unit is S^{-1})(87).

Usually, the TCR affinity (essentially measured by the equilibrium constant, K, and $K=K_a/K_d$) correlates with the stability of the complex measured by the rate constant of dissociation (K_d) or half-life ($t_{1/2}$): the higher the affinity, the longer the complex half-life. The value of $t_{1/2}$ can be either measured directly or calculated from the dissociation rate constant K_d, i.e., $K_d= 0.693/ t_{1/2}$.

Traditionally, the affinity of an antibody or TCR for its ligand is expressed as a dissociation equilibrium constant K_D ($K_D=K_d/K_a$, $S^{-1}/ M^{-1}\times S^{-1}=M$, thus its unit is M), which describes the concentration of antigen (for antibody) or ligand (pMHC, for TCR) that is required to occupy the binding sites of half the antibody or TCR molecules present in the system. A smaller K_D indicates a stronger or higher affinity interaction. The affinity of the TCR for peptide-MHC complexes is quite low, much lower than that of most antibodies. In the few T cells that have been analyzed in detail, the dissociation equilibrium constant (K_D) of TCR interactions with peptide-MHC complexes varies from $\sim10^{-5}$ to $\sim10^{-7}$ M, while the dissociation constants of

immunoglobulin is frequently < 10^{-9} M (88, 89). The off rate of this interaction is also rapid, because the half-life of TCR-pMHC interaction is on the order of only 1 to 10 seconds. These are the likely reasons why accessory molecules are needed to stabilise the adhesion of T cells to APCs, thus allowing biological responses to be initiated.

Since the TCR affinity is determined by the ratio between the dissociation and the association rate constant ($K_D=K_d/K_a$), and K_d inversely correlates with the half-live of the interaction ($K_d= 0.693/ t_{1/2}$), TCR-pMHC complexes with the same affinity may have different half-lives. Indeed, in some circumstances the half-life, not the affinity of the complex, may determine the nature and magnitude of the T-cell response.

Why do some T cell responses depend primarily on the kinetics of the TCR-pMHC reaction (i.e. K_{off}, $t_{1/2}$), while others depend on the reaction's equilibrium dissociation constant (affinity, K_D)? This distinction can be explained based on the assumption that only a fraction of the TCR-pMHC encounters produce "activated" TCRs, and that triggering of downstream signaling events requires accumulation of a critical number of productively engaged TCRs. The concept of productive engagement assumes that a TCR-pMHC complex persists for a critical time sufficient for the receptors to become activated. This critical time may be different for various manifestations of the T cell responses. Thus, both the number of ligated TCR and the half-life of TCR-pMHC complexes or bonds are meaningful. For a high affinity reaction, practically every TCR-pMHC bond will persist longer than the required critical time and the magnitude of the response will depend on the affinity-determined number of ligated TCRs. Such T cell responses are driven by the reaction's equilibrium (affinity) binding constant. In contrast, in the case of low-affinity reactions only a small fraction of the TCR-pMHC bonds last long enough. For such reactions variations in the half-life of the TCR-pMHC complex become a more important parameter than TCR affinity. For instance, two reactions with comparably low affinities would produce similar numbers of ligated TCRs. However, the probability to accumulate the critical number of productively engaged TCRs would be higher for reaction with smaller K_{off} or longer TCR-pMHC half-life. Thus, T cell responses induced by low-affinity reactions may be limited by the kinetics of pMHC dissociation from the TCR rather than by the

TCR-pMHC reaction's affinity. Under these conditions T cell responses are kinetically dominated.

II.9 functional outcomes of TCR triggering with altered peptide ligands (APLs)

Through introducing single amino acid substitution, one can generate series of peptide analogs which only differ from the original immunogenic peptide with just one residue. In different antigen system, these analogs have been tested for their capacity to stimulate a variety of T cell responses in panels of T cells specific for the same ligand. From such analysis, MHC-anchor residue and TCR contact site can be mapped for a certain peptide antigen. TCR contact residues can be further classified as " primary " or " secondary " TCR contact site. The primary TCR contact residue is represented by an amino acid which makes a major contribution in establishing strong interaction between MHC:peptide complex and different TCRs of the same peptide specificity. Secondary residues were defined as the other TCR contact sites, which were unique to each TCR in the population, and they were assumed to play a lesser role in the overall interaction between TCR and ligand. Initially, Sloan-Lancaster and Allen defined the term "altered peptide ligand (APL)" to describe analogs of immunogenic peptides in which the TCR contact sites have been manipulated (90). In broad terms, any peptide that serves as a TCR ligand in which substitutions of a single or multiple amino acids lead to changes in functional outcome of TCR signaling may be considered an APL.

Based on the functional outcome, TCR ligands can be classified as agonists, partial agonists, antagonists, superagonists and null peptides (89, 91). In principle, an agonist peptide induces the full functional repertoire of a given T cell, for example, cytolysis, cytokine and chemokine release, proliferation and upregulation of surface receptors. On the other hand, partial agonists are APLs with amino acid substitution in one of the TCR or MHC contact residues that have a distinct signaling "footprint" and induce only some, not all, T cell functions. Antagonists usually do not induce any specific function, but are engaged by the TCR and can inhibit responses normally stimulated by agonist if both ligands are present at the same time. Superagonists are APLs that induce stronger functional activation in specific T cells than the original

immunogenic peptide does. Engagement of null ligands has no functional consequences for the T cell because their binding affinity is below the threshold for TCR signaling. Also, it has been shown that a subgroup of TCR-APLs selectively induces apoptosis in CD4+ T cells (92).

It is now certain that kinetic parameters are important. In particular, the off rate (and thus the half-life) of the monomeric TCR-MHC/peptide interaction plays a decisive role in determining the final MHC density accumulated into the mature immunological synapse (IS, a commonly-observed structure formed in T cells after antigen stimulation; it is believed to play a role in T cell activation. I will come to the disscussion of IS in section IV of this thesis), and hence the extent of T cell activation. The wide range of biological activities displayed by altered peptide ligands has been correlated with the half-life ($t_{1/2}$) of the TCR-MHC-peptide interactions, in agreement with the "kinetic model" of T cell activation. According to this model, optimal ligands induce the longest interaction between MHC-peptide and the TCR (longest $t_{1/2}$), while all other potential ligands sustain this trimolecular complex for lesser time. The $t_{1/2}$ of the trimolecular complex determines whether the full immunological synapse formation develops between the T cell and APC. On the other hand, APLs with shorter $t_{1/2}$ (partial agonists) elicits only partial T cell triggering, which may be adequate for some effector functions, but are inefficient to form a full mature immulogical synapse, which is required for the most complex functions such as T cell proliferation. Engagement of null ligands has no functional consequences for the T cell because their binding affinity is below the threshold for TCR signaling.

III. TCR-mediated signalling

The T cell receptor complex and co-receptor (CD4 or CD8) are associated with Src-family protein kinases, Fyn and Lck respectively (93, 94). T cell activation by antigen-presenting cells results in the activation of protein tyrosine kinases (PTKs) that associate with the CD3 and TCRζ subunits and the co-receptor CD4 or CD8. It has now been firmly established that members of the Src, ZAP (ζ-associated protein)-70/Syk, Tec and Csk families of nonreceptor PTKs play a crucial role in T cell activation (95-101). Activation of PTKs following TCR engagement results in the

recruitment and tyrosine phosphorylation of enzymes such as phospholipase Cγ1 (PLCγ1) and Vav as well as critical adaptor proteins such as LAT (linker for activation of T cells), Src-homology domain (SH2)-containing leukocyte protein 76(SLP-76) and Cbl. These proximal activation events lead to reorganization of the cytoskeleton as well as transcriptional activation of multiple genes leading to T lymphocyte proliferation, differentiation and/or effector function. The details of these signal transduction pathways will be discussed in the following part of this section.

Upon binding of the pMHC ligand to the TCR and co-receptor, the TCR, CD4 or CD8 coreceptor and the protein tyrosine phosphotase CD45 are brought together. This allows the CD45 tyrosine phosphatase to remove inhibitory phosphate groups and thereby activate Lck and Fyn. As described before, CD3-γ, -δ and -ε each contain one ITAM and TCRζ contains three ITAM; The two critical tyrosines of each TCRζ are phosphorylated by the activated Src family kinase Lck and/or Fyn (81, 96), resulting in the generation of differentially phosphorylated TCRζ isoforms, p21 and p23, which have been observed predominantly in resting and activated T cells respectively. It has also been shown that p21 and p23 contain predominantly singly and dual phosphorylated ITAMs, respectively. More importantly, it has been demonstrated that there exists a highly ordered sequential phosphorylation of all the six tyrosines of 3 TCRζ ITAMs and that full phosphorylation of all six tyrosines of the TCRζ was dependent on the strength of TCR occupancy (102).

Phosphorylation of the TCRζ chains enables them to bind the cytosolic PTK ZAP-70 through the two SH2 domains of ZAP-70 (99, 103, 104), the bound ZAP-70 becomes a substrate for the adjacent Lck, and as a result, ZAP-70 is tyrosine phosphorylated, acquires its own tyrosine kinase activity (95, 97, 105). Once activated, ZAP-70 can also autophosphorylate itself. The cruitial function of ZAP-70 in TCR signal transduction has previously been established from studies in ZAP-70 deficient mice and humans with a genetic deficiency in ZAP-70. It is well-documented that ZAP-70 plays two crucial roles: firstly in the phosphorylation of PLCγ1, SLP-76 and LAT (106-108); secondly in TCR-induced Ca^{2+} mobilization, activation of the transcription factor NFAT (nuclear factor of activated T cells) and IL-2 production (108).

After the membrane adapter protein LAT and the cytosolic adaptor protein SLP-76 become tyrosine phosphorylated by ZAP-70, the cytosolic enzyme PLCγ1 is recruited to the plasma membrane and then phosphorylated by ZAP-70 (106, 109, 110). PLCγ1 thus become active and catalyzes the hydrolysis of a plasma membrane phospholipid called phosphatidylinositol 4,5-biphosphate (PIP2), generating two PIP2 breakdown products, inositol 1,4,5-triphosphate (IP3) and diacyglucerol (DAG)(111). These two breakdown products then activate two distinct signaling pathways in T cells. IP3 diffuses through the cytosol to the endoplasmic reticulum, where it binds to its receptor and stimulates release of Ca^{2+} store, causing a rapid rise (over a few minutes) in the cytosolic free Ca^{2+} ion concentration, from a resting level of about 100 nM to a peak of 600 to 1000 nM. In addition, a plasma membrane Ca^{2+} channel is opened in response to as yet incompletely understood TCR-generated signals, producing an influx of extracellular Ca^{2+}. This influx of extracellular Ca^{2+} allows the T cell to sustain an increase in cytosolic free Ca^{2+} for over an hour. Cytosolic free Ca^{2+} acts as a signaling molecule by binding to a ubiquitous Ca^{2+}-dependnet regulatory protein called calmodulin. Calcium-calmodulin complexes then activate the protein serine/threonine phosphatase calcineurin, which dephosphorylate the transcription factor NFAT, thereby uncovering a nuclear localization signal that allows NFAT to translocate from the cytoplasm to the nucleus (112). NFAT is a transcription factor required for the expression of IL-2, IL-4, TNF, and other cytokine genes. Once in the nucleus, NFAT binds to consensus-binding sequences in the promoter and/or enhancer regions of IL-2, IL-4 and other cytokine genes, usually in association with other transcription factors, such as AP-1. On the other hand, DAG, the second breakdown product of PIP2, activates the enzyme PKC (which has several isofroms) that also participates in the generation of active transcription factors, such as NF-κB. NF-κB is involved in T cell activation, contributing to IL-2 transcription (113). NF-κB also activates the transcriptions of genes whose products protect cells from apoptotic.

Besides the calcineurin and PKC signaling pathway, another important signaling pathway in T cell activation is the MAPK (mitogen-activated protein kinase) pathway, which is also called the Ras and Rac pathway (114). Ras molecules compose a family

of 21 KD guanine nucleotide-binding proteins with intrinsic GTPase activity, and Ras activation links cell membrane receptors with downstream signaling pathways. Ras is loosely attached to the plasma membrane through covalently attached lipids. In its inactive form, the guanine nucleotide-binding site is occupied by guanine diphosphate (GDP). When the bound GDP is replaced by GTP, Ras undergoes a conformational change and becomes an allosteric activator of various cellular enzymes. Following TCR triggering, ZAP-70 is activated; as mentioned earlier, it then phosphorylates the adaptor protein LAT. LAT then binds and recruits to the membrane additional SH2 domain-containing adaptor proteins, such as Grb-2. Once attached to the complex, Grb-2 itself is tyrosine phosphorylated by ZAP-70, and it recruits to the membrane the Ras GTP/GDP exchange factor Sos (son of sevenless). Sos catalyzes GTP for GDP exchange on Ras. This generates the GTP-bound form of Ras (Ras.GTP), which then function as an allosteric activator of the cascade of MAP kinase. This enzyme cascade involves the sequential activation of at least three different kinases (Raf, MEK-1, ERK-1, 2), each of which phosphorylates (and thus activates) the next enzyme in the cascade. The MAP kinase pathway ultimately leads to the activation of an enzyme called the extracellular receptor-activated kinase (ERK), which is the prototypic member of the family of MAP kinasees. The activated ERK phosphorylates a protein called Elk, and phosphorylated Elk stimulates transcription of Fos, a component of the activation protein-1 (AP-1) transcription factor. Another enzyme, called phosphatidylinositol-3 kinase (PI-3 kinase), also appears to play a role in the Ras-MAP kinase pathway, but the exact function of PI-3 kinase in T cells is not well known. In parallel with the catalysis of Ras.GTP exchange through recruitment of Grb-2 and Sos, the phosphorylated LAT and SLP-76 adaptor proteins also recruit and activate a GTP exchange protein called Vav that acts on another small 21 KD guanine nucleotide-binding protein called Rac. Rac.GTP initiates a parallel MAP kinase cascade (Mekk, Jnkk), resulting in the activation of the C-Jun N-terminal Kinase (JNK). JNK is sometimes called stress-activated protein (SAP) kinase. Activated JNK then phsphorylates c-Jun, the second component of the AP-1 transcription factor. A related member of the SAP kinase family is called p38, and it too may play a role in T cell responses.

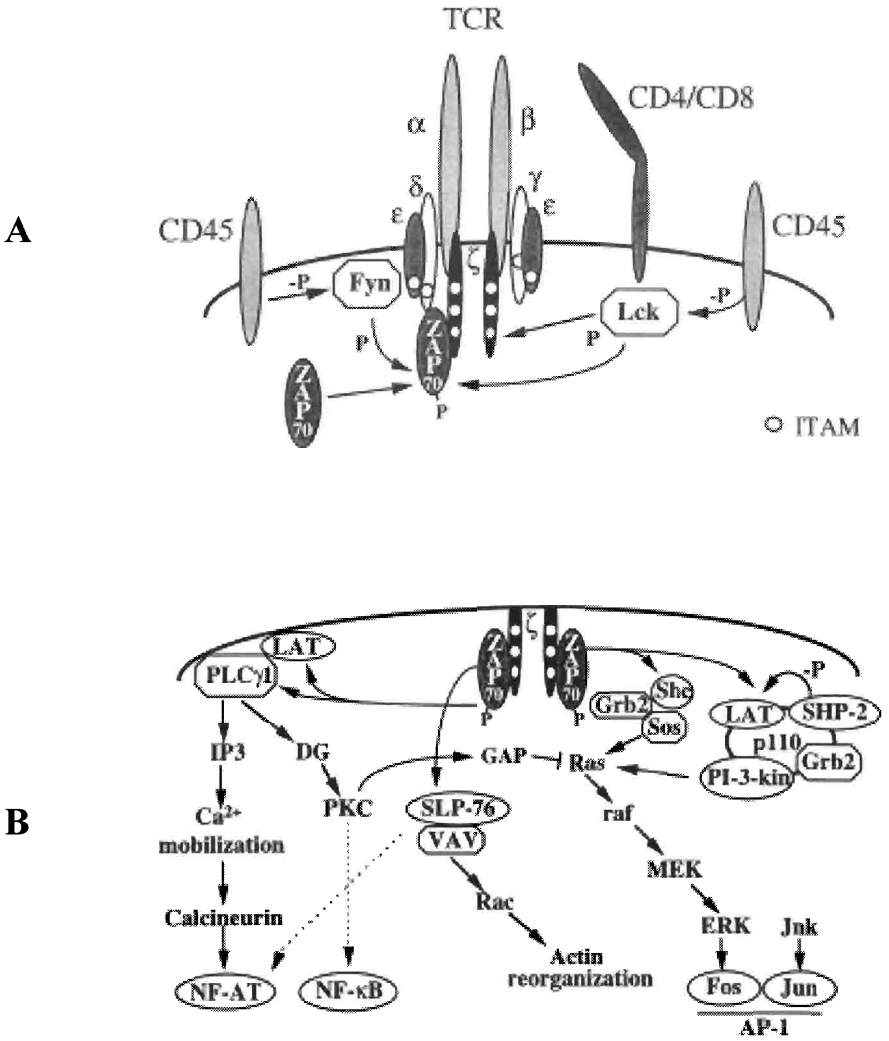


Figure 5. TCR signalling. **(A)** Early signalling of the T cell receptor. Trigger of the TCR together with the co-receptor CD4 or CD8 results in the recruitment of the PTKs (Fyn, Lck) and ZAP-70 kinase, which are activated by dephosphorylation/phosphorylation as indicated. **(B)** Complexicity of TCR signalling. Activation of the kinases as indicated in (A) results in the activation of several pathways that lead to transcriptional activation or actin reorganization. Adapted from Medema J. P. & Borst J., Human Immunology 1999, 60(5): 403-411.

