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THE RECOGNITION PATTERN OF GAMMADELTA T CELLS

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1. ABSTRACT

The main function of immune system is to recognize and respond to foreign antigens. During the course of evolution, the body has successfully developed four kinds of mechanisms to recognize antigens, including pattern recognition mediated by macrophages, missing-self and induced-self recognition for NK cells, antigen specific recognition for alphabeta T cells and B cells and "broad-spectrum specific" recognition for gammadelta T cells. The three formers have made great progress these years. However, details of antigen recognition by gammadelta T cells are still mysterious. Gammadelta T cells, a class of T cells only existing in primates, differ from alphabeta T cells in TCR diversity, the structure of TCR-CD3 complex, the tissue distribution, the antigens that they recognize and the way involved in recognition. Here, we shed light on the recognition mechanism of gammadelta T cells against several known antigens and discuss the possible response pattern along the research historical process. We put forward a recognition hypothesis for gammadelta TCR that is conformational recognition based on germline encoded recognition. The key germline encoded amino acids dominate the "putative binding box" and are responsible for recognition. Meanwhile, after gammadelta T cells are activated, several other molecules such as CD69, CD16, 2B4, NKG2D also participate in inducing cytotoxicity of activated gammadelta T cells. Obviously, the illustration of recognition mechanism for gammadelta T cells will help to comprehensively understand the whole immune system and design the higher effective multi-epitope vaccines for tumor and infection immunity.

2. INTRODUCTION

Gammadelta T cells, a class of T cells only existing in primates, differ from alphabeta T cells in TCR diversity, the structure of TCR-CD3 complex, the tissue distribution, the antigens that they recognize and the way involved in recognition. Unlike alphabeta T cells, there are only eight Vgamma and eight Vdelta gene segments (Vgamma1.2, Vgamma1.3, Vgamma1.4, Vgamma1.5, Vgamma1.8, Vgamma2, Vgamma3, Vgamma4/Vdelta1, Vdelta2, Vdelta3, Vdelta4, Vdelta5, Vdelta6, Vdelta7, Vdelta8) (1). Nevertheless, gammadelta TCR repertoire is more diverse than or at least as that of alphabeta TCR due to usage of three Ddelta fragments and the translation of Ddelta sequences in all three possible reading frames and N nucleotides insertion. Among them, two major subsets exist in human. Whereas Vdelta1 gammadelta T cells mainly present in normal epithelial tissue and increase in the peripheral blood of HIV and malarial infected individuals (2-5), Vgamma2Vdelta2 (also termed Vgamma9Vdelta2) gammadelta T cells mainly distribute in peripheral blood and expand in vivo during bacterial and parasitic infections (6-18). Although gammadelta T cells only take up a small proportion of human T lymphocytes, a large body of evidence indicate that they play an important role in the first line of immune defense. However, the detailed recognition mechanism remains unclear. With the establishment of antigen-specific gammadelta T cell lines and/or clones, people have accumulated lots of data about the feature of antigen responsive gammadelta TCR. CDR3 length analysis and available TCR structures (19-21) indicate that the recognition of gammadeltaTCR is more like antibody than alphabeta TCR, that is MHC-unrestricted direct recognition. Factually, there have been lots of reviews about recognition against known antigens since the discovery of gammadelta T cells (22-29). Some of the areas will be revisited in more detail in this review and expanded upon in light of recent findings. We also compare the difference of proliferation and cytotoxicity effects between activated and native gammadelta T cells. On the basis of available recognition data, we put forward a recognition hypothesis for gammadelta TCR that is conformational recognition based on germline encoded recognition. The key germline encoded amino acids dominate the "putative binding box" and are responsible for interaction with antigens. Meanwhile, after gammadelta T cells are activated, several other molecules such as CD69, CD16, 2B4, NKG2D also participate in inducing cytotoxicity of activated gammadelta T cells. Obviously, the illustration of recognition mechanism for gammadelta T cells will help to comprehensively understand the whole immune system and design the higher effective multi-epitope vaccines for tumor and infection immunity.

3. NON-PEPTIDE ANTIGENS

3.1 Mycobacterium tuberculosis and phosphoantigens

Among the known antigens for gammadelta T cells, the most attractive ones are non-peptide antigens. Several years ago, it had been found that gammadelta T cells increased in mycobacterial infection and in vitro when PBMC were cultured with killed whole Mycobacterium tuberculosis strain H37Ra (M. tb). It's unnecessary for prior exposure to mycobacterial Ag as gammadelta T cells expanded in the PBMC from either PPD^+ or PPD^- individuals or even newborn (30). Such M. tuberculosis-induced gammadelta T cells mainly involved Vgamma9Vdelta2 T cells. Later, Mycobacterium tuberculosis specific gammadelta T cell lines were established which required antigen-presenting cells such as blood monocytes for optimal proliferation. Before adding live Mycobacterium tuberculosis, prefixation or pretreatment of monocytes with cytochalasin D, an inhibitor of phagocytosis by inhibiting actin polymerization, inhibited the proliferation of Mycobacterium tuberculosis specific gammadelta T cell lines, indicating that phagocytosis and processing by monocytes of Mycobacterium tuberculosis were required for presentation of antigens to gammadelta T cell lines (31). However, as for the effect of Brefeldin A that inhibits transport from the endoplasmic reticulum to the trans-Golgi network, it led to different results. While Brefeldin A attenuated the cytotoxicity of Mycobacterium tuberculosis specific gammadelta T cell lines against monocytes incubated with live Mycobacterium tuberculosis (31), it didn't affect the proliferation of Mycobacterium tuberculosis specific gammadelta T cell lines in response to Mycobacterium tuberculosis (32), indicating that the proliferation and cytotoxicity of gammadelta T cells, at least Mycobacterium tuberculosis specific gammadelta T cell lines may apply distinct antigen processing pathways.

In vitro experiments, freshly negatively isolated peripheral blood gammadelta T cells alone failed to proliferate in response to killed Mycobacterium tuberculosis without the autologous or allogeneic PBMC as accessory cells or even in the presence of T cell-depleted (E-) autologous feeder cells (33,34). Autologous $CD4^+$ T cells or IL-2 could recover the proliferation of these negatively selected gammadelta T cells. Meanwhile, endogenously produced IFN-gamma was pivotal for the activation of gammadelta T cells by Mycobacterium tuberculosis as neutralizing anti-IFN-gamma inhibited the responsiveness (34). However, it's controversial for $CD4^+$ T cell precise functional mechanism. Klaus Pechhold *et al* proved that the helper effect of $CD4^+$ T cells was mediated by soluble factors by virtue of double chamber experiments (34). But such experiment couldn't negate the contribution of cell-cell contact as the data obtained from co-culture was much bigger (8.9) than the one from separate culture (5.0), although they both caused marked increase of Vgamma9Vdelta2 T cells in response to killed M. tb. In fact, LUIS M. VILA *et al* further proved that the expansion of Vgamma9Vdelta2 T cell clones induced by the acetone-precipitable fraction of Mycobacterium tuberculosis (AP-MT) were accessory cell dependent but independent of professional antigen-presenting cells and that a certain subset of memory helper T cells ($CD4^+CD45RO^+CD7^-$ alphabetaT cells) determined this helper function which was based on cell-cell contact (35). IL-2 itself had no function in this system perhaps because they used Vgamma9Vdelta2 T cell clones instead of freshly negatively isolated gammadelta T cells. The results accorded with the finding that anti-IL-2 receptor

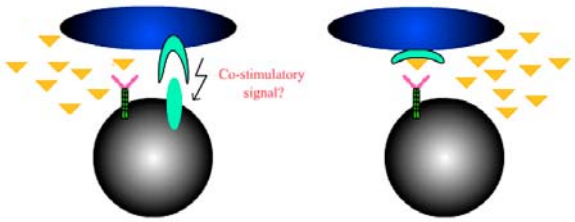

Figure 1. Proliferation of Vgamma9Vdelta2 T cells in response to mycobacterial Ag.

antibody couldn't block the proliferation of some Vgamma9Vdelta2 T cell clones against AP-MT in the presence of accessory cells (36), indicating the different sensitivity or reactivity of freshly negatively isolated gammadelta T cells and gammadelta T cell clones against IL-2. Interestingly, Craig T. Morita *et al* found that most $CD4^-CD8^-$ and $CD8^+$ Vgamma9Vdelta2 T cell clones responded to mycobacterial Ag in the absence of accessory cells and that T cell- T cell contact was required for recognition, although at low levels. Meanwhile, they also discovered that some $CD4^+$ Vgamma9Vdelta2 T cell clones needed accessory cells for proliferation (37). Notably, in any case, optimal proliferation of Vgamma9Vdelta2 T cell lines or clones needed accessory cells and cell-cell contact (37,38).

