Immunol Res (2011) 50:49–68
DOI 10.1007/s12026-010-8173-y

On the logic of restrictive recognition of peptide by the T-cell antigen receptor

Melvin Cohn

Published online: 8 October 2010

Abstract This essay provides an analysis of the inadequacy of the current view of restrictive recognition of peptide by the T-cell antigen receptor. A competing model is developed, and the experimental evidence for the prevailing model is reinterpreted in the new framework. The goal is to contrast the two models with respect to their consistency, coverage of the data, explanatory power, and predictability.

Keywords MHC-encoded restricting element · TCR model · Alloreactivity · Restricted reactivity · Structure–function relationships

Introduction

Given the sharp differences in how the data are viewed, an effort to analyze restrictive recognition of peptide by the TCR that is based on the general principles of genetics and structure might prove to be of value. The Tritope Model derived from these considerations will be compared with the Standard Model, and illustrative crucial experiments will be reinterpreted in the alternative framework.

The phenomenon

The descendent from the original observation of Zinkernagel and Doherty [1] is "restrictive recognition of peptide." This means the allele-specific and peptide-specific interaction of the TCR with the MHC-encoded restricting element (R) that presents peptide (P) (i.e., a [PR]-complex). A model of TCR function is one that explains how these two interactions, one specific for the allelic determinant expressed on R and the other, for peptide (P) bound to it, are integrated to signal the cell. The site on the TCR that engages the allele-specific determinant will be referred to as "anti-R." The site on the TCR that specifically engages the peptide will be referred to as "anti-P."

The focus on a requirement for recognition of a polymorphic allele-specific determinant by the TCR has all but disappeared from the literature. In its place are metaphors that obscure the logic of the phenomenon and the inevitability of the conclusions to be derived from it. For this reason, I cannot use the standard nomenclature.

Major histocompatibility complex (MHC) is a region of the chromosome that encodes among other things, the restricting elements (R) recognized in large measure via their allele-specific determinants by the TCR. We need the term MHC or its equivalent if one wishes to refer to this region. The use of MHC as an abbreviation for a restriction element (R) has led to lapses in clarity as we will see when considering "alloreactivity" versus "allorestriction" or when we wish to deal with the genetics of the haplotype.

An account of the standard model

All of the models of TCR signaling prior to the discovery that its ligand was a [PR]-complex were disproven because they tried to explain the recognition of cell-bound antigen, when, in fact, the preoccupation of the TCR was with

M. Cohn (✉)
Conceptual Immunology Group, The Salk Institute For Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA
e-mail: cohn@salk.edu

intracellular antigen [2]. Had this been appreciated the role of peptide as a specificity element might well have been predicted. In any case, the rationale that surrounded these early models was tacitly carried over to yield what I will refer to as the Standard Model [3] of today.

Initially the TCR was modeled after the BCR and pictured as having a single combining site that recognized an interaction determinant or new antigenic determinant (NAD) formed as a meld between the antigen and the restricting element [4]. When it was shown that the "antigen" was a peptide, it was not viewed as a challenge to this model. The NAD was simply considered to be a derivative of the [PR]-complex. The Standard Model of today is a Gaussian distribution of thinking around this concept that remains based on the BCR role model. The clearest statement of this dominant position is owed to Collins and Riddle [5] who make the prediction "that the BCR genes would function as TCR if expressed on thymocytes and subjected to the mechanisms of thymic selection [5]."

Under the Standard Model, the repertoire of the TCR is hesitantly treated as being random, from which positive selection and negative selection extract peptide- and allele-specific recognition of the NAD. The individual TCRs in the repertoire are viewed as having specificities referred to as ranging from peptide-centric to MHC-centric (initially dubbed "slip-and-slide[3]"), and metaphors (bias, obsession, predilection, etc. for MHC) are used to explain the necessity to account for the germline-selection for allele-specific recognition of R. The Standard Model requires that specific recognition of peptide be obligatory for all TCR signaling interactions whether it be positive selection, alloreactivity, effector function or negative selection. As this leads to contradictions, the specific recognition of peptide at low affinity is assumed to signal via the TCR-positive selection and at high affinity, negative selection. This assumption as we will see has powered a good deal of crucial experimental work, the interpretation of which is dependent on the implications of the terms "low and high affinity." To be meaningful, "low affinity" must imply a signal via the TCR to the T-cell that results from a specific recognitive interaction with the NAD and is unique to positive selection. This is to be distinguished from another distinctly different signal via the TCR initiated by a "high affinity" interaction with the NAD unique to negative selection. Two distinct signals via the TCR, one for each affinity range, are implied under the Standard Model. Alloreactivity becomes a violation of the law of restrictive reactivity [6] and is assumed to be due to degeneracy of recognition of the NAD that in some unexplained way maintains specificity for and defines the same alleles of R that restrict host reactivity.

