Review

Manipulation of MHC-I/TCR Interaction for Immune Therapy

Qingjun Liu[1, 2] and Bin Gao[2, 3]

Adoptive immunotherapy involving the transfer of autologous tumor or virus-reactive T lymphocytes has been demonstrated to be effective in the eradication of cancer and virally infected cells. Identification of MHC-restricted antigens and progress in generation of adaptive immune responses have provided new direction for such treatment for severe pathologies such as cancer and autoimmune diseases. Here we review the latest development about the molecular basis of MHC-I/TCR interaction, and its manipulation including enhanced MHC-I expression, modification of peptide and engineered TCR for clinical applications such as vaccine design, tumor therapy and autoimmune diseases. *Cellular & Molecular Immunology*. 2008;5(3):171-182.

Key Words: MHC-I, TCR, MHC-I/TCR interaction, immune therapy

Introduction

The cellular immune response is orchestrated by T cells. These cells recognize antigenic peptides presented on the surface of host cells by major histocompatibility complex (MHC) molecules. There are two types of MHC molecules: MHC class I molecules (MHC-I) and MHC class II molecules (MHC-II). MHC-II are only found on "professional antigen-presenting cells" (APCs) such as dendritic cells (DCs), B-cells, macrophages. These molecules present exogenous antigen to helper T cells (Th-cells). By contrast, MHC-I are type I membrane glycoproteins found on all nucleated cells and mainly present peptides of endogenous proteins to $CD8^+$ cytotoxic T cells (CTLs). T cells scan the peptide repertoire on the cell surface of the target cell and eventually kill cells that present non-self peptides derived from endogenous pathogens or mutated proteins, which provides an important protection against virus infection and tumor mutation since antigens from tumor and virus pathogens are largely produced inside cells. Since CTLs can be activated by cells that infected by virus or tumor and in turn the activated CTLs kill infected or mutated cells, many efforts have been made to enhance the killing ability of CTLs to clear certain virus infection or tumor which otherwise can not be cured by serological method and operative treatment. Here we will review current literature about the molecular basis of MHC-I/TCR (αβ T-cell receptor) interaction from MHC-I, peptides to TCR, and it's manipulation including enhanced MHC-I expression, modification of peptide and engineered TCR for clinical applications such as vaccine design, tumor therapy and autoimmune diseases.

MHC-I/TCR interaction

The recognition of CTLs for non-self target, e.g., pathogen infected cells or mutant cells, is through the interaction between the receptor on T cell and peptide loaded MHC molecules on target cell. Both TCR and peptide loaded MHC-I molecules are undergoing sophisticated procedure to appear on respective cells to interact with each other for triggering effective T cell responses.

MHC class I complex

MHC-I is a membrane anchored protein composed of two subunits with a short bound peptide. The membrane spanning protein, called heavy chain is approximately 350 amino acids in length, with about 75 amino acids at the carboxylic end comprising the transmembrane and cytoplasmic portions. The remaining about 270 amino acids, are divided into three

[1]Institute of Biotechnology, Academy of Military Medical Sciences, Beijing 100071, China;

[2]Institute of Microbiology, Chinese Academy of Sciences (CAS), Beijing 100101, China;

[3]Corresponding to: Dr. Bin Gao. Institute of Microbiology, Chinese Academy of Sciences (CAS), Beijing 100101, China. Tel: +86-10-6480-7408. Fax: +86-10-6480-7338. E-mail: bgao2008@gmail.com

Received Mar 7, 2007. Accepted May 7, 2008.

Abbreviations: MHC, major histocompatibility complex; MHC-I, MHC class I molecules; MHC-II, MHC class II molecules; CTL, $CD8^+$ cytotoxic T cell; APC, antigen-presenting cell; DC, dendritic cell; β_2m, beta-2 microglobulin; IFN-γ, interferon-gamma; TAP, transporter associated with antigen processing; ER, endoplasmic reticulum; CRT, calreticulin; BiP, immunoglobulin binding protein; TCR, T-cell receptor; pMHC-I, peptide-MHC class I; CDR, complementarity determining region; TIL, tumor-infiltrating lymphocyte; PBMC, peripheral blood mononuclear cell; TAA, tumor associated antigen.

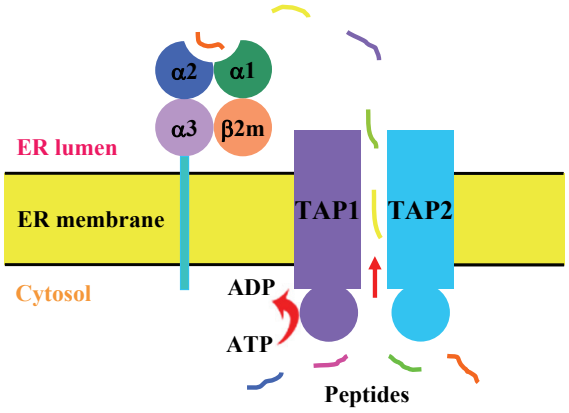

Figure 1. Transportation of intracellular peptides by TAP. TAP heterodimer formed by TAP1 and TAP2 is required for transportation of intracellular peptides from cytosol to ER. TAP has a heteromeric "peptide pump" which possesses an ATP binding cassette and 6 to 8 transmembrane helical segments.

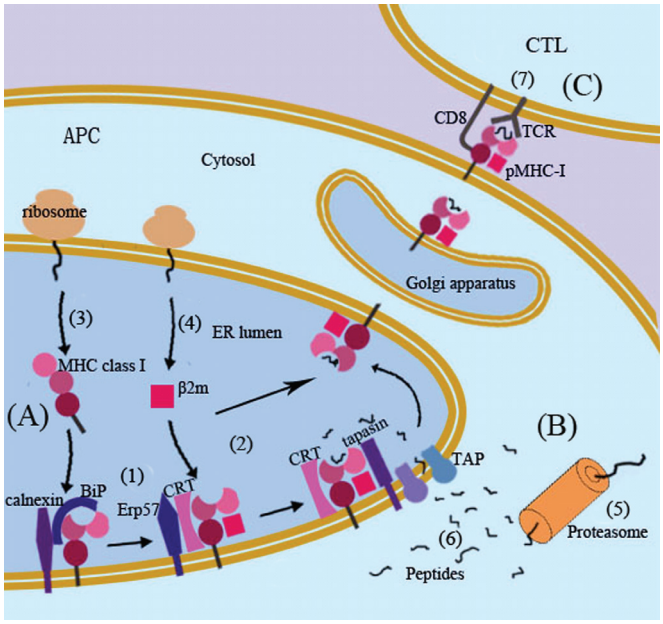

Figure 2. Manipulation of MHC-I/TCR interaction for immune intervention. (A) Enhancement of MHC-I expression. (1) The chaperones such as GRP94, CRT, hsp70 and hsp90 can escort peptides into MHC-I antigen processing and presentation pathway; (2) Cytokines such as IL-2, IFN-γ, GM-CSF and TNF-α can increase the expression of pMHC-I on the cell surface; (3) and (4) Pre- assembled MHC-I also can promote the folding and trafficking of pMHC-I and expression on the surface of the cell. (B) Modification of peptide. (5) Substitution of amino acids of peptide greatly increases the binding affinity without interfering with the peptide recognition; (6) The epitope linked to a degron signal regulating degradation by the 26S protesome enhances the pMHC-I presentation and T cell activation. (C) Engineering TCR. (7) CTLs transduced with high avidity TCR specific to the epitope have potential to kill tumor cells or the cells infected by virus.

domains labeled Alpha-1 (α1), Alpha-2 (α2) and Alpha-3 (α3), with α1 being close to the amino terminus and α3 located to the membrane. The second portion of the molecule is a small globular, unvaried protein called β-2 microglobulin (β_2m). It associates primarily with the α3 domain of heavy chain and is necessary for the stability of MHC-I. The bound peptide about 8~10 amino acids in length sits within the groove formed by heavy chain, and determines the specificity of MHC-I. The proteins encoded by each MHC-I allele may vary up to 20 amino acids making them highly polymorphic. Variability plots (1) of amino acid sequences of MHC-I molecule shows that the variation arising from genetic polymorphism is restricted to the amino-termini for MHC-I, α1 and α2 domain. These polymorphic residues lie largely at sites within and around the peptide-binding groove of MHC-I, and therefore determine the peptide binding properties of each MHC-I allele.