A great deal of indirect evidence indicated that the Vgamma9Vdelta2 TCR mediated the responsiveness of Vgamma9Vdelta2 T cells against mycobacterial Ag (38-44). Direct evidence for Vgamma9Vdelta2 TCR mediated recognition was raised by Francois Davodeau *et al.* By virtue of gene tranfection experiment, they demonstrated that almost all (50/53) clones expressing a Vgamma9JPC1gamma/Vdelta2 TCR (or Vgamma2Jgamma1.2C1gamma/Vdelta2 TCR) recognized mycobacterial Ag, while none of the $Vgamma9^+Vdelta2^-$ TCR, $Vgamma9^-Vdelta2^+$ TCR, $Vgamma9^-Vdelta2^-$ TCR or Vgamma9J2C2gamma/Vdelta2 TCR expressing clones were responsive. Those expressing Vgamma9J1C1gamma/Vdelta2 TCR clones were weakly or nonreactive (45). All of them indicated that only some certain Vgamma9Vdelta2 TCR sequence either germline fragment and/or reconstructed CDR3 region were responsible for recognizing mycobacterial Ag, which was in agreement with the different responsiveness of Vgamma9Vdelta2 T cell clones. In that case, the clones expressing some certain Vgamma9Vdelta 2TCR that have strong affinity with mycobacterial Ag may proliferate without additional accessory cells, while those clones with low affinity Vgamma9Vdelta2 TCR may require accessory cells for optimal proliferation. However, it can't determine whether free mycobacterial Ag itself binds with TCR and accessory cells offer co-stimulatory signals or certain presenting molecules on accessory cells present mycobacterial Ag to Vgamma9Vdelta2 TCR (Figure 1). Of note, it's necessary for the continuous presence of the mycobacterial Ag in proliferation phase and for TNF production since they couldn't be pulsed on accessory cells and simple wash would abolish its activity(37,46). Classical MHC I and II and CD1 molecules were not the presenting modules (37,46,47). However, in fact, a lot of other cells including EBV-transformed B cells, allogeneic PBMC, hemopoietic and fibroblastic murine cells were all able to function as $CD4^+CD45RO^+CD7^-$ alphabetaT cells(37,38). Importantly, whether these cells all apply the same helper mechanism remains unclear. Among them, both fixed and unfixed EBV-transformed B cells were capable of inducing the proliferative responses of APC-dependent Vgamma9Vdelta2 T cell clones against mycobacterial Ag (37), which lended support to the former presenting hypothesis. Further, although Guido Sireci *et al* also showed that fixed PBMC could induce the proliferation of Vgamma9Vdelta2 T cell clone (G4) against mycobacterial Ag. However, due to the limited information about the characteristic feature of the clone, whether APC-dependent or independent, it's difficult to give the firm evidence that fixed PBMC did function in this system in that they didn't make the related control about the contribution of mycobacterial Ag alone and the same clone (G4) could produce significant IFN-gamma and IL-4 in response to mycobacterial Ag without additional APC (38). In any case, mycobacterial Ag could induce Vgamma9Vdelta2 T cells to proliferate, produce cytokines or even their cytotoxicity against a broad set of target cells including hemopoietic and fibroblastic human and murine cells or even Fas-positive and Fas-negative xenogeneic cells (46). With respect to the extensive cytotoxicity, the exact mechanism is unclear. But at least two possible explanations could be offered. One is the Vgamma9Vdelta2 TCR-independent nonspecific Nk-like cytotoxicity. Alternatively, presenting molecules are extensively expressed on many cells.

With the development of research, several antigens derived from Mycobacterium tuberculosis had been discovered and isolated. These antigens were divided into two groups, collectively referred to as phosphantigens which were different from conventional protein antigens and resistant to protease but sensitive to phosphatase. One was the isoprenoid-PP including isopentenyl-PP, dimethylallyl-PP, farnesyl-PP, and geranyl-PP (37,39,43,48). The other was called TUBag (49,50), of which the major active component was identified as 3-formyl-1-butyl pyrophosphate (51). At the same time, other phosphoantigen derivatives and synthetic analogues with different activity potency were also identified (40,43,52-56) (Figure 2). Many Vgamma9Vdelta2 T cell clones exhibited cross-reactive fashion against these antigens (40,57,58). Of important, with respect to the bioactivity or antigenicity of these non-peptide phosphate complexes, first, the phosphate moiety was essential for their antigenicity as cleavage of the terminal phosphate by alkaline phosphatase completely abolished the stimulatory activity and the stimulating intensity of monophosphate Ag was weaker than that of related pyrophosphate Ag (59,60). By contrast, another research group discovered that partial hydrolysis of the pyrophosphate Ag (BrHPP) and simultaneous release of stoichiometric amounts of Pi could be detected during the stimulating experiment of Vgamma9Vdelta2 T cells and that incubating with dephosphorylation-inhibiting drugs abolished the responsiveness of gammadelta T cells against BrHPP, indicating the hydrolysis of the pyrophosphate moiety was

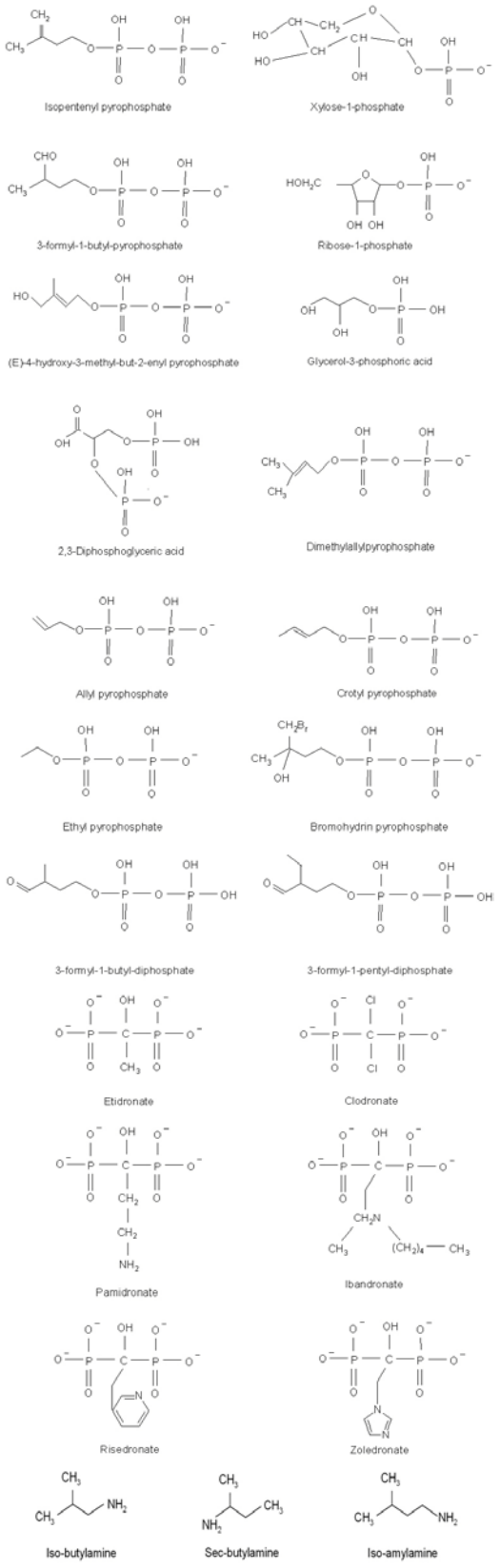

Figure 2. Structure of major nonpeptide ligands (naturally occurring and synthetic antigens).

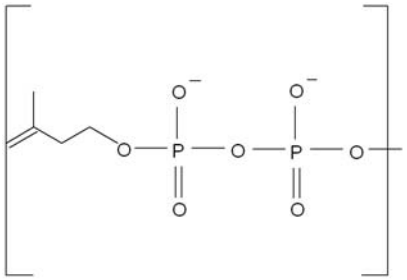

Figure 3. Isoprenoid unit.

required for the stimulating bioactivity (52). Paradoxically, both progressive phosphate degradation with cells and alkaline phosphatase treatment resulted in irreversible loss of phosphantigen bioactivity (52,61). Second, the carbon chain closest to the pyrophosphate moiety was of great importance for antigenicity. For less than four carbons, double bond didn't affect bioactivity (60). Nevertheless, for more than five carbons, a core isoprenoid unit would be required for antigenicity (Figure 3). Either adding to the pyrophosphate end with large chemical groups such as AMP or adding to the alkyl chain end of the unit with aromatic chains wouldn't alter bioactivity significantly (60). What' more, others without core unit also had potential activity if substituted with polarizable groups such as (-CH2Br) and (-CH2I) at C3 station (52).