In short, following ligand binding to TCR, four different signal transduction pathways are initiated that result in the activation of four enzymes: the Ras pathway leading to activation of ERK, the Rac pathway leading to activation of JNK, the PLCγ1-Ca^{2+}-dependnet pathway leading to activation of calcineurin, and the PLCγ1-DAG-dependnet pathway leading to activation of PKC. These pathways contribute to the activation of transcription factors AP-1, NFAT and NF-κB respectively, resulting in the expression of genes encoding proteins needed for T cell clonal expansion, differentiation, and effector functions. A simplified scheme of early and downstream signalling events following TCR triggering is shown in **Figure 5**.

Another important aspect in T cell activation is the signaling delivered by costimulatory molecules (115, 116). It is well-documented that costimulatory molecules prevent the occurrence of anergy in naïve T cells; they can lower the signaling threshold required for T cell activation, and costimulation also increases the expression level of the anti-apoptotic proteins Bcl-X_L in T cells following TCR triggering (117-119). Biochemically, CD 28 triggering can activate PI-3 kinase and facilitate GTP exchange in Ras, resulting in activation of the MAP kinase pathway. CD28 also provides an independent pathway for the activation of Vav, and the associated Rac pathway. Costimulators may function in T cell activation by increasing the level of the same signal transduction pathways that are triggered by the TCR (i.e., costimulatory signals may interact directly with and augment TCR signaling). Alternatively, costimulators may activate distinct signaling pathways that converge with those activated by the TCR, or they may function by activating novel signal transduction pathways that are unrelated to TCR signals. These possibilities are not mutually exclusive and are not yet resolved.

In addition, TCR engagement results in polarized reorganization of the cytoskeleton and the formation of TCR caps. Recent studies implicate SLP-76 as a critical scaffold in these processes. TCR-induced tyrosine phosphorylation of SLP-76 results in the formation of a trimolecular SLP-76-Vav-Nck complex (120). In turn, Nck binds through its SH3 domain to two effector proteins of Rho-family GTPases, i.e. the serine-threonine kinase Pak (p21-activated kinase) and the actin-binding WASP (Wiscott-Aldrich syndrome protein). Vav- and WASP-deficient mice studies indicate

both Vav and WASP are critical for polarized actin reorganization, receptor capping and TCR-induced proliferation (121-124). Therefore, it appears that SLP-76 functions as a scaffold to efficiently induce Rho-family small-GTPase-dependent activation of Pak and WASP—leading to polarized F-actin formation as well as receptor caps which in turn, may be important for efficient TCR signaling.

IV. Models of sustained TCR triggering and T cell activation

Throughout the last decade, many experimental data accumulated during T cell biologists' pursue for understanding T cell activation. Biochemical analyses have provided much important information. It is known that within seconds of pMHC engagement, the TCR initiates a tyrosine phosphorylation cascade that triggers multiple branching signaling pathways. These early signals may be sufficient to trigger some effector functions, such as lysis of target cells by CTL. In contrast, more complex functions, such as T cell proliferation, require TCR engagement and signaling for many minutes or hours. The mechanism of sustained TCR engagement, however, is not well understood. Different models were proposed to explain how exactly TCR occupancy by pMHC ligand leads to proximal signaling and eventually sustained downstream signaling events and full T cell activation. Among these models the serial triggering model, the kinetic proof-reading model are the two best known models.

IV.1 The serial triggering model

The idea of serial engagement of the TCR was first proposed by Lanzavecchia and colleagues to explain how T cells could be activated by 100 specific peptide–MHC complexes on an APC (125). They propose that a single MHC–peptide complex can trigger up to 100 TCRs, thus explaining how a minimal number of peptide–MHC complexes could result in activation of a T cell. Assuming that receptor engagement results in internalization, they measured the number of surface TCRs after stimulation and found a correlation between the internalization of 8000 receptors and full scale T cell activation (126). Using T cells containing two different TCRs, they showed that

internalization is specific, as it requires specific antigen recognition. Because of differences in half-lives, it is not surprising that a single MHC–peptide complex can trigger multiple TCRs. MHC–peptide complexes can have half-lives on the order of hours (127), while the association of the TCR with MHC–peptide has a half-life of seconds (128). Thus, assuming that unliganded TCRs can be recruited and held together in the contact area, the high chemical off rate of the TCR–MHC complex will result in TCR–MHC partner swapping. Although partner swapping has not been directly demonstrated for the TCR–MHC interaction, it has been demonstrated for the CD2–CD58 interaction (129). Thus, the potency of any particular peptide–MHC complex will be related to both its overall affinity and its off rate. The affinity (2D K_D) of the TCR for the peptide–MHC complex will determine the minimal number of receptors that can be engaged at equilibrium. The off rate will determine how many additional TCRs can be engaged by a single peptide–MHC complex.

IV.2 The kinetic model

At the same time, McKeithan and McConnell and colleagues separately proposed models of T cell activation that they call "kinetic proofreading"(130) or "kinetic editing" models (131), respectively. These models were proposed to explain how a low affinity receptor, the TCR, could distinguish between small differences in antigen. The TCR, for example, which makes contact with only a few amino acid side chains of the antigenic peptide, can easily distinguish single, conservative amino acid changes in the peptide (132). These models propose that small differences in receptor affinity correlate with differences in the duration of receptor engagement. Because T cell activation requires the assembly of multiple layers of proteins to the phosphorylated TCR (133), a certain amount of time is required to assemble the correct signaling complex. Thus, the formation of the complete complex will require stimuli that exceed a certain threshold of strength and duration. Altered peptide ligands (APL), which are slightly lower affinity ligands for the TCR (134), stimulate the TCR but are unable to sustain signaling long enough to generate the complete signaling complex. Thus, the system allows the TCR to respond in an off or on fashion to antigenic stimuli within a very narrow affinity range. These models were a

breakthrough in our understanding of TCR signaling.

A key feature of these kinetic models is the importance of signal duration. But these models do not address exactly how signal duration is achieved, assuming simply that signal duration is directly related to receptor affinity. Because of issues of TCR size and affinity, it seems likely that signal duration will be closely related to the stability of the cell–cell contact formed. Extracellular binding events, which are largely dependent on 2D affinities, will therefore determine the duration of TCR signalling.

Consistent with the kinetic proofreading model, it has been shown that TCR-peptide-MHC (pMHC) binding affinities that distinguish the process of positive selection and T cell activation exhibit an extremely narrow window (135). For example, affinities with K_D values of around 100 μM appear to be sufficient for positive selection, while affinities with K_D values of 1-10 μM elicit either negative selection or activation of peripheral T cells (89). It has been suggested that it is the affinity of a TCR-pMHC interaction (136) or the dissociation rate of the TCR from the pMHC (128, 134, 137) that determines the biological activity of a T cell. In the ¨ kinetic proofreading model¨, as mentioned earlier, it has been proposed that TCR-pMHC lifetimes of dissociation ($t_{1/2}$) in the range of about 10 to 40 s yield full signaling and T cell activation, while shorter lifetimes yield partial signaling and antagonist effects (130, 131, 134). According to the serial triggering theory, for an efficient serial engagement of multiple TCRs by a few cognate pMHC ligands, each individual TCR needs to bind and dissociate from the ligand with fast enough kinetics to allow the next TCR to be engaged. This results in a sustained TCR signal. Also, an additional requirement for a productive TCR signal is a minimal half-life for the TCR-pMHC interaction, as postulated by the kinetic proofreading model. According to this theory, premature dissociation of the TCR from the pMHC ligand would result in incomplete signaling, preventing T cell activation. In support of this model it has been shown that, in general, T cell activation correlates with the half-life of the TCR-pMHC interaction. Thus, for an agonist pMHC ligands, the half-life of TCR interaction is above a certain threshold, whereas for antagonist pMHC ligands the half-life must be below this threshold. These antagonist pMHC ligands are unable to engage the TCR with sufficient stability to allow completion of a productive signaling cascade.

However, the strict positive correlation between the affinity or half-life of a TCR-pMHC interaction and T cell activation is not always observed (138). It has been found that some peptide variants with a longer half-life or higher affinity induce reduced response in T cells bearing the specific TCRs (139-141). But because of the very limited number of available examples, this discrepancy has not been conclusively resolved yet.

IV.3 The immunological synapse (IS)

Four to five years ago, several independent imaging studies of antigen-specific T cell junctions have revealed the formation of a specialised contact, termed the immunological synapse (IS)(142-144). The mature immunological synapse is defined by a specific bull's eye pattern of receptor segregation with a central cluster of TCRs surrounded by a ring of integrin family adhesion melecules (e.g. LFA-1)(144). The immunological synapse usually forms within 15 minutes of T cell-APC contact. When both the T cell and APC contact regions were analyzed by confocal microscopy, it was detected that the center of the bull's eye or cSMAC (central supramolecular activation cluster) is enriched for TCR and peptide/MHC complexes, and the ring of the bull's eye or the peripheral SMAC (pSMAC) contains the integrin LFA-1 and its counterreceptor ICAM-1. The central SMAC also contains the signaling molecules on the T cell cytoplasmic side of the IS, including PKC θ and Lck. The T cell side of the pSMAC contains the integrin-associated cytoskelettal proteins including talin. The formation of the IS requires the intact T cell cytoskeleton, it is an active and dynamic process which actually begins as an inverted structure with a central adhesion cluster surrounded by a ring of engaged TCR. The final stage of IS formation is the stabilization of the central cluster of MHC-peptide complexes, which correlates with sustained parallel engagement of TCR by at least 60 MHC-peptide complexes. Maintenance of the stabilized IS for greater than 1hr is well correlated with full T cell activation. Based on image studies, it has been proposed that physiological T cell activation can be divided into a series of temporal stages: T cell polarization, initial adhesion, IS formation (initial signaling), and IS maturation (sustained signaling)(145).

The discovery of IS formation during T cell activation and the established concept of IS seem to provide a good model to explain the mechanism of sustained TCR engagement and signaling. As suggested from the recent study by Grakoui et al. (144), sustained engagement faces several barriers that are overcome by formation of the immunological synapse. (i) The barrier of the small size of the TCR and MHC-peptide complexes in relation to large glycoproteins is overcome by forcing a ring of T cell membrane against the substrate early in the synapse formation process. Close apposition was maintained as the captured MHC-peptide complexes were transported to the center of the synapse. (ii) The barrier of low affinity is overcome by the tight apposition of this ring of membrane to the substrate that confines the interaction to an attoliter (10^{-18} liter) volume. (iii) The barrier of rare MHC-peptide complexes is overcome by actively concentrating available complexes by greater than 100-fold in the center of the synapse. This concentration may have been accomplished by active cytoskeletal transport of engaged TCRs that associate with the actin cytoskeleton after interaction with MHC-peptide complexes. The concentration of MHC-peptide complexes in the central cluster may also drive formation of TCR-MHC-peptide oligomers that could account for the cluster stabilization stage. (iv) Finally, the barrier of T cell migration is overcome by the cytoskeletal organization that is an integral part of synapse formation. The organization of the immune synapse is radially symmetric and inward directed, such that force vectors cancel and the cells remain in place. Thus, the immunological synapse organization is synonymous with the antigen-dependent stop signal. The data of Grakoui A. et al. also support a role for the immunological synapse in the discrimination of potential antigenic ligands by the TCR. The TCR exhibits an exquisite specificity for its antigen as demonstrated by the significantly different biological outcomes induced by ligands that differ by only a single amino acid. Examination of TCR interaction with MHC-peptide by a panel of altered peptide ligands showed a direct relationship between the process of immunological synapse formation and T cell proliferation. Furthermore, all densities of agonist MHC-peptide complexes that induced T cell proliferation also triggered a central cluster with greater than or equal to 60 molecules per square micrometer of accumulated MHC-peptide complexes. As the number of accumulated MHC-peptide complexes was "locked in" in the final stage of immunological synapse formation, the

T cell could thus set a specific threshold to determine whether to commit to the program of T cell activation.

Consistent with the kinetic model of T cell activation, Grakoui A. et al. found that the $t_{1/2}$ of the TCR-MHC-peptide interaction correlates with the number of MHC-peptide complexes in the cluster. One process that may contribute to kinetic discrimination is the recruitment of $p56^{lck}$ to the engaged TCR by CD4. The time required to recruit CD4 to the engaged TCR may provide a kinetic window that determines the ability of antigenic peptide complexes to trigger early signals. In contrast, there is no evidence that CD4 participates directly in stabilizing the TCR-MHC-peptide interaction. As for the requirement of coreceptors in the formation of IS, Kupfer and colleagues(146) have recently demonstrated that naïve CD8+ T cells require CD8 molecules in order to form a c-SMAC. This differs from recently activated CD4+ T cells, in which engagement of CD4 is not an absolute requirement for c-SMAC formation(147). The formation of a cluster of MHC-peptide complexes, the size of which is determined by early kinetic discrimination, provides a link between early signaling and later T cell commitment.

This quantitative analysis reveals how the formation of an immunological synapse provides the machinery to integrate cell surface events into the T cell activation decision. Central cluster formation is based on a transport process sensitive to MHC-peptide strength and number. The intrinsically transient interaction of TCR and MHC-peptide complex is stabilized in the immunological synapse. The MHC-peptide complex must be of sufficient density and potency to attain the stable central cluster, to fully activate the T cell. Thus, building an immunological synapse provides a high-order molecular mechanism to assess MHC-peptide strength with respect to three stages of synapse formation: junction formation (stopping), MHC-peptide transport, and cluster stabilization.

In summary, many experimental observations convincingly demonstrated the involvement of the IS during T cell activation, and studies on IS formation seem to suggest it allows for T cell receptor (TCR) clustering and for sustained signalling in T cells for many hours, leading to T cell activation. However, up till now, most of the work on IS formation and T cell activation are correlation studies, i.e. when T cell is

fully activated, a mature IS is observed; when T cell is partially or weakly activated, IS formation is either disturbed or disorganized, no mature IS is formed; when T cell is not activated (i.e. in the case of null peptide), no IS formation can be observed. Whether IS formation is really an important, indispensable signalling event or it is just a companying event that simply co-proceed with TCR signalling, or it actually plays some other roles than TCR signalling remains unclear. Recently, a work by Andrew Shaw's group (148) has clearly shown that TCR-mediated tyrosine kinase signalling in naive T cells occurrs primarily at the periphery of the synapse and is largely abated before mature immunological synapses is formed. These data suggest that many hours of TCR signalling are not required for T cell activation. These observations challenge current ideas about the role of immunological synapses in T cell activation. Moreover, the same group also demonstrates that in T cells from CD2AP knockout mice, no TCR clustering is seen even after 30 minutes of triggering; also, TCR downregulation is defective in these T cells. However, these T cells are hyperreactive instead of hyporeactive or nonresponsive following TCR triggering (149). All these data together suggest that IS probably is not involved in initiating or sustaining TCR signalling, it actually is important in initiating TCR internalization. Also, serial triggering of TCR might serve other functions than signalling. More data need to be generated from different system to resolve the role of IS in T cell activation.

V. Regulation of mature T cell apoptosis by TCR triggering

V.1 Apoptotic pathways in T lymphocytes

Following TCR engagement with peptide-MHC complex, the expression of a number of immediate early genes is induced in T cells. These early events dictate the eventual outcomes of TCR stimulation in different T cell populations. For thymocytes, TCR signals can lead either to apoptosis or to positive selection, whereas TCR stimulation of peripheral T cells usually results in T cell activation. Resting mature T lymphocytes are activated when triggered via their specific TCR to elicit an appropriate immune response. In contrast, for preactivated peripheral T cells repeated

stimulation can also lead to cell death (activation-induced cell death, AICD). Along with cell death induced by growth factor deprivation, AICD followed by the elimination of useless or potentially harmful cells preserves homeostasis, leads to termination of cellular immune responses.

Development of peptide-MHC tetramers to stain T cells on the basis of TCR specificity, and of intracellular cytokine staining assay have greatly assisted our understanding of the dynamic of CD8+ T cell response in a more precisely quantitative way. For example, following infection with the natural pathogen LCMV, the number of naïve CD8+ T cells that recognize LCMV epitopes increases 2-10 fold by eight days after exposure. Notably, most of the CD8+ T-cell expansion is antigen-specific. All these antigen-specific T cells may go through 13 or more divisions within 8 days, producing massive amounts of effector cells. The rare, antigen-specific, naïve CD8+ T cells present before infection can divide 13-15 times during this time, so that their frequency may go from 1 in 10^4-10^5 to more than 50% the $CD8^+$ T cell population (150, 151). Such rapid proliferation leads to the production of enormous numbers of effectors capable of killing infected cells and releasing cytokines upon antigen encounter. Such a massive CD8+ T cell response is not unique to LCMV infection, as large numbers of antigen-specific T cells are generated in other human and animal infections, such as HIV (7), EBV(152), HSV(153), influenza virus (154). During the few days after antigen has been cleared, 90-99% of the effector T cells die, leaving an expanded population of memory pool (155). The original naïve CD8+ T cell pool is left intact, except for the promotion of the pathogen-specific members. What regulates the decline and contraction of the overwheming numbers of memory cells is not well understood yet. There is evidence that not all the massive apoptosis of the effector cells can be attributed to the role of Fas/FasL (also called CD95/CD95L) or TNF family member.

Since it is a prerequisite for successful attempts to modulate AICD, the molecular mechanisms controlling the induction or prevention of AICD have developed into a scientific topic of great interest to immunologists. Over the past 7-9 years, it was suggested that the CD95/CD95L system plays a major role in the induction and regulation of programmed cell death in T lymphocytes (156-161). It was also shown

that AICD in many cases reflects a CD95-dependnent cell death where CD95L is transiently induced by activation of the T cells via the antigen-specific TCR (162). A commonly considered scenario proceeds as follows: upon stimulation of activated T cells via the CD3/TCR complex, associated with tyrosine phosphorylation and activation of TCR-sensitive transcription factors, CD95L mRNA and cell surface expression are rapidly induced. CD95L binds to the surface-expressed CD95 molecule on the same or on neighbouring cells and triggers CD95-dependent apoptosis. Due to membrane folding or, alternatively, CD95 cleavage by metalloproteases, CD95/CD95L interactions result in both autocrine "suicide" and paracrine "fratricide". Although the role of the CD95/CD95L system in the induction of T cell apoptosis is unambiguous in different in vitro model systems, it is evident that other molecules could also be important. Therefore, it is unlikely that all T cell apoptosis involves the Fas/FasL system. Under certain circumstances, TNFα and TRAIL can act as a mediator of apoptosis in mature T lymphocytes. In addition, CD95-independent apoptosis has been reported for T cells triggered through the alternative pathway of T cell activation via the CD2 molecule.