MHC class I antigen processing and presentation

Antigenic peptides loaded onto MHC-I for interaction with TCR on CTLs are generated in the cytosol by the 26S proteasome. Its 20S proteasome core consists of four seven-membered rings composed of 14 different subunits belonging to either the α-type or β-type subfamilies in eukaryotes. Two activators PA28-α and PA28-β, which are inducible by interferon-gamma (IFN-γ), activate the latent 20S proteasome, generating peptides by digestion of intracellular antigens, which are then presented to T cells.

When the peptides generated in the cytosol, they need to be transported into ER, where MHC-I are assembled. Two MHC-linked genes, TAP1 and TAP2, are responsible for such function. These genes encode the polypeptides that form a heterogenic "peptide pump" and possess an ATP binding cassette and 6 to 8 transmembrane helical segments (2) (Figure 1). The peptide transportation procedure can be divided into TAP-dependent classical pathway and TAP-independent pathway according to the requirement of TAP.

The classical TAP-dependent MHC-I antigen processing and presentation pathway

The classical TAP-dependent MHC-I antigen processing and presentation depends on a transporter PSF1, (TAP1 and TAP2 were primitively named as PSF1 and PSF2 respectively), to mediate entry of the cytosolic peptides into ER where they bind to class I heavy chains and promote their stable assembly with β_2m. PSF1 is associated with a complementary transporter chain, which is polymorphic and is encoded by the PSF2 gene (3-5). Efficient antigen presentation needs intact expression of the TAP1/2 molecules. TAP-deficient cells lack the transporter for MHC-I restricted peptides to enter the ER and therefore present only peptides derived from leader sequences (6) or exogenous peptides. The absence of ER localized peptides in these cells results in the accumulation of empty MHC-I in the ER-Golgi intermediate compartments as well as on the cell surface. The empty MHC-I can be rapidly loaded by available peptides

(7). Murine TAP2-defective RMA-S cell line required 2-3 hour incubation and approximately 10 times higher doses of Sendai virus to reach the same level of killing as the RMA parental line. After transfection of RMA-S cells with the murine TAP1/2 gene, Sendai virus antigen presentation was restored to levels of the RMA wild-type line (8). In 2001, Norbury et al. reported that minor antigen derived peptide could be transferred by host DCs for MHC-I presentation *via* TAP-dependent manner (9). Inhibition of peptide transport by downregulation of TAP-1 is a potential strategy of malignant cells to evade immune surveillance (10). US6, a recombinant soluble version of product of a human cytomegalovirus immune-evasion gene, blocks peptide translocation by TAP. The MHC-I of human cells expressed US6 dramatically reduced (11-13). To evade the host's immune response, herpes simplex virus employs the immediate early gene product ICP47 (IE12) to suppress antigen presentation to CTLs by inhibition of TAP (14). The ability of TAP to translocate antigenic peptides from the cytosol to the lumen of the ER for presentation on MHC-I in HIV-1-infected cells is abolished (15).

The short peptide epitope recognized by TCR, is usually 8~10 amino acids long, in conjunction with MHC-I. The mechanisms of peptide transport from the cytosol to ER and subsequent assembly with MHC-I are relatively well understood. The antigen processed and presented to the cell surface was illustrated in Figure 2. In general, endogenously synthesized foreign antigens such as viral proteins or tumor specific proteins are degraded into peptide fragments in the cytosol through an ATP-dependent proteolytic system started by ubiquitin conjugation (16). Newly synthesized and rapidly degraded polypeptides by proteasomes, plus so-called defective ribosomal products (DRiPs), constitute a large fraction of MHC-I restricted epitopes (17). The fragmented peptides are further translocated into ER through TAP and trimmed peptides of 8~10 amino acids that fit into the groove of MHC-I groove with the help of various chaperons such as tapasin, calnexin and calreticulin (CRT) (18-21). These MHC-I associated peptides are rapidly transferred through Golgi apparatus to the plasma membrane and stimulate T cells bearing CD8 molecules.

TAP-independent class I pathway

As mentioned above, TAP2-deficient mouse RMA-S cells, human 721.174 and T2 cells express little cell surface MHC-I and fail to present most viral and other MHC-I- associated peptides (22); furthermore, viral inhibitors of TAP function cause low MHC-I cell surface expression and poor peptide antigen presentation. For these reasons, MHC-I on TAP-deficient cells appear to be "empty" or devoid of tightly bound peptides. Despite their obvious defects in MHC-I antigen processing, TAP-deficient cells do express peptide associated MHC-I on the surface. For example, sub-physiologic temperature incubation significantly enhances MHC-I expression on TAP-deficient cells, especially for mouse MHC-I (23). The epitopes presented inculde ER signal sequence in these cells. Shi et al. also reported that antigen presentation of TAP-independent allorecognition of HLA-B7 was enhanced at sub-physiologic temperatures (24). In some instances, CTLs recognize TAP-deficient cells that express viral proteins (8, 25-29) or MHC-I epitopes expressed by transfected minigene constructs (30, 31). Peptides that are eluted from HLA-B7 molecules transfected on TAP2-deficient T2 cells have a typical HLA-B7-binding sequence motif; and the ratio of peptide to HLA-B7 heavy chain is the same as that in TAP2 and TAP1 competent cells (32). Smith and Lutiz observed that HLA-A2 and HLA-B7 molecules utilize distinct TAP-independent peptide supply, which may allow immune recognition of intracellular pathogens that interfere with TAP-dependent peptide transportation (32).

The endosomal peptides generated TAP-independently are likely to bound to recycling MHC-I which are often found in the endosomes of DCs (33, 34). Indeed, a unique endosome-to-cytosol transport pathway which permits selectively delivery of internalized antigens to the cytosol has been demonstrated (35).

TCR/CD3 complex

There are two classes of T-cell receptors, which are either composed of subunits αβ or γδ. Since γδ appears earlier in thymic ontogeny than αβ, γδ receptor is sometimes referred to as TCR1 and αβ receptor as TCR2. In general, γδ T-cells make up only 0.5-15% of the T cells in peripheral blood in human, and seem to be biased toward the recognition of certain types of microbial antigens. In this review, we discuss only TCRαβ for its important function in removal of tumor cells and the cells infected by virus. TCR is composed of two, disulfide-linked polypeptide chains, α and β, each having separate constant and variable domains much like immuno-globulin. Most CTLs possess both TCR and CD8 molecules on their surfaces. These TCRs are able to recognize peptides when they are expressed in complexes with MHC-I. The variable domain contains three complementarity determining regions (CDRs) that are responsible for antigen recognition. Genetic diversity is ensured in a manner analogous to that for immunoglobulin. Thus, just as the B-cell surface immuno-globulin provides antigen specificity to its B-cell, the TCR allows T-cells to recognize their particular antigenic moiety. Upon recognition of a specific antigen, the signal is passed to the CD3 molecule and then into the T-cell, prompting T-cell activation and the release of lymphokines. In all immuno-competent T-cells, the antigen receptor is noncovalently but still closely linked with CD3 in a complex. CD3 is a TCR associated complex of transmembrane polypeptides and contains invariant γδ chains which closely link to TCR recognition units, two molecules of CD3ε, plus the disulfide-linked ζ-ζ dimer. The total complex therefore has the structure TCR2-CD3γδε2-ζ2. The ITAM tyrosine motifs are on the bottom of CD3ζ and associate with protein tyrosine kinases thereby transducing signal generated by ligand bound to the TCR (36).

Molecular basis of MHC-I/TCR interaction

Interaction between TCR and MHC-I plays a very important role in the activities ranging from the thymic selection of

CTLs, proliferation and differentiation of T cells after activated by APCs, and to the killing of target cells in the effective stage (37). TCR/pMHC-I engagement is referred to as the immunological synapse, wherein not only TCRs but also co-receptors and additional CD3 interact to form the signaling-competent, supramolecular complex. There are many factors involved in such sophisticated interaction but the density of molecules involved, affinity and avidity of interaction and the peptides associated with MHC-I are critical for such interaction.

Since the first crystal structure determinations of TCR bound to pMHC-I in 1996, the comprehension about molecular basis of MHC-I/TCR interaction is gradually elucidated. MHC-I has an architecture of a seven-stranded β-sheet which represents the floor of the binding groove, and the sides formed by α1 and α2 helices that straddle the β-sheet. The 8~10 residues peptide in an extended conformation with the termini is buried in specificity pockets that differ from allele to allele (38, 39). This binding mode leaves the upward-pointing peptide side chains available for direct interaction with the TCR (40). Longer peptides can either bind by extension at the C terminus (41) or, due to the fixing of their termini, bulge out of the binding groove, providing additional surface area for TCR recognition (42, 43). Thus, the bound peptide is more accessible for TCR inspection in MHC-I due to its ability to bulge out of the groove (40). The TCRs bind pMHC-I with relatively low affinity (~1-100 μM) through CDRs present in their variable domains. Usually, the Vα domain is located to the position above the N terminal half of the peptide, whereas the Vβ domain is covered over the C-terminal portion of the peptide. TCRs with their Vα domains located closer to the N terminus of the peptide exhibited CD8-dependent signaling (44). Peptide contacts are made primarily through the CDR3 loops, which exhibit the greatest degree of genetic variability.