Recently, great success about the recognition mechanism against pyrophosphoantigen was achieved by several research groups. First, site-directed mutagenesis of JgammaP fragment demonstrated that two positively charged N-terminal lysine residues (K108 and K109) in the Vgamma9 chain CDR3 region encoded by the germline JgammaP gene determined the recogniton of monoethyl pyrophosphate (EtPP), isobutyl amine (IBA) and pamidronate (57). Changing either of the two lysine residues to "E" amino acid abolished responsiveness (57). In contrast to this, rhesus monkey Vgamma9Vdelta2 T cells responded to many nonpeptide antigens and shared similar responsive rank order with human even lysine residue (K108) in human was altered to "R" amino acid in rhesus monkey (62). Noteworthy, "R" and "K" amino acid belong to alkaline type, while "E" is acidic one. Such distinct characteristic may be responsible for the different reactivity. With the structure of Vgamma9Vdelta2 TCR being deciphered, the CDR-containing surface characteristics were clarified, which exhibited more jags when compared with the flatter surfaces of alphabeta TCRs (21). Meanwhile, the possible phosphantigen-binding positively charged site was predicted, which was formed by Arg 59 of CDR2gamma, Lys 109 of CDR3gamma, and Arg 51 of CDR2delta (21). Interestingly, chimeric TCRs that paired human Vgamma9 with rhesus monkey Vdelta2 and rhesus monkey Vgamma9 with human Vdelta2 retained reactivity to nonpeptide Ags (62). Comparison the Vgamma9Vdelta2 TCR sequences between human and rhesus monkey exhibits that they both share the latter two amino acids (gammaK109 and deltaR51), except for the 59 amino acid of CDR2gamma which is substituted with oppositely charged amino acid "E" in rhesus monkey (62), suggesting Arg 59 of CDR2gamma isn't absolutely pivotal for recognition. Last year, Seiji Yamashita *et al* demonstrated that substitution K109 with "A" amino acid only partially affected the response to EtPP with essentially no effect on that to IBA and pamidronate, while substitution Arg 51 of CDR2delta with "A" amino acid completely abrogated the responsiveness (63). Second, gene transfection research demonstrated that substitution of the "W" in the CDR3 region of Ag-reactive Vgamma9/JgammaP TCR with "GN" from that of the Ag-nonreactive Vgamma9/JgammaP TCR abrogated responsiveness. While substitution of the JgammaP part of the Ag-nonreactive Vgamma9/GN/JgammaP TCR with Jgamma1.3 fragment remained reactivity against another phosphate Ag in the mycobacterial supernatant, although it lost responsiveness against EPP and IPP (64). Backup proof for the importance of the N-encoded CDR3 region in Vgamma9 chain came from the in vitro stimulation experiment with IPP (65). Although IPP induced polyclonal expansion of Vgamma9Vdelta2 T cells, it failed to stimulate any clone. In fact, some certain Vgamma9/JgammaP sequences in normal control (more than once) would be absent in stimulated sample, while other specific Vgamma9/JgammaP sequences (more than once) were present in stimulated but not control samples, indicating stimulation with IPP did have somewhat selectivity. Of note, the major type C1 in normal control but not in stimulated samples didn't have the "E" amino acid encoded by JgammaP gene. Meanwhile, the CDR3 length of C1 was relative shorter than those in stimulated samples (65). Consistent with this, another report displayed that a Vgamma9/JgammaP/Vdelta2 TCR transfecting J.RT3-T3.5 cell KG81D2-27 didn't respond with monoethyl pyrophosphate (EtPP), isobutyl amine (IBA), although remained weak yet significant response against pamidronate pulsed APC (57). One of the possible explanations is the difference of CDR3 region in Vgamma9/JgammaP chain which is relative longer than those of reacting ones so that may destroy the conformation of predicted Ag-binding pocket. Of course , there is a possibility that amino acid buildup in Vgamma9 chain CDR3 region such as "Vgamma9/GN/JgammaP" may also affect the conformaion of phosphoantigen-binding pocket so that influence the reactivity of Vgamma9Vdelta2 T cells. Finally, since pairing of Vgamma9 and Vdelta2 is essential for recognition, it's likely that the CDR3 region of Vdelta2 may affect responsiveness, although it isn't involved in the predicted Ag-binding pocket. Actually, surging evidence indicated that the Vgamma9Vdelta2 T cells predominating in adult human peripheral blood was induced by Ag-driven selection (66). There is a conserved hydrophobic residue (leucine, valine or isoleucine) at position 97 encoded by N-region of Vdelta2/Jdelta junction (67). Substitution deltaL97 with "A" amino acid totally abolished the responsiveness (63).

3.2 Aminobisphosphonate

During the research process of mycobacterial Ag, other non-peptide small antigens including aminobisphosphonate and other bisphosphonates, alkylamine, and hexamethylene diisocyanate were

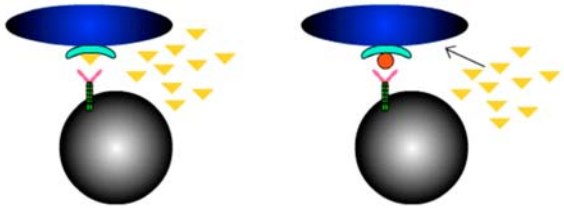

Figure 4. Recognition pattern of gammadelta T cells against pamidronate.

identified (68-75). Most of these antigens share the same characteristic that they all contain phosphate group and/or amino group and are able to induce Vgamma9Vdelta2 T cells to proliferate and/or cytotoxicity of activated Vgamma9Vdelta2 T cells against tumor target cells, especially hematopoietic origin.

Consistent with mycobacterial phosphantigens, the responsiveness of purified Vgamma9Vdelta2 T cells against aminobisphosphonate such as pamidronate are either IL-2 or cell-dependent (71,76). Unlike mycobacterial phosphoantigens, the bioactivity of pamidronate could be maintained only in the presence of human monocytes (76). However, whether the precise functioning cell type in monocytes is the same as for mycobacterial Ags ($CD4^+CD45RO^+CD\ 7^-$ alphabetaT cells) remains unclear. Further, the exact helper mechanism is unknown. Recently, it had been found that aminobisphosphonates such as zoledronic acid, ibandronate, alendronate and pamidronate stimulated the proliferation of Vgamma9Vdelta2 T cells in human PBMC cultures by an indirect pathway-disrupting the mevalonate pathway by inhibiting farnesyl diphosphate synthase and thus leading to the accumulation of isoprenoid lipids (eg IPP) which accounted for the bioactivity, while non-aminobisphosphonate such as clodronate and etidronate that don't inhibit farnesyl diphosphate synthase had no effect (72,77-79). By contrast, another group argued that bisphosphonate clodronate could enhance the proliferation and cytotoxicity of positively selected gammadelta T cells induced by IL-2 (73,80). Gene transfection experiment demonstrated that Vgamma9Vdelta2 TCR also mediated the recognition (72). Importantly, aminobisphosphonate could be pulsed on a lot of tumor target cell lines of human origin or even normal human monocytes and induce the cytotoxicity of human Vgamma9Vdelta2 T cell lines or clones, while tumor cell lines of nonhuman origins pulsed with pamidronate fail to activate human Vgamma9Vdelta2 T cells without exception (71,72,76,81). LFA-1/ICAM adhesive interaction was necessary but not sufficient for stimulating Vgamma9Vdelta2 T cells to proliferate and produce IFN-gamma by pamidronate-pulsed human tumor cells as anti-LFA-1 antibody inhibited the reactivity and some ICAM-1^{low} human tumor cells such as LK-2 were able to induce a much stronger activation of Vgamma9Vdelta2 T cells after transfected with ICAM-1 (82). However, other ICAM-1^{low} human tumor cells such as MKN45 remained weak stimulating capacity even when transfected with ICAM-1 (82). Interestingly, expression of human ICAM-1 failed to simultaneously transfer bioactivity to ICAM-1^{low} murine tumor cells (82), indicating direct recognition against pamidronate is more possible for cytotoxicity than the inducing recognition as washing would remove isoprenoid lipids induced by pamidronate (Figure 4).

3.3 Alkylamine

Alkylamine was another newly identified antigen for Vgamma9Vdelta2 T cells. Many human pathogens produce a number of biologically active alkylamine (8,83-86). Lots of food including tea, white and red wine and apples all contain alkylamines or the precursor of alkylamines (69). PBMC cultured with alkylamine resulted in the proliferation of Vgamma9Vdelta2 T cells and IL-2 release (69). Alkylamine-primed PBMC led to the expansion and IFN-gamma production of Vgamma9Vdelta2 T cells in response to iso-butylamine rechallenge and heat-killed bacteria and LPS independent of Vgamma9Vdelta2 TCR but dependent of IL-12 (68). Like pyrophosphoantigens, cell-cell contact and continuous presence of alkylamine were necessary for response which was directly mediated by Vgamma9Vdelta2 TCR (69,87). ELISpot technology demonstrated that the optimal IFN-gamma production of Vgamma9Vdelta2 T cell clones required accessory cells such as human EBV-B cells and spinner Hela cells but not human erythrocytes or rat and mouse myeloma B cells against alkylamines and IPP, although Vgamma9Vdelta2 T cell clones could "present" alkylamines to themselves and induced suboptimal response (87). Notably, alkylamines also present in various human secretions and fluids such as blood and urine of tea drinker, breast milk and amniotic fluid (88,89). The important thing is whether and how these physiological alkylamines affect Vgamma9Vdelta2 T cells in vivo. Arati B. Kamath *et al* demonstrated that tea drinking induced a Vgamma9Vdelta2 T cell recall response to non-peptide antigens and enhanced the response of Vgamma9Vdelta2 T cells to heat killed bacteria, although failed to alter the absolute numbers of Vgamma9Vdelta2 T cells in PBMC (68), which partially indicated the immunological benefit of tea drinking. Like pyrophosphoantigens and bisphosphonates, not all alkylamines have bioactivity. Structure analysis of several antigenic and non-antigenic alkylamines indicated that a straight or branched alkyl chain of two to five carbons with a single primary amine group as the only substituent was active, while alkylamines with one carbon or more than five carbons, or any substituent other than the primary amino group had no the effect (69). Compared with negatively charged pyrophosphoantigens, alkylamines contain a positively charged amino group. If Vgamma9Vdelta2 TCR indeed bound with both of them, it may apply two different sites or the same site with distinct mechanism. Because the three critical residues, gammaK108, deltaR51, deltaL97 are also important for alkylamine recognition. Seji Yamashita *et al* predicted a model for their recognition. Pyrophosphoantigens combined with Vgamma9Vdelta2 TCR by virtue of the interactions of negatively charged pyrophosphate group with two adjacent cationic residues (gammaK108 and deltaR51) and of hydrocarbon chain with short aliphatic side chain of deltaL97 via hydrophobic interaction, while alkylamine antigens bound with Vgamma9Vdelta2 TCR at the same site via bridging effect connecting two positively charged groups, amino group and deltaR51 (63).