The theoretical framework

Some basic concepts

How is the recognition of allele-specificity determined?

There is no way that a somatic selection process can preferentially extract from a random repertoire those TCRs that recognize the allele-specific determinants on R. The allele-specific recognition of R by the TCR must be germline-selected. The anti-R repertoire of the TCR must be nonrandom, anti-the-allele-specific-determinants of the species. The alleles of R that are defined by the TCR are the same whether they are mapped by restrictive reactivity or alloreactivity. Germline-selection for allele-specific recognition must have a target site on the TCR upon which it operates, termed here, *anti-R*. All that experiment can do is define the precise germline-selected allele-specific structures on R and on the anti-R site of the TCR that interact to determine allele-specificity (see "Reinterpreting illustrative data").

How is the recognition of peptide determined?

The recognition of peptide must be determined by a somatically generated random repertoire. In order to select for and maintain germline-encoded specificities, the selecting epitope would have to remain essentially invariant. This is not the case for protein epitopes involved in the interactive selection between hosts and pathogens. In the case of the BCR repertoire which has a germline-selected component that is diversified somatically, its specificity is largely anti-carbohydrate [7, 8]. Carbohydrate is a much more stable epitope over evolutionary time than protein because, in order to change its structure, the specificity of a synthesizing enzyme must be changed and that is sufficiently rare. Consequently, the TCR anti-P repertoire is entirely somatically generated and random with respect to the recognition of self and nonself. As the only parts of the TCR that are varied somatically are the junctional regions, $(NDN)_{\beta}$ of the β-subunit and N_{α} of the α-subunit, it can be logically concluded that the anti-P site is determined by complementation between them. Needless to say, deletions and insertions in the bordering V and J of the junction must also have an effect on the anti-P site.

Thus, two targets of evolutionary selection on the TCR are defined, germline-selected anti-R and the mechanism for somatically generating anti-P. This conclusion is logically derived from the observation of "restrictive recognition of peptide" and the pattern of variation of the TCR.

The consequences for a TCR–PR interaction

How are allele-specific determinants distributed on R-elements?

Molecular morphology presented us with a structure of [PR] in which the peptide (P) is anchored in a groove formed between two α-helical domains of the restricting element (R) (reviewed in [9]). For Class I restricting elements (RI), the two domains are referred to as α_1 and α_2; for Class II restricting elements (RII), the two domains are β_1 and α_1. For simplicity of nomenclature, I will refer to RIα_2 and RIIβ_1 as the West (W) domains and RIα_1 and RIIα_1 as the East domains. It was further observed for several cases that Vα docks on the West domain and Vβ on the East domain (reviewed in [9]). This was anticipated and can be generalized. If the pool of Vα and Vβ gene segments encoded random docking then half of the naive TCRs would be nonfunctional because they docked on the same domain. More important is an unappreciated consequence of the docking relationships. The allele-specific determinants must be expressed one per domain, West and East, potentially two per R-element. The anti-R sites on the Vαs of the TCR recognize the family of allele-specific determinants on the West domains of R, whereas the anti-R sites on the Vβs recognize the allele-specific determinants on the East domains of R. The key here is that the V-gene segment pool, Vα plus Vβ, acts as a single family recognizing the allele-specific determinants of the species [3, 10, 11]. Each functionally distinct V-gene segment in the pool of Vα plus Vβ encodes recognition of an allele-specific determinant on R.