The concentration of pMHC-I complexes on the surface of APCs or target cells is an influencing factor for TCR docking. Antigen presentation cells possess the highest concentration of pMHC-I complexes, with about 5×10^5 molecules per cell. In contrast, fibroblasts, muscle cells, and liver hepatocytes carry much lower quantities, sometimes 100 or fewer per cell. It was estimated that APC pulsed with high concentration (20 μM) of peptide displays ~7500 pMHC-I complex, and with low concentration (50 nM) display only ~100 pMHC-I complex (45). Normally, each nucleated cell expresses 4~6 different kinds of MHC-I. It turns out that 75% or so of peptides originate in the cytosal and most of them will be in low abundance, about 100~400 copies per cell. Thus proteins expressed with unusual abundance, such as oncofetal proteins in tumors and viral antigen in infected cells, should be readily detected by resting T-cells. The critical number of pMHC-I complexes required per target cell for the activation of CTL responses varies with different combinations of pMHC-I complexes and CTL clones from several thousand complexes to fewer than ten per target cell (46). For example, the amount of complexes of SC-Kd and peptide 170-179 of HLA-CW3 which coated onto the beads for priming CTL response was calculated to be between 2.5×10^4 and 5×10^4 complexes (47). T cells are capable of detecting as few as 3~10 pMHC-I molecules on a target cell and have ability to specifically recognize low level of viral or tumor pMHC-I, even in the presence of orders of magnitude less than self-pMHC-I molecules (48). Tumor cell susceptibility to CTL-mediated lysis may base on the level of specific pMHC-I expression rather than on the total level of tumor associated antigen (TAA) expression (49).

Due to genetic recombination events each CTL expresses a unique TCR which only binds to a specific pMHC-I complex. CTLs which recognize self-peptides are removed in the thymus or tolerized after their release from the thymus. The kinetics and affinity of interaction between TCR and pMHC-I and the densities of TCR and pMHC-I on the cell surface are critical for different outcomes during thymic selection. In general, it is thought that agonist pMHC that cause negative selection, have higher affinities and, in particular, slower off-rates than partial or weak agonists and antagonists that cause positive selection (50). In light of mechanism in thymic selection, it is speculated that the kinetics and affinity of interaction between TCR and pMHC-I and the densities of TCR and pMHC-I on the cell surface are important for the activation of T cells and killing of target cells.

The accessory molecule CD8 bound to the α3 domain of the MHC-I (51) provides further force for the interaction beween TCR and pMHC-I. The proportion of the proximal and distal CD8 and TCR molecules can determine the capacity of a T-cell to respond to APCs carrying either low or high levels of cognate pMHC-I complexes (52). The CTL attracts target cell expressing pMHC-I also utilizes other antigen-independent major adhesion molecules, such as ICAM-1/2, LFA-1/2 and CD2. It is speculated that the TCR regularly 'scan' the MHC-I when the binding energy between TCR and MHC-I is sufficient to allow the TCR to temporarily dock onto the pMHC-I complex and interrogate the peptide, as long as there are sufficient energetically favorable contacts between them, then signaling through the TCR complex can occur. It is likely that two TCR molecules with two MHC-peptide moieties can form a complex that might trigger T-cell signaling. The high avidity eptiopes may contribute to the higher activity of CTL. Terasawa et al. reported that an agonist epitope designated PSA-3A ("A" for agonist) of the PSA-3 CTL epitope enhanced binding to the MHC-I A2 allele as well as enhanced stability of the pMHC-I complex and induced higher levels of T-cell activation (53).

TCR exhibits a degree of cross-reactivity to bind and respond to different pMHC-I ligands (54, 55). The mechanisms of cross-activity generally accepted include molecular mimicry (56) and conformation adjustments. Significant changes in TCR CDR3 loops can take place upon binding (57-59) and CDR3 can occupy different conformations depending upon which ligand is bound (60, 61). Gagnon et al. suggested that the combined role that protein dynamics can play in TCR cross-reactivity. TCR recognition of ligand can be achieved through diverse and complex molecular

mechanisms produced occur simul- taneously in the interface, not limited to molecular mimicry and CDR loop shifts (62). Recently, it has been reported that the cross-reactivity correlated with a shrinking, increasingly hydrophobic TCR-ligand interface (63), and a few conserved amino acids in CDR1 and CDR2 were involved in the engagement (63, 64).

Modification of MHC-I/TCR interaction for immune manipulation

With the better understanding of MHC-I/TCR interaction, one of the therapeutic strategies for immune manipulation is the modification of MHC-I/TCR interaction to enhance the activity of CTLs specific to target cells. Strategies in enhancing MHC-I expression, identification and modification of epitopes, engineering TCR, have been extensively adopted (Figure 2).

Enhancing MHC-I expression

CTLs can be activated through recognizing the MHC-I expressed on the target cells or APCs. The more pMHC-I an APC expresses, the better CTL may be activated. It has been reported that chaperones and cytokines can enhance MHC-I expression. Of course MHC-I expression will increase if the efficiency of folding of MHC class I complex improves.

The chaperones in protein folding and trafficking can enhance the antigen expression on the surface of APCs, so one of strategies to treat the diseases of tumor or virus is to utilize some chaperones as adjuvant of vaccine. The resident ER chaperone proteins GRP94 (gp96) and CRT can escort tumor-derived peptides into the endogenous antigen presentation pathway of APC (65, 66). Heat shock proteins (HSPs) hsp70 and hsp90 have a similar property which they chaperone peptides in antigen presentation and are necessary for priming CTL responses (67-70). Biswas et al. investigated that the sequence 1~355 of GRP94 sufficiently augments peptide presentation to T cells (71). CRT has been reported to have an effect of upregulating MHC-I presentation *in vitro* and *in vivo* (72).

Certain cytokines have been long known to be efficient for promoting MHC-I expression. It is reported that, when cells bind cytokines such as IFN-γ, the transcription of MHC-I and β_2m, and the linked genes for processing peptides (proteasome and peptide transporter genes) are markedly increased. The peptides supplied to MHC-I increase with enhanced MHC-I expression (73, 74). Comparing with their control cells, cytokine-treated cell lines showed an increase in Her2 (369)-A2 epitope density (49). Macrophage colony-stimulating factor (GM-CSF) has also been reported to increase greatly the expression of MHC-1 (H-2K^d) on tumor cells (75), so has TNF-α (76).

Pre-assembled MHC-I has been reported to enhance the folding and trafficking of pMHC-I and expression on the surface of the cell. MHC-I expressed on the cells are associated with a large number of different peptides so that the density of a given pMHC-I complex is relatively low. Mottez et al. took a single antigenic peptide genetically attach to the MHC-I and expressed in cells. The cells were recognized by specific T cells and could prime CTL *in vitro*. The availability of MHC-I bound to a single peptide (77) and the peptide β_2m fusion (78, 79) provide strategies to facilitate the production of pMHC-I complexes. Tafuro et al. (80) demonstrated that the peptides directly linked to β_2m could complex with human MHC-I heavy chain in TAP or β_2m negative cells. Furthermore, the peptide-fused to β_2m/HLA class I complex had 2.5 times higher expression and significantly higher binding ability with a specific TCR (81).