4. CLASSICAL MHC MOLECULES AND MHC-LIKE MOLECULES ANTIGENS

4.1 Classical MHC molecules

As alphabeta T cells, MHC and non-classical MHC (also termed MHC class Ib) restricted recognition were both observed for gammadelta T cells (36,90-94). In that case, peptide-MHC or peptide-MHC class Ib molecule complex mediated the recognition. However, the cases are very limited. In the majority of situations, the recognition is MHC-unrestricted, although gammadelta T cells are able to respond to MHC and MHC class Ib molecules themselves as antigens. Initial studies performed in both mouse and human systems showed us that certain gammadelta T cells may recognize self, allogeneic MHC and MHC-like molecules (95-107). However, limited information was available about the structure of the recognition receptors and their fine specificity. Striking evidence was provided by Barbara L. Rellahan *et al* that junctional sequences influenced the specificity of gammadelta TCR against classical MHC II molecules (108). Under that condition, two cell lines with the same rearranged Vgamma1.2Jgamma2/Vdelta5Ddelta2Jdelta1 gene elements, differing the V(D)J junctional regions of gamma and delta genes, had individual specificity for I-A^d and I-$E^{k,b,s}$, respectively (108). Of note, the specificity against IE^k was most likely independent of peptide bound to the IE molecule. Purified IE^k alone was sufficient to activate reactive LBK5 which was derived from the peripheral lymph nodes of athymic B10(H-2^b) nu/nu mice by in vitro stimulation of T cells with H-2-congenic B10.BR(H-2^k) splenic antigen-presenting cells (109). Recent research identified that the epitope was centered around the polymorphic residues 67 and 70 of the IE beta-chain and N-linked glycosylation at residue 82 of the IE alpha-chain profoundly influenced the recognition (110).

4.2 T10/T22

Besides classical MHC molecules, several non-classical MHC ones recognized by gammadelta T cells were documented. Although class Ib molecules are relatively nonpolymorphic, they are similar in amino acid sequence and probably protein structure to class I, and both classes of molecules associate with beta2m. In mouse, non-classical MHC molecules $T10^k$,$T10^b$, and $T10^{129}$, as well as $T22^b$ (also named $T27^b$) but not $T10^d$ allele were stimulatory to G8 and KN6 (26,109,111). G8 and KN6 belong to different subsets, the former is Vgamma2/Valpha11.1 generated by immunizing BALB/c nude mice with B10.BR spleen cells, while the latter is Vgamma4/Vdelta5 derived from normal C57BL/6 thymocytes (96,101). It seems that the two gammadelta T cells recognize different peptides loaded on the same class Ib molecules. However, it's equivocal for the putative peptide. Whereas peptide was likely involved in the interaction of KN6 TCR with T22 since mutations in the putative floor of the peptide groove altered recognition, the recognition of G8 TCR against T10 and T22 had nothing to do with peptide (109,112-115). T22 mRNA is expressed in almost all cell types, while the T10 expression seems to be restricted to only a subset of cells (e.g. spleen, thymus, and peritoneal exudates cells) (111). Generally, T region-encoded Ags are poorly expressed on the cell surface and mitogenic or antigenic activation can enhance T10/T22 expression (26,116). Moreover, the affinity of T10/T22 and G8 gammadelta TCR interaction is high (117,117). All of them indicate that the alteration of T10/T22 experssion on the cell surface of lymphocytes may provide a mechanism by which gammadelta T cells specific for T10/T22 to regulate immune cells (117). The possible regulation role of gammadelta T cells is supported by the finding that a population of gammadelta T cells recognizing T10/T22 can also be found in the spleen and iIEL of normal unimmunized mice (117). Although purified T10/mousebeta2m could activate G8, the ability of plate bound T10/mousebeta2m to stimulate G8 was about 10-fold lower than that of cells expressing T10 perhaps because of its relative instability (114). In spite of the independence of peptide, T10/T22 may present other nonpeptide antigens such as carbohydrates which may help to stabilize T10/T22 expression on the cell surface and then promote stimulation (118).

4.3 Thymus leukemia antigens

Mouse thymus leukemia (TL) antigens also belong to the family of MHC class Ib and are restricted to the intestines in all mouse strains as well as the thymus of TL^+ strains (eg., A-strain and BALB/c mice) (119). However, even in TL^- strains (e.g., C57BL/6 (B6) and C3H/He (C3H)) mice, malignant transformation of T cell causes TL to be expressed. TL-specific gammadelta T cell clones were established by Kunio Tsujimura (120). Although not all clones were derived from a single cell, they could be devided into two groups (CD8alphabeta and DN). Either anti-gammadelta TCR or CD3 antibodies blocked both CTL activities. However, anti-CD8 antibodies only abolished the cytotoxicity of CD8alphabeta gammadelta T cell clones but didn't affect that of DN gammadelta T cell clones. Because TL can bind CD8 molecule, such cytotoxic heterogeneity may be due to the difference of killing mechanism as that of alphabeta CTL (121,122). Most, if not all, of these clones were derived from adult thymus with extensive CDR3 sequence heterogeneity (120). Recently, Kunio Tsujimura displayed that $T3^b$-TL tetramers without antigenic peptides were able to bind to TL-restricted CTL dependent of CD3, indicating the interaction between gammadelta TCR and TL may be indepenent of peptide, although positive and negative evidence for the presenting function, at least TAP-dependent pathway, were both provided (123-125).

4.4 CD1C

CD1 family of proteins as novel antigen-presenting molecules are encoded by genes located outside of MHC. CD1 proteins can present a variety of foreign lipids and glycolipids by directly binding the hydrophobic alkyl portions of these antigens and positioning the polar or hydrophilic head groups of bound lipids and glycolipids for highly specific interactions with TCR (126,127). According to the expression and function of CD1 proteins, they can be divided into two groups. One is group I CD1 including human CD1A, B, and C and their homologues in other mammals. Group II contains human CD1D and its close homologues in other species. With respect to

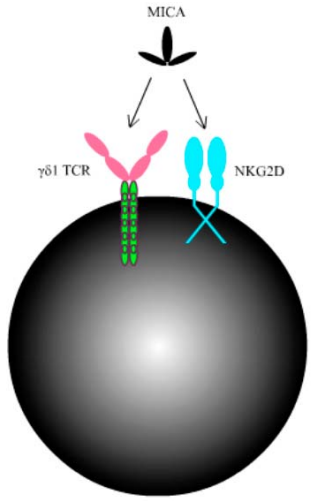

Figure 5. MICA delivers double signals through Vdelta1 gammadelta TCR and NKG2D.

CD1C, it's expressed on langerhans DC, interstitial DC, monocyte derived DC, and mature DC. Moreover, CD1C is also expressed in lymph node mantle zones and germinal centers, in marginal zone B cells of spleen, and on a subpopulation of circulating B cells in fetal and adult human peripheral blood (127). Initial studies showed that CD1C was recognized by two DN gammadelta T cells (IDP2 gammadelta T cell line Vgamma9Jgamma2/Vdelta1Jdelta1 and J2B7 gammadelta T cell clone Vgamma3 or Vgamma4Jgamma2/Vdelta1Jdelta1) (100,128-132). In contrast to IDP2 which was derived from one immunodeficient patient, J2B7 was from normal human peripheral blood by nonspecific mitogenic stimulation. Gene transfection experiments further confirmed the interaction between gammadelta TCR and CD1C (131,133). Recently, another research group also established two CD1C-specific gammadelta T cell line (JR.2 Vgamma2/Vdelta1Jdelta1 and XV.1 Vgamma1.4/Vdelta1Jdelta1). Both gammadelta T cell lines were able to proliferate in response to $CD1C^+$ presenter cells, released Th1 cytokines, produced granulysin, and lysed $CD1C^+$ targets through perforin- and Fas-mediated pathway in the absence of additional lipid or glycolipid antigens (133). However, it's unclear whether these gammadelta T cell lines are responding to CD1C molecule alone or to CD1C molecule containing a self-lipid molecule as all antigens are $CD1C^+$ cells. Importantly, all CD1C-restricted gammadelta T cell lines belong to Vdelta1 gammadelta subset, the major population in tissues such as intestine and the spleen. However, the frequency of these CD1C-reactive gammadelta T cell lines was very low based on large screening experiment. Although CD1C was capable of presenting glycolipid and CD1C-restricted alphabeta T cells mediated antimicrobial immunity (134-137), whether these CD1C-reactive gammadelta T cells are representative of autoreactive gammadelta T cells or some primary resident Vdelta1 gammadelta T cells in vivo indeed are able to respond to lipid or glycolipid antigens presented by CD1C should be addressed. Indeed, microbial lipid antigens could be recognized by Vdelta1 gammadelta T cell and dendritic cells, IL-12, adhesion (LFA3/CD2, LFA1/ICAM1) and costimulatory (MICB/NKG2D) molecules but not CD1C were required for optimal activation (138).

4.5 MICA/B

Recently, another MHC class I chain-related genes (MIC) were identified (139,140), of which only MICA and MICB encode expressed transcripts that are antigens for some subsets of Vdelta1 gammadelta T cells (141). Similar to MHC-I molecules, MIC is highly polymorphic and contains alpha1, alpha2, and alpha3 domains but is not associated with beta2-microglobulin and doesn't exhibit conventional class I peptide binding (142-145). They are expressed on a variety of cells including intestinal epithelium, endothelial cells, fibroblasts, activated T cells and tumor cells especially epithelial derived tumor cells and can be regulated by promoter heat shock elements similar to those of HSP70 genes, which leads to the concept that MIC is probably a 'marker of stress' in the epithelia (144,146-150). MICA was recognized by some subsets of human intestinal epithelial Vdelta1 gammadelta T cells through Vdelta1 gammadelta TCR and NKG2D, both of which were needed for response as either Vdelta1 gammadelta TCR mAb or NKG2D mAb was able to decrease reactivity (141,151,152). In spite of the structural similarity between MICA and MHC-I molecules, such MICA-specific response was independent of peptide, which was confirmed by our studies as immobilized MICA could expand human Vdelta1 gammadelta T cells in vitro (153). Gene transfection experiments showed that MICA-specific response was mediated by certain delta1 chain and combination of delta1 and gamma chain (152). Cross-combination of these responding Vdelta1 gammadelta TCR would abolish reactivity (152). By contrast, our experiments indicated that shuffled single-chain Vdelta1 gammadelta TCR derived from MICA-specific Vdelta1 gammadelta T cells was still able to bind MICA in vitro (JianQin Zhao in preparation).