The Fas apoptosis pathway has been studied extensively since the discovery of this molecule a decade ago. Like many forms of apoptosis induced through other pathways, Fas-mediated apoptosis involves the activation of a series of cystein proteases that cleave proteins after the aspartic acid residues and have therefore been called caspases (163). The ligand for this molecule, FasL, is in the form of trimers and when they bind, they induce trimerization of the Fas molecule. The cytoplasmic tail of Fas possess a motif know as death domain, which is a protein-protein interaction domain. Following the crosslinking of Fas by FasL, an adaptor protein FADD binds to the death domain in the cytoplasmic tail of Fas. FADD in turn interact with the protein pro-caspase 8. Pro-caspase 8 undergoes autocatalytic activation and is able to cleave and activate the downstream effector caspases, for example caspase 3 (164). At the end of this pathway a caspase-activated DNAse (CAD) enters the nucleus and cleaves DNA into the 200 base pair fragments characteristic of apoptotic cell death (165, 166). A scheme of Fas-mediated apoptotic pathway is illustrated in **Figure 6**.

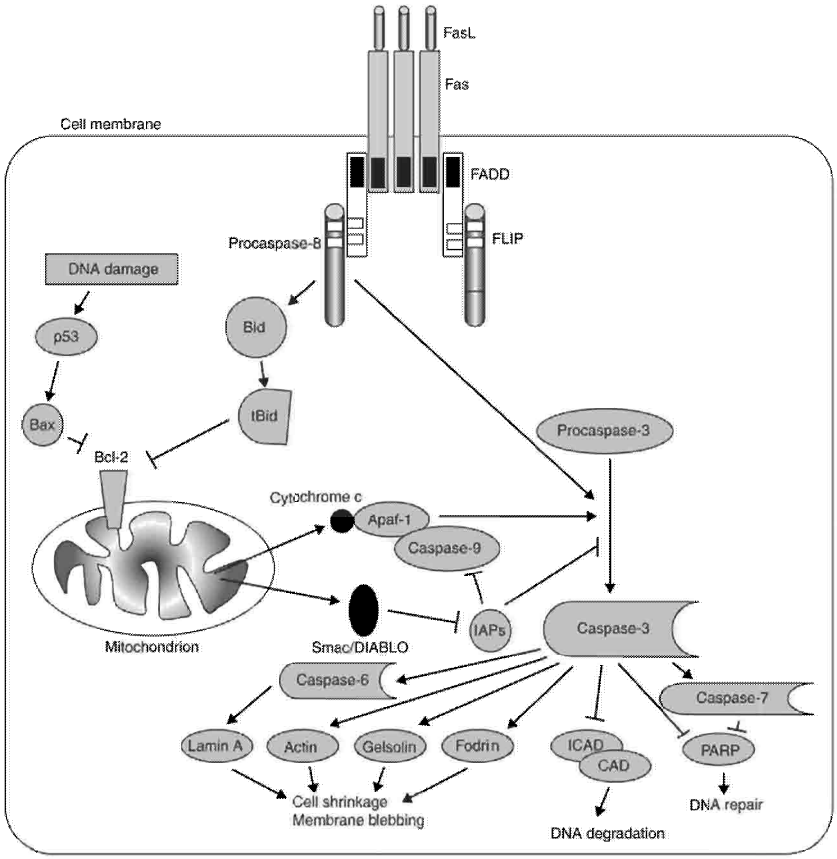


Figure 6. The death receptor-dependent apoptosis pathway as typified by Fas(CD95). Oligomerization of Fas by FasL (on the same cell or a neighbouring cell) induces recruitment of adaptor protein FADD to the cytoplasmic tail of Fas by their mutual death domains (DD, black boxes). The opposite end of FADD contains death effector domains (DED, white boxes) that allows recruitment of either procaspase-8 or FLIP. The latter contains a mutation in the enzymatic domain (black line)rendering it enzymatically inactive. Caspase-8 can cleave the BH3-only protein Bid and the resulting truncated Bid (tBid) can inactivate Bcl-2 in the mitochondria membrane (as can Bax, following DNA damage). This allows the release of cytochrome c, which clusters with Apaf-1 and caspase-9 in the presence of dATP to activate caspase-9. Smac/DIABLO is also released from the mitochondria and inactivates IAPs (inhibitor of apoptosis). Active caspase-9 can cleave abd activate procaspase-3 to its active form, leading to activation of other caspases, breakdown of several cytoskeletal proteins and degradation of the DNAse inhibitor, ICAD, therebyreleasing CAD. Adapted from Budd R. C., Current Opinion in Immunology 2001, 13: 356-362.

In preactivated mature T lymphocytes, AICD can be triggered via the TCR/CD3 antigen receptor complex. Monoclonal anti-TCR or anti-CD3 antibodies have been widely used to study AICD in T cells, because it was confirmed by many studies that crosslinking of the TCR/CD3 complex by mAbs triggered AICD not only in immature thymocytes (167), transformed T cells (168, 169), T cell hybridomas (170) but also in normal T lymphocytes (171-173). Usually, resting peripheral T lymphocytes are resistant to AICD triggered by anti-CD3/TCR mAbs; instead, these cells respond to CD3/TCR signaling by the induction of proliferative response. Under in vitro culture conditions, it takes several days of stimulation before freshly isolated mature peripheral T cells acquire sensitivity towards AICD (174-176). The development of AICD sensitivity at least in part correlates with CD95 surface expression and CD95 sensitivity and is regulated by cellular inhibitors of the CD95-signalling cascade, termed FLICE-inhibitory proteins, c-FLIPs (177-179). In response to anti-TCR mAbs, AICD is induced both in T cells expressing the conventional $\alpha\beta$ TCR and in T cells expressing the $\gamma\delta$ TCR (180). The induction of AICD sensitivity of mature T cells is also influenced by several parameters. Interestingly, the T cell growth factor IL-2 has been implicated in the priming of T lymphocytes for AICD (181, 182). It was demonstrated that the role of IL-2 in enhancing the Fas-mediated AICD is to increase the transcription and surface expression of Fas ligand and suppress transcription and expression of FLIP, the inhibitor of Fas-mediated apoptosis (183). Also, the ligation of CD4 molecules through mAb crosslinking has also been shown to regulate AICD triggered via TCR/CD3 complex (184, 185), though the role of CD4 in the regulation of AICD is more complex: while for resting CD4+ T cells crosslinking of CD4 primes the T cell for apoptosis, the opposite was observed for activated CD4+ T lymphocytes. It was shown that ligation of CD4 inhibits subsequent AICD of human CD4 T cells triggered through CD3/TCR, due to the prevention of upregulation of FasL (186, 187).

Taken together, there are many examples where AICD has been successfully triggered in normal preactivated T lymphocytes by mAbs against CD3 or TCR. However, one should keep in mind that the strength (and probably the quality) of the signal generated by anti-CD3/TCR mAb versus aitigenic peptide presented by appropriate

MHC molecules or by superantigens that ligate MHC molecules to certain TCR chains may be quite different (188, 189). Therefore, importantly, it has been well-documented that AICD can be initiated by TCR ligands that are naturally encountered by T lymphocytes including superantigens and conventional peptide antigens (reviewed in Ref. 189).

Although the exact molecular mechanism underlying the differential outcome of CD3 signaling (proliferative response or AICD) in individual populations are still not understood, mutational studies of the TCRζ chain ITAMs and othe functional studies clearly indicated that the principal signaling components include the respective chains of the TCR/CD3/ζ complex(190), the Src family PTK ($p56^{Lck}$ and $p59^{fyn}$)(191-193), ZAP-70 kinase(194), PKC-θ (195, 196), calcineurin (195, 196) and downstream signaling molecules (197). In this scenario, the non-receptor tyrosine kinases play a cruicial role in TCR/CD3 triggered AICD. It was demonstrated that AICD, in contrast to CD95 mAb-triggered death, strictly depends on PTK activity and can be blocked efficiently using inhibitors for Src kinases such as Herbimycin (191, 192). As has been suggested by a number of different in vitro and in vivo studies, the complex regulation of CD95L expression involves a whole bunch of molecules including Myc and Max (198), Nur77 (199, 200), Egr-3 (201), ALG-2 and ALG-3 (202), GILZ (203), NFAT (204, 205), RAR (206), NF-κB (207), RORγ1 (208) and many others. However, the mechanism of how these different gene products play in concert to regulate CD95L expression following antigen receptor stimulation are still poorly understood. The regulation of AICD and CD95L expression is further complicated by the fact that ligation of other (co-stimulatory) surface molecules such as CD4 (186, 187) or CD28 (118, 209) is able to modulate the TCR signal to abrogate AICD as do certain cytokines such as IL-6(210), IL-7 (211), TGF-β (212).

In addition to the above-discussed antigen-driven apoptosis of T cells (AICD), another major form of T cell apoptosis is induced by lymphokine withdrawl. Usually they are also called passive T cell apoptosis to distinguish from the antigen-driven, active T cell apoptosis. The active and passive forms of apoptosis have molecular differences. Passive apoptosis requires new protein synthesis, is strongly inhibitede by

Bcl-2 and related molecules, and may involve mitochondria apoptosis mechanisms rather than death cytokines (TNF, TRAIL, FasL etc). Active apoptosis requires TCR stimulation, and involves death cytokines such as TNF and FasL, is independent of new protein sysnthesis after death, and is inefficiently inhibited by Bcl-2. Passive apoptosis can be prevented by several T cell growth cytokines that all use the common gamma chain IL-2, IL-4, IL-7 and IL-15. Active apoptosis can occur at concentrations of these cytokines that cause proliferation, but IL-2 causes the greatest proliferation and susceptibility to active apoptosis (Reviewed in Ref. 213).

Mitochondria play an important role in the induction of apoptosis and in part also regulate CD95-induced apoptosis (214, 215). It is now clear that in response to apoptosis-inducing signals mitochondria release cytochrome c, which forms a complex with Apaf-1 and caspase 9 and catalyzes the activation of caspase 9 (216). Although the mitochondria have a regulatory role in the pathways induced by many apoptotic stimuli, their role in death receptor-induced apoptosis seems to depend on the cell type tested. In so-called type 1 cells, where sufficient amounts of caspase 8 are activated by CD95, mitochondria are not required and the caspase cascade can be triggered directly. In contrast, type 2 cells display only marginal initial caspase 8 activation and require the mitochondria to amplify the signal. Most studies in vitro suggest that T cells are type 1 cells, in which CD95-induced apoptosis is not blocked by overexpression of Bcl-X_L(215), Bcl-2 (217), or absence of the mediator of mitochondria-initiated apoptosis, i.e. Apaf-1 (218) or caspase-9.

V.2 The role of different apoptotic pathways in the regulation of T cell death in vivo

Although from the above discussion it seems clear that several apoptosis pathways (death receptor-mediated, lymphokine withdrawal-mediated) can operate during the process of T cell apoptosis, we should always remember that most of these data are obtained through in vitro studies, and the majority of them use anti-CD3/TCR antibodies to mimic the physiological stimulation (i.e. TCR triggering by pMHC on APCs) in vivo. Though as model system these studies have provided many useful information about the molecular signalling pathways involved in T cell apoptosis, the

in vivo significance of all these pathways need to be evaluated carefully using animal model systems. In deed, in vivo studies have provided many important informations and significantly improved our understanding of T cell death in vivo. In a study performed by Pircher and co-workers, it is demonstrated that transgenic mice that constitutively express high levels of the anti-apoptotic Bcl-X_L or Bcl-2 protein in their T cells display similar clonal downsizing after a viral infection as wild mice, though expression of Bcl-X_L or Bcl-2 does prevent LCMV peptide-induced peripheral deletion of mature CD8+ T cells in vivo and apoptosis of activated LCMV-specific effector T cells in vitro (219). These data suggest distinct apoptosis signaling pathways exist in CD8+ T cells, one can be inhibited by Bcl-X_L or Bcl-2 (probably involves mitochondria-mediated apoptotic pathway), and another one can not be blocked (probably involves death receptors-mediated pathway).

More importantly, several recent in vivo studies strongly suggest that withdrawal of lymphokines and AICD mediated by death receptors cannot account for all instances of T cell death. Experiments with mice deficient in Fas or FasL (220-224) or lacking both Fas and TNFR1 demonstrate that these molecules are not essential for the termination of specific immune responses (225, 226), suggesting the existence of active but death receptor-independent mechanisms of T cell death. Furthermore, superantigen-induced deletion of mature T lymphocytes in vivo does not require Fas or TNFR (227), and the development of transplantation tolerance under conditions of costimulatory blockade involves triggering and proliferation of specific T cells followed by their elimination in a Fas-independent manner (228, 229). However, in these two cases T cell activation does not involve the recognition of specific MHC-peptide ligands and does not lead to productive immune response and establishment of memory. It is not known whether death receptor-independent apoptotic programs can be directly initiated in mature T lymphocytes by recognition of MHC-peptide complexes on the surface of APCs. Therefore, we decided to explore this possibility in our model system based on EBV-specific A11-restircted CTL clones and analyze modulation of T cell apoptosis by the affinity of MHC/TCR interactions.

VI. Modulation of activation and apoptotic programs in mature T cells by variations in the affinity in MHC: TCR interaction

It has been well-documented in different independent systems that the altered, weaker TCR signal generated from TCR triggering with APLs correlates with either a lower TCR affinity or higher dissociation rate (and hence shorter half-life), causing a partial activation (89). For antagonists, it has been shown that they have a lower on-rate and higher off-rate compared with the original agonistic peptide (134). Therefore, it is reasonable to simply consider partial agonists or antagonists as low affinity ligands or kinetically unstable ligands for TCR, and superagonists as high affinity ligands.

The focus of my studies was to characterize the modulation of activation as well as apoptotic programs in mature T cells by TCR:pMHC affinity. As suggested by many studies, modulation of T cell activation can be achieved in two principally different ways: through structural modificantion of TCR ligand or by changing the context of TCR triggering by the same ligand. The first can be accomplished by introducing amino acid substitutions in either the specific peptide (90) or the relevant MHC molecule (230). This can result in the generation of functionally different TCR ligands refered to as partial agonists, antagonists, superagonists (90). The second phenomenon is observed when the same TCR ligand is presented to the specific T cell by different APCs (231, 232) or in different lymphokine melieu (233, 234). Our work has been concentrated on partially agonistic peptide ligands (partial agonists). In the literature, partial activation was shown to engage only some of the signal transduction pathways operating in the course of full scale T cell activation (235). Partially agonistic peptides can selectively induce cytolytic function of CTLs (236), Fas-mediated cytotoxicity (237, 238), early activation events without lymphokine production or cell proliferation (239), secretion of some lymphokines without detectable production of others (240, 241), as well as induce anergy in specific T cells (242-245).

In **Paper I** (246) we analyzed the effect of partially agonistic peptides on the activation and survival of CTL clones specific for a highly immunogenic HLA A11-

restricted peptide epitope derived from the EBV nuclear Ag-4, IVTDFSVIK (designated IVT). Several analogues with substitutions of TCR contact residues were able to trigger cytotoxic activity without induction of IL-2 mRNA and protein or T cell proliferation. Triggering with these partial agonists in the absence of exogenous IL-2 resulted in down-regulation of the cytotoxic potential of the specific CTLs. Analysis of early T cell activation events, induction of Ca^{2+} influx, and acid release did not reveal significant differences between the two types of analogue peptides.

Besides activation, apoptosis is another possible outcome of TCR engagement. In the same study, we found that one partially agonistic analogue selectively triggered apoptosis as efficiently as the original epitope, subdividing the partial agonists into apoptosis-inducing and non-inducing ligands. These results demonstrate that some partial agonists can dissociate the induction of CTL death from CTL activation.

It has been reported that some APLs can selectively trigger apoptosis in mouse $CD4^+$ T cells by expression of FasL and TNF mRNA without concomitantly inducing IL-2, IL-3 and IFN-γ production (92). These apoptosis-inducing ligands did not elicit a common pattern of tyrosine phoshorylation of the TCR-associated signal transduction chains, suggesting that they are low affinity TCR ligands. Interestingly, some other analogues do not induce cytokine production and apoptosis directly by themselves, but deliver " competence to die" signal. These partial agonists can regulate T cell deletion in vivo even when Fas or TNF is provided by T cells of unrelated specificity, but they do not cause the liver necrosis that is associated with T cell elimination by the full agonist (247). Thus, modulation of TCR affinity can lead to selective signalling and regulate T cell deletion and immune damage in vivo and may be important for peripheral tolerance.

In **Paper II** (248) we further characterized the apoptotic programs induced by the immunogenic peptide and its partially agonistic analogues in the IVT-specific CTL clones. Our major finding is that CTL triggering with partially agonistic peptide ligands can initiate death receptor dependent and independent programs in the effector cells. In contrast to classical AICD, death receptors are not essential for the elimination of CTLs activated with partially agonistic peptides. In addition, death

receptor independent apoptosis requires caspases other than caspase 3 and 8. Induction of anti-apoptotic Bcl-2 and Bcl-X_L expression is associated with resistance to this form of apoptosis. Also, IL-2 enhances classical and inhibits death receptor independent AICD. We concluded that TCR triggering not accompanied by IL-2 production may result in elimination of T-lymphocytes in death receptor independent manner. Our data demonstrated that engagement of TCR by MHC-peptide complexes could trigger diverse apoptotic programs of AICD and that the choice between these programs is determined by the agonistic potency of MHC-peptide ligand.