The consequence of this is that the total number of functionally distinct allele-specific determinants associated with the West domains of R is less than the number of Vα-gene segments, and with the East domains of R is less than the number of Vβ-gene segments. In mouse, this number is <80 allelic determinants for West domains and <20 for East domains. It is likely that a sizable proportion of the V-gene segments are nonfunctional or redundant in recognition. How these determinants are distributed among the MHC-haplotypes is an important overlooked question as we will see when we discuss alloreactivity versus allorestriction. In the present context, the revealing of over 10^3 alleles of R based on amino acid sequence [5, 12] is an aside. The functional allele-specific determinants are defined by restrictive and allo-recognition (reactivity). The functional allele-specific determinants are polymorphic and comprise a subset of the total amino acid sequence differences.

How does the TCR police the [PR]-complexes of the host?

T-cells via their TCRs are constantly surveilling the cells of the host looking for peptides derived from intracellular foreigners. They do this by docking on the allele-specific determinant which exposes the TCR anti-P site to react with the bound P. If P is recognized, the T-cell is signaled (Signal[1]); if not, the T-cell disengages from the target and looks elsewhere. The mechanism regulating this on–off process is an important yet to be solved problem.

What is the role of thymic selection?

The function of positive selection is to sort the anti-R repertoire so that all of the functional T-cells of the individual restrictively recognize appropriate host alleles. The function of negative selection is to sort the anti-P repertoire by purging anti-self P recognition leaving the residue, anti-nonself P, to protect the host from infection.

Negative selection per force operates via restrictive recognition of peptide. Peptide is acting as a specificity element, the recognition of which is required to deliver a signal via the TCR to the T-cell (Signal[1]). The sorting of the anti-P repertoire requires that its level of discrimination be sufficient to distinguish self-P from nonself-P.

There is a negative selection process that operates on anti-R to cull inappropriate specificities as will be discussed later. This should not create confusion with the negative selection process that sorts the anti-P repertoire as part of the self–nonself discrimination and requires restrictive recognition of self-peptide. Both pathways result in delivery of the same Signal[1] to the T-cell.

Positive selection, on a priori grounds, only requires recognition of the host allele-specific determinant. Peptide in this context is acting as a structural (not specificity) element of the [PR]-complex necessary for its stability and conformation, in particular the expression of the allele-specific determinant. No requirement for specific recognition of peptide as a concomitant of the selection for recognition of host alleles is implied. The interaction of the anti-R site with the allele-specific determinant on R is necessary and sufficient to initiate positive selection [13, 14]. The so-called low affinity at which positive selection operates is a characteristic of the germline-selected R–anti-R interaction. The TCR delivers restrictively only one P–anti-P-dependent signal (Signal[1]) to the T-cell.

The two corollaries to this framework

1. As single V-domains, Vα or Vβ, are positively selected to recognize host R-elements, each entrains a complementing subunit, Vβ or Vα, which recognizes the nonhost or allo-alleles. The consequence is a TCR that has three specific combining sites (Tritope), anti-P, anti-host-R, anti-allo-R. Two of the sites, anti-host-R and anti-P, are required to be engaged for restrictive recognition of peptide. Engagement of the third site, anti-allo-R, is responsible for alloreactivity, which, like positive selection, is peptide unspecific [15, 16].
2. The existence of two distinct interactions, one for restrictive and the other for allogeneic reactivity, that result in the same signal via the TCR to the T-cell (Signal[1]) necessitates two ligand-binding orientations

(restrictive and allogeneic) that initiate the same Signal[1]. These two signaling orientations occur within a fixed TCR-[PR] docking, Vα on the West, Vβ on the East.

Thus, we arrive at a three site TCR Model (Tritope) that delivers a single Signal [1] initiated from either of two orientations. This framework derived almost entirely deductively leaves us with a paradoxical question. The individual never encounters allo-alleles, so what problems did evolutionary selection solve by having a TCR with a second signaling orientation that is P-unspecific and tolerigenic (Signal[1])?