Noteworthily, MICA can deliver NKG2D-dependent costimulatory signal 2 to Vdelta1 gammadelta T cells (Figure 5). Structure analysis showed that similar surfaces on each NKG2D monomer interacted with different surfaces on either the alpha1 or alpha2 domains of MICA allele 001 and that only one polymorphic position 129 appeared to dominate the major binding capacity to NKG2D despite the highly polymorphic nature of MIC genes (154,155). Similarly, our unpublished data indicated that the cytotoxicity of MICA-specific Vdelta1 gammadelta T cells was mainly blocked by alpha2 domain mAb. Moreover, although primate MIC molecules share relatively few identical amino acids in potentially accessible regions of their alpha1alpha2 domains, they were recognized by diverse MICA/B-restricted human intestinal epithelial gammadelta T cells (156), indicating a single conserved interaction site may be in the alpha2 domain. Further, MICA-NKG2D can enhance the antigen-depended effector function of Vgamma9Vdelta2 T cells in peripheral blood (157). In contrast, NKG2D was able to directly mediate the

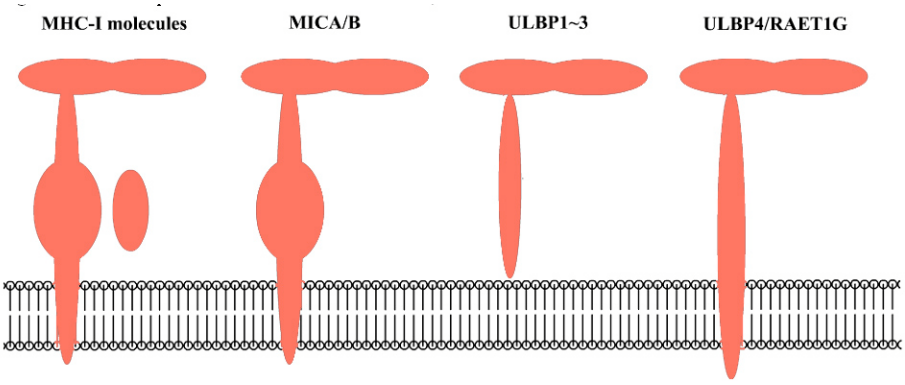

Figure 6. Structure comparison between MHC-I molecules, MIC and ULBPs.

cytotoxicity of activated $CD8^+$ T cells independent of TCR under some conditions(158-160), suggesting another signal pathway can be used to elicit cytotoxicity of $CD8^+$ CTL. Recently, a novel ligand family (ULBP) of NKG2D in human were identified (161-165). ULBPs contain alpha1 and alpha2 domains, but no alpha3 domain. Of them, ULBP1, 2 and 3 are GPI-linked membrane proteins, while ULBP4 and RAET1G contain predicted transmembrane and cytoplasmic domains (165,166) (Figure 6). Because of the sequence and structure similarity of the extracellular domains between MICA and ULBPs, ULBPs may function as antigens for some Vdelta1 gammadelta T cell subsets (166). Indeed, this year, Alessandro Poggi *et al* discovered that Vdelta1 gammadelta T lymphocytes from B-CLL patients recognized ULBP3 expressed on leukemic B cells (167), which lends support to the antigen hypothesis.

5. HEAT SHOCK PROTEINS

5.1. Heat shock proteins in mouse and rat

Heat shock proteins (HSP) are widely distributed in nature, which are highly conserved among prokaryotes and eukaryotes and are known to be involved in cytoprotection against various stress conditions and immune responses to various pathogens and tumors. Heat shock proteins are categorized into different families based on their approximate molecular weight.

Several gammadelta T cell hybridomas derived from neonatal mouse thymus and spleen could spontaneously produce IL-2, which was mediated by gammadelta TCR (168-170). These spontaneously reactive gammadelta T cell hybridomas all responded to PPD in the presence or absence of additional accessory cells such as splenic cells, although accessory cells somewhat increased the PPD-specific response. Analysis of gammadelta TCR showed that these hybridomas subsets mainly consisted of Vgamma1JCgamma4/Vdelta6 (including Vdelta6.3 and Vdelta6(pλ12)) displaying extensive heterogeneity, most of which used the Ddelta2 gene segment (169). However, the pairing of Vgamma1JCgamma4 and Vdelta6 was not sufficient for the response since some Vgamma1JCgamma4/Vdelta6 expressing hybridomas were nonreactive, indicating CDR3 region of gammadelta TCR may in some ways affect recognition. In addition, other Vgamma1JCgamma4/Vdelta6$^-$ also possessed the PPD-reactive characteristic, although the quantity of them was very small (169). Because hsp65 is the major protein component in PPD and known to be an immunodominant antigen that contains many stimulatory epitopes for B cells and alphabeta T cells, purified recombinant hsp65 derived from Mycobacterium bovis was used as stimulator and was proved to be also able to stimulate the PPD-reactive gammadelta T cell hybridomas and gammadelta T cell lines derived from BCG-immune individual dependent on the presence of splenic cells, although the potency was weaker than that elicited by PPD (91,168). Willi Born *et al* identified that one peptide (L180-196), corresponding to amino acids 180 to 196 of M.leprae hsp65 and identical to M. tuberculosis and M. bovis hsp65 in this region strongly stimulated several PPD-reactive gammadelta T cell hybridomas, while other peptides responded weakly (171). Interestingly, one of the two strongest responsive hybridomas (Vgamma1JCgamma4/Vdelta6.3) also responded to L181-195 with the similar potency, while the other failed (171). Further, synthesized peptides of equal length corresponding to the same region in homologous proteins of several distant species including human, yeast and E.coli were also able to stimulate the two strongest responsive hybridomas, although the stimulatory capacity was much weaker (171). In vivo experiments demonstrated that repeated priming mice with L180-196 resulted in the frequency increase of generating PPD, rHSP60 (derived from mycobacterium bovis) and L180-196 responsive hybridomans. However, most of them consisted of Vgamma1JCgamma4/Vdelta6.1, compared with those derived from normal murines (172). Further truncated peptide stimulation experiments indicated that the responsive hierarchies of several PPD-reactive hybridomas were different from each other. Minimal seven amino acids at position 181-187 especially the two amino acids (F181 and L183) were required for the stimulatory bioactivity (173). Direct evidence for TCR mediated recognition came from gene transfection which demonstrated that introducing Vgamma1JCgamma4/Vdelta6(pλ12) TCR derived from a PPD-reactive hybridoma into TCR negative recipient cell transfered the response against short peptides. Further, the non-reactive hybridoma with nonpermissive Vgamma6/Vdelta6 TCR obtained the response when transfected with the same Vgamma1JCgamma4 chain

(173), indicating the Vgamma1JCgamma4 chain was principally responsible for recognition.

Another heat shock cognate protein (#067 molecule), detected by mAb067, was a heat-inducible transformation-associated cell surface Ag that was expressed on the activated H-ras oncogene-transformed rat fibrosarcoma W31, but not its parental nontransformed fibroblast WFB (174). The immunoprecipitate made with mAb067 and W31 cell lysates reacted with anti-rat 70kDa heat shock cognate (HSC) mAb, TG5E, indicating that 067-defined Ag may be a rat 70kDa HSC (175). $CD4^-CD8^-$ T cells (DNT cells) negatively isolated from spleen and PBL of live BCG-primed rats showed cytotoxicity against W31 cells and RNK16 cells (another #067 molecule positive rat lymphoma), which was completely blocked by mAb067 and anti-CD3 mAb, suggesting both #067 molecule and gammadelta TCR were involved in the recognition (175). Since computer analysis of the three-dimensional structure of 70kDa HSC and MHC class I molecule suggest that they have very similar three-dimensional structures, although these molecules have only 24% of amino acid sequence identities (176), indicating that #067 molecule may function as presenting element and then the complex formed by #067 molecule and peptide is recognized by DNT cells. In fact, brefeldin A, which blocks the complex formation between MHC class I molecule and peptides in the ER, also inhibited the cytotoxicity, but didn't affect the expression of #067 molecule itself on W31 cells (175). Moreover, although DNT cells lysised W31 cultured in a conventional 5% FCS (5%W31), they failed to kill W31 when cultured in 1% FCS (1%W31) even if the expression of #067 molecule was the same as the former. However, pulsing 1%W31 with trifluoroacetic acid (TFA)-extracted Ags from the former could recover the reactivity (177). Further, pronase K treatment of Ags also abolished the cytotoxicity (177). Importantly, hsp70 was indeed able to bind to the HLA-A31-restricted gastric tumor antigenic peptide, F4.2 (178). HSP70 associated peptides could elicit specific cancer immunity, while treatment with ATP to remove low-molecular weight material resulted in loss of antigenicity (179). In addition, another member of hsp70 family PBP72/74 also possessed the ability of binding peptides and functioned as an antigen presentation molecule (180). Recently, the same research group successfully established #067 molecule-reactive gammadelta T cell hybridomas and found that they preferentially used TCRs with the Vgamma1/Vdelta6 family, while those non-reactive ones displayed more diversity (178,181). Vgamma1/Vdelta6 gene usage itself was not sufficient for the recognition as non-reactive hybridomas also used it. Comparison of the CDR3 regions between reactive and non-reactive Vgamma1/Vdelta6 using hybridomas indicated that the length variation rather than amino acid composition was important for reactivity (178,181).