As a continuation, the molecular basis of different outcomes of CTLs stimulation with immunogenic and partially agonistic peptide ligands was analysed in **Paper III.** The role of MHC:peptide/TCR affinity in the regulation of T cell activation was characterized using tetramer technology. A panel of CTL clones, bulk CTL cultures and PBMC isolated from HLA A11-positive EBV-infected individuals were analysed for their ability to bind tetramer complexes assembled with either the immunogenic IVT peptide or the partially agonistic Y5 peptide, and tetramer binding was compared with the activity of the peptides in functional assays. Our results demonstrated that the A11 complexes assembled with the partial agonist dissociated from the surface of IVT-specific CTLs with a faster kinetics as compared with complexes containing the immunogenic peptide. We also showed that the efficiency of CTL recognition correlates with the stability of interaction between the specific TCR and MHC:peptide complex. Tetramer binding and secretion of INFγ were shown to be compatible with T-cell activation by partially agonistic peptides. Therefore, MHC:TCR interactions of low affinity may contribute to the results of T-cell monitoring performed with these assays.

As far as modulation of T cell activation and apoptosis by peptide lignads with higher affinity are concerned, there are few examples in the literature. Previously, various models have predicted that activation is limited to a narrow window of affinities (or dissociation rates) for the TCR-pMHC interaction and that above or below this window, T cells will fail to undergo activation. In a recent work by Holler et al., the authors examined the activity of a T cell that expresses a TCR with 300-fold greater affinity than even the highest affinity interaction ever studied, the 2C TCR and

QL9/L^d (K_D=3 μM). The study was made possible by using a mutant TCR from CTL 2C (K_D=10 nM) that was engineered for higher affinity by a process of yeast surface display and directed evolution. Their results show that despite a 300-fold higher affinity and a 45-fold longer off-rate compared with the wild-type TCR, T cells that expressed the mutant TCRs were activated by the peptide. In fact, activation could be detected at significantly lower peptide concentrations than with T cells that expressed the wild-type TCR. These findings indicate that: even with an affinity as high as 10 nM and a dissociation lifetime of 1,650 s, T cells exhibited enhanced, peptide-specific activity (249). This suggests that the kinetic or equilibrium "optimum" models probably need to be revised.

As for the modulation of T cell apoptosis by high affinity TCR ligands, Berzofsky's group has shown that following stimulation with a supraoptimal peptide-MHC, apoptotic death occurs in specific CTLs. The death is mediated by TNF and involves its binding to TNF-RII. However, although TNF and TNF-RII are necessary for induction of apoptotic death of CTL in this model, under usual conditions they are not sufficient. One finding of the study is that the supraoptimal TCR ligand decreased level of Bcl-2 protein in CTLs and thus renders them competent to die by exposure to TNF. These findings suggest that a decrease in Bcl-2 may account for the TNF-α-mediated apoptotic death triggered by supraoptimal peptide-MHC stimulation (250).

In conclusion, our results indicate that the affinity of TCR/MHC:peptide interaction determines the strength of TCR signalling, extent of CTL activation as well as the apoptotic pathway that operates in CTLs in the course of AICD. Our data defined a new alternative apoptotic pathway operating in T cells, which is TCR-triggered and death receptor-independent, openning new possibility for studying the molecular mechanisms regulating T cell apoptosis in vivo. It is conceivable that this type of analogues can play an important role in the modulation of anti-tumor or anti-viral CTL immune responses in vivo. In fact, naturally-occuring partially agonistic self peptide ligands, functionally similar to analogues characterized in our model system, have been found for MART-1-specific CTLs (251), although the apoptosis-inducing activity of these analogues have not been studied. It is also imaginable that immune responses against genetically unstable pathogens (such as HIV) could be abolished by

viral epitope variants with partially agonistic properties. In addition, partially agonistic peptide analogues may also allow the development of therapeutic approaches aiming to selectively down-regulate harmful manifestations of immunity such as autoimmune and allergic reactions or damage accompanying excessive immune responses against viruses and bacteria.

VII. The role of TCR/MHC affinity in the selection of the T cell repertoire

In addition to modulation of apoptosis and activation programs, TCR affinity has been reported to play a role in the selection of TCR repertoires in several studies (252-254). Therefore, we also studied the role of TCR affinity in this process.

Since the discovery of the genetic organization of the TCR genes, a large amount of data regarding the expressed TCR repertoire, in health and disease, has accumulated. By the use of monoclonal antibodies specific for V gene products, it was demonstrated that the level of expression is non-random (255). Furthermore, a biased usage of certain TCR Vα/β genes towards both CD4+ as well as CD8+ T cell subsets has been reported (255, 256). Moreover, among both healthy individuals and patients with various diseases, exceptionaly selective usage of certain TCR V genes has been found (257-261). In many instances, these expansions were demonstrated to be clonal or oligoclonal.

Evidence for restricted TCR usage has also been reported when the CDR3 region of TCR has been investigated. In particular, frequent oligoclonal populations are normally detected in CD8+ T cells as well as in many different chronic diseases (259, 262, 263). Most of our knowledge of TCR repertoire evolution after immunization comes from systems where a highly restricted T cell population responds to a dominant epitope. When antigen-driven T cell expansion was studied, analysis of TCR composition in response to dominant or sub-dominant epitopes reveals certain patterns of TCR usage. T cells of a given MHC class II-peptide complex specificity generally express TCRs that exhibit limited diversity (264-266). This limitation concerns in most cases both TCR α and TCR β chains, and for each chain both the V

and J segments and the amino acid composition of the junctional region. Interestingly, from different individuals T cells with identical TCR α and/or β chains have been isolated at a relatively high frequencies, suggesting a highly selective pressure for TCR repertoire shaping against certain epitopes. Similar levels of constraints on V gene usage and CDR3 diversity have been detected in MHC class I restricted responses (267-270).

Several mechanisms have been suggested to account for this phenomenon. First, Mariansky and Casanova proposed that endogenous peptides structurally related to the immunogenic epitope induce deletion of potentially peptide reactive T-cells thereby narrowing the specific TCR usage (271). Several studies provided indirect support for this model. However, it was later shown that, at least in some cases, deletion of a highly conserved TCR caused by cross-reactivity to self results in diversification of TCR usage by peptide specific T cells (272). Second, it was suggested that competition of clones with different TCR affinity for the specific peptide epitope could select for a very limited set of high affinity clones (267, 273, 274). Such a mechanism may be especially relevant for peptides presented on the surface of APCs at low copy numbers. Third, interclonal competition between T-cells specific for different peptides was suggested to account for the phenomenon of immunodominance and could also contribute to the selection of restricted TCR repertoires (275-278). Fourth, the process of positive selection in the thymus and subsequent shaping of the repertoire in the periphery have been shown to significantly affect the composition of TCR repertoire in mice (279). Therefore, restriction of TCR usage could also result from very efficient central or preferential selection of certain T-cell clones which accidentally express high affinity receptor for the immunogenic peptide in question. Finally, it is conceivable that structural constrains may account for the selection of restricted TCR repertoires in response to certain peptide epitopes. Although the compositions of TCR repertoires expressed by antigen specific T-cells have been analysed in a number of animal models as well as in healthy and diseased humans, there is no conclusive evidence as to which of the above mentioned mechanisms is the major driving force in the selection of restricted peptide specific TCR repertoires. Nevertheless, knowledge of such a mechanism would be important for our general understanding of T-cell selection and differentiation and may be also

relevant for vaccine design and monitoring of immunity because T-cell populations with restricted TCR repertoire may be more easily subverted by antigenic variation in the specific CTL epitope and less efficient in controlling genetically unstable microorganisms.

In our previous studies performed in vitro, we have demonstrated that CTL response specific to the HLA A11 restricted peptide epitope derived from the amino acid residues 399-408 (AVFDRKSDAK, referred thereafter as AVF-peptide) of the Epstein-Barr virus (EBV) nuclear antigen 4 (EBNA 4) exhibits an extraordinary conserved TCR usage characterized by selective expression of Vβ 3, one of the two structurally related Vβ chains and highly conserved structure of CDRs3 (267). In some A11 positive EBV carriers, the amino acid motifs and the length of the CDR3 of AVF-specific TCRs were identical. In contrast, a broad repertoire utilizing different combinations of TCR-α/β V and J segments and CDRs3 was detected both in any given individual and at the level of population in response to the immunodominant IVT epitope (EBNA4 416-424, IVTDFSVIK). In **paper IV**, we have investigated the restriction of TCR usage among AVF-specific CTL responses using tetramer technology. In agreement with these earlier findings, ex vivo analysis of AVF-specific CTLs using AVF-containing HLA A11 polymeric complexes revealed the same degree of conservation of the AVF-specific response both in healthy virus carriers and in the course of primary EBV infection. Tetramer binding and dissociation experiments performed with AVF-specific CTLs or CTLs expressing a very diverse set of TCRs and specific to another immunodominant A11-restricted EBV-derived peptide epitope did not support a model of affinity driven selection of restricted TCR repertoires. Characterization of individuals that fail to mount responses to the immunodominant A11-restricted CTL epitope but efficiently respond to the AVF-peptide argued against interclonal competition as the reason for the observed TCR conservation. Collectively, our data confirm the existence of naturally induced peptide-specific CTL responses with highly restricted TCR usage. Our data do not support a major role for affinity of MHC:TCR interaction in the selection of structurally conserved TCR-repertoires and suggest that specific structural features of the AVF-peptide may be responsible for the restricted TCR usage by AVF-specific CTLs

VIII. Conclusions

In conclusion, our results indicate that the affinity of TCR/MHC:peptide interaction determines the strength of TCR signalling, extent of CTL activation as well as the apoptotic pathways that operate in CTLs in the course of AICD. However, the affinity of TCR/MHC:peptide interaction does not appear to play a major role in the selection of restricted TCR repertoire in the model system analyzed in this study.

Acknowledgements

I would like to express my sincere gratitude to all those who have helped me during my Ph. D studies. Especially I would like to thank

My dear wife Daorong, for her love, understanding, tolerance, encouragement and care of my life ever since we married; my son Chunsheng Alexander Wei; I am so lucky to have you two, thank you for having brought tremendous happiness, joy and hope into my life. Also, my family in China, especially my father and mother, for your unconditional love, support and understainding all the time.

My supervisors-

Maria, for giving me the chance to start research work in your group since 1996. I must say that I am really proud of having worked in such a brilliant group. Thank you for all your support, help, trust, encouragement and very helpful discussion all these years. Your extreme dedication to science, wide expertise in both bench techniques and literature knowledge are excellent examples for my future career. Also, many thanks to you for your warm help during Daorong's pregnancy and after the birth of my son (by the way, you picked the English name Alexander for him). In addition, I will always remember all those happy parties in your place where food, wine, music and entertainments were so good. Gracia!!

Victor, for your direct supervision over my experiments all these years, for teaching me all the basic techniques from the beginning, and sharing with me your wide expertise in many fields, not only in T cell biology or immunology, but also in biochemistry and virology. Your extremely helpful discussion always guides me out of difficult situations during experiments. Without your guidance and help, all the work in this thesis would have been impossible. Your help did not stop there. Thank you for picking Daorong from Arlanda in spring 1998 when I had to took an exam in a course, and many other help when I had difficulties in study or life. Also, thank you for all the happy time when we had dinner together either at your place or in restaurants (I still remember the flavour of the real Russian caviar and mushroom soup). Spacibo!!

It is also obligatory for me to say thank you to George, for inviting me to Sweden in 1996, for keeping an interest in my work even if I did not work in the field of tumor biology, and also for wrting a very strong recommendation letter for me when I applied for postdoc positions in the states. Your never-ending enthusiam to science, your broad expertise in science and persistent pursuit in scientific truth have been and will continue to be my spiritual lamp in my scientific career.

Many thanks also to all the former and present members of the Maria group for kindness and help: Pedro Otavio for great technical tips when I just joined the group, for discussion about life and jokes, for taking me as your friend; Jelena Levitskaya for making the lab a warm place at that time, for kind help with experiments and for being a good friend in my difficult time in the beginning of my study; Terisita, for always being kind and ready for help, for teaching me Italian and learning Chinese from me, for true friendship all the time; LiQi for always being a good and warm-hearted friend, for sharing happy and unhappy time during study life, for happy chatting in Chinese, and for delicious Chinese food and nice party at your place; Anatoly for understanding, for sharing with me your scientific knowledge and wisdom of life, and for friendship; Piri for always being helpful and kind to me, for sharing reagents and great help during application for thesis defence; Farhat for making our lifves colourful during your one-year stay in the group; Kristina Lindsten for many kind help including translation of Swedish and many others, for visiting us in the Karolinska Hospital (together with Nico, Stijn, to whom I would like to say thank you very much too here) after Alexander was born, for knitting the blanket for him(together with Anna, Xu Hong, Jelena petrovich, Simona, to whom I also owe " Many thanks ") , and above all for friendship, tack så mycket!! Nico for teaching me how to play squash, how to use the fluorometer and fluoromicroscope, for nice talks late in the evening in the lab when he just arrived in Sweden, and for sharing with me his terrific beer in a party at his place; Ainars for help with experimental techniques, for playing football together and for your patient and valuable help with my fighting with computers during thesis writing; Anna for friendship, for detailed description about California especially San Diego, for very helpful advice when I was applying postdoc positions, for sharing protocols and reagents, and for nice chat about life and

future; Stijn for playing football together, for pleasant chat when we were drinking beer together at MTC pub after playing football, and for sharing reagents, protocols; Many thanks particularly to the member of the Levitsky group: Elena, Mikael and Andre for understanding, help in the lab, sharing reagents and protocols, nice scientific and non-scientific discussions, and for being friends; Mariane for all the help with ordering reagents, translating Swedish and many other help and kindness; Yvonne for help with arranging the tickets, reimbursement during my trip to Keystone and other help, and for the hand-made hat for Alexander; Victoria for teaching me a little Spanish and learning Chinese from me, for interesting talk in the lunchroom and for the nice dinner at your place; Vaya for being friend, and also the tasteful sweets you brought from Greek; Lisette for pleasant talk about my son and your nephew, for understanding and for your liking of Alexander; Iris for pleasant talks about your children and my son, nice chat in the lunch room and for kindly bring marker pens, folders from the " white house" for me; Sara for many nice, friendly chat in the lunch room; Anders for sharing with me your expertise in virology and T cell activation.

Former and current prefect of MTC, Professor Ingemar Ernberg and Professor Klas Kärre, for making MTC a very pleasant place to work in. Professor Mikael Jondal, Professor Klas Kärre for valuable time and very helpful suggestions during my half-time control, and also Prof. Peter Liljeström for valuable time and kindly being my subject examiner, I benefited a lot from reading the books you recommended.

All co-authors in my publications or submitted manuscript (included in this thesis or not): Craig Beeson (University of Washington), Hideo Yagita (Jutendo University School of Medicine), Mikael Uhlin, Jeff Alexander and Alessandro Sette (Epimmune, San Diego), Kristin Hallermalm and Jelena Levitskaya (CCK), Farideh Sabril.

All my Chinese friends in Stockholm, especially Xu Yunhe and Wu Weiping, Wang Hong and Chen Rong, LiQi Li and Guosheng Wu, Xu Hong and Ma Zuheng, Hu Hu, Bian Zhao and Zheng Li, Shi Yu and He Zhong, Wei Yudan, for friendship, understanding, encouragement and many kind help. You have made my life outside science much happier and enjoyable. Thank you so very much to you all!!

Immunologists in the MTC who have helped me in different ways, either sharing

reagenrts or their knowledge and expertise. People form Hans-Gustav's group: Benedict Chambers, Robert Wallin, Erica for advice on mouse Vβ staining, Rickard Glas for protocol on mouse T cell expansion and culture, Jelena for advice on mouse T cell stimulation; People from Klas Kärre group: Jacob Mickaelson for sharing reagents, his expertise in tetramer and FACS on mouse spleenocyte; Sofia and Håkan for sharing their protocol of intracellular staining of IFN-γ for mouse splenocytes, and Peter himself for protocols for mouse NK cell activation, and some other technical help. Noemy in Eva's group, for sharing reagents, protocols and particularly for pleasant chat on bus 507 and in the corridor. Adanane Achour for funny jokes and infecting me with his happiness in the corridor or elevators.

All the Chinese colleagues in MTC whom I got acquainted here all these years, Tao Wen, Qian Wang, LiFu Hu, Jiezhi Zou, Zhao Bian, Fu Chen, Yintong Xue, Gu Xiaogang, Liying Chen, Wang Zhaohui, Xiangning Zhang, Qin Lizeng, Li Yinghua, and Anquan Liu, for all the understanding and support.

My former supervisors and teachers in my home university, Tongji Medical University, in Wuhan, China: Professor Zhien Wu, Associate Professor Zhang Minxuan, Professor He Yehua, and many other teachers who have helped me extablish the basis of my medical knowledge and scientific research.

My best friends in China, particularly Tang Zhouping, Peng Xiaodong, Liang Jinjun and Zhou Jianlin, for all the valuable support and help. My good friends in the United States, Jun Yang, Xia Bu and Xiaoyu Li, Yong Lin and Yan Zhang, Weizheng Wei and Quan Li, Shujuan Chen, for long-lasting friendship, encouragement and kind help.

References

1. Janeway, C.A., P. Travers, M. Walport, and J.D. Capra. 1999. Immunobiology: the immune system in health and disease. 4th ed. Current Biology Publications, London.

2. Banyer, J.L., N.H. Hamilton, I.A. Ramshaw, and A.J. Ramsay. 2000. Cytokines in innate and adaptive immunity. *Rev. Immunogenet.* 2, no. 3:359-373.

3. Lo, D., L. Feng, L. Li, M.J. Carson, M. Crowley, M. Pauza, A. Nguyen, and C.R. Reilly. 1999. Integrating innate and adaptive immunity in the whole animal. *Immunol. Rev.* 169:225-239.

4. Davis, M.M., and P.J. Bjorkman. 1988. T-cell antigen receptor genes and T-cell recognition. *Nature* 334, no. 6181:395-402.

5. Goulder, P.J., R.E. Phillips, R.A. Colbert, S. McAdam, G. Ogg, M.A. Nowak, P. Giangrande, G. Luzzi, B. Morgan, A. Edwards, A.J. McMichael, and S. Rowland-Jones. 1997. Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. *Nat. Med.* 3, no. 2:212-217.

