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Suboptimal engagement of the T-cell receptor by a variety of peptide–MHC ligands triggers T-cell anergy

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Summary

T cells recognize antigen via the T-cell receptor (TCR) and produce a spectrum of responses that range from activation to anergy or cell death. The variety of outcomes may be dictated by the strength of the signals transmitted upon cognate recognition of the TCR. The physiological outcome of TCR engagement is determined by several factors, including the avidity of the ligand for TCR, the duration of engagement, and the presence and nature of accessory molecules present on antigen-presenting cells (APCs). In this review, we discuss a model of anergy induced by presentation of low densities of peptide–major histocompatibility complex (MHC) ligand in CD4$^+$ T cells and compare it to anergy induced by altered peptide ligands in an effort to identify a unifying mechanism. We suggest that altered peptide ligand (APL) and low densities of agonist ligands induce anergy by engaging less than optimal numbers of TCRs. The physiological impacts of anergy in memory CD4$^+$ T cells are discussed.

Keywords: altered peptide ligand; avidity; memory T cells; regulation; T-cell receptor

Introduction

Tolerance is a hallmark of the immune system and can be achieved through multiple mechanisms. Elimination of self-reactive cells during thymic development (negative selection),[1] regulation by specialized regulatory T cells (Tregs),[2,3] and anergy are among the strategies evolved to inhibit reactivity against self. Anergy has been defined as a state of unresponsiveness in T cells associated with lack of proliferation and cytokine production, and is reversible by interleukin (IL)-2.[4] While negative selection takes place in the thymus, regulation by Tregs and T-cell anergy mostly take place in the periphery, and are likely to target memory T cells.

Anergy was originally discovered in clonal T cells that were stimulated with cognate antigens (signal 1) in the absence of costimulation (signal 2).[5] However, later investigations led to the discovery that T-cell anergy could also be generated in the presence of signal 2 but upon recognition of variants of cognate peptides that carried mutations at their T-cell receptor (TCR) contact residues.[6–8] Such variants were named 'antagonist' ligands, as they were effective when mixed with the cognate or 'agonist' ligands at concentrations far in excess of that of the agonist, hence resembling pharmacological antagonists.[6,9] Another group of ligands, 'partial agonists', were also discovered that could stimulate T cells to produce some but not all effector responses.[7,10] The term 'altered peptide ligand' (APL) was adopted to accommodate both antagonist and partial agonist peptides.[11]

APL-induced T-cell anergy; conformational model

Nearly 15 years of detailed investigations devoted to the understanding of the mechanism of APL function resulted in a wealth of information regarding TCR signalling at the membrane interface and the nature of intracellular signalling molecules. All studied antagonist and partial agonist peptide variants were thought to have identical anchor residues to the agonist peptides, and hence form stable complexes with MHC molecules. Thus it was logical to assume that their differences from the agonist must

lie in the topology of the APL–MHC–TCR interface, leading to a qualitative model for T-cell activation or anergy.[6–9,12] According to this model, T-cell signalling is initiated by the conformational changes induced at the complementary determining region 3 (CDR3) of the TCR upon engagement by the peptide–MHC (pMHC) ligand. The topology of the APL–MHC complexes induces signals in T cells that cause anergy rather than activation induced by the agonist–MHC ligand. Despite the appeal of this model, three-dimensional studies of three singly substituted peptide variants of the human T cell lymphotropic virus-1 (HTLV-1) Tax peptide bound to human leucocyte antigen (HLA)-A2 with the A6 TCR and the wild-type agonist peptide showed near-identical structures with no indications of conformational differences in the TCR induced by antagonist peptide.[13,14] The lack of correlation between structural changes and the type of T-cell signals induced provided direct evidence against different ligand-induced conformational changes in the $\alpha\beta$-TCR.