There are two related problems:

1. Under the Tritope Model, each V-domain is expressed in two orientations in the unselected thymic population. One orientation is functional in restrictive reactivity, and the other manifests itself in alloreactivity. If the V-domain in the allo-orientation is specific for a host allele, then that TCR must be ridded by negative selection. The restrictive orientation is positively selected as functional. One would expect to find in the unselected $CD4^+CD8^+$thymic population, two TCRs that share in common a V-domain, one TCR positively selected by a host allele, the other TCR negatively selected by the same host allele. Such a finding would make the Tritope Model inevitable. I will analyze potential examples that suggest this prediction in the section Alloreactivity reflects a functional role.
2. Roughly 10–20% of positively selected TCRs entrain a subunit that is also specific for a host allele. This would result in a large population of mixed reactivity, T-helpers, and cytotoxic T-cells that recognize both RI and RII elements of the host, as well as T-cells reactive with combinations of two different host RI and RII-elements. These TCRs that could well be debilitating are ridded by deletional interactions in the entrained or unselected allo-orientation, which is peptide unspecific (discussed in [2], page 1434).

Alloreactivity indirectly reveals a normal critical function. This represents a change in my thinking from alloreactivity being of no functional significance to the individual [2, 17] and therefore unselectable, to its reflecting an important role in sorting the anti-R repertoire (see "The experimental input", "Alloreactivity reflects a functional role").

A closer look at signaling

It is generally observed that ligand-receptor signaling interactions function in an affinity threshold-dependent manner. In the case of the TCR, the affinity threshold for negative selection is quite sharp as has been shown by Palmer and associates in a series of elegant experiments [18, 19]. Negative selection is dependent on the delivery of Signal[1] via the TCR that is tolerigenic. When above the Palmer affinity threshold, Signal[1] is delivered via the TCR to the T-cell and requires that both anti-R and anti-P be engaged. At below this threshold, when positive selection operates, only anti-R needs be engaged and no signal via the TCR to the T-cell need be envisaged. The signal for positive selection is initiated by R, as the TCR has no way of knowing whether its restriction specificity is for RI or RII. Only R has that information. At an occupancy level below the threshold for positive selection, where neither anti-R nor anti-P is productively engaged, the T-cells die-by-neglect. In essence, if anti-R is engaged, negative or positive selection occurs dependent on whether anti-P is engaged. If anti-R is not engaged, the thymic T-cells simply turn over (death-by-neglect). These two interactions P–anti-P plus R–anti-R and R–anti-R alone are separated by a sharp threshold distinguishing negative from positive selection. The sharp threshold for negative selection originates from the cooperative binding of R and P to their respective sites on the TCR to restrictively deliver Signal[1].

This raises the question as to the origin of the signal for positive selection which is postulated to require that only R–anti-R be engaged. Lineage commitment involves two pathways. A thymic precursor marked by expression of $CD4^+8^+$ has the potential to differentiate into either the 4^+8^- T-helper lineage or the 4^-8^+ cytotoxic lineage. The decision between two pathways requires two signals. These two signals can be arranged in one of two ways.

If no signal is delivered to the T-cell via the TCR on binding to the allele-specific determinant on R, then the two signals must be initiated by R. TCR binding to RI triggers the 4^-8^+ cytotoxic T-cell pathway, whereas binding to RII triggers the 4^+8^- T-helper pathway.

If a signal to the T-cell passes via the TCR upon interaction with R, this must trigger a default pathway, either 4^+8^- or 4^-8^+. In this case, binding to the appropriate R would initiate a signal that diverts this default pathway to the other one. The revealing studies from Singer's laboratory [20] favor this second model. The binding of the TCR to RI or RII initiates a signal via the TCR that results in the expression of the IL-7R receptor by the T-cell. This is a default pathway to the 4^+8^- T-helper lineage. Upon interaction with IL-7, this default pathway is diverted to the 4^-8^+ cytotoxic lineage.

In both models, a signal via R is required. If signaling via the TCR to the T-cell is insufficient to distinguish the choice of pathways by the 4^+8^+thymic precursor, then the TCR and R must switch roles. The TCR must be acting as

the ligand for R acting as a receptor on the thymic positively selecting cell to deliver a discriminatory signal via a pathway independent of the TCR [14].