Modification of peptides

Modification of a peptide epitope associated with MHC-I is another practical method to enhance the interaction between MHC-I and TCR, increase epitope's immunogenicity and affinity as well as the specificity and spectrum. It is reported that CTLs induced by *in vitro* sensitization using the modified peptide exhibited better recognition of the native peptide compared with CTLs raised with the native peptide (82). Substitutions of amino acids of a peptide epitope, for example, can greatly increase the binding affinity without interfering with the peptide recognition (83-86). Most of the immunogenic melanoma and melanocyte differentiation antigen peptides have a moderate or relatively low affinity for the HLAA*0201 molecule, in contrast to viral peptides that have high binding affinity for their corresponding MHC-I (87-90). As mentioned above, the amino acid of the peptides bound by HLA-A2, is Leucine or Methionine at P2, which identified as preferred anchor residues. With one or two amino acid substitutions of gp100-derived peptides, the binding affinity to the HLA-A*0201 molecule increased (90). In the gp100:209-217 peptide, the relative binding affinity of the Leucine modification instead of Threonine at P2 resulted in a peptide with a 52-fold higher binding affinity than that of the native peptide. Furthermore, when Threonine was replaced by Methionine at P2, this modified peptide bound to HLA-A2 with a approximately 9-fold greater affinity and has a approximately 7-fold slower dissociation rate at physiological temperature was more immunogenic *in vitro* and *in vivo* (91). Webb et al. replaced certain residues of the SIINFEKL epitope with the corresponding β-amino acid, the MHC-I binding and immunogenicity of the peptide are improved (92).

Engineering TCR

One of the best ways to increase the interaction between MHC-I/TCR is to have a high affinity TCR expressed on T cell. Improved interaction ability and specificity of T cell can be used to kill tumor or cells infected by some virus more efficiently (93).

Johnson and colleagues derived 24 MART-1:27-35 (MART-1) melanoma antigen-reactive tumor-infiltrating lymphocyte (TIL) clones from the tumors of five patients. One of them showed very high avidity against MART-1 expressing tumors. Expression of this highly avid TCR in patient PBMCs had the potential to induce tumor regression (94). p53 protein is markedly up-regulated in a high

proportion of human malignancies, Cohen et al. isolated TCRαβ from a murine CTL clone that recognized the human p53 (264-272) epitope with high affinity. These genes were cloned into a retroviral vector that mediated high efficiency gene transfer into primary human lymphocytes. The p53 TCR-transduced lymphocytes were able to specifically recognize peptide-pulsed APCs as well as HLA-A2.1 positive cells transfected with either wild-type or mutant p53 protein, and kill a broad spectrum of human tumor cell lines and fresh human tumor cells (95). When several TCR genes were co-transferred into a T cell, some TCR were stronger in terms of cell surface expression and replaced weak TCR on the cell surface, resulting in exchange of antigen specificity (96). Morgan and colleagues isolated the TCRαβ from a highly avid anti-gp100 CTL clone. The lymphocytes transduced with this TCR could lyse melanoma tumor cell lines (97). These results suggest that lymphocytes genetically engineered to express specific TCR may be of value in the adoptive immunotherapy of patients.

TCR with high avidity can be obtained not only by direct cloning, but also from different display technologies such as yeast, phage and mammalian cell surface display (98). High-affinity, peptide-specific TCRs could be generated by mutations in CDR1, CDR2 or CDR3 (99): a single point mutation in the CDR3β loop of the 2C TCR (Gly95Arg) increased its affinity by a factor of 1000 to the QL9/Ld pMHC, most likely due to direct electrostatic interaction of the TCR arginine side chain with an aspartate residue at P8. The cysteine-modified TCRs, which form dimmers easily by additional interchain disulfide bond, were more highly expressed on the surface of human lymphocytes compared with their wild-type counterparts and were able to mediate higher levels of cytokine secretion and specific lysis when co-cultured with specific tumor cell lines (100). To decrease the proportion of mispairing between α and β chain, Thomas and colleagues developed a chimeric, partially humanized double-chain TCR construct by exchanging mouse αβ constant (C) with human αβ regions (101). In contrast, Biswas et al. used directed evolution method to generate a panel of high affinity TCRs for TSST-1, allowing the mutation serve as potential leads toward the development of therapeutic agents for superantigen-mediated disease (102).

Applications of manipulation of interaction between MHC-I/TCR

Enhanced interaction between MHC-I and TCR can be employed to develop the efficient therapy for tumor, virus infection and autoimmune disease.

Vaccine design

MHC-I density, the binding affinity to TCR on APC can have great impact on efficiency of presentation of antigen to immune system. Improvement of these properties can be used for vaccine design.

The chaperones and cytokines promote the expression of specific pMHC-I on APCs and can be coadministrated as adjuvants with other immunogens. An allogeneic prostate carcinoma cell line, LNCaP transduced with the IL-2 and IFN-γ genes, could express IL-2 and IFN-γ. In a phase I trial, the irradiated cells were transferred into HLA-A2-matched patients with hormone refractory prostate cancer (HRPC) (103). Two of the six patients produced effective anti-tumor response. Zhao et al. (72) used chaperone calreticulin for up-regulating MHC-I presentation in a DNA vaccine. They constructed DNA vaccines by employing different lengths of CRT chimerically linked to antigen HPV6bE7 of human papillomavirus (HPV) and investigated the immunological effects of these vaccines. The result showed that recombinant CRT180 or CRT120 with HPV6bE7 vaccines could elicit more efficient E7-specific immune response than that by HPV6bE7 alone. Administration of exogenous IL-2 following cell transfer markedly augmented the efficacy of adoptive therapy (104, 105) and the dosage for clinical application was identified (106). Moreover, IL-2-transduced lymphocytes also grew in an autocrine fashion and remained responsive to antigen (107).

The modification of T cell peptide can also be considered in vaccine design. A modified version of the gp100 (209-217) HLA-A2 binding peptide, P2 Methionine replaced by Threonine has been demonstrated to produce greater immunogenicity *in vitro* and *in vivo* due to the enhanced stability of the pMHC-I complex (91). Tumor antigens are often taken up by APCs, especially DCs (108), which thereafter stimulate CTLs or $CD4^+$ helper cells. Some colorectal cancer (CRC) patients vaccinated with DCs pulsed with tumor lysates of CRC showed specific anti-tumor immune responses (108). Three common gp100 epitopes have been identified from different patients: G9154 (KTWG QYWQV), G9209 (ITDQVPFSV), and G9280 (YLEPGP VTA). After five weekly restimulations with either the native G9209 or G9280 peptide, melanoma-reactive CTLs could only be induced from two of seven patients. However, amino acid substitutions in these peptides enabled the induction of melanoma-reactive CTLs from all seven patients (90). The degron signal regulating degradation by the 26S protesome on the DRiPs pathway was introduced into the designation of epitope vaccines. Xiang et al. designed an autologous oral DNA vaccine containing the murine ubiquitin gene fused to minigenes encoding epitopes gp100 (25-33) and TRP-2 (181-188), which could break peripheral T cell tolerance by effective antigen processing and presentation (109). When the degron signal linked with N terminal of protein galactosidase, the MHC-I presentation of the protein was enhanced and elevated T cell activation evoked (110). Andreas Goldwith et al. demonstrated that an antigen conjugated to a degron signal was lead into defective ribosomal product pathway and the antigen was efficiently processed and specific $CD8^+$ T cell activation against a dominant MHC-I epitope in the protein was elevated both *in vitro* and *in vivo* (111).

Tumor adoptive therapy

Adoptive T cell immunotherapy to tumor has been demonstrated to be a successful strategy for the treatment of

cancer. There are several reasons to promote the development of adoptive T cell immunotherapy: 1) The method of *in vitro* culturing specific T cells had been developed (105, 112); 2) High avidity CTLs have superior antitumor efficacy *in vitro* and *in vivo* (113); 3) Gene delivery vectors are developed to transduce lymphocytes, such as retrovirus vector (114-116) and lentivector (117); 4) Adoptive T cell therapy overcomes the difficulty of isolating antigen-specific T lymphocytes for individual patients and could break the tolerance of self over-expressed peptides in tumor cell (118, 119).

In experimental treatment of tumor, both primary human lymphocytes and tumor infiltrating lymphocytes were conferred with reactivity to antigen and tumor cell lines *in vitro* after retroviral tranducing with high avidity TCR specific to MART-1 (120). Helmut et al. described that mouse CTLs introduced with a TCR$\alpha\beta$ genes specific for influenza nucleoprotein (NP) peptide by a retroviral vector, had a potency to reject tumor expressing this peptide *in vivo* (121). Lymphocytes retrovirally transduced TCR specific to gp100 significantly slowed tumor development in a murine melanoma model (122). Johnson et al. confirmed that when PBMCs and TILs were transduced with the high aividity TCRs specific to MART-1:27-35, they would induce melanoma regression *in vitro* and *in vivo* (94). T lymphocytes tranduced with retrovirally TCRs specific to WT1 could kill tumor lines endogenously expressing WT1 and surpress human leukaemia cells implanted in NOD/SCID mice (117, 123).