Kinetic proof-reading

Another set of experiments with the aim of kinetic measurement of the interaction of soluble TCRs with soluble MHC proteins in complex with APLs provided new insights into the mechanism of T-cell activation. Because of the extremely low affinities integral to the ternary complexes of TCR–pMHC [dissociation constant (K_d) values of $\sim 10^{-4}$–10^{-6} M, and lifetimes of ~1–100 seconds],[15,16] the majority of those data were collected using surface plasmon resonance (SPR) instruments which provided real-time measurements at high sensitivity.[17,18] Those studies showed that the agonist peptide–MHC complexes dissociated at slower rates than the partial agonists and antagonists, and partial agonists dissociated at slower rates than the antagonist peptides, although the antagonist dissociation could not be measured because the dissociation rates exceeded the instrument capacity.[15,19] Those findings, despite multiple exceptions, were overwhelmingly accepted and led to the dogma that the determinant of T-cell activation or anergy was the lifetime of the TCR–pMHC ligand. These findings suggested the 'kinetic discrimination' or 'kinetic proof reading' model for T-cell recognition and activation.[20–23] It was proposed that agonist ligands lead to T-cell activation because of a longer duration of TCR engagement, and partial agonists could only stimulate some effector functions in T cells because of somewhat faster dissociation from the TCR. Elegant studies provided evidence for correlations between the dissociation rate of TCR–peptide–MHC ternary complexes and the different effector functions, with faster dissociation rates leading to the expression of fewer T-cell activation markers and/or functions.[24–26]

Incomplete phosphorylation of the cascade of signalling molecules downstream of the TCR has been the best available biochemical explanation for the mechanism of APL function consistent with the kinetic discrimination theory. However, kinetic discrimination does not consistently explain the effects of all APLs, particularly the antagonist ligands studied. A challenge to the theory is that it does not offer a certain threshold common to all agonists, partial agonists or antagonists with similar effects, and experimental data do not support a unifying concept.[13,14,27–29] Because of extremely fast dissociation rates for antagonist peptides, and lack of biochemical data suggesting measurable cellular effects of antagonist peptides, it is difficult to place them together with other partial agonists with measurable lifetimes and/or biochemical effects at the cellular level.

Another challenge to the kinetic proof-reading theory comes from work with peptide ligands that dissociated rapidly from the MHC and induced T-cell anergy. Work by Mirshahidi *et al.* [30] postulated that, if the lifetime of the ternary complexes of TCR–pMHC was the determining factor for the induction of anergy or activation, then peptides that dissociate rapidly from the MHC should have similar effects to APLs and should induce anergy. An agonist peptide such as $HA_{306–318}$ from HA1 of the influenza virus was converted to a peptide that formed only short-lived complexes with HLA-DR1 by substituting tyrosine (Y) at position 308, the main anchor residue for binding to HLA-DR1, with a small or polar amino acid.[30–32] When such peptides were tested for the induction of activation or anergy in clone-1 T cells,[6] the results were mostly consistent with the principles of the kinetic proof-reading theory, as short-lived peptides induced anergy. Notably, however, one of the short-lived peptides, HA_{Y308A}, triggered both activation and anergy but in the 10- to 100-fold concentration range. Interestingly, when similar peptide titrations were applied to the agonist $HA_{306–318}$ peptide, the same trend was observed; higher doses of the agonist peptide induced activation and lower doses induced anergy.

Agonist peptides at low densities induce anergy

The finding that, in the presence of signal 2, agonist ligands induced T-cell anergy when presented at low densities on APCs strongly suggested that neither the lifetime of the TCR–pMHC nor the quality or topology of the pMHC presented to T cells is the best determinant of T-cell activation or anergy. The observation highlighted a quantitative model for T-cell activation. It showed that the overall avidity of peptide–MHC–TCR ternary complexes rather than the affinity of the individual molecular complex is the critical factor for T-cell responsiveness. Other laboratories also found that presentation of low densities of agonist peptide–MHC complexes induced T-cell anergy.[30,33–38]

The magnitude of TCR engagement is a critical predictor of T-cell anergy or activation

Antigen recognition by TCR engagement as detected by internalization and degradation has been established as a correlate of T-cell activation.[39,40] Korb *et al.*[34] demonstrated that, at low doses of antigen, fewer than 10 complexes of peptide–MHC were presented per APC and engaged ~1000 TCRs in T-cell clones, which caused T-cell anergy as defined by a lack of T-cell proliferation and IL-2 production. Although Sykulev *et al.*[41] reported that presentation of three pMHC complexes induced cytotoxic killing of target cells in a $CD8^+$ T-cell clone, and Irvine *et al.*[42] showed that a single pMHC can induce a transient calcium release in clonal T cells. However, neither result negates the findings of Korb *et al.*[34] because CTL killing requires little stimulation,[43] and calcium release is an early activation marker. In an attempt to compare the number of TCRs down-regulated upon interaction with the inhibitory doses of agonists, short-lived peptides, or APLs, it became clear that, regardless of differences in structural properties, all inhibitory stimulation resulted in down-regulation of ~1000 TCR molecules, whereas stimulatory doses of agonists or short-lived ligands down-regulated > 4000 TCRs.[30] Further experiments comparing the short-lived peptides with agonist peptides for induction of early and late activation markers confirmed expression of similar activation markers by both categories of peptides at doses that induced anergy and/or activation. Short-lived peptides induced similar changes in surface expression of two early indicators of T-cell activation, CD25 (IL-2R) and CD69, compared with that induced by a tolerogenic dose of the agonist $HA_{306–318}$.