6. Rowland-Jones, S., T. Dong, P. Krausa, J. Sutton, H. Newell, K. Ariyoshi, F. Gotch, S. Sabally, T. Corrah, J. Kimani, K. MacDonald, F. Plummer, J. Ndinya-Achola, H. Whittle, and A. McMichael. 1998. The role of cytotoxic T-cells in HIV infection. *Dev. Biol. Stand.* 92:209-214.

7. Wilson, J.D., G.S. Ogg, R.L. Allen, C. Davis, S. Shaunak, J. Downie, W. Dyer, C. Workman, S. Sullivan, A.J. McMichael, and S.L. Rowland-Jones. 2000. Direct visualization of HIV-1-specific cytotoxic T lymphocytes during primary infection. *Aids* 14, no. 3:225-233.

8. Kaul, R., F.A. Plummer, J. Kimani, T. Dong, P. Kiama, T. Rostron, E. Njagi, K.S. MacDonald, J.J. Bwayo, A.J. McMichael, and S.L. Rowland-Jones. 2000. HIV-1-specific mucosal CD8+ lymphocyte responses in the cervix of HIV-1-resistant prostitutes in Nairobi. *J. Immunol.* 164, no. 3:1602-1611.

9. Rowland-Jones, S.L., T. Dong, L. Dorrell, G. Ogg, P. Hansasuta, P. Krausa, J. Kimani, S. Sabally, K. Ariyoshi, J. Oyugi, K.S. MacDonald, J. Bwayo, H. Whittle, F.A. Plummer, and A.J. McMichael. 1999. Broadly cross-reactive HIV-specific

cytotoxic T-lymphocytes in highly-exposed persistently seronegative donors. *Immunol. Lett.* 66, no. 1-3:9-14.

10. Rowland-Jones, S.L., T. Dong, K.R. Fowke, J. Kimani, P. Krausa, H. Newell, T. Blanchard, K. Ariyoshi, J. Oyugi, E. Ngugi, J. Bwayo, K.S. MacDonald, A.J. McMichael, and F.A. Plummer. 1998. Cytotoxic T cell responses to multiple conserved HIV epitopes in HIV-resistant prostitutes in Nairobi. *J. Clin. Invest.* 102, no. 9:1758-1765.

11. Rowland-Jones, S.L., S. Pinheiro, R. Kaul, P. Hansasuta, G. Gillespie, T. Dong, F.A. Plummer, J.B. Bwayo, S. Fidler, J. Weber, A. McMichael, and V. Appay. 2001. How important is the 'quality' of the cytotoxic T lymphocyte (CTL) response in protection against HIV infection? *Immunol. Lett.* 79, no. 1-2:15-20.

12. Rickinson, A.B., and D.J. Moss. 1997. Human cytotoxic T lymphocyte responses to Epstein-Barr virus infection. *Annu. Rev. Immunol.* 15:405-431.

13. Heslop, H.E., M. Perez, E. Benaim, R. Rochester, M.K. Brenner, and C.M. Rooney. 1999. Transfer of EBV-specific CTL to prevent EBV lymphoma post bone marrow transplant. *J. Clin. Apheresis.* 14, no. 3:154-156.

14. Gustafsson, A., V. Levitsky, J.Z. Zou, T. Frisan, T. Dalianis, P. Ljungman, O. Ringden, J. Winiarski, I. Ernberg, and M.G. Masucci. 2000. Epstein-Barr virus (EBV) load in bone marrow transplant recipients at risk to develop posttransplant lymphoproliferative disease: prophylactic infusion of EBV-specific cytotoxic T cells. *Blood* 95, no. 3:807-14.

15. Gahn, B., G. Hunt, C.M. Rooney, and H.E. Heslop. 2002. Immunotherapy to reconstitute immunity to DNA viruses. *Semin. Hematol.* 39, no. 1:41-47.

16. Chen, F.E., G. Aubert, P. Travers, I.A. Dodi, and J.A. Madrigal. 2002. HLA tetramers and anti-CMV immune responses: from epitope to immunotherapy. *Cytotherapy* 4, no. 1:41-48.

17. Riddell, S.R., K.S. Watanabe, J.M. Goodrich, C.R. Li, M.E. Agha, and P.D. Greenberg. 1992. Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. *Science* 257, no. 5067:238-241.

18. Walter, E.A., P.D. Greenberg, M.J. Gilbert, R.J. Finch, K.S. Watanabe, E.D. Thomas, and S.R. Riddell. 1995. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N. Engl. J. Med.* 333, no. 16:1038-44.

19. Zaia, J.A., J.G. Sissons, S. Riddell, D.J. Diamond, M.R. Wills, A.J. Carmichael, M.P. Weekes, M. Gandhi, C.L. Rosa, M. Villacres, S. Lacey, S. Markel, and J. Sun. 2000. Status of Cytomegalovirus Prevention and Treatment in 2000. *Hematology (Am Soc Hematol Educ Program)*:339-355.

20. Bertoletti, A., and M.K. Maini. 2000. Protection or damage: a dual role for the virus-specific cytotoxic T lymphocyte response in hepatitis B and C infection? *Curr. Opin. Microbiol.* 3, no. 4:387-392.

21. Singhal, S., J.C. Shaw, J. Ainsworth, M. Hathaway, G.M. Gillespie, H. Paris, K. Ward, D. Pillay, P.A. Moss, and D.J. Mutimer. 2000. Direct visualization and quantitation of cytomegalovirus-specific CD8+ cytotoxic T-lymphocytes in liver transplant patients. *Transplantation* 69, no. 11:2251-2259.

22. Gillespie, G.M., M.R. Wills, V. Appay, C. O'Callaghan, M. Murphy, N. Smith, P. Sissons, S. Rowland-Jones, J.I. Bell, and P.A. Moss. 2000. Functional heterogeneity and high frequencies of cytomegalovirus-specific CD8(+) T lymphocytes in healthy seropositive donors. *J. Virol.* 74, no. 17:8140-8150.

23. Mullins, D.W., T.N. Bullock, T.A. Colella, V.V. Robila, and V.H. Engelhard. 2001. Immune responses to the HLA-A*0201-restricted epitopes of tyrosinase and glycoprotein 100 enable control of melanoma outgrowth in HLA-A*0201-transgenic mice. *J. Immunol.* 167, no. 9:4853-4860.

24. Yamshchikov, G., L. Thompson, W.G. Ross, H. Galavotti, W. Aquila, D. Deacon, J. Caldwell, J.W. Patterson, D.F. Hunt, and C.L. Slingluff, Jr. 2001. Analysis of a natural immune response against tumor antigens in a melanoma survivor: lessons applicable to clinical trial evaluations. *Clin. Cancer Res.* 7, no. 3 Suppl:909s-916s.

25. Romero, P., P.R. Dunbar, D. Valmori, M. Pittet, G.S. Ogg, D. Rimoldi, J.L. Chen, D. Lienard, J.C. Cerottini, and V. Cerundolo. 1998. Ex vivo staining of metastatic lymph nodes by class I major histocompatibility complex tetramers reveals high numbers of antigen-experienced tumor-specific cytolytic T lymphocytes. *J. Exp. Med.* 188, no. 9:1641-1650.

26. Gottschalk, S., H.E. Heslop, and C.M. Roon. 2002. Treatment of Epstein-Barr virus-associated malignancies with specific T cells. *Adv. Cancer. Res.* 84:175-201.

27. Liu, Z., B. Savoldo, H. Huls, T. Lopez, A. Gee, J. Wilson, M.K. Brenner, H.E. Heslop, and C.M. Rooney. 2002. Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes for the prevention and treatment of EBV-associated post-transplant

lymphomas. *Recent Results Cancer Res.* 159:123-133.

28. Haque, T., C. Taylor, G.M. Wilkie, P. Murad, P.L. Amlot, S. Beath, P.J. McKiernan, and D.H. Crawford. 2001. Complete regression of posttransplant lymphoproliferative disease using partially HLA-matched Epstein Barr virus-specific cytotoxic T cells. *Transplantation* 72, no. 8:1399-1402.

29. Savoldo, B., H.E. Heslop, and C.M. Rooney. 2000. The use of cytotoxic t cells for the prevention and treatment of epstein-barr virus induced lymphoma in transplant recipients. *Leuk. Lymphoma* 39, no. 5-6:455-464.

30. Yewdell, J., L.C. Anton, I. Bacik, U. Schubert, H.L. Snyder, and J.R. Bennink. 1999. Generating MHC class I ligands from viral gene products. *Immunol. Rev.* 172:97-108.

31. Niedermann, G., E. Geier, M. Lucchiari-Hartz, N. Hitziger, A. Ramsperger, and K. Eichmann. 1999. The specificity of proteasomes: impact on MHC class I processing and presentation of antigens. *Immunol. Rev.* 172:29-48.

32. Watts, C., and S. Powis. 1999. Pathways of antigen processing and presentation. *Rev. Immunogenet.* 1, no. 1:60-74.

33. Smyth, M.J., J.M. Kelly, V.R. Sutton, J.E. Davis, K.A. Browne, T.J. Sayers, and J.A. Trapani. 2001. Unlocking the secrets of cytotoxic granule proteins. *J. Leukoc. Biol.* 70, no. 1:18-29.

34. Barry, M., J.A. Heibein, M.J. Pinkoski, S.F. Lee, R.W. Moyer, D.R. Green, and R.C. Bleackley. 2000. Granzyme B short-circuits the need for caspase 8 activity during granule-mediated cytotoxic T-lymphocyte killing by directly cleaving Bid. *Mol. Cell. Biol.* 20, no. 11:3781-3794.

35. Hayashida, M., H. Kawano, T. Nakano, K. Shiraki, and A. Suzuki. 2000. Cell death induction by CTL: perforin/granzyme B system dominantly acts for cell death induction in human hepatocellular carcinoma cells. *Proc. Soc. Exp. Biol. Med.* 225, no. 2:143-150.

36. Hishii, M., J.T. Kurnick, T. Ramirez-Montagut, and F. Pandolfi. 1999. Studies of the mechanism of cytolysis by tumour-infiltrating lymphocytes. *Clin. Exp. Immunol.* 116, no. 3:388-394.

37. Yasukawa, M., H. Ohminami, J. Arai, Y. Kasahara, Y. Ishida, and S. Fujita. 2000. Granule exocytosis, and not the fas/fas ligand system, is the main pathway of cytotoxicity mediated by alloantigen-specific CD4(+) as well as CD8(+) cytotoxic T

lymphocytes in humans. *Blood* 95, no. 7:2352-2355.

38. Nagata, S., and P. Golstein. 1995. The Fas death factor. *Science* 267, no. 5203:1449-1456.

39. Parham, P. 1999. Virtual reality in the MHC. *Immunol. Rev.* 167:5-15.

40. Campbell, R.D., and J. Trowsdale. 1993. Map of the human MHC. *Immunol. Today* 14, no. 7:349-352.

41. Parham, P., E.J. Adams, and K.L. Arnett. 1995. The origins of HLA-A,B,C polymorphism. *Immunol. Rev.* 143:141-180.

42. Trowsdale, J., I. Hanson, I. Mockridge, S. Beck, A. Townsend, and A. Kelly. 1990. Sequences encoded in the class II region of the MHC related to the 'ABC' superfamily of transporters. *Nature* 348, no. 6303:741-744.

43. Abbas, A.K., A.H. Lightman, and J.S. Pober. 2000. Cellular and molecular immunology. 4th ed. W. B. Saunders Company, Philadelphia.

44. Tamada, K., and L. Chen. 2000. T lymphocyte costimulatory molecules in host defense and immunologic diseases. *Ann. Allergy Asthma Immunol.* 85, no. 3:164-175; quiz 175-177.

45. Harris, N.L., and F. Ronchese. 1999. The role of B7 costimulation in T-cell immunity. *Immunol. Cell. Biol.* 77, no. 4:304-311.

46. Braud, V.M., D.S. Allan, and A.J. McMichael. 1999. Functions of nonclassical MHC and non-MHC-encoded class I molecules. *Curr. Opin. Immunol.* 11, no. 1:100-108.

47. Pamer, E., and P. Cresswell. 1998. Mechanisms of MHC class I--restricted antigen processing. *Annu. Rev. Immunol.* 16:323-358.

48. Solheim, J.C. 1999. Class I MHC molecules: assembly and antigen presentation. *Immunol. Rev.* 172:11-19.

49. Michalek, M.T., E.P. Grant, C. Gramm, A.L. Goldberg, and K.L. Rock. 1993. A role for the ubiquitin-dependent proteolytic pathway in MHC class I-restricted antigen presentation. *Nature* 363, no. 6429:552-554.

50. Rock, K.L., C. Gramm, L. Rothstein, K. Clark, R. Stein, L. Dick, D. Hwang, and A.L. Goldberg. 1994. Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. *Cell* 78, no. 5:761-771.

51. Lupas, A., A.J. Koster, and W. Baumeister. 1993. Structural features of 26S

and 20S proteasomes. *Enzyme Protein* 47, no. 4-6:252-273.

52. Cardozo, C. 1993. Catalytic components of the bovine pituitary multicatalytic proteinase complex (proteasome). *Enzyme Protein* 47, no. 4-6:296-305.

53. Cardozo, C., and R.A. Kohanski. 1998. Altered properties of the branched chain amino acid-preferring activity contribute to increased cleavages after branched chain residues by the "immunoproteasome&quot. *J. Biol. Chem.* 273, no. 27:16764-16770.

54. Driscoll, J., M.G. Brown, D. Finley, and J.J. Monaco. 1993. MHC-linked LMP gene products specifically alter peptidase activities of the proteasome. *Nature* 365, no. 6443:262-264.

55. Rammensee, H.G., K. Falk, and O. Rotzschke. 1993. Peptides naturally presented by MHC class I molecules. *Annu. Rev. Immunol.* 11:213-244.

56. Heemels, M.T., T.N. Schumacher, K. Wonigeit, and H.L. Ploegh. 1993. Peptide translocation by variants of the transporter associated with antigen processing. *Science* 262, no. 5142:2059-2063.

57. Schumacher, T.N., D.V. Kantesaria, M.T. Heemels, P.G. Ashton-Rickardt, J.C. Shepherd, K. Fruh, Y. Yang, P.A. Peterson, S. Tonegawa, and H.L. Ploegh. 1994. Peptide length and sequence specificity of the mouse TAP1/TAP2 translocator. *J. Exp. Med.* 179, no. 2:533-540.

58. Momburg, F., J. Roelse, G.J. Hammerling, and J.J. Neefjes. 1994. Peptide size selection by the major histocompatibility complex-encoded peptide transporter. *J. Exp. Med.* 179, no. 5:1613-1623.

59. Momburg, F., J. Roelse, J.C. Howard, G.W. Butcher, G.J. Hammerling, and J.J. Neefjes. 1994. Selectivity of MHC-encoded peptide transporters from human, mouse and rat. *Nature* 367, no. 6464:648-651.

60. Androlewicz, M.J., and P. Cresswell. 1994. Human transporters associated with antigen processing possess a promiscuous peptide-binding site. *Immunity* 1, no. 1:7-14.

61. van Endert, P.M. 1999. Genes regulating MHC class I processing of antigen. *Curr. Opin. Immunol.* 11, no. 1:82-88.

62. Rammensee, H.G., T. Friede, and S. Stevanoviic. 1995. MHC ligands and peptide motifs: first listing. *Immunogenetics* 41, no. 4:178-228.

63. den Haan, J.M., and M.J. Bevan. 2001. Antigen presentation to CD8+ T cells:

cross-priming in infectious diseases. *Curr. Opin. Immunol.* 13, no. 4:437-441.

64. Heath, W.R., and F.R. Carbone. 2001. Cross-presentation, dendritic cells, tolerance and immunity. *Annu. Rev. Immunol.* 19:47-64.

65. Heath, W.R., and F.R. Carbone. 1999. Cytotoxic T lymphocyte activation by cross-priming. *Curr. Opin. Immunol.* 11, no. 3:314-318.

66. Bjorkman, P.J., M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, and D.C. Wiley. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 329, no. 6139:506-512.

67. Bjorkman, P.J., M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, and D.C. Wiley. 1987. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature* 329, no. 6139:512-518.

68. Madden, D.R. 1995. The three-dimensional structure of peptide-MHC complexes. *Annu. Rev. Immunol.* 13:587-622.

69. Young, A.C., S.G. Nathenson, and J.C. Sacchettini. 1995. Structural studies of class I major histocompatibility complex proteins: insights into antigen presentation. *Faseb. J.* 9, no. 1:26-36.

70. Saper, M.A., P.J. Bjorkman, and D.C. Wiley. 1991. Refined structure of the human histocompatibility antigen HLA-A2 at 2.6 A resolution. *J. Mol. Biol.* 219, no. 2:277-319.

71. Moroy, T., and H. Karsunky. 2000. Regulation of pre-T-cell development. *Cell. Mol. Life. Sci.* 57, no. 6:957-975.

72. Nemazee, D. 2000. Receptor selection in B and T lymphocytes. *Annu. Rev. Immunol.* 18:19-51.

73. Grawunder, U., R.B. West, and M.R. Lieber. 1998. Antigen receptor gene rearrangement. *Curr. Opin. Immunol.* 10, no. 2:172-180.

74. Krangel, M.S., C. Hernandez-Munain, P. Lauzurica, M. McMurry, J.L. Roberts, and X.P. Zhong. 1998. Developmental regulation of V(D)J recombination at the TCR alpha/delta locus. *Immunol. Rev.* 165:131-147.

75. Willerford, D.M., W. Swat, and F.W. Alt. 1996. Developmental regulation of V(D)J recombination and lymphocyte differentiation. *Curr. Opin. Genet. Dev.* 6, no. 5:603-609.

76. Chothia, C., D.R. Boswell, and A.M. Lesk. 1988. The outline structure of the T-cell alpha beta receptor. *EMBO J.* 7, no. 12:3745-3755.