The antibody with binding specificity of TCRs to MHC-I associated with MAGE-3 peptide has been transduced into T cells, which confers the specific killing activity of T cells against HLA-A1$^+$, MAGE-3$^+$ melanoma cells (124). Transducing TCR into CD4$^+$ T cells also resulted in long-term tumor protection since CD4$^+$ T cells produced high levels of IL-2 expanded *in vivo*, providing help for CTLs-mediated tumor rejection (125), which can be used for establishment of long-term tumor immunity (126).

Morgan et al. have published a promising clinical trial result using TCR gene therapy in clinic trial that two patients demonstrated a sustained objective regression of their metastatic melanoma (127). In the trial, patients were treated with self PBMC transduced with genes of TCR specific to MART-1 from a TIL clone of a cancer patient who had a complete regression of metastatic melanoma after adoptive cell transfer previously. Although the generation of tumor-reactive T lymphocytes by transfer of specific TCR genes into autologous or allogenic lymphocytes is feasible, the response rate observed in TCR gene therapy trial is low only 2 out 17 were completely responsed. The selection of well-expressed and more epitope coverage TCRs with high affinity would improve such treatment (128, 129).

Autoimmune diseases

In certain circumstances body's immune system attacks itself by mistake, which causes autoimmune diseases. One of possible mechanisms is that CTLs activated by autoantigen or cross-stimulation start to attack itself and produce serious pathological outcome. Type I diabetes, also known as insulin-dependent diabetes mellitus (IDDM) results from the destruction of insulin-producing pancreatic β cells presumably involved with reactive CTLs (130). The development of autoimmune diabetes in NOD mice involves prevalent recruitment of CTLs recognizing epitopes of islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP). Administration of peptide analogs of IGRP206-214, the dominant epitope, reduced disease incidence but only under conditions that selective high-avidity T-cell clones were deleted (131). Repeated treatment of pre-diabetic NOD mice with soluble NRP-A7 peptide blunts the avidity maturation of the NRP-A7-reactive CTLs population by selectively deleting those clones expressing T-cell receptors with the highest affinity and lowest dissociation rates for pMHC-I binding. This inhibits the local production of T cells that are cytotoxic to β cells, and halts the progression from severe insulitis to diabetes (132).

Down-regulation of pMHC-I on the surface of target cells can be an effective way to prevent the attack by auto-reactive T cells. (NZBxNZW)F1 mice spontaneously develop with age an autoimmune disease that resembles the human disease, systemic lupus erythematosus (SLE). Since experimentally induced SLE depended on the expression of MHC-I, mice deficient in β_2m did not express MHC-I on cell surface and were resistant to the induction of experimental SLE (131). Live attenuated strains of human cytomegalovirus are under development as vaccines to prevent birth defects resulting from congenital infections. These strains encode four proteins that inhibit surface expression of MHC-I, presumably to evade CTL recognition and, perhaps, attenuate induction of immunity (132), the evasion strategy used by virus might be useful in treatment for autoimmune diseases caused by auto-reactive CTLs.

Incomplete deletion of autoreactive T cell populations of relatively high avidity can contribute to the development of pathogenic autoimmunity in the periphery (133). Hess et al. reported that MHC-I tetramers coupled ribosome-inactivating protein (RIP) saporin (SAP) toxin could kill antigen-specific CTLs (134). This strategy could be used to selectively eradicate pathogenic clonotypes expressing self-destructive TCRs.

Summary

pMHC-I/TCR interaction plays a fundamental role in the immune therapy of for viral infections, tumors and autoimmune diseases. Scientists have made great effort to elucidate the molecular basis involving this interaction and manipulate MHC-I/TCR interaction for immune therapy based on the knowledge of MHC-I antigen processing and presentation and the molecular mechanisms pMHC-I/TCR interaction.

The strategies have been demonstrated to be effective so far include: the enhancement of the expression of specific pMHC-I on the APC with elavated cytokines and chaperones, as well as the activities of proteasomes in the path of MHC-I antigen processing and presentation, or the delivery of a

pre-assembled MHC-I into APC. Increasing the stability, or spectrum, or immunogenicity of pMHC appeared on the surface of APC by replacement of some amino acids in a given peptide, or linking other peptides or molecules to the peptide. The engineered TCRs possessing better solubility or specific affinity to pMHC-I also exhibit potential application value in tumor therapy. The manipulation of pMHC-I/TCR interaction mentioned above greatly increases the efficiency of the therapy for viral infection and tumor. Conversely, the blockage therapy for those autoimmune diseases characterized as killing normal cells expressing self pMHC-I by CTLs, may be implemented by neutralizing CTLs or decreasing the expression self pMHC-I, as well as deleting autoreactive T cells using the modified peptides with higher affinity than self peptide.

The therapeutic strategy targeting MHC/TCR interaction has been demonstrated to be effective in fighting several viral infection clinically but its effectiveness for tumour treatment is only limited to very few individual cases. The applications for dealing with autoimmune diseases have shown some promise results pre-clinically but its usefulness as a clinical intervention remains to be seen. However, the growing knowledge of antigen processing, regulation of T cell responses, the improvement of gene delivery method and novel methodologies for the enhancement of pMHC expression and TCR recognition would make the manipulation of pMHC/TCR interaction targeting at both $CD4^+$ helper T cells and CTLs a powerful therapy for unmet clinical implications such as infection, tumor and autoimmune diseases.

Acknowledgement

We thank Miss Yi Mei for making figure 2.

Reference

1. Johnson-Tardieu JM, Walworth EW, Cornelius JG, Ye X, Schuster SM, Peck AB. Autoimmune diabetes-prone NOD mice express the Lyt2α (Lyt2.1) and Lyt3α (Lyt3.1) alleles of CD8. Immunogenetics. 1996;43:6-12.
2. Abele R, Tampe R. The ABCs of immunology: Structure and function of TAP, the transporter associated with antigen processing. Physiology. 2004;19:216-224.
3. Spies T, DeMars R. Restored expression of major histo-compatibility class I molecules by gene transfer of a putative peptide transporter. Nature. 1991;351:323-324.
4. Arnold D, Driscoll J, Androlewicz M, Hughes E, Cresswell P, Spies T. Proteasome subunits encoded in the MHC are not generally required for the processing of peptides bound by MHC class I molecules. Nature. 1992;360:171-174.
5. Spies T, Cerundolo V, Colonna M, Cresswell P, Townsend A, DeMars R. Presentation of viral antigen by MHC class I molecules is dependent on a putative peptide transporter heterodimer. Nature. 1992;355:644-646.
6. Henderson RA, Michel H, Sakaguchi K, et al. HLA-A2.1-associated peptides from a mutant cell line: a second pathway of antigen presentation. Science. 1992;255:1264-1266.
7. Raposo G, van Santen HM, Leijendekker R, Geuze HJ, Ploegh HL. Misfolded major histocompatibility complex class I molecules accumulate in an expanded ER-Golgi intermediate compartment. J Cell Biol. 1995;131:1403-1419.
8. Zhou X, Glas R, Momburg F, Hammerling GJ, Jondal M, Ljunggren HG. TAP2-defective RMA-S cells present Sendai virus antigen to cytotoxic T lymphocytes. Eur J Immunol. 1993;23:1796-1801.
9. Norbury CC, Princiotta MF, Bacik I, et al. Multiple antigen-specific processing pathways for activating naive CD8+ T cells *in vivo*. J Immunol. 2001;166:4355-4362.
10. Cromme FV, Airey J, Heemels MT, et al. Loss of transporter protein, encoded by the TAP-1 gene, is highly correlated with loss of HLA expression in cervical carcinomas. J Exp Med. 1994;179:335-340.
11. Ahn K, Gruhler A, Galocha B, et al. The ER-luminal domain of the HCMV glycoprotein US6 inhibits peptide translocation by TAP. Immunity. 1997;6:613-621.
12. Lehner PJ, Karttunen JT, Wilkinson GW, Cresswell P. The human cytomegalovirus US6 glycoprotein inhibits transporter associated with antigen processing-dependent peptide translocation. Proc Natl Acad Sci U S A. 1997;94:6904-6909.
13. Hengel H, Koopmann JO, Flohr T, et al. A viral ER-resident glycoprotein inactivates the MHC-encoded peptide transporter. Immunity. 1997;6:623-632.
14. Aisenbrey C, Sizun C, Koch J, et al. Structure and dynamics of membrane-associated ICP47, a viral inhibitor of the MHC I antigen-processing machinery. J Biol Chem. 2006;281:30365-30372.
15. Kutsch O, Vey T, Kerkau T, Hunig T, Schimpl A. HIV type 1 abrogates TAP-mediated transport of antigenic peptides presented by MHC class I. Transporter associated with antigen presentation. AIDS Res Hum Retroviruses. 2002;18:1319-1325.
16. Goldberg AL, Rock KL. Proteolysis, proteasomes and antigen presentation. Nature. 1992;357:375-379.
17. Schubert U, Anton LC, Gibbs J, Norbury CC, Yewdell JW, Bennink JR. Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. Nature. 2000;404:770-774.
18. Cresswell P, Ackerman AL, Giodini A, Peaper DR, Wearsch PA. Mechanisms of MHC class I-restricted antigen processing and cross-presentation. Immunol Rev. 2005;207:145-157.
19. Cresswell P, Bangia N, Dick T, Diedrich G. The nature of the MHC class I peptide loading complex. Immunol Rev. 1999;172: 21-28.
20. Day PM, Yewdell JW, Porgador A, Germain RN, Bennink JR. Direct delivery of exogenous MHC class I molecule-binding oligopeptides to the endoplasmic reticulum of viable cells. Proc Natl Acad Sci U S A. 1997;94:8064-8069.
21. Burrows GG, Ariail K, Celnik B, et al. Variation in H-2K (k) peptide motif revealed by sequencing naturally processed peptides from T-cell hybridoma class I molecules. J Neurosci Res. 1996;45:803-811.
22. York IA, Rock KL. Antigen processing and presentation by the class I major histocompatibility complex. Annu Rev Immunol. 1996;14:369-396.
23. Ljunggren HG, Stam NJ, Ohlen C, et al. Empty MHC class I molecules come out in the cold. Nature. 1990;346:476-480.
24. Shi Y, Smith KD, Lutz CT. TAP-independent MHC class I peptide antigen presentation to alloreactive CTL is enhanced by target cell incubation at subphysiologic temperatures. J Immunol. 1998;160:4305-4312.
25. Esquivel F, Yewdell J, Bennink J. RMA/S cells present endo-genously synthesized cytosolic proteins to class I-restricted cytotoxic T lymphocytes. J Exp Med. 1992;175:163-168.
26. Hosken NA, Bevan MJ. An endogenous antigenic peptide