Low-density TCR ligand and APLs and intracellular signalling

A major signalling defect triggered by APL stimulation was the partial phosphorylation of CD3z and the lack of ZAP-70 (protein-tyrosine kinase) recruitment to the signalling complex.[6,8,34] A comparison of the phosphorylation patterns of TCR signalling components initiated by low doses of agonist peptide or inhibitory doses of short-lived peptides indicated similar degrees and patterns of phosphorylation to those of the APL. T cells pretreated with low doses of agonist peptide or inhibitory doses of short-lived peptides exhibited partial phosphorylation of CD3ζ and below-detection levels of pZAP-70, pLAT (linker for activation of T cells) and pSLP-76 (the adapter protein SH2 domain-containing leukocyte protein of 76 kDa). However, Fyn was fully phosphorylated, consistent with the phosphorylation pathway seen in anergy induced by traditional APLs.[44–46]

All three forms of ligands that induced anergy; APLs, low doses of agonist peptides, or fast dissociating peptides led to inhibition of IL-2 synthesis. IL-2 gene transcription is initiated by the formation of active (c-Fos and c-Jun) activating protein 1 (AP-1):nuclear factor (NF)-AT and NF-κB complexes, each an end product of different TCR/CD3-linked signalling modules. A low level of signalling transmitted by the engagement of 1000 TCR–CD3 complexes might be sufficient to activate some but not all of the components of this transcription complex and consequently may lead to a failure of IL-2 transcription. Another similarity among the three forms of ligand was the transient phosphorylation of Vav, a known regulator of rearrangement of the actin cytoskeleton and capping of the TCR.[46–50] Vav-deficient T cells were shown to be defective in TCR-induced actin polymerization.[51,52] Low densities of agonist peptides or short-lived peptides induced a transient phosphorylation of Vav in T cells treated with inhibitory doses of peptides, which was accompanied by no detectable actin polymerization.

Overall, the clear trend emerging is that short-lived peptide–MHC complexes and low densities of long-lived agonist peptides both induce T-cell anergy through engagement of fewer TCRs. Thus, a common trigger for the induction of anergy could be engagement of a limited number of TCRs by a variety of ligands.

A unifying model explaining how APLs, agonist ligands and short-lived peptides induce anergy

Engagement of ~1000 TCRs together with costimulatory molecules is not sufficient to deliver the sustained signalling necessary for T-cell proliferation and cytokine production.[53] We suggest that all forms of ligand discussed above induce anergy by engaging less than optimal numbers of TCRs. Short-lived variants of agonist peptides may deliver negative signals to the T cells specific for the agonist peptide–MHC complexes by two synergistic mechanisms, both promoting presentation of few effective peptide–MHC complexes for the engagement of TCRs: first, the short-lived peptide–MHC complexes continuously dissociate, leaving only a few pMHCs on the surface of the APCs, and secondly, because of the lack of a strong anchor residue, the surface of the bound peptide remains flexible, and can only signal a percentage of the T cells that recognize the particular topology of the complex, as described by Kersh *et al.*[54]

We propose that the traditional antagonist peptide ligands might simply function by occupying the majority of the available MHC molecules on the APCs, and reducing the number of active agonist peptide–MHC complexes to tolerogenic levels. This explanation is consistent with the characteristics of antagonists that have high affinity for MHC molecules and work only at high concentrations relative to the agonist ligand.[55] Experimental evidence from Hampl *et al.*[56] supports this explanation, demonstrating that CD4 augments the response of a T cell to agonist but not antagonist ligands.