77. Hedrick, S.M., and L.L. Sharp. 1998. T-cell fate. *Immunol. Rev.* 165:95-110.

78. Saito, T., and N. Watanabe. 1998. Positive and negative thymocyte selection. *Crit. Rev. Immunol.* 18, no. 4:359-370.

79. Griesser, H., and T.W. Mak. 1994. The T-cell receptor--structure, function, and clinical application. *Hematol Pathol* 8, no. 1-2:1-23.

80. Exley, M., C. Terhorst, and T. Wileman. 1991. Structure, assembly and intracellular transport of the T cell receptor for antigen. *Semin. Immunol.* 3, no. 5:283-297.

81. Wange, R.L., and L.E. Samelson. 1996. Complex complexes: signaling at the TCR. *Immunity* 5, no. 3:197-205.

82. Ding, Y.H., K.J. Smith, D.N. Garboczi, U. Utz, W.E. Biddison, and D.C. Wiley. 1998. Two human T cell receptors bind in a similar diagonal mode to the HLA-A2/Tax peptide complex using different TCR amino acids. *Immunity* 8, no. 4:403-411.

83. Garboczi, D.N., P. Ghosh, U. Utz, Q.R. Fan, W.E. Biddison, and D.C. Wiley. 1996. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. *Nature* 384, no. 6605:134-141.

84. Garcia, K.C., M. Degano, L.R. Pease, M. Huang, P.A. Peterson, L. Teyton, and I.A. Wilson. 1998. Structural basis of plasticity in T cell receptor recognition of a self peptide-MHC antigen. *Science* 279, no. 5354:1166-1172.

85. Garcia, K.C., M. Degano, R.L. Stanfield, A. Brunmark, M.R. Jackson, P.A. Peterson, L. Teyton, and I.A. Wilson. 1996. An alphabeta T cell receptor structure at 2.5 A and its orientation in the TCR-MHC complex. *Science* 274, no. 5285:209-219.

86. Rudolph, M.G., and I.A. Wilson. 2002. The specificity of TCR/pMHC interaction. *Curr. Opin. Immunol.* 14, no. 1:52-65.

87. Segel, I.H. 1976. Biochemical calculations: How to solve mathematical problems in general biochemistry. 2nd ed. John Wiley & Sons, Inc., New York.

88. Eisen, H.N., Y. Sykulev, and T.J. Tsomides. 1996. Antigen-specific T-cell receptors and their reactions with complexes formed by peptides with major histocompatibility complex proteins. *Adv. Protein. Chem.* 49:1-56.

89. Davis, M.M., J.J. Boniface, Z. Reich, D. Lyons, J. Hampl, B. Arden, and Y. Chien. 1998. Ligand recognition by alpha beta T cell receptors. *Annu. Rev. Immunol.* 16:523-544.

90. Sloan-Lancaster, J., and P.M. Allen. 1996. Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology. *Annu. Rev. Immunol.* 14:1-27.

91. Germain, R.N., and I. Stefanova. 1999. The dynamics of T cell receptor signaling: complex orchestration and the key roles of tempo and cooperation. *Annu. Rev. Immunol.* 17:467-522.

92. Combadiere, B., C.R. e Sousa, R.N. Germain, and M.J. Lenardo. 1998. Selective induction of apoptosis in mature T lymphocytes by variant T cell receptor ligands. *J. Exp. Med.* 187, no. 3:349-355.

93. Samelson, L.E., A.F. Phillips, E.T. Luong, and R.D. Klausner. 1990. Association of the fyn protein-tyrosine kinase with the T-cell antigen receptor. *Proc. Natl. Acad. Sci. U. S. A.* 87, no. 11:4358-4362.

94. Straus, D.B., and A. Weiss. 1992. Genetic evidence for the involvement of the lck tyrosine kinase in signal transduction through the T cell antigen receptor. *Cell* 70, no. 4:585-593.

95. Chan, A.C., M. Dalton, R. Johnson, G.H. Kong, T. Wang, R. Thoma, and T. Kurosaki. 1995. Activation of ZAP-70 kinase activity by phosphorylation of tyrosine 493 is required for lymphocyte antigen receptor function. *EMBO J.* 14, no. 11:2499-2508.

96. Iwashima, M., B.A. Irving, N.S. van Oers, A.C. Chan, and A. Weiss. 1994. Sequential interactions of the TCR with two distinct cytoplasmic tyrosine kinases. *Science* 263, no. 5150:1136-1139.

97. Kong, G., M. Dalton, J.B. Wardenburg, D. Straus, T. Kurosaki, and A.C. Chan. 1996. Distinct tyrosine phosphorylation sites in ZAP-70 mediate activation and negative regulation of antigen receptor function. *Mol. Cell. Biol.* 16, no. 9:5026-5035.

98. Schaeffer, E.M., J. Debnath, G. Yap, D. McVicar, X.C. Liao, D.R. Littman, A. Sher, H.E. Varmus, M.J. Lenardo, and P.L. Schwartzberg. 1999. Requirement for Tec kinases Rlk and Itk in T cell receptor signaling and immunity. *Science* 284, no. 5414:638-641.

99. Zenner, G., T. Vorherr, T. Mustelin, and P. Burn. 1996. Differential and multiple binding of signal transducing molecules to the ITAMs of the TCR-zeta chain. *J. Cell. Biochem.* 63, no. 1:94-103.

100. Cloutier, J.F., and A. Veillette. 1999. Cooperative inhibition of T-cell antigen

receptor signaling by a complex between a kinase and a phosphatase. *J. Exp. Med.* 189, no. 1:111-121.

101. Schmedt, C., K. Saijo, T. Niidome, R. Kuhn, S. Aizawa, and A. Tarakhovsky. 1998. Csk controls antigen receptor-mediated development and selection of T-lineage cells. *Nature* 394, no. 6696:901-904.

102. Kersh, E.N., A.S. Shaw, and P.M. Allen. 1998. Fidelity of T cell activation through multistep T cell receptor zeta phosphorylation. *Science* 281, no. 5376:572-575.

103. Osman, N., S.C. Lucas, H. Turner, and D. Cantrell. 1995. A comparison of the interaction of Shc and the tyrosine kinase ZAP-70 with the T cell antigen receptor zeta chain tyrosine-based activation motif. *J. Biol. Chem.* 270, no. 23:13981-13986.

104. Koyasu, S., A.G. Tse, P. Moingeon, R.E. Hussey, A. Mildonian, J. Hannisian, L.K. Clayton, and E.L. Reinherz. 1994. Delineation of a T-cell activation motif required for binding of protein tyrosine kinases containing tandem SH2 domains. *Proc. Natl. Acad. Sci. U. S. A.* 91, no. 14:6693-7.

105. Wange, R.L., R. Guitian, N. Isakov, J.D. Watts, R. Aebersold, and L.E. Samelson. 1995. Activating and inhibitory mutations in adjacent tyrosines in the kinase domain of ZAP-70. *J. Biol. Chem.* 270, no. 32:18730-18733.

106. Zhang, W., J. Sloan-Lancaster, J. Kitchen, R.P. Trible, and L.E. Samelson. 1998. LAT: the ZAP-70 tyrosine kinase substrate that links T cell receptor to cellular activation. *Cell* 92, no. 1:83-92.

107. Peterson, E.J., J.L. Clements, N. Fang, and G.A. Koretzky. 1998. Adaptor proteins in lymphocyte antigen-receptor signaling. *Curr Opin Immunol* 10, no. 3:337-44.

108. Williams, B.L., K.L. Schreiber, W. Zhang, R.L. Wange, L.E. Samelson, P.J. Leibson, and R.T. Abraham. 1998. Genetic evidence for differential coupling of Syk family kinases to the T-cell receptor: reconstitution studies in a ZAP-70-deficient Jurkat T-cell line. *Mol. Cell. Biol.* 18, no. 3:1388-1399.

109. Finco, T.S., T. Kadlecek, W. Zhang, L.E. Samelson, and A. Weiss. 1998. LAT is required for TCR-mediated activation of PLCgamma1 and the Ras pathway. *Immunity* 9, no. 5:617-626.

110. Williams, B.L., B.J. Irvin, S.L. Sutor, C.C. Chini, E. Yacyshyn, J. Bubeck Wardenburg, M. Dalton, A.C. Chan, and R.T. Abraham. 1999. Phosphorylation of

Tyr319 in ZAP-70 is required for T-cell antigen receptor-dependent phospholipase C-gamma1 and Ras activation. *EMBO J.* 18, no. 7:1832-1844.

111. Lee, S.B., and S.G. Rhee. 1995. Significance of PIP2 hydrolysis and regulation of phospholipase C isozymes. *Curr. Opin. Cell. Biol.* 7, no. 2:183-189.

112. Guse, A.H. 1998. Ca2+ signaling in T-lymphocytes. *Crit. Rev. Immunol.* 18, no. 5:419-448.

113. Cantrell, D. 1996. T cell antigen receptor signal transduction pathways. *Annu. Rev. Immunol.* 14:259-274.

114. Rincon, M. 2001. MAP-kinase signaling pathways in T cells. *Curr. Opin. Immunol.* 13, no. 3:339-345.

115. Greenfield, E.A., K.A. Nguyen, and V.K. Kuchroo. 1998. CD28/B7 costimulation: a review. *Crit. Rev. Immunol.* 18, no. 5:389-418.

116. Holdorf, A.D., O. Kanagawa, and A.S. Shaw. 2000. CD28 and T cell co-stimulation. *Rev. Immunogenet.* 2, no. 2:175-184.

117. Noel, P.J., L.H. Boise, J.M. Green, and C.B. Thompson. 1996. CD28 costimulation prevents cell death during primary T cell activation. *J. Immunol.* 157, no. 2:636-642.

118. Radvanyi, L.G., Y. Shi, H. Vaziri, A. Sharma, R. Dhala, G.B. Mills, and R.G. Miller. 1996. CD28 costimulation inhibits TCR-induced apoptosis during a primary T cell response. *J. Immunol.* 156, no. 5:1788-1798.

119. Boise, L.H., A.J. Minn, P.J. Noel, C.H. June, M.A. Accavitti, T. Lindsten, and C.B. Thompson. 1995. CD28 costimulation can promote T cell survival by enhancing the expression of Bcl-XL. *Immunity* 3, no. 1:87-98.

120. Bubeck Wardenburg, J., R. Pappu, J.Y. Bu, B. Mayer, J. Chernoff, D. Straus, and A.C. Chan. 1998. Regulation of PAK activation and the T cell cytoskeleton by the linker protein SLP-76. *Immunity* 9, no. 5:607-616.

121. Holsinger, L.J., I.A. Graef, W. Swat, T. Chi, D.M. Bautista, L. Davidson, R.S. Lewis, F.W. Alt, and G.R. Crabtree. 1998. Defects in actin-cap formation in Vav-deficient mice implicate an actin requirement for lymphocyte signal transduction. *Curr. Biol.* 8, no. 10:563-572.

122. Fischer, K.D., Y.Y. Kong, H. Nishina, K. Tedford, L.E. Marengere, I. Kozieradzki, T. Sasaki, M. Starr, G. Chan, S. Gardener, M.P. Nghiem, D. Bouchard, M. Barbacid, A. Bernstein, and J.M. Penninger. 1998. Vav is a regulator of

cytoskeletal reorganization mediated by the T-cell receptor. *Curr. Biol.* 8, no. 10:554-562.

123. Fischer, K.D., K. Tedford, and J.M. Penninger. 1998. Vav links antigen-receptor signaling to the actin cytoskeleton. *Semin. Immunol.* 10, no. 4:317-327.

124. Snapper, S.B., F.S. Rosen, E. Mizoguchi, P. Cohen, W. Khan, C.H. Liu, T.L. Hagemann, S.P. Kwan, R. Ferrini, L. Davidson, A.K. Bhan, and F.W. Alt. 1998. Wiskott-Aldrich syndrome protein-deficient mice reveal a role for WASP in T but not B cell activation. *Immunity* 9, no. 1:81-91.

125. Valitutti, S., S. Muller, M. Cella, E. Padovan, and A. Lanzavecchia. 1995. Serial triggering of many T-cell receptors by a few peptide-MHC complexes. *Nature* 375, no. 6527:148-151.

126. Viola, A., and A. Lanzavecchia. 1996. T cell activation determined by T cell receptor number and tunable thresholds. *Science* 273, no. 5271:104-106.

127. Nelson, C.A., S.J. Petzold, and E.R. Unanue. 1994. Peptides determine the lifespan of MHC class II molecules in the antigen-presenting cell. *Nature* 371, no. 6494:250-252.

128. Matsui, K., J.J. Boniface, P. Steffner, P.A. Reay, and M.M. Davis. 1994. Kinetics of T-cell receptor binding to peptide/I-Ek complexes: correlation of the dissociation rate with T-cell responsiveness. *Proc. Natl. Acad. Sci. U. S. A.* 91, no. 26:12862-12866.

129. Dustin, M.L., L.M. Ferguson, P.Y. Chan, T.A. Springer, and D.E. Golan. 1996. Visualization of CD2 interaction with LFA-3 and determination of the two-dimensional dissociation constant for adhesion receptors in a contact area. *J. Cell. Biol.* 132, no. 3:465-474.

130. McKeithan, T.W. 1995. Kinetic proofreading in T-cell receptor signal transduction. *Proc. Natl. Acad. Sci. U. S. A.* 92, no. 11:5042-5046.

131. Rabinowitz, J.D., C. Beeson, D.S. Lyons, M.M. Davis, and H.M. McConnell. 1996. Kinetic discrimination in T-cell activation. *Proc. Natl. Acad. Sci. U. S. A.* 93, no. 4:1401-1405.

132. Evavold, B.D., J. Sloan-Lancaster, B.L. Hsu, and P.M. Allen. 1993. Separation of T helper 1 clone cytolysis from proliferation and lymphokine production using analog peptides. *J. Immunol.* 150, no. 8 Pt 1:3131-3140.

133. Chan, A.C., and A.S. Shaw. 1996. Regulation of antigen receptor signal

transduction by protein tyrosine kinases. *Curr. Opin. Immunol.* 8, no. 3:394-401.

134. Lyons, D.S., S.A. Lieberman, J. Hampl, J.J. Boniface, Y. Chien, L.J. Berg, and M.M. Davis. 1996. A TCR binds to antagonist ligands with lower affinities and faster dissociation rates than to agonists. *Immunity* 5, no. 1:53-61.

135. Alam, S.M., P.J. Travers, J.L. Wung, W. Nasholds, S. Redpath, S.C. Jameson, and N.R. Gascoigne. 1996. T-cell-receptor affinity and thymocyte positive selection. *Nature* 381, no. 6583:616-620.

136. Sykulev, Y., A. Brunmark, M. Jackson, R.J. Cohen, P.A. Peterson, and H.N. Eisen. 1994. Kinetics and affinity of reactions between an antigen-specific T cell receptor and peptide-MHC complexes. *Immunity* 1, no. 1:15-22.

137. Kersh, G.J., E.N. Kersh, D.H. Fremont, and P.M. Allen. 1998. High- and low-potency ligands with similar affinities for the TCR: the importance of kinetics in TCR signaling. *Immunity* 9, no. 6:817-826.

138. al-Ramadi, B.K., M.T. Jelonek, L.F. Boyd, D.H. Margulies, and A.L. Bothwell. 1995. Lack of strict correlation of functional sensitization with the apparent affinity of MHC/peptide complexes for the TCR. *J. Immunol.* 155, no. 2:662-673.

139. Sykulev, Y., Y. Vugmeyster, A. Brunmark, H.L. Ploegh, and H.N. Eisen. 1998. Peptide antagonism and T cell receptor interactions with peptide-MHC complexes. *Immunity* 9, no. 4:475-483.

140. Baker, B.M., S.J. Gagnon, W.E. Biddison, and D.C. Wiley. 2000. Conversion of a T cell antagonist into an agonist by repairing a defect in the TCR/peptide/MHC interface: implications for TCR signaling. *Immunity* 13, no. 4:475-484.

141. Kalergis, A.M., N. Boucheron, M.A. Doucey, E. Palmieri, E.C. Goyarts, Z. Vegh, I.F. Luescher, and S.G. Nathenson. 2001. Efficient T cell activation requires an optimal dwell-time of interaction between the TCR and the pMHC complex. *Nat. Immunol.* 2, no. 3:229-234.

142. Monks, C.R., H. Kupfer, I. Tamir, A. Barlow, and A. Kupfer. 1997. Selective modulation of protein kinase C-theta during T-cell activation. *Nature* 385, no. 6611:83-86.

143. Monks, C.R., B.A. Freiberg, H. Kupfer, N. Sciaky, and A. Kupfer. 1998. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* 395, no. 6697:82-86.

144. Grakoui, A., S.K. Bromley, C. Sumen, M.M. Davis, A.S. Shaw, P.M. Allen,

and M.L. Dustin. 1999. The immunological synapse: a molecular machine controlling T cell activation. *Science* 285, no. 5425:221-227.

145. Bromley, S.K., W.R. Burack, K.G. Johnson, K. Somersalo, T.N. Sims, C. Sumen, M.M. Davis, A.S. Shaw, P.M. Allen, and M.L. Dustin. 2001. The immunological synapse. *Annu. Rev. Immunol.* 19:375-396.

146. Potter, T.A., K. Grebe, B. Freiberg, and A. Kupfer. 2001. Formation of supramolecular activation clusters on fresh ex vivo CD8+ T cells after engagement of the T cell antigen receptor and CD8 by antigen-presenting cells. *Proc. Natl. Acad. Sci. U. S. A.* 98, no. 22:12624-12629.

147. Wulfing, C., C. Sumen, M.D. Sjaastad, L.C. Wu, M.L. Dustin, and M.M. Davis. 2002. Costimulation and endogenous MHC ligands contribute to T cell recognition. *Nat. Immunol.* 3, no. 1:42-7.

148. Lee, K.H., A.D. Holdorf, M.L. Dustin, A.C. Chan, P.M. Allen, and A.S. Shaw. 2002. T cell receptor signaling precedes immunological synapse formation. *Science* 295, no. 5559:1539-1542.