bypasses the class I antigen presentation defect in RMA-S. J Exp Med. 1992;175:719-729.
27. Zhou X, Glas R, Liu T, Ljunggren HG, Jondal M. Antigen processing mutant T2 cells present viral antigen restricted through H-2K^b. Eur J Immunol. 1993;23:1802-1808.
28. Khanna R, Burrows SR, Moss DJ, Silins SL. Peptide transporter (TAP-1 and TAP-2)-independent endogenous processing of Epstein-Barr virus (EBV) latent membrane protein 2A: implications for cytotoxic T-lymphocyte control of EBV-associated malignancies. J Virol. 1996;70:5357-5362.
29. de la Salle H, Houssaint E, Peyrat MA, et al. Human peptide transporter deficiency: importance of HLA-B in the presentation of TAP-independent EBV antigens. J Immunol. 1997;158:4555-4563.
30. Zweerink HJ, Gammon MC, Utz U, et al. Presentation of endogenous peptides to MHC class I-restricted cytotoxic T lymphocytes in transport deletion mutant T2 cells. J Immunol. 1993;150:1763-1771.
31. Bacik I, Cox JH, Anderson R, Yewdell JW, Bennink JR. TAP (transporter associated with antigen processing)-independent presentation of endogenously synthesized peptides is enhanced by endoplasmic reticulum insertion sequences located at the amino- but not carboxyl-terminus of the peptide. J Immunol. 1994;152:381-387.
32. Smith KD, Lutz CT. Peptide-dependent expression of HLA-B7 on antigen processing-deficient T2 cells. J Immunol. 1996;156: 3755-3764.
33. Gromme M, Uytdehaag FG, Janssen H, et al. Recycling MHC class I molecules and endosomal peptide loading. Proc Natl Acad Sci U S A. 1999;96:10326-10331.
34. MacAry PA, Lindsay M, Scott MA, Craig JI, Luzio JP, Lehner PJ. Mobilization of MHC class I molecules from late endosomes to the cell surface following activation of CD34-derived human Langerhans cells. Proc Natl Acad Sci U S A. 2001;98:3982-3987.
35. Rodriguez A, Regnault A, Kleijmeer M, Ricciardi-Castagnoli P, Amigorena S. Selective transport of internalized antigens to the cytosol for MHC class I presentation in dendritic cells. Nat Cell Biol. 1999;1:362-368.
36. M.Roitt I, Delves PJ. Roitt's Essential Immunolgy (ed Tenth). Beijing: Higher Education Press; 2002.
37. Chen W, Cai MY, Wei DP, Wang X. Pivotal molecules of MHC I pathway in human primary hepatocellular carcinoma. World J Gastroenterol. 2005;11:3297-3299.
38. Fremont DH, Matsumura M, Stura EA, Peterson PA, Wilson IA. Crystal structures of two viral peptides in complex with murine MHC class I H-2K^b. Science. 1992;257:919-927.
39. Madden DR, Garboczi DN, Wiley DC. The antigenic identity of peptide-MHC complexes: a comparison of the conformations of five viral peptides presented by HLA-A2. Cell. 1993;75:693-708.
40. Rudolph MG, Stanfield RL, Wilson IA. How TCRs bind MHCs, peptides, and coreceptors. Annu Rev Immunol. 2006;24:419-466.
41. Stern LJ, Wiley DC. Antigenic peptide binding by class I and class II histocompatibility proteins. Structure. 1994;2:245-251.
42. Tynan FE, Borg NA, Miles JJ, et al. High resolution structures of highly bulged viral epitopes bound to major histo-compatibility complex class I. Implications for T-cell receptor engagement and T-cell immunodominance. J Biol Chem. 2005; 280:23900-23909.
43. Speir JA, Stevens J, Joly E, Butcher GW, Wilson IA. Two different, highly exposed, bulged structures for an unusually long peptide bound to rat MHC class I RT1-Aa. Immunity. 2001; 14:81-92.
44. Buslepp J, Wang H, Biddison WE, Appella E, Collins EJ. A correlation between TCR Vα docking on MHC and CD8 dependence: implications for T cell selection. Immunity. 2003; 19:595-606.
45. Valitutti S, Muller S, Cella M, Padovan E, Lanzavecchia A. Serial triggering of many T-cell receptors by a few peptide-MHC complexes. Nature. 1995;375:148-151.
46. Kageyama S, Tsomides TJ, Sykulev Y, Eisen HN. Variations in the number of peptide-MHC class I complexes required to activate cytotoxic T cell responses. J Immunol. 1995;154:567-576.
47. Iris Motta, Yu-Chun Lone, Kourilsky P. *In vitro* induction of naive cytotoxic T lymphocytes with complexes of peptide and recombinant MHC class I molecules coated onto beads: role of TCR/ligand density. Eur. J. Immunol. 1998;28:3685-3695.
48. Krogsgaard M, Davis MM. How T cells 'see' antigen. Nat Immunol. 2005;6:239-245.
49. Weidanz JA, Nguyen T, Woodburn T, et al. Levels of specific peptide-HLA class I complex predicts tumor cell susceptibility to CTL killing. J Immunol. 2006;177:5088-5097.
50. Holmberg K, Mariathasan S, Ohteki T, Ohashi PS, Gascoigne NR. TCR binding kinetics measured with MHC class I tetramers reveal a positive selecting peptide with relatively high affinity for TCR. J Immunol. 2003;171:2427-2434.
51. Schlom J, Hodge JW. The diversity of T-cell co-stimulation in the induction of antitumor immunity. Immunol Rev. 1999;170: 73-84.
52. Pecht I, Gakamsky DM. Spatial coordination of CD8 and TCR molecules controls antigen recognition by CD8$^+$ T-cells. FEBS Lett. 2005;579:3336-3341.
53. Terasawa H, Tsang KY, Gulley J, Arlen P, Schlom J. Identification and characterization of a human agonist cytotoxic T-lymphocyte epitope of human prostate-specific antigen. Clin Cancer Res. 2002;8:41-53.
54. Wilson DB, Wilson DH, Schroder K, et al. Specificity and degeneracy of T cells. Mol Immunol. 2004;40:1047-1055.
55. Maverakis E, van den Elzen P, Sercarz EE. Self-reactive T cells and degeneracy of T cell recognition: evolving concepts-from sequence homology to shape mimicry and TCR flexibility. J Autoimmun. 2001;16:201-209.
56. Lang HL, Jacobsen H, Ikemizu S, et al. A functional and structural basis for TCR cross-reactivity in multiple sclerosis. Nat Immunol. 2002;3:940-943.
57. Reiser JB, Gregoire C, Darnault C, et al. A T cell receptor CDR3β loop undergoes conformational changes of unprecedented magnitude upon binding to a peptide/MHC class I complex. Immunity. 2002;16:345-354.
58. Kjer-Nielsen L, Clements CS, Purcell AW, et al. A structural basis for the selection of dominant αβ T cell receptors in antiviral immunity. Immunity. 2003;18:53-64.
59. Garcia KC, Degano M, Pease LR, et al. Structural basis of plasticity in T cell receptor recognition of a self peptide-MHC antigen. Science. 1998;279:1166-1172.
60. Ding YH, Baker BM, Garboczi DN, Biddison WE, Wiley DC. Four A6-TCR/peptide/HLA-A2 structures that generate very different T cell signals are nearly identical. Immunity. 1999;11: 45-56.
61. Reiser JB, Darnault C, Gregoire C, et al. CDR3 loop flexibility contributes to the degeneracy of TCR recognition. Nat Immunol. 2003;4:241-247.
62. Gagnon SJ, Borbulevych OY, Davis-Harrison RL, et al. T cell receptor recognition *via* cooperative conformational plasticity. J Mol Biol. 2006;363:228-243.