5.2. Heat shock proteins in human

Because mouse and rat Vdelta6 and Vdelta7 genes belong to the same subfamily which corresponds to the human Vdelta1 gene (182,183), it remains the possibility for the recognition of human gammadelta TCR (eg Vdelta1 TCR) to hsp. Fifteen years ago, it had been found that the rabbit hsp58 antiserum rasied by injection of hsp58 isolated from mitochondria of Hela cells blocked the expansion of human gammadelta T cells in PBMC stimulated with some extracts from mycobacteria and E. coli (184). Vgamma9Vdelta2 T cell lines GD1 and one $CD4^-CD8^-$ Vgamma9Vdelta2 T cell clone (GD1 clone)derived from the same BCG-immuned individual responded to PPD and purified mycobacterial hsp65, which was dependent on autologous APC (91,185). The GD1 clone also responded to recombinant human P1 protein (also termed mitochondrial hsp60) that is the homologue of mycobacterial hsp65, but only in the presence of APC (185). In addition, both peripheral blood gammadelta T cells and the Vgamma9Vdelta2 T cell clones were able to respond to Daudi, which was suppressed by the hsp58 antiserum and anti-human hsp60 mAb (186,187). Hsp58 antiserum could also block the response of Vgamma9Vdelta2 T cell clones to RPMI 8226 (188). Human oligodendrocytes and fetal astrocytes, glial cells expressing hsp60 on their surface, stimulated the expansion of gammadelta T cells mainly bearing Vdelta2 in PBMC with the addition of exogenous IL-2 (189). gammadelta T cell lines derived from the proliferation lysised oligodendrocytes, Daudi and RPMI 8226. In cold target competition assays, Daudi and RPMI 8226 effectively competed for lysis with the oligodendrocytes, suggesting hsp60 may be imvovled in gammadelta T cell-induced lysis of oligodendrocytes (190). More recently, Laad et al demonstrated that gammadelta T cells negatively isolated from peripheral blood of oral cancer patients could lysis Daudi cells, autologous and allogenic oral tumor cells, which was blocked by mAb against framework of gammadelta TCR or Vgamma9 and two mAbs against distinct epitopes of mycobacterial hsp65 (191). One year later, another group demonstrated that gammadelta T cells selected in the same way from peripheral blood of oesophageal tumor patients also displayed cytotoxicity against Daudi cells, autologous and allogenic oesophageal tumor cells (192). Not only the above gammadelta TCR and Vgamma9 mAbs but anti-Vdelta1 and anti-hsp70 mAb, recognizing one of the epitopes on mycobacterial hsp65, inhibited their cytotoxicity, although the anti-Vdelta1 mAb showed the relative lower effect as the lower frequency of Vdelta1 gammadelta T cells in the subset (192).

Human HSP70 family is composed of several similar proteins which are localized in various intracellular compartments. Among them, hsp70 protein is constitutively located within the cytoplasm and expressed on the cell surface when stress-induced. The cell surface expression of hsp70 on heat-stressed tumor cells but not on unstressed tumor cells increased their susceptibility to lysis by $CD4^-CD8^-$ Vgamma9Vdelta2 T cell clones derived from autologous blood lymphocytes. Both anti-Vdelta2 mAb and anti-hsp70 mAb recognizing human hsp70 could weaken the response (193). Further, the cell surface expression of hsp72 (another member of hsp70 family in human) on human colon carcinoma cells CX2 enhanced their sensitivity to lysis mediated by adherent NK cells (194). Although the receptor on NK cells is unclear, it's possible that gammadelta T cells and NK cells may share the same recognizing receptor as they have much comparability in some cases. Therefore, it's difficult to completely exclude the possibility that other receptor on Vgamma9Vdelta2 T

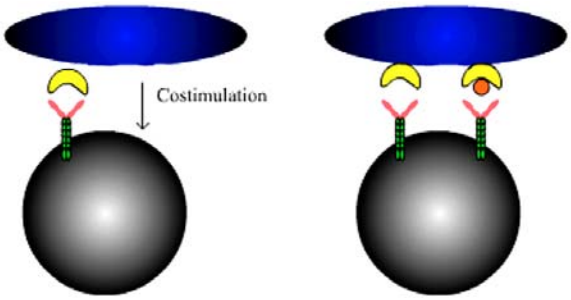

Figure 7. Recognition pattern of gammadelta T cells against HSP.

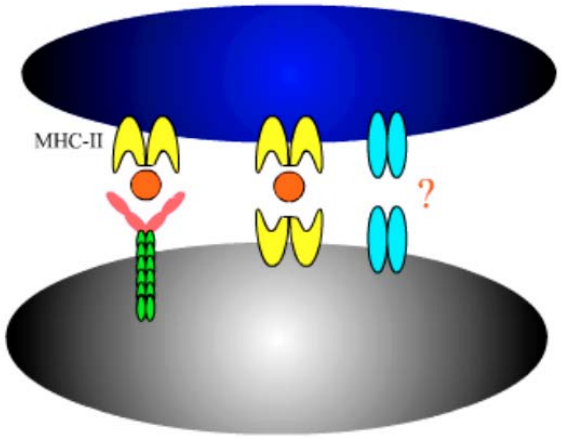

Figure 8. Recognition pattern of gammadelta T cells against SEA.

cell clones also recognize hsp70. Finally, grp 75 protein, a member of the hsp70 family, was able to function as the presenting molecule for gammadelta CTL. In that case, gammadelta T cells responded to the complex formed by processed peptide from tumor Ig and grp 75 protein (195).

To sum up, it appears that gammadelta T cells recognize hsps in free from and those on cell surface by different mechanisms. For the former, gammadelta T cells directly recognize a certain epitope. For the latter, either direct or peptide-hsp complex recognition may be involved in the response (Figure 7).

6. STAPHYLOCOCCAL ENTEROTOXIN A

Target cells coated with staphylococcal enterotoxin A (SEA) were sensitive to Vgamma9 gammadelta T cell clones. Both anti-MHC class II and anti-gammadelta TCR receptor monoclonal antibodies inhibited the response. However, Vgamma9 gammadelta T cell clones couldn't lysis MHC-class II negative cells that had been preincubated with SEA. It's interesting that these Vgamma9 gammadelta T cell clones killed SEA coated target cell with matched and mismatched MHC class II (196). Later researchers found that some Vgamma9 gammadelta T cell clones including Vgamma9Vdelta2 T cell clones LH32/PH3 and Vgamma9Vdelta1 T cell clones LH22/PH20 required the specific human serum containing the SEA-antibody to lysis SEA coated EBV-B cells, while others such as Vgamma9Vdelta1 T cell clones AH21/MH4 and Vgamma9$^-$Vdelta1$^+$ T cell clones PH21/LH18 and Vgamma9$^-$Vdelta1$^-$Vdelta2$^-$ T cell clones AH1 killed SEA and/or SEE-coated target cells independent of the serum (197). They argued that the lytic activity of gammadelta T cell clones was mediated by two pathways: an antibody dependent and an antibody independent pathway. By contrast, the same cell clones used by Rust *et al* didn't have the feature (196), which may be due to the different effector:target ratio (50:1 VS 20:1). Vgamma9 gammadelta T cell clones were also able to proliferate when stimulated by SEA no matter whether they added rIL-2 in proliferation experiment (198). Notably, responsiveness to SEA varied between these Vgamma9Vdelta2 T cell clones. Both CD4$^+$ Vgamma9Vdelta2 T cell clones (HF.2 and JN.23) responded to SEA, as five of seven CD4$^-$CD8$^-$ Vgamma9Vdelta2 T cell clones under the same culture condition (198). Similar results were also achieved by Hergen Spits *et al* who demonstrated that some certain gammadelta T cell clones (CD4$^-$CD8$^-$ Vdelta3 and CD4$^-$ CD8$^-$ Vdelta1) proliferated in response to SEA or SEB in the presence of autologous irradiated MHC class II$^+$ EBV-B cells as accessory cells (199). Nevertheless, it can't conclude that such proliferation effect is definitely mediated by gammadelta TCR since SEA or SEB induces the proliferation of CD3$^-$ MHC class II$^+$ CD56$^+$ NK cell clones by engaging MHC-II molecule and these gammadelta T cell clones also express MHC class II molecules (199). Direct evidence was put forward by Elwyn Y. Loh *et al* who demonstrated that the Vgamma9 portion of the receptor, independent of the J region and C region or the delta-chain was responsible for IL-2 production in response to SEA coated autologous LCL (200). In 2001, the more detailed SEA recognition mechanism by Vgamma9 TCR was expounded. The amino-terminal region especially residues 20-27 and 64 played an important role in determining Vgamma9 TCR recognition of SEA (198). Hypothetical Vgamma9-SEA complex model indicated that predicted binding was between the CDR1, CDR2, and HV4 regions of Vgamma9 and the cleft region between the beta-grasp and beta-barrel motifs of SEA, leaving the CDR3 region of Vgamma9 outside (198). Therefore, it seems that the diversity of the CDR3 region of Vgamma9 affects the response potency by influencing the putative conformational binding box, which is corresponding to the proliferation difference of gammadelta T cell clones. Further, it should be noted that the proliferation responsiveness required the accessory cells. Since these Vgamma9 gammadelta T cell clones express MHC-II, they can present SEA as "superantigen" to themselves theoretically. However, the absolute necessity of accessory cells indicates the importance of accessory singals. Finally, since SEA or SEB can induce the expansion of CD3$^-$ MHC-class II$^+$ CD56$^+$ NK cell clones, it's likely that SEA-MHC-class II pathway can function fully alone in Vgamma9 gammadelta T cell clones as in CD3$^-$ MHC-class II$^+$ CD56$^+$ NK cell clones (Figure 8).