149. Shaw, A.S. 2002. Immunological synapse formation and TCR signal transduction. 2002 Keystone symposia: T lymphocyte activation, differentiation and death, Keystone, Colorado, USA.

150. Butz, E.A., and M.J. Bevan. 1998. Massive expansion of antigen-specific CD8+ T cells during an acute virus infection. *Immunity* 8, no. 2:167-175.

151. Murali-Krishna, K., J.D. Altman, M. Suresh, D.J. Sourdive, A.J. Zajac, J.D. Miller, J. Slansky, and R. Ahmed. 1998. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity* 8, no. 2:177-187.

152. Callan, M.F., L. Tan, N. Annels, G.S. Ogg, J.D. Wilson, C.A. O'Callaghan, N. Steven, A.J. McMichael, and A.B. Rickinson. 1998. Direct visualization of antigen-specific CD8+ T cells during the primary immune response to Epstein-Barr virus In vivo. *J. Exp. Med.* 187, no. 9:1395-1402.

153. Cose, S.C., C.M. Jones, M.E. Wallace, W.R. Heath, and F.R. Carbone. 1997. Antigen-specific CD8+ T cell subset distribution in lymph nodes draining the site of herpes simplex virus infection. *Eur. J. Immunol.* 27, no. 9:2310-2316.

154. Flynn, K.J., G.T. Belz, J.D. Altman, R. Ahmed, D.L. Woodland, and P.C. Doherty. 1998. Virus-specific CD8+ T cells in primary and secondary influenza pneumonia. *Immunity* 8, no. 6:683-691.

155. Goldrath, A.W., and M.J. Bevan. 1999. Selecting and maintaining a diverse T-cell repertoire. *Nature* 402, no. 6759:255-262.

156. Russell, J.H., B. Rush, C. Weaver, and R. Wang. 1993. Mature T cells of autoimmune lpr/lpr mice have a defect in antigen-stimulated suicide. *Proc. Natl. Acad. Sci. U. S. A.* 90, no. 10:4409-4413.

157. Dhein, J., H. Walczak, C. Baumler, K.M. Debatin, and P.H. Krammer. 1995. Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). *Nature* 373, no. 6513:438-441.

158. Ju, S.T., D.J. Panka, H. Cui, R. Ettinger, M. el-Khatib, D.H. Sherr, B.Z. Stanger, and A. Marshak-Rothstein. 1995. Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation. *Nature* 373, no. 6513:444-448.

159. Russell, J.H. 1995. Activation-induced death of mature T cells in the regulation of immune responses. *Curr. Opin. Immunol.* 7, no. 3:382-388.

160. Van Parijs, L., and A.K. Abbas. 1996. Role of Fas-mediated cell death in the regulation of immune responses. *Curr. Opin. Immunol.* 8, no. 3:355-361.

161. Sytwu, H.K., R.S. Liblau, and H.O. McDevitt. 1996. The roles of Fas/APO-1 (CD95) and TNF in antigen-induced programmed cell death in T cell receptor transgenic mice. *Immunity* 5, no. 1:17-30.

162. Janssen, O., A. Stocker, R. Sanzenbacher, H.H. Oberg, M.A. Siddiqi, and D. Kabelitz. 2000. Differential regulation of activation-induced cell death in individual human T cell clones. *Int. Arch. Allergy. Immunol.* 121, no. 3:183-193.

163. Thornberry, N.A. 1999. Caspases: a decade of death research. *Cell Death Differ.* 6, no. 11:1023-1027.

164. Ashkenazi, A., and V.M. Dixit. 1998. Death receptors: signaling and modulation. *Science* 281, no. 5381:1305-1308.

165. Enari, M., H. Sakahira, H. Yokoyama, K. Okawa, A. Iwamatsu, and S. Nagata. 1998. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* 391, no. 6662:43-50.

166. McIlroy, D., H. Sakahira, R.V. Talanian, and S. Nagata. 1999. Involvement of caspase 3-activated DNase in internucleosomal DNA cleavage induced by diverse apoptotic stimuli. *Oncogene* 18, no. 31:4401-4408.

167. Smith, C.A., G.T. Williams, R. Kingston, E.J. Jenkinson, and J.J. Owen. 1989. Antibodies to CD3/T-cell receptor complex induce death by apoptosis in immature T

cells in thymic cultures. *Nature* 337, no. 6203:181-184.

168. Mercep, M., J.A. Bluestone, P.D. Noguchi, and J.D. Ashwell. 1988. Inhibition of transformed T cell growth in vitro by monoclonal antibodies directed against distinct activating molecules. *J. Immunol.* 140, no. 1:324-335.

169. Takahashi, S., H.T. Maecker, and R. Levy. 1989. DNA fragmentation and cell death mediated by T cell antigen receptor/CD3 complex on a leukemia T cell line. *Eur. J. Immunol.* 19, no. 10:1911-1919.

170. Odaka, C., H. Kizaki, and T. Tadakuma. 1990. T cell receptor-mediated DNA fragmentation and cell death in T cell hybridomas. *J. Immunol.* 144, no. 6:2096-2101.

171. Russell, J.H., C.L. White, D.Y. Loh, and P. Meleedy-Rey. 1991. Receptor-stimulated death pathway is opened by antigen in mature T cells. *Proc. Natl. Acad. Sci. U. S. A.* 88, no. 6:2151-2155.

172. Bensussan, A., G. Leca, N. Corvaia, and L. Boumsell. 1990. Selective induction of autocytotoxic activity through the CD3 molecule. *Eur. J. Immunol.* 20, no. 12:2615-2619.

173. Janssen, O., S. Wesselborg, and D. Kabelitz. 1992. Immunosuppression by OKT3--induction of programmed cell death (apoptosis) as a possible mechanism of action. *Transplantation* 53, no. 1:233-234.

174. Wesselborg, S., O. Janssen, and D. Kabelitz. 1993. Induction of activation-driven death (apoptosis) in activated but not resting peripheral blood T cells. *J. Immunol.* 150, no. 10:4338-4345.

175. Radvanyi, L.G., G.B. Mills, and R.G. Miller. 1993. Religation of the T cell receptor after primary activation of mature T cells inhibits proliferation and induces apoptotic cell death. *J. Immunol.* 150, no. 12:5704-5715.

176. Salmon, M., D. Pilling, N.J. Borthwick, N. Viner, G. Janossy, P.A. Bacon, and A.N. Akbar. 1994. The progressive differentiation of primed T cells is associated with an increasing susceptibility to apoptosis. *Eur. J. Immunol.* 24, no. 4:892-899.

177. Irmler, M., M. Thome, M. Hahne, P. Schneider, K. Hofmann, V. Steiner, J.L. Bodmer, M. Schroter, K. Burns, C. Mattmann, D. Rimoldi, L.E. French, and J. Tschopp. 1997. Inhibition of death receptor signals by cellular FLIP. *Nature* 388, no. 6638:190-195.

178. Peter, M.E., F.C. Kischkel, C.G. Scheuerpflug, J.P. Medema, K.M. Debatin, and P.H. Krammer. 1997. Resistance of cultured peripheral T cells towards

activation-induced cell death involves a lack of recruitment of FLICE (MACH/caspase 8) to the CD95 death-inducing signaling complex. *Eur. J. Immunol.* 27, no. 5:1207-1212.

179. Peter, M.E., and P.H. Krammer. 1998. Mechanisms of CD95 (APO-1/Fas)-mediated apoptosis. *Curr. Opin. Immunol.* 10, no. 5:545-551.

180. Janssen, O., S. Wesselborg, B. Heckl-Ostreicher, K. Pechhold, A. Bender, S. Schondelmaier, G. Moldenhauer, and D. Kabelitz. 1991. T cell receptor/CD3-signaling induces death by apoptosis in human T cell receptor gamma delta + T cells. *J. Immunol.* 146, no. 1:35-39.

181. Lenardo, M.J. 1991. Interleukin-2 programs mouse alpha beta T lymphocytes for apoptosis. *Nature* 353, no. 6347:858-861.

182. Wang, R., A.M. Rogers, B.J. Rush, and J.H. Russell. 1996. Induction of sensitivity to activation-induced death in primary CD4+ cells: a role for interleukin-2 in the negative regulation of responses by mature CD4+ T cells. *Eur. J. Immunol.* 26, no. 9:2263-2270.

183. Refaeli, Y., L. Van Parijs, C.A. London, J. Tschopp, and A.K. Abbas. 1998. Biochemical mechanisms of IL-2-regulated Fas-mediated T cell apoptosis. *Immunity* 8, no. 5:615-623.

184. Newell, M.K., L.J. Haughn, C.R. Maroun, and M.H. Julius. 1990. Death of mature T cells by separate ligation of CD4 and the T-cell receptor for antigen. *Nature* 347, no. 6290:286-289.

185. Banda, N.K., J. Bernier, D.K. Kurahara, R. Kurrle, N. Haigwood, R.P. Sekaly, and T.H. Finkel. 1992. Crosslinking CD4 by human immunodeficiency virus gp120 primes T cells for activation-induced apoptosis. *J. Exp. Med.* 176, no. 4:1099-1106.

186. Oberg, H.H., R. Sanzenbacher, B. Lengl-Janssen, T. Dobmeyer, S. Flindt, O. Janssen, and D. Kabelitz. 1997. Ligation of cell surface CD4 inhibits activation-induced death of human T lymphocytes at the level of Fas ligand expression. *J Immunol* 159, no. 11:5742-9.

187. Sanzenbacher, R., D. Kabelitz, and O. Janssen. 1999. SLP-76 binding to p56lck: a role for SLP-76 in CD4-induced desensitization of the TCR/CD3 signaling complex. *J. Immunol.* 163, no. 6:3143-3152.

188. Gonzalo, J.A., E. Baixeras, A. Gonzalez-Garcia, A. George-Chandy, N. Van Rooijen, C. Martinez, and G. Kroemer. 1994. Differential in vivo effects of a

superantigen and an antibody targeted to the same T cell receptor. Activation-induced cell death vs passive macrophage-dependent deletion. *J. Immunol.* 152, no. 4:1597-1608.

189. Kabelitz, D., and O. Janssen. 1997. Antigen-induced death of T-Lymphocytes. *Front. Biosci.* 2:d61-77.

190. Combadiere, B., M. Freedman, L. Chen, E.W. Shores, P. Love, and M.J. Lenardo. 1996. Qualitative and quantitative contributions of the T cell receptor zeta chain to mature T cell apoptosis. *J. Exp. Med.* 183, no. 5:2109-2117.

191. Anel, A., M. Buferne, C. Boyer, A.M. Schmitt-Verhulst, and P. Golstein. 1994. T cell receptor-induced Fas ligand expression in cytotoxic T lymphocyte clones is blocked by protein tyrosine kinase inhibitors and cyclosporin A. *Eur. J. Immunol.* 24, no. 10:2469-2476.

192. Oyaizu, N., S. Than, T.W. McCloskey, and S. Pahwa. 1995. Requirement of p56lck in T-cell receptor/CD3-mediated apoptosis and Fas-ligand induction in Jurkat cells. *Biochem. Biophys. Res. Commun.* 213, no. 3:994-1001.

193. Gonzalez-Garcia, A., R.B. L, E. Leonardo, I. Merida, A.C. Martinez, and A.C. Carrera. 1997. Lck is necessary and sufficient for Fas-ligand expression and apoptotic cell death in mature cycling T cells. *J. Immunol.* 158, no. 9:4104-12.

194. Eischen, C.M., B.L. Williams, W. Zhang, L.E. Samelson, D.H. Lynch, R.T. Abraham, and P.J. Leibson. 1997. ZAP-70 tyrosine kinase is required for the up-regulation of Fas ligand in activation-induced T cell apoptosis. *J. Immunol.* 159, no. 3:1135-1139.

195. Villalba, M., S. Kasibhatla, L. Genestier, A. Mahboubi, D.R. Green, and A. Altman. 1999. Protein kinase ctheta cooperates with calcineurin to induce Fas ligand expression during activation-induced T cell death. *J. Immunol.* 163, no. 11:5813-5819.

196. Villunger, A., N. Ghaffari-Tabrizi, I. Tinhofer, N. Krumbock, B. Bauer, T. Schneider, S. Kasibhatla, R. Greil, G. Baier-Bitterlich, F. Uberall, D.R. Green, and G. Baier. 1999. Synergistic action of protein kinase C theta and calcineurin is sufficient for Fas ligand expression and induction of a crmA-sensitive apoptosis pathway in Jurkat T cells. *Eur. J. Immunol.* 29, no. 11:3549-3561.

197. Latinis, K.M., L.L. Carr, E.J. Peterson, L.A. Norian, S.L. Eliason, and G.A. Koretzky. 1997. Regulation of CD95 (Fas) ligand expression by TCR-mediated

signaling events. *J. Immunol.* 158, no. 10:4602-4611.

198. Bissonnette, R.P., A. McGahon, A. Mahboubi, and D.R. Green. 1994. Functional Myc-Max heterodimer is required for activation-induced apoptosis in T cell hybridomas. *J. Exp. Med.* 180, no. 6:2413-2418.

199. Liu, Z.G., S.W. Smith, K.A. McLaughlin, L.M. Schwartz, and B.A. Osborne. 1994. Apoptotic signals delivered through the T-cell receptor of a T-cell hybrid require the immediate-early gene nur77. *Nature* 367, no. 6460:281-284.

200. Woronicz, J.D., B. Calnan, V. Ngo, and A. Winoto. 1994. Requirement for the orphan steroid receptor Nur77 in apoptosis of T-cell hybridomas. *Nature* 367, no. 6460:277-281.

201. Mittelstadt, P.R., and J.D. Ashwell. 1998. Cyclosporin A-sensitive transcription factor Egr-3 regulates Fas ligand expression. *Mol. Cell. Biol.* 18, no. 7:3744-3751.

202. Vito, P., E. Lacana, and L. D'Adamio. 1996. Interfering with apoptosis: Ca(2+)-binding protein ALG-2 and Alzheimer's disease gene ALG-3. *Science* 271, no. 5248:521-525.

203. D'Adamio, F., O. Zollo, R. Moraca, E. Ayroldi, S. Bruscoli, A. Bartoli, L. Cannarile, G. Migliorati, and C. Riccardi. 1997. A new dexamethasone-induced gene of the leucine zipper family protects T lymphocytes from TCR/CD3-activated cell death. *Immunity* 7, no. 6:803-812.

204. Latinis, K.M., L.A. Norian, S.L. Eliason, and G.A. Koretzky. 1997. Two NFAT transcription factor binding sites participate in the regulation of CD95 (Fas) ligand expression in activated human T cells. *J. Biol. Chem.* 272, no. 50:31427-31434.

205. Li-Weber, M., O. Laur, A. Hekele, J. Coy, H. Walczak, and P.H. Krammer. 1998. A regulatory element in the CD95 (APO-1/Fas) ligand promoter is essential for responsiveness to TCR-mediated activation. *Eur. J. Immunol.* 28, no. 8:2373-2383.

206. Yang, Y., S. Minucci, K. Ozato, R.A. Heyman, and J.D. Ashwell. 1995. Efficient inhibition of activation-induced Fas ligand up-regulation and T cell apoptosis by retinoids requires occupancy of both retinoid X receptors and retinoic acid receptors. *J. Biol. Chem.* 270, no. 31:18672-18677.

207. Kasibhatla, S., L. Genestier, and D.R. Green. 1999. Regulation of fas-ligand expression during activation-induced cell death in T lymphocytes via nuclear factor

kappaB. *J. Biol. Chem.* 274, no. 2:987-992.

208. He, Y.W., M.L. Deftos, E.W. Ojala, and M.J. Bevan. 1998. RORgamma t, a novel isoform of an orphan receptor, negatively regulates Fas ligand expression and IL-2 production in T cells. *Immunity* 9, no. 6:797-806.

209. Collette, Y., A. Benziane, D. Razanajaona, and D. Olive. 1998. Distinct regulation of T-cell death by CD28 depending on both its aggregation and T-cell receptor triggering: a role for Fas-FasL. *Blood* 92, no. 4:1350-1363.

210. Ayroldi, E., O. Zollo, L. Cannarile, D.A. F, U. Grohmann, D.V. Delfino, and C. Riccardi. 1998. Interleukin-6 (IL-6) prevents activation-induced cell death: IL-2-independent inhibition of Fas/fasL expression and cell death. *Blood* 92, no. 11:4212-4219.

211. Amos, C.L., A. Woetmann, M. Nielsen, C. Geisler, N. Odum, B.L. Brown, and P.R. Dobson. 1998. The role of caspase 3 and BclxL in the action of interleukin 7 (IL-7): a survival factor in activated human T cells. *Cytokine* 10, no. 9:662-668.

212. Genestier, L., S. Kasibhatla, T. Brunner, and D.R. Green. 1999. Transforming growth factor beta1 inhibits Fas ligand expression and subsequent activation-induced cell death in T cells via downregulation of c-Myc. *J. Exp. Med.* 189, no. 2:231-239.

213. Lenardo, M., K.M. Chan, F. Hornung, H. McFarland, R. Siegel, J. Wang, and L. Zheng. 1999. Mature T lymphocyte apoptosis--immune regulation in a dynamic and unpredictable antigenic environment. *Annu. Rev. Immunol.* 17:221-253.

214. Green, D.R., and J.C. Reed. 1998. Mitochondria and apoptosis. *Science* 281, no. 5381:1309-1312.

215. Scaffidi, C., S. Fulda, A. Srinivasan, C. Friesen, F. Li, K.J. Tomaselli, K.M. Debatin, P.H. Krammer, and M.E. Peter. 1998. Two CD95 (APO-1/Fas) signaling pathways. *EMBO J.* 17, no. 6:1675-1687.

216. Li, P., D. Nijhawan, I. Budihardjo, S.M. Srinivasula, M. Ahmad, E.S. Alnemri, and X. Wang. 1997. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91, no. 4:479-489.

217. Strasser, A., A.W. Harris, D.C. Huang, P.H. Krammer, and S. Cory. 1995. Bcl-2 and Fas/APO-1 regulate distinct pathways to lymphocyte apoptosis. *EMBO J.* 14, no. 24:6136-6147.