63. Dai S, Huseby ES, Rubtsova K, et al. Crossreactive T Cells spotlight the germline rules for αβ T cell-receptor interactions with MHC molecules. Immunity. 2008;28:324-334.
64. Marrack P, Scott-Browne JP, Dai S, Gapin L, Kappler JW. Evolutionarily conserved amino acids that control TCR-MHC interaction. Annu Rev Immunol. 2008;26:171-203.
65. Freedman RS, Platsoucas CD. Immunotherapy for peritoneal ovarian carcinoma metastasis using ex vivo expanded tumor infiltrating lymphocytes. Cancer Treat Res. 1996;82:115-146.
66. Berwin B, Hart JP, Rice S, et al. Scavenger receptor-A mediates gp96/GRP94 and calreticulin internalization by antigen-presenting cells. EMBO J. 2003;22:6127-6136.
67. Binder RJ, Kelly JB, 3rd, Vatner RE, Srivastava PK. Specific immunogenicity of heat shock protein gp96 derives from chaperoned antigenic peptides and not from contaminating proteins. J Immunol. 2007;179:7254-7261.
68. Binder RJ, Srivastava PK. Peptides chaperoned by heat-shock proteins are a necessary and sufficient source of antigen in the cross-priming of $CD8^+$ T cells. Nat Immunol. 2005;6:593-599.
69. Melief CJ. Escort service for cross-priming. Nat Immunol. 2005;6:543-544.
70. SenGupta D, Norris PJ, Suscovich TJ, et al. Heat shock protein-mediated cross-presentation of exogenous HIV antigen on HLA class I and class II. J Immunol. 2004;173:1987-1993.
71. Biswas C, Sriram U, Ciric B, Ostrovsky O, Gallucci S, Argon Y. The N-terminal fragment of GRP94 is sufficient for peptide presentation *via* professional antigen-presenting cells. Int Immunol. 2006;18:1147-1157.
72. Zhao KJ, Cheng H, Zhu KJ, et al. Recombined DNA vaccines encoding calreticulin linked to HPV6bE7 enhance immune response and inhibit angiogenic activity in B16 melanoma mouse model expressing HPV 6bE7 antigen. Arch Dermatol Res. 2006.
73. Liu G, Zheng W, Chen X. Molecular cloning of proteasome activator PA28-β subunit of large yellow croaker (Pseudosciana crocea) and its coordinated up-regulation with MHC class I α-chain and β2-microglobulin in poly I:C-treated fish. Mol Immunol. 2007;44:1190-1197.
74. Kloetzel PM. The proteasome and MHC class I antigen processing. Biochim Biophys Acta. 2004;1695:225-233.
75. Wang L, Qi X, Sun Y, Liang L, Ju D. Adenovirus-mediated combined P16 gene and GM-CSF gene therapy for the treatment of established tumor and induction of antitumor immunity. Cancer Gene Ther. 2002;9:819-824.
76. Batten P, Yacoub MH, Rose ML. Effect of human cytokines (IFN-γ, TNF-α, IL-1β, IL-4) on porcine endothelial cells: induction of MHC and adhesion molecules and functional significance of these changes. Immunology. 1996;87:127-133.
77. Mottez E, Langlade-Demoyen P, Gournier H, et al. Cells expressing a major histocompatibility complex class I molecule with a single covalently bound peptide are highly immunogenic. J Exp Med. 1995;181:493-502.
78. Uger RA, Chan SM, Barber BH. Covalent linkage to β2-microglobulin enhances the MHC stability and antigenicity of suboptimal CTL epitopes. J Immunol. 1999;162:6024-6028.
79. White J, Crawford F, Fremont D, Marrack P, Kappler J. Soluble class I MHC with β2-microglobulin covalently linked peptides: specific binding to a T cell hybridoma. J Immunol. 1999;162: 2671-2676.
80. Tafuro S, Meier UC, Dunbar PR, et al. Reconstitution of antigen presentation in HLA class I-negative cancer cells with peptide-β2m fusion molecules. Eur J Immunol. 2001;31:440-449.
81. Shen C, Chang CC, Zhang J, et al. Structural and functional characterization of peptide-β2m fused HLA-A2/MART1 (27-35) complexes. Biochem Biophys Res Commun. 2006;342:57-65.
82. Denkberg G, Klechevsky E, Reiter Y. Modification of a tumor-derived peptide at an HLA-A2 anchor residue can alter the conformation of the MHC-peptide complex: probing with TCR-like recombinant antibodies. J Immunol. 2002;169:4399-4407.
83. Lipford GB, Bauer S, Wagner H, Heeg K. Peptide engineering allows cytotoxic T-cell vaccination against human papilloma virus tumour antigen, E6. Immunology. 1995;84:298-303.
84. Pogue RR, Eron J, Frelinger JA, Matsui M. Amino-terminal alteration of the HLA-A*0201-restricted human immuno-deficiency virus pol peptide increases complex stability and *in vitro* immunogenicity. Proc Natl Acad Sci U S A. 1995;92: 8166-8170.
85. Sloan-Lancaster J, Allen PM. Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology. Annu Rev Immunol. 1996;14:1-27.
86. Slansky JE, Rattis FM, Boyd LF, et al. Enhanced antigen-specific antitumor immunity with altered peptide ligands that stabilize the MHC-peptide-TCR complex. Immunity. 2000;13: 529-538.
87. Chen W, Khilko S, Fecondo J, Margulies DH, McCluskey J. Determinant selection of major histocompatibility complex class I-restricted antigenic peptides is explained by class I-peptide affinity and is strongly influenced by nondominant anchor residues. J Exp Med. 1994;180:1471-1483.
88. Sette A, Vitiello A, Reherman B, et al. The relationship between class I binding affinity and immunogenicity of potential cytotoxic T cell epitopes. J Immunol. 1994;153:5586-5592.
89. Kawakami Y, Eliyahu S, Jennings C, et al. Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with *in vivo* tumor regression. J Immunol. 1995;154:3961-3968.
90. Parkhurst MR, Salgaller ML, Southwood S, et al. Improved induction of melanoma-reactive CTL with peptides from the melanoma antigen gp100 modified at HLA-A*0201-binding residues. J Immunol. 1996;157:2539-2548.
91. Borbulevych OY, Baxter TK, Yu Z, Restifo NP, Baker BM. Increased immunogenicity of an anchor-modified tumor-associated antigen is due to the enhanced stability of the peptide/MHC complex: implications for vaccine design. J Immunol. 2005;174:4812-4820.
92. Webb AI, Dunstone MA, Williamson NA, et al. T cell determinants incorporating β-amino acid residues are protease resistant and remain immunogenic *in vivo*. J Immunol. 2005; 175:3810-3818.
93. Weinhold M, Sommermeyer D, Uckert W, Blankenstein T. Dual T cell receptor expressing CD8+ T cells with tumor- and self-specificity can inhibit tumor growth without causing severe autoimmunity. J Immunol. 2007;179:5534-5542.
94. Johnson LA, Heemskerk B, Powell DJ, Jr., et al. Gene transfer of tumor-reactive TCR confers both high avidity and tumor reactivity to nonreactive peripheral blood mononuclear cells and tumor-infiltrating lymphocytes. J Immunol. 2006;177:6548-6559.
95. Cohen CJ, Zheng Z, Bray R, et al. Recognition of fresh human tumor by human peripheral blood lymphocytes transduced with a bicistronic retroviral vector encoding a murine anti-p53 TCR. J Immunol. 2005;175:5799-5808.
96. Sommermeyer D, Neudorfer J, Weinhold M, et al. Designer T cells by T cell receptor replacement. Eur J Immunol. 2006;36: 3052-3059.
97. Morgan RA, Dudley ME, Yu YY, et al. High efficiency TCR gene transfer into primary human lymphocytes affords avid