Anergy induced by low-density pMHC is physiological and occurs *in vivo*

To study T-cell anergy induced by low-density agonist presentation, Mirshahidi *et al.*[57] used several transgenic mouse models, including an HLA-DR1 transgenic strain, populated with heterogeneous CD4 T cells.[33] Use of heterogeneous CD4 T cells is particularly important because of recent concerns about the effects of intraclonal competition among abnormally large numbers of clonally identical T cells in adoptive transfer experiments.[58–61] Mirshahidi *et al.* provided evidence that low-avidity engagement of T cells by low densities of agonist pMHC led to the induction of anergy in memory rather than naïve $CD4^+$ T cells *in vivo*.[30,33,62] *In vivo* results using short-lived peptides confirmed data obtained using T-cell clones. It was consistently observed that, in comparison to the agonist peptide, 10- to 100-fold higher doses of the short-lived peptide HA_{Y308A} were necessary to induce anergy in memory T cells in HLA-DR1 transgenic mice.[30] Use of short-lived peptides for induction of anergy *in vivo* has advantages over the traditional APL because, unlike the APL which is strictly specific for a single clonal TCR, short-lived peptides can interact with several T-cell clones specific for a given peptide–MHC complex *in vivo* and tolerize HLA-DR1 transgenic mice. Because APLs tolerize single T-cell clones, their clinical relevance is limited when used for the treatment of pathological self-reactivity *in vivo*. Indeed, clinical trials with APLs have raised important questions regarding their use in immunotherapy.[63,64]

Why anergy in memory T cells?

Memory T cells have generally been accepted to have a lower antigenic threshold, respond more rapidly to antigen, and be less dependent on the second signal for activation,[65–71] although there are opposing views.[72–76] Memory T cells are thus also capable of damaging host tissues as a result of cross-reactivity, unless their reactivation is strictly regulated.[77] Activation-induced cell death and regulatory T cells have evolved to regulate activated T cells;[78] however, less is known about the regulation of memory T cells. We suggest that anergy induced by low densities of pMHC ligand might be a clever way adopted by the immune system to accomplish this task.

Memory $CD4^+$ T cells undergo anergy upon presentation of low levels of antigen by follicular (B2 B) cells, and not by dendritic cells (DCs).[62] We propose that a reduced level of antigen/MHC expression on the surface of B cells might be a mechanism evolved to signal to memory T cells of the 'end of an infection' so that cells stop proliferating and secreting inflammatory cytokines.[79] Following the termination of infection, the antigen load is gradually diminished with inflammation. At that point, B cells bearing specific high-affinity antigen receptors would preferentially capture antigen and present it to the central memory $CD4^+$ T cells. B cells would act as the APC of choice when antigen falls to non-threatening low levels.[80] Thus, it is logical to hypothesize that B cells signal memory T cells to undergo anergy. A feature of anergy as described here is its reversibility upon encounter with IL-2 and antigen, a condition that is met during the onset of an infection, when a surge of inflammatory responses from the innate immune system coincides with the release of multiple cytokines, including IL-2.[81] In support of this hypothesis are our previous reports that encounter with low densities of antigen in the presence of IL-2 does not lead to the induction of anergy, and that anergized cells when incubated or treated with IL-2 and antigen are no longer anergized.[30,33,34]

Concluding remarks

We have presented a simple quantitative model for T-cell activation and anergy that accommodates all the different categories of peptide studied to date. We propose that, regardless of the nature of peptide–MHC ligand, as long as 1000 TCR–CD3 complexes are engaged the biological effect is a state of long-term unresponsiveness that is reversible by IL-2. Furthermore, we have shown that anergy in memory T cells is physiological as it occurs *in vivo* and in a heterogeneous population of $CD4^+$ T lymphocytes. Importantly, we suggest that induction of anergy in memory T cells might be a mechanism for the regulation of memory T cells, preventing them from damaging self tissues by cross-reactivity. A better understanding of this phenomenon could also help to reveal the underlying mechanisms for viral and tumour surveillance. Many viruses and several tumours are known to decrease expression of cell surface MHC class II.[82] Also, some tumour-associated peptides bind poorly to the MHC molecules.[83] The reduced surface expression of MHC and/or low-affinity peptide–MHC complexes leads to the presentation of low densities of specific peptide–MHC complexes, which would induce anergy in memory T cells specific to the viral or tumour-derived antigens.

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Disclosures

None.

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