218. Yoshida, H., Y.Y. Kong, R. Yoshida, A.J. Elia, A. Hakem, R. Hakem, J.M. Penninger, and T.W. Mak. 1998. Apaf1 is required for mitochondrial pathways of

apoptosis and brain development. *Cell* 94, no. 6:739-750.

219. Petschner, F., C. Zimmerman, A. Strasser, D. Grillot, G. Nunez, and H. Pircher. 1998. Constitutive expression of Bcl-xL or Bcl-2 prevents peptide antigen-induced T cell deletion but does not influence T cell homeostasis after a viral infection. *Eur. J. Immunol.* 28, no. 2:560-569.

220. Razvi, E.S., Z. Jiang, B.A. Woda, and R.M. Welsh. 1995. Lymphocyte apoptosis during the silencing of the immune response to acute viral infections in normal, lpr, and Bcl-2-transgenic mice. *Am. J. Pathol.* 147, no. 1:79-91.

221. Lohman, B.L., E.S. Razvi, and R.M. Welsh. 1996. T-lymphocyte downregulation after acute viral infection is not dependent on CD95 (Fas) receptor-ligand interactions. *J. Virol.* 70, no. 11:8199-8203.

222. Ehl, S., U. Hoffmann-Rohrer, S. Nagata, H. Hengartner, and R. Zinkernagel. 1996. Different susceptibility of cytotoxic T cells to CD95 (Fas/Apo-1) ligand-mediated cell death after activation in vitro versus in vivo. *J. Immunol.* 156, no. 7:2357-2360.

223. Teh, S.J., J.P. Dutz, B. Motyka, and H.S. Teh. 1996. Fas (CD95)-independent regulation of immune responses by antigen-specific CD4-CD8+ T cells. *Int. Immunol.* 8, no. 5:675-681.

224. Zimmermann, C., M. Rawiel, C. Blaser, M. Kaufmann, and H. Pircher. 1996. Homeostatic regulation of CD8+ T cells after antigen challenge in the absence of Fas (CD95). *Eur. J. Immunol.* 26, no. 12:2903-2910.

225. Nguyen, L.T., K. McKall-Faienza, A. Zakarian, D.E. Speiser, T.W. Mak, and P.S. Ohashi. 2000. TNF receptor 1 (TNFR1) and CD95 are not required for T cell deletion after virus infection but contribute to peptide-induced deletion under limited conditions. *Eur. J. Immunol.* 30, no. 2:683-688.

226. Reich, A., H. Korner, J.D. Sedgwick, and H. Pircher. 2000. Immune down-regulation and peripheral deletion of CD8 T cells does not require TNF receptor-ligand interactions nor CD95 (Fas, APO-1). *Eur. J. Immunol.* 30, no. 2:678-682.

227. Hildeman, D.A., T. Mitchell, T.K. Teague, P. Henson, B.J. Day, J. Kappler, and P.C. Marrack. 1999. Reactive oxygen species regulate activation-induced T cell apoptosis. *Immunity* 10, no. 6:735-744.

228. Li, Y., X.C. Li, X.X. Zheng, A.D. Wells, L.A. Turka, and T.B. Strom. 1999. Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of

alloreactive T cells and induction of peripheral allograft tolerance. *Nat. Med.* 5, no. 11:1298-1302.

229. Li, X.C., Y. Li, I. Dodge, A.D. Wells, X.X. Zheng, L.A. Turka, and T.B. Strom. 1999. Induction of allograft tolerance in the absence of Fas-mediated apoptosis. *J. Immunol.* 163, no. 5:2500-2507.

230. Sun, R., S.E. Shepherd, S.S. Geier, C.T. Thomson, J.M. Sheil, and S.G. Nathenson. 1995. Evidence that the antigen receptors of cytotoxic T lymphocytes interact with a common recognition pattern on the H-2Kb molecule. *Immunity* 3, no. 5:573-582.

231. Corinti, S., D. Medaglini, C. Prezzi, A. Cavani, G. Pozzi, and G. Girolomoni. 2000. Human dendritic cells are superior to B cells at presenting a major histocompatibility complex class II-restricted heterologous antigen expressed on recombinant Streptococcus gordonii. *Infect. Immun.* 68, no. 4:1879-1883.

232. Toes, R.E., E.I. van der Voort, S.P. Schoenberger, J.W. Drijfhout, L. van Bloois, G. Storm, W.M. Kast, R. Offringa, and C.J. Melief. 1998. Enhancement of tumor outgrowth through CTL tolerization after peptide vaccination is avoided by peptide presentation on dendritic cells. *J. Immunol.* 160, no. 9:4449-4456.

233. Van Parijs, L., V.L. Perez, A. Biuckians, R.G. Maki, C.A. London, and A.K. Abbas. 1997. Role of interleukin 12 and costimulators in T cell anergy in vivo. *J. Exp. Med.* 186, no. 7:1119-1128.

234. Becker, J.C., and E.B. Brocker. 1994. Prevention of anergy induction in cloned T cells by interleukin 12. *Exp. Dermatol.* 3, no. 6:283-289.

235. Chau, L.A., J.A. Bluestone, and J. Madrenas. 1998. Dissociation of intracellular signaling pathways in response to partial agonist ligands of the T cell receptor. *J. Exp. Med.* 187, no. 10:1699-1709.

236. Hollsberg, P., W.E. Weber, F. Dangond, V. Batra, A. Sette, and D.A. Hafler. 1995. Differential activation of proliferation and cytotoxicity in human T-cell lymphotropic virus type I Tax-specific CD8 T cells by an altered peptide ligand. *Proc. Natl. Acad. Sci. U. S. A.* 92, no. 9:4036-4340.

237. Brossart, P., and M.J. Bevan. 1996. Selective activation of Fas/Fas ligand-mediated cytotoxicity by a self peptide. *J. Exp. Med.* 183, no. 6:2449-2458.

238. Cao, W., S.S. Tykodi, M.T. Esser, V.L. Braciale, and T.J. Braciale. 1995. Partial activation of CD8+ T cells by a self-derived peptide. *Nature* 378, no.

6554:295-298.

239. Rabinowitz, J.D., C. Beeson, C. Wulfing, K. Tate, P.M. Allen, M.M. Davis, and H.M. McConnell. 1996. Altered T cell receptor ligands trigger a subset of early T cell signals. *Immunity* 5, no. 2:125-135.

240. Evavold, B.D., and P.M. Allen. 1991. Separation of IL-4 production from Th cell proliferation by an altered T cell receptor ligand. *Science* 252, no. 5010:1308-1310.

241. Windhagen, A., C. Scholz, P. Hollsberg, H. Fukaura, A. Sette, and D.A. Hafler. 1995. Modulation of cytokine patterns of human autoreactive T cell clones by a single amino acid substitution of their peptide ligand. *Immunity* 2, no. 4:373-380.

242. Tsitoura, D.C., W. Holter, A. Cerwenka, C.M. Gelder, and J.R. Lamb. 1996. Induction of anergy in human T helper 0 cells by stimulation with altered T cell antigen receptor ligands. *J. Immunol.* 156, no. 8:2801-2808.

243. Sloan-Lancaster, J., B.D. Evavold, and P.M. Allen. 1994. Th2 cell clonal anergy as a consequence of partial activation. *J. Exp. Med.* 180, no. 4:1195-1205.

244. Sloan-Lancaster, J., B.D. Evavold, and P.M. Allen. 1993. Induction of T-cell anergy by altered T-cell-receptor ligand on live antigen-presenting cells. *Nature* 363, no. 6425:156-159.

245. Ryan, K.R., and B.D. Evavold. 1998. Persistence of peptide-induced CD4+ T cell anergy in vitro. *J. Exp. Med.* 187, no. 1:89-96.

246. Wei, C.H., C. Beeson, M.G. Masucci, and V. Levitsky. 1999. A partially agonistic peptide acts as a selective inducer of apoptosis in CD8+ CTLs. *J. Immunol.* 163, no. 5:2601-2609.

247. Combadiere, B., C. Reis e Sousa, C. Trageser, L.X. Zheng, C.R. Kim, and M.J. Lenardo. 1998. Differential TCR signaling regulates apoptosis and immunopathology during antigen responses in vivo. *Immunity* 9, no. 3:305-313.

248. Wei, C.H., H. Yagita, M.G. Masucci, and V. Levitsky. 2001. Different programs of activation-induced cell death are triggered in mature activated CTL by immunogenic and partially agonistic peptide ligands. *J. Immunol.* 166, no. 2:989-995.

249. Holler, P.D., A.R. Lim, B.K. Cho, L.A. Rund, and D.M. Kranz. 2001. CD8(-) T cell transfectants that express a high affinity T cell receptor exhibit enhanced peptide-dependent activation. *J. Exp. Med.* 194, no. 8:1043-1052.

250. Alexander-Miller, M.A., M.A. Derby, A. Sarin, P.A. Henkart, and J.A.

Berzofsky. 1998. Supraoptimal peptide-major histocompatibility complex causes a decrease in bc1-2 levels and allows tumor necrosis factor alpha receptor II-mediated apoptosis of cytotoxic T lymphocytes. *J. Exp. Med.* 188, no. 8:1391-1399.

251. Loftus, D.J., P. Squarcina, M.B. Nielsen, C. Geisler, C. Castelli, N. Odum, E. Appella, G. Parmiani, and L. Rivoltini. 1998. Peptides derived from self-proteins as partial agonists and antagonists of human CD8+ T-cell clones reactive to melanoma/melanocyte epitope MART1(27-35). *Cancer Res.* 58, no. 11:2433-2439.

252. Savage, P.A., J.J. Boniface, and M.M. Davis. 1999. A kinetic basis for T cell receptor repertoire selection during an immune response. *Immunity* 10, no. 4:485-492.

253. Busch, D.H., and E.G. Pamer. 1999. T cell affinity maturation by selective expansion during infection. *J. Exp. Med.* 189, no. 4:701-710.

254. Malherbe, L., C. Filippi, V. Julia, G. Foucras, M. Moro, H. Appel, K. Wucherpfennig, J.C. Guery, and N. Glaichenhaus. 2000. Selective activation and expansion of high-affinity CD4+ T cells in resistant mice upon infection with Leishmania major. *Immunity* 13, no. 6:771-782.

255. van den Beemd, R., P.P. Boor, E.G. van Lochem, W.C. Hop, A.W. Langerak, I.L. Wolvers-Tettero, H. Hooijkaas, and J.J. van Dongen. 2000. Flow cytometric analysis of the Vbeta repertoire in healthy controls. *Cytometry* 40, no. 4:336-345.

256. Grunewald, J., C.H. Janson, and H. Wigzell. 1991. Biased expression of individual T cell receptor V gene segments in CD4+ and CD8+ human peripheral blood T lymphocytes. *Eur. J. Immunol.* 21, no. 3:819-822.

257. Grunewald, J., R. Ahlberg, A.K. Lefvert, H. DerSimonian, H. Wigzell, and C.H. Janson. 1991. Abnormal T-cell expansion and V-gene usage in myasthenia gravis patients. *Scand. J. Immunol.* 34, no. 2:161-168.

258. Grunewald, J., M. Jeddi-Tehrani, H. Dersimonian, R. Andersson, and H. Wigzell. 1992. A persistent T cell expansion in the peripheral blood of a normal adult male: a new clinical entity? *Clin. Exp. Immunol.* 89, no. 2:279-284.

259. Callan, M.F., N. Steven, P. Krausa, J.D. Wilson, P.A. Moss, G.M. Gillespie, J.I. Bell, A.B. Rickinson, and A.J. McMichael. 1996. Large clonal expansions of CD8+ T cells in acute infectious mononucleosis. *Nat. Med.* 2, no. 8:906-11.

260. Gigliotti, D., A.K. Lefvert, M. Jeddi-Tehrani, S. Esin, V. Hodara, R. Pirskanen, H. Wigzell, and R. Andersson. 1996. Overexpression of select T cell receptor V beta gene families within CD4+ and CD8+ T cell subsets of myasthenia

gravis patients: a role for superantigen(s)? *Mol. Med.* 2, no. 4:452-459.

261. Grunewald, J., R. Andersson, L. Rydberg, D. Gigliotti, C. Schaufelberger, G.K. Hansson, and H. Wigzell. 1994. CD4+ and CD8+ T cell expansions using selected TCR V and J gene segments at the onset of giant cell arteritis. *Arthritis. Rheum.* 37, no. 8:1221-1227.

262. Alam, A., N. Lambert, J. Lule, H. Coppin, B. Mazieres, C. de Preval, and A. Cantagrel. 1996. Persistence of dominant T cell clones in synovial tissues during rheumatoid arthritis. *J. Immunol.* 156, no. 9:3480-3485.

263. Grunewald, J., T. Hultman, A. Bucht, A. Eklund, and H. Wigzell. 1995. Restricted usage of T cell receptor V alpha/J alpha gene segments with different nucleotide but identical amino acid sequences in HLA-DR3+ sarcoidosis patients. *Mol. Med.* 1, no. 3:287-296.

264. Boitel, B., M. Ermonval, P. Panina-Bordignon, R.A. Mariuzza, A. Lanzavecchia, and O. Acuto. 1992. Preferential V beta gene usage and lack of junctional sequence conservation among human T cell receptors specific for a tetanus toxin-derived peptide: evidence for a dominant role of a germline-encoded V region in antigen/major histocompatibility complex recognition. *J. Exp. Med.* 175, no. 3:765-777.

265. Breiteneder, H., R. Friedl-Hajek, C. Ebner, S. Schenk, G. Fischer, D. Kraft, and O. Scheiner. 1996. Sequence comparisons of the CDR3 hyper-variable loops of human T cell receptors specific for three major T cell epitopes of the birch pollen allergen Bet v 1. *Mol. Immunol.* 33, no. 13:1039-1048.

266. Cibotti, R., J.P. Cabaniols, C. Pannetier, C. Delarbre, I. Vergnon, J.M. Kanellopoulos, and P. Kourilsky. 1994. Public and private V beta T cell receptor repertoires against hen egg white lysozyme (HEL) in nontransgenic versus HEL transgenic mice. *J. Exp. Med.* 180, no. 3:861-872.

267. Campos-Lima, P.O., V. Levitsky, M.P. Imreh, R. Gavioli, and M.G. Masucci. 1997. Epitope-dependent selection of highly restricted or diverse T cell receptor repertoires in response to persistent infection by Epstein-Barr virus. *J. Exp. Med.* 186, no. 1:83-89.

268. Casanova, J.L., P. Romero, C. Widmann, P. Kourilsky, and J.L. Maryanski. 1991. T cell receptor genes in a series of class I major histocompatibility complex-restricted cytotoxic T lymphocyte clones specific for a Plasmodium berghei

nonapeptide: implications for T cell allelic exclusion and antigen-specific repertoire. *J. Exp. Med.* 174, no. 6:1371-1383.

269. Casanova, J.L., J.C. Cerottini, M. Matthes, A. Necker, H. Gournier, C. Barra, C. Widmann, H.R. MacDonald, F. Lemonnier, B. Malissen, and et al. 1992. H-2-restricted cytolytic T lymphocytes specific for HLA display T cell receptors of limited diversity. *J. Exp. Med.* 176, no. 2:439-447.

270. Casanova, J.L., F. Martinon, H. Gournier, C. Barra, C. Pannetier, A. Regnault, P. Kourilsky, J.C. Cerottini, and J.L. Maryanski. 1993. T cell receptor selection by and recognition of two class I major histocompatibility complex-restricted antigenic peptides that differ at a single position. *J. Exp. Med.* 177, no. 3:811-820.

271. Casanova, J.L., and J.L. Maryanski. 1993. Antigen-selected T-cell receptor diversity and self-nonself homology. *Immunol Today* 14, no. 8:391-394.

272. Burrows, S.R., S.L. Silins, D.J. Moss, R. Khanna, I.S. Misko, and V.P. Argaet. 1995. T cell receptor repertoire for a viral epitope in humans is diversified by tolerance to a background major histocompatibility complex antigen. *J. Exp. Med.* 182, no. 6:1703-1715.

273. Fasso, M., N. Anandasabapathy, F. Crawford, J. Kappler, C.G. Fathman, and W.M. Ridgway. 2000. T cell receptor (TCR)-mediated repertoire selection and loss of TCR vbeta diversity during the initiation of a CD4(+) T cell response in vivo. *J. Exp. Med.* 192, no. 12:1719-1730.

274. McHeyzer-Williams, L.J., J.F. Panus, J.A. Mikszta, and M.G. McHeyzer-Williams. 1999. Evolution of antigen-specific T cell receptors in vivo: preimmune and antigen-driven selection of preferred complementarity-determining region 3 (CDR3) motifs. *J. Exp. Med.* 189, no. 11:1823-1838.

275. Grufman, P., E.Z. Wolpert, J.K. Sandberg, and K. Karre. 1999. T cell competition for the antigen-presenting cell as a model for immunodominance in the cytotoxic T lymphocyte response against minor histocompatibility antigens. *Eur. J. Immunol.* 29, no. 7:2197-2204.

276. Wolpert, E.Z., P. Grufman, J.K. Sandberg, A. Tegnesjo, and K. Karre. 1998. Immunodominance in the CTL response against minor histocompatibility antigens: interference between responding T cells, rather than with presentation of epitopes. *J. Immunol.* 161, no. 9:4499-4505.

277. Kedl, R.M., W.A. Rees, D.A. Hildeman, B. Schaefer, T. Mitchell, J. Kappler,

and P. Marrack. 2000. T cells compete for access to antigen-bearing antigen-presenting cells. *J. Exp. Med.* 192, no. 8:1105-1113.

278. Roy-Proulx, G., M.C. Meunier, A.M. Lanteigne, S. Brochu, and C. Perreault. 2001. Immunodomination results from functional differences between competing CTL. *Eur. J. Immunol.* 31, no. 8:2284-2292.

279. Correia-Neves, M., C. Waltzinger, D. Mathis, and C. Benoist. 2001. The shaping of the T cell repertoire. *Immunity* 14, no. 1:21-32.