With repect to those reactiving Vgamma9$^-$ T cell clones, one possible reason is that the SEA-MHC-II pathway mediates the reactivity. Alternatively, there is a possibility for Vgamma9$^-$ TCR mediated recognition, since

it may bind to different "superantigen" epitope (if existed) or form similar conformational binding box as that of Vgamma9 TCR interacting with the same SEA portion.

7. TETANUS TOXOID

In 1989, Danuta kozbor *et al* found that three $CD8^+$ gammadelta T cell clones derived from hyperimmunized donor with tetanus toxoid proliferated and produced IFN-gamma when stimulated with tetanus toxoid in the presence of autologous APC, which was enhanced by IL-2. By using APC coming from different donors, they discovered that the association between the expression of DR4 on unrelated APC and the ability to present tetanus toxoid to these gammadelta T cell clones was highly significant (92). Several years later, another lab found that three $CD4^-CD8^-$ Vgamma9Vdelta2 T cell clones isolated from the synovial fluid of a patient with early rheumatoid arthritis proliferated in response to both mycobacterial antigen AP-MT and tetanus toxoid and that the peptide 1235-1246 of tetanus toxoid determined the stimulating effect (36,201). MHC restriction patterns experiments indicated that tetanus toxoid was presented by donors of the HLA-DRw53 serotype which is a supertype shared by all individuals with the haplotypes DR4, DR7, or DR9. The response could be blocked by anti-CD3 and anti-Vdelta2 monoclonal antibodies and intercellular adhesion was required for proliferation since anti-LFA 1alpha mAb could also block it. It's noteworthy that anti-IL-2 receptor antibody blocked the activation by tetanus toxoi, which was consistent with the former experiment, indicating that such reactivity was IL-2 dependent (36). Meanwhile, they also found that some other Vgamma9Vdelta2 T cell clones failed to respond to tetanus toxoid, indicating that CDR3 regions of Vgamma9Vdelta2 TCR were responsible for recognition. Recently, Adam V. Wisnewski *et al* documented that tetanus toxoid could stimulate the increase of $CD4^-CD8^-$ Vgamma8 T cells in airway biopsy-derived T cell lines in the presence of IL-2 and autologous APC, while mitogens or the allergen DerP2 didn't generate similar results under the same culture conditions (202). Sequence analysis demonstrated that these $CD4^-CD8^-$ Vgamma8 T cells preferentially used Vgamma8JP2 rearrangement and that 87% of the functional RT-PCR clones displayed identical CDR3 sequence, suggesting an antigen dependence for $CD4^-CD8^-$ Vgamma8 T cell growth (202). Another report showed that a high proportion of Vgamma8 T cells distributed in the inflamed synovial tissue and fluid (203). The interesting question is whether such Vgamma8 T cells distributing in the inflamed synovial fluid can also respond to tetanus toxoid, since the responsive Vgamma9Vdelta2 T cell clones are all from the inflamed synovial fluid. Taken together, it seems that tetanus toxoid presented by HLA-DRw53 can be recognized by both Vgamma8 TCR and Vgamma9Vdelta2 TCR, only they bind to different epitopes.

8. OTHER ANTIGENS

Several other protein and phospholipid antigens such as tumor immunoglobulin, herpes simplex virus type 1 transmembrane glycoprotein (GI), cardiolipin and beta2-glycoprotein 1 complex were also documented (204-207). However, these responsive gammadelta T cells are limited to some clones or hybridomas and the exact recognition mechanism is still unknown, though they are all MHC-unrestricted direct recognition.

9. NK-LIKE CYTOTOXICITY AND TARGET CELLS

Many early experiments indicated that gammadelta T cell clones, although established from different derivation and method, displayed a broad spectrum of TCR-independent cytotoxicity against tumor cells of distinct histologic origin including NK-sensitive target cells such as K562, which was named non-MHC-restricted NK-like cytotoxicity (100,188,208-216), while freshly isolated peripheral blood gammadelta T cells lacked such cytotoxic activity, though they could form conjugates with target cells such as K562 and NK-resistant HL-60-R (217,218). However, NK-like cytotoxicity varied among different gammadelta T cell clones. Jitsukawa *et al* discovered that almost all $Vgamma9^+$ gammadelta T cell clones showed strong NK-like cytotoxicity, while $Vgamma9^-$ gammadelta T cell clones displayed large varieties, although they also held the normal potential of lysis (100). Moreover, another group observed that not $CD56^-$ Vdelta1 gammadelta T cell clones but $CD56^+$ Vdelta1 gammadelta T cell clones had NK-like cytotoxicity (214). Further, although both anti-CD3 and anti-gammadelta TCR didn't affect NK-like cytotoxicity in most cases, they were able to enhance the cytotoxicity of $CD16^+$ gammadelta T cell clones and that of gammadelta T cell clones against $CD16^+$ target cells through ADCC pathway (210). It's noteworthy that NK-like cytotoxicity was IL-2 dependent and blocked by IL-4(213). Subsequent culture of these IL-4 isolated clones in IL-2 generated anti-K562 cytolytic avtivity (213). Interestingly, with the development of culture, gammadelta T cell clones gradually lost NK-like cytotoxicity, while retained the ability to lyse Daudi and Molt-4 (44,215,219), suggesting NK-like cytotoxicity is associated with the state of gammadelta T cells and that the killing threshold is lower than native gammadelta T cells so that it's relatively easy to be elicited. Alternatively, the activation molecules expressed on gammadelta T cell clones mediated NK-like cytotoxicity. With the identification of NKG2D, it's suggested that NKG2D-ligand pathway may also contribute to the NK-like cytotoxicity as K562 cells express RAET1G (220). However, NKG2D-ligand pathway alone seems not sufficient for NK-like cytotoxicity since MICA can only enhance the antigen-depended effector function of Vgamma9Vdelta2 T cell clones by engaging NKG2D and that most NKG2D positive Vdelta1 gammadelta T cell clones and Vdelta2 gammadelta T cell lines fail to lyse Daudi and ULBP3 positive B-CLL, respectively (167). In fact, 2B4-CD48 (also termed TCT.1) pathway is also partially responsible for NK-like cytotoxicity (221-226).

Although the effect of anti-CD3 and anti-gammadelta TCR antibodies in NK-like cytotoxicity was controversial based on the balance between negative signal, spatial blocking and cross-linking stimulation, they blocked the cytotoxicity against Daudi cells in general, suggesting

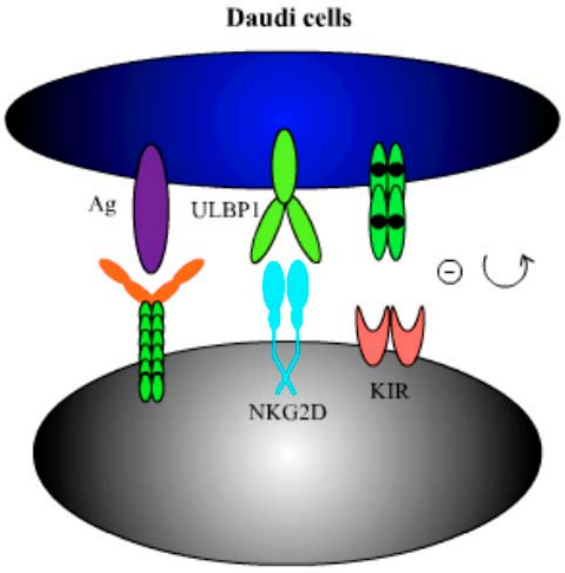

Figure 9. Recognition of gammadelta T cells against Daudi cells.

gammadelta TCR was mediated in lytic activity. Of note, Daudi reactive gammadelta TCR appeared to be limited to most of Vgamma9$^+$Vdelta2$^+$ TCR and only a small part of Vgamma9$^+$Vdelta1$^+$ TCR (215,219). These gammadelta T cell clones not only displayed cytotoxicity against Daudi cells but also were able to proliferate in response to Daudi cells, whereas failed to proliferate against K562 (219), suggesting that the mechanisms for proliferation and NK-like cytotoxicity of gammadelta T cell clones are different. In other words, gammadelta TCR pathway is required for proliferation, while NK-like cytotoxicity is TCR-independent. With respect to multifold reactive gammadelta TCR, they may form similar three-dimensional conformation box recognizing the same antigen epitope. Therefore, the cytotoxicity of gammadelta T cell clones against Daudi cells may include two pathways (TCR-dependent specific cytotoxicity and TCR-independent NK-like cytotoxicity). Daudi but not other lines of B-LCL also induced increased percentages of native gammadelta T cells in PBL and IL-2 could enhance the proliferative stimulation (186,187,227,228). The proliferative response gammadelta T cells from PBL by Daudi cells was inhibited by antiserum to the mammalian groEL related heat shock proteins (hsp58 or hsp60) and anti-hsp60 mAb, which didn't affect the cytotoxicity of gammadelta T cell clones against Daudi cells (186,187). Both of them immunoprecipitated a molecule of corresponding molecular size from the surface of Daudi cells (186,187). The antigens for gammadelta T cells didn't require presentation by conventional polymorphic or beta2M-associated MHC molecules (186). Notably, Daudi and mycobacteria reactive Vgamma9$^+$Vdelta2$^+$ T cells were overlapping. Sequence analysis of Daudi reactive Vgamma9$^+$Vdelta2$^+$ T cell clones displayed that they were mainly restricted to gammadelta T cells expressing Vgamma9JPC1gammaVdelta2 TCR (45), which was also demonstrated by gene transfection (229). Recently, Gober *et al* discovered that endogenous mevalonate metabolites appeared to mediate the TNF-alpha production by gammadelta T cell clones against Daudi and YMB-1 cells, a solid breast carcinoma (230), suggesting both hsp and endogenous mevalonate metabolites may participate in the recognition of Vgamma9JPC1gammaVdelta2 TCR. With the identification of NKG2D, it's suggested that NKG2D-ligand pathway may also contribute to the activation of gammadelta T cells, in that ULBP1 is highly expressed on Daudi cells (220). Moreover, missing HLA class I expression on Daudi cells (231,232) downregulated the negative signal via KIR and then partially accounted for the activation (233-237) (Figure 9). Nevertheless, KIR-HLA class I negative pathway appeared to be restriced in cytotoxic CD4$^-$ Vgamma9$^+$Vdelta2$^+$ T cell clones since CD4$^+$ Vgamma9$^+$Vdelta2$^+$ T cell clones with a T helper cell phenotype were KIR negative, which retained the ability to respond to HLA class I positive Daudi cells (233,234).