recognition of melanoma tumor antigen glycoprotein 100 and does not alter the recognition of autologous melanoma antigens. J Immunol. 2003;171:3287-3295.
98. Richman SA, Kranz DM. Display, engineering, and applications of antigen-specific T cell receptors. Biomol Eng. 2007;24:361-373.
99. Chlewicki LK, Holler PD, Monti BC, Clutter MR, Kranz DM. High-affinity, peptide-specific T cell receptors can be generated by mutations in CDR1, CDR2 or CDR3. J Mol Biol. 2005;346: 223-239.
100. Cohen CJ, Li YF, El-Gamil M, Robbins PF, Rosenberg SA, Morgan RA. Enhanced antitumor activity of T cells engineered to express T-cell receptors with a second disulfide bond. Cancer Res. 2007;67:3898-3903.
101. Stanislawski T, Voss RH, Lotz C, et al. Circumventing tolerance to a human MDM2-derived tumor antigen by TCR gene transfer. Nat Immunol. 2001;2:962-970.
102. Biswas C, Moza B, Sundberg EJ, Kranz DM. Characterization of T cell receptors engineered for high affinity against toxic shock syndrome toxin-1. J Mol Biol. 2005;353:308-321.
103. Brill TH, Kubler HR, von Randenborgh H, et al. Allogeneic retrovirally transduced, IL-2- and IFN-γ-secreting cancer cell vaccine in patients with hormone refractory prostate cancer--a phase I clinical trial. J Gene Med. 2007;9:547-560.
104. Greenberg PD, Cheever MA. Effector mechanisms operative in adoptive therapy of tumor-bearing animals: implications for the use of interleukin-2. J Biol Response Mod. 1984;3:455-461.
105. Cheever MA, Thompson DB, Klarnet JP, Greenberg PD. Antigen-driven long term-cultured T cells proliferate *in vivo*, distribute widely, mediate specific tumor therapy, and persist long-term as functional memory T cells. J Exp Med. 1986;163: 1100-1112.
106. Margolin KA, Doroshow JH, Akman SA, et al. Phase I trial of interleukin-2 plus doxorubicin. J Immunother Emphasis Tumor Immunol. 1993;14:70-76.
107. Treisman J, Hwu P, Minamoto S, et al. Interleukin-2-transduced lymphocytes grow in an autocrine fashion and remain responsive to antigen. Blood. 1995;85:139-145.
108. Tamir A, Basagila E, Kagahzian A, et al. Induction of tumor-specific T-cell responses by vaccination with tumor lysate-loaded dendritic cells in colorectal cancer patients with carcinoembryonic-antigen positive tumors. Cancer Immunol Immunother. 2007;56:2003-2016.
109. Xiang R, Lode HN, Chao TH, et al. An autologous oral DNA vaccine protects against murine melanoma. Proc Natl Acad Sci U S A. 2000;97:5492-5497.
110. Grant EP, Michalek MT, Goldberg AL, Rock KL. Rate of antigen degradation by the ubiquitin-proteasome pathway influences MHC class I presentation. J Immunol. 1995;155: 3750-3758.
111. Goldwich A, Hahn SS, Schreiber S, et al. Targeting HIV-1 Gag into the defective ribosomal product pathway enhances MHC class I antigen presentation and $CD8^+$ T cell activation. J Immunol. 2008;180:372-382.
112. Cheever MA, Klarnet JP, Peace DJ, Greenberg PD. Antigen-specific cultured T cells can mediate tumor therapy and provide long-term immunologic memory *in vivo*. Prog Clin Biol Res. 1987;244:49-58.
113. Zeh HJ, 3rd, Perry-Lalley D, Dudley ME, Rosenberg SA, Yang JC. High avidity CTLs for two self-antigens demonstrate superior *in vitro* and *in vivo* antitumor efficacy. J Immunol. 1999; 162:989-994.
114. Rosenberg SA, Aebersold P, Cornetta K, et al. Gene transfer into humans--immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med. 1990;323:570-578.
115. Culver K, Cornetta K, Morgan R, et al. Lymphocytes as cellular vehicles for gene therapy in mouse and man. Proc Natl Acad Sci U S A. 1991;88:3155-3159.
116. Cornetta K, Nguyen N, Morgan RA, et al. Infection of human cells with murine amphotropic replication-competent retroviruses. Hum Gene Ther. 1993;4:579-588.
117. Stauss HJ, Thomas S, Cesco-Gaspere M, et al. WT1-specific T cell receptor gene therapy: Improving TCR function in transduced T cells. Blood Cells Mol Dis. 2008;40:113-116.
118. Sadovnikova E, Stauss HJ. Peptide-specific cytotoxic T lymphocytes restricted by nonself major histocompatibility complex class I molecules: reagents for tumor immunotherapy. Proc Natl Acad Sci U S A. 1996;93:13114-13118.
119. Xue S, Gillmore R, Gao L, et al. Use of the allogeneic TCR repertoire to enhance anti-tumor immunity. J Biol Regul Homeost Agents. 2004;18:131-133.
120. Hughes MS, Yu YY, Dudley ME, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. Hum Gene Ther. 2005;16:457-472.
121. Kessels HW, Wolkers MC, van den Boom MD, van der Valk MA, Schumacher TN. Immunotherapy through TCR gene transfer. Nat Immunol. 2001;2:957-961.
122. Abad JD, Wrzensinski C, Overwijk W, et al. T-cell receptor gene therapy of established tumors in a murine melanoma model. J Immunother (1997). 2008;31:1-6.
123. Xue SA, Gao L, Hart D, et al. Elimination of human leukemia cells in NOD/SCID mice by WT1-TCR gene-transduced human T cells. Blood. 2005;106:3062-3067.
124. Chames P, Willemsen RA, Rojas G, et al. TCR-like human antibodies expressed on human CTLs mediate antibody affinity-dependent cytolytic activity. J Immunol. 2002;169:1110-1118.
125. Morris EC, Tsallios A, Bendle GM, Xue SA, Stauss HJ. A critical role of T cell antigen receptor-transduced MHC class I-restricted helper T cells in tumor protection. Proc Natl Acad Sci U S A. 2005;102:7934-7939.
126. Morris E, Hart D, Gao L, Tsallios A, Xue SA, Stauss H. Generation of tumor-specific T-cell therapies. Blood Rev. 2006; 20:61-69.
127. Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science. 2006;314:126-129.
128. Jorritsma A, Gomez-Eerland R, Dokter M, et al. Selecting highly affine and well-expressed TCRs for gene therapy of melanoma. Blood. 2007;110:3564-3572.
129. Stauss HJ, Cesco-Gaspere M, Thomas S, et al. Monoclonal T-cell receptors: new reagents for cancer therapy. Mol Ther. 2007;15:1744-1750.
130. Yoon JW, Jun HS. Cellular and molecular roles of β cell autoantigens, macrophages and T cells in the pathogenesis of autoimmune diabetes. Arch Pharm Res. 1999;22:437-447.
131. Maree AF, Santamaria P, Edelstein-Keshet L. Modeling competition among autoreactive $CD8^+$ T cells in autoimmune diabetes: implications for antigen-specific therapy. Int Immunol. 2006;18:1067-1077.
132. Amrani A, Verdaguer J, Serra P, Tafuro S, Tan R, Santamaria P. Progression of autoimmune diabetes driven by avidity maturation of a T-cell population. Nature. 2000;406:739-742.
133. Han B, Serra P, Yamanouchi J, et al. Developmental control of CD8 T cell-avidity maturation in autoimmune diabetes. J Clin Invest. 2005;115:1879-1887.
134. Hess PR, Barnes C, Woolard MD, et al. Selective deletion of

antigen-specific $CD8^+$ T cells by MHC class I tetramers coupled to the type I ribosome-inactivating protein saporin. Blood. 2007; 109:3300-3307.