Similarly, Molt-4 cells were sensitive to lysis by many gammadelta T cell clones, but not able to stimulate the expansion of gammadelta T cells (44,215,219,238). Although one of these reactive gammadelta T cell line IDP2 (Vgamma9Jgamma2/Vdelta1Jdelta1) seemed to be CD1C-dependent (131,211), other Vgamma9$^+$Cgamma1Vdelta T cell clones were CD1C-independent but TCR-dependent, in that not anti-CD1C but anti-TCR antibody partially inhibited killing of Molt-4 (44). Of course, Vgamma9$^+$ is not indispensable for lysis of Molt-4 as many Vgamma9$^-$ also kill Molt-4 (219), suggesting two pathways (TCR-dependent specific cytotoxicity and TCR-independent NK-like cytotoxicity) are both involved in the cytotoxicity against Molt-4 similar with that of Daudi cells. However, TCR-pathway failed to stimulate the proliferation, indicating that the threshold of proliferation is higher than that of cytotoxicity of gammadelta T cell clones and that the interaction between gammadelta TCR and antigen on Molt-4 cell surface is lower than that between gammadelta TCR and antigen on Daudi cells. Similarly, on account of the high expression of ULBP1 and 2, NKG2D-ligand pathway may be also involved in the response of gammadelta T cells against Molt-4 (220).

Finally, gammadelta T cells also showed HLA-unrestricted cytotoxicity against virus-infected cells (236,239,240), activated macrophages (241,242), damaged, stressed, or transformed keratinocytes (243-245), and other tumor cells including neuroblastoma, lung carcinomas, lymphoma, myeloma, colorectal cancer, oesophageal tumor and pancreatic tumor cells (80,192,246-251). However, interestingly, although activated CD4$^+$ T cells were not sensitive to lysis by gammadelta T cells, they were able to stimulate gammadelta T cells to proliferate (252-254).

10. OTHER ACTIVATING PATHWAYS

It is of important for gammadelta T cells that besides classical gammadelta TCR signal pathway, other ways seem to be involved in activation and can directly activate gammadelta T cells.

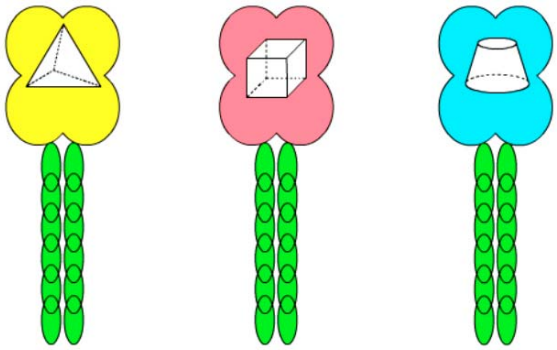

Figure 10. Putative conformational binding box.

The first is IL-2 and IL-2R complex signal pathway. IL-2R comprises at least three subunits encoded by different genes: IL-2Ralpha sharing homology with IL-15Ralpha, IL-2Rbeta being shared with IL-15R and IL-2Rgamma which also participates in the formation of IL-4, IL-7, IL-9 and IL-15 receptors (255). Isolated beta chain binds IL-2 with a characteristic low affinity, while individual alpha chain binds IL-2 with a higher affinity. By contrast, IL-2Rgamma alone has no measurable affinity (255,256). IL-2Rbeta and IL-2Rgamma subunits form an intermediate affinity receptor and will converts into an authentic high affinity receptor when co-express with IL-2Ralpha (257-259). In human PBMC, the expression of CD25 (IL-2R alpha chain) is comparablely low on resting gammadelta and alphabeta T cells, while IL-2R beta chain expression is much higher on resting gammadelta T cells than that on alphabeta T cells (260). Short-term culture to high dose of rIL-2 selectively stimulated gammadelta T cells but not alphabeta T cells to express activation antigens (CD69, CD25 and HLA-DR), while low dose of IL-2 also induced CD69 expression. Long-term exposure to high concentrations of rIL-2 alone (2 weeks) could selectively cause the increase in the proportion of the resting gammadelta T cells in PBMC. However, not anti-IL-2R alpha chain antibody but anti-IL-2R beta chain antibody blocked the expansion (260)

Second, CD69 as an activation antigen appeared to be able to directly mediate the cytotoxicity of Vdelta1 gammadelta and Vdelta2 gammadelta T cell clones but not alphabeta T cell clones against P815 through cross-linking with anti-CD69 antibody without TCR-CD3 signals (261), which leads to considering the possibility for a putative ligand on target cells.

Third, ligation CD16 (Fcgamma receptor III) with immobilized anti-CD16 mAb or IgG-coated cells triggered activated Vgamma9Vdelta2 T cells which acquired CD16 expression to secrete TNF-alpha (262). More recently, it has been discovered that $CD16^+$ Vdelta2 gammadelta T cells ($Vdelta2^+$ T_{EMRA} cells) also exist in healthy human peripheral blood though at low frequency and that anti-CD16 mAb induced $Vdelta2^+$ T_{EMRA} cell clone ($CD62L^-$ $CD27^-$ $CD45RA^+$ $CD16^+$) to produce IFN-gamma. Furthermore, positively selected $Vdelta2^+$ T_{EMRA} cell subsets derived from preactivated Vgamma9Vdelta2 T cells with BrHPP phosphoantigen showed moderate lytic activity against Daudi cells. However, because this observation is based on activated gammadelta T cells (263), whether those native $Vdelta2^+$ T_{EMRA} cell subsets present in blood have such capacity is open to doubt. Importantly, CD16 can directly trigger NK cell cytotoxicity by engaging putative CD16 antigen on target cell surface, although the potency is relative low as the activating signals can not override the inhibition mediated by the class I MHC molecules (264).

Finally, the facts that laminin receptor on human lung cancer cells is capable of deliver immediate activating signals independent of gammadelta TCR and that vitronectin receptor on gammadelta T cells serves as an accessory molecule for gammadelta T cells activation were also reported (248,265,266). In contrast, vitronectin receptor can use the TCR/CD3 signal transduction pathway mediated by the ζ-chain to directly induce cytokine production of alphabeta TCR-negative hybridoma (267).

11. CONCLUDING REMARKS

It's not long since the accidental discovery of gammadelta T cells. Surging evidence indicate their important roles in anti-infection immunity, immune surveillance, immune modulation, and bridging innate and adaptive immune response. However, the precise recognition pattern is still mysterious not only because of recognition heterogeneity but the relative difficulty for obtaining enough purified native gammadelta T cells and the difference between gammadelta T cell clones and native gammadelta T cells. Therefore, it should be careful in deducting the effect features from gammadelta T cell clones to native gammadelta T cells. Recently, immortalized gammadelta T cells provide a novel experimental system for helping to illuminate recognition mechanism (268-270).

It has been suggested that "the analysis of antigen recognition may be the key to the understanding of the unique functions of gammadelta T cells" (271). Successfully, lots of protein and non protein antigens were identified. Unlike alphabeta T cells which are MHC-restriced recognition, gammadelta T cells directly recognize antigens independent of MHC molecules in most cases. What's the most important is the difference of eliciting mechanism accounting for proliferation and cytotoxicity. It seems that gammadelta TCR is absolutely required for expansion of gammadelta T cells, but not completely necessary for cytotoxicity (eg NK-like cytotoxicity). When activated by antigens through engaging gammadelta TCR, gammadelta T cells employ both gammadelta TCR and other molecules such as CD69, CD16, 2B4, MHC-II to deliver inducing signals. Further, although gammadelta TCR can respond to some free form antigens, cell-cell contact definitely enhances the reactive potency. With respect to the interaction between gammadelta TCR and antigens, most of the cases appear to be conformational recognition based on germline encoded recognition and CDR3 sequences including length and composing somewhat affect the interaction by directly or indirectly influencing the "putative binding box" which is

formed by several key germline encoded amino acids (Figure 10). The hypothesis is supported by the finding of conservation of TCR conformation in mice epidermal gammadelta cells with disrupted primary Vgamma gene usage (272). Factually, we are attemptting to establish phage displayed library of single-chain gammadelta TCR to validate the hypothesis.

Down-regulation of MHC-I molecules in progressive deterioration damages alphabeta T cells mediated specific immune response, which makes us consider the application of gammadelta T cells for anti-tumor and anti-infection immunity based on the MHC-unrestricted recognition pattern and the character of mediating both specific and nonspecific cytotoxicity synchronously. In the future, a novel complex vaccine designed against B cells, alphabeta and gammadelta T cells may be more beneficial than conventional vaccine in that it can comprehensively mobilize the whole immune system.

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