At the experimental level, it is ill-defined how an IL-7 signal distinguishes the cells interacting with RI versus RII. However, extrapolating from the study of Alves et al. [21], consider a T-cell expressing both IL-7R and an RI-restricted TCR that is on a default pathway to becoming a $CD4^+$ helper. A thymic selecting cell that expresses RI only and upon interaction with this TCR secretes IL-7 active over a short range would divert the T-cell to the correct $CD8^+$ cytotoxic lineage with acceptable discrimination of class.

The TCR is now viewed as capable of transmitting two distinct signals to the T-cell. The signal for negative selection (Signal[1]) is allele- and peptide-specific. The discriminatory signal for positive selection is allele-specific but peptide-unspecific. This latter signal is also required for survival in the periphery and for homeostatic proliferation [22].

In sum then, given a germline-selected affinity of R–anti-R, the cooperative affinity of the TCR-[PR] interaction depends on the P–anti-P interaction. This has a threshold above which the TCR acting as a receptor delivers Signal[1] to the T-cell and below which the TCR acting as a ligand in the R–anti-R interaction triggers the positively selecting thymic cell to signal the differentiation of the precursor $CD4^+CD8^+$ T-cells to $CD4^+$RII-restricted T-helpers and $CD8^+$ RI-restricted T-killers. Postulated is that the peptide is not functioning as a specificity element during positive selection and that the discriminatory signal for the determination of effector class is initiated by R acting as a receptor upon binding its ligand, the TCR.

What can one anticipate from a system of germline-selection involving a set of V_α- and V_β-gene segments encoding recognition of a set of R-elements?

The pool of V_α- and V_β-gene segments is being selected upon to recognize R-elements. This informs the T-cell that it is dealing with intracellularly derived peptide. The R-elements are selected upon to present peptide. This presentation requires anchoring via recognition by R of several of the amino acid side chains of P.

Why wasn't the recognition of R by the TCR an invariant in which a common determinant was seen by a single $V\alpha V\beta$ pair? Why high polymorphism and low polygeneism of R?

This is a complex question having diverse consequences, but for our purpose here, it might be assumed that pathogen mimicry of the postulated unique site on R recognized by the TCR would inactivate the immune system. Whatever the interactive selection pressure driving allele-specific recognition of R [17], several consequences of the process are to be anticipated.

1. As V-gene segments evolve by duplication, mutation and selection, a proportion of them will be nonfunctional or redundant. In mouse, the functionally distinct V-gene segments in the V_α-locus is <80 and in the V_β-locus, <20.
2. The recognition of an allele-specific determinant by a given V will range from specific for a given R-element (defined by sequence) to "crossreactive" (sequence distinguishable R-elements sharing an allele-specific determinant). In mouse, each MHC-haplotype expresses roughly 10 allele-specific determinants, 2 per R (K,D,L, A, E). Clearly, different MHC-haplotypes can share to varying extents allele-specific determinants. It would be expected that Class I R and Class II R which are associated with different effector functions will not share allele-specific determinants (see, "The experimental input"). Within a given RI or RII class and between MHC-haplotypes, a range of distributions of shared allele-specific determinants is expected.
3. In mouse, the West domains seen by Vα will express many more polymorphic alleles than the East domains seen by $V\beta$.

What is the relationship between the alleles of R defined by specificity of R for anchor residues on the bound peptide and by allele-specific TCR recognition of R?

Given that the selection pressure on R-elements is to bind distinct families of peptides and on pathogens to escape recognition of their peptides, a family of alleles of R is defined by the specificity of anchoring pockets. Given that the selection pressure on the TCR is to recognize determinants on R that are unique (restrictive), a relationship between the two interactions is to be anticipated, namely that the allele-specific determinant seen by the TCR is derived as a consequence of the binding of peptide anchor residues by the recognitive pockets in the peptide-binding groove of the R-element. Therefore, sequence variation in the pocket or in the bound peptide would be expected to affect the expression of the allele-specific determinant seen by the TCR. The interpretation of the data is critically dependent on this point as changes in peptide sequence affecting anchoring can result in changes in expression of the allele-specific determinant and, consequently, the binding of a given [PR]-complex to the TCR. There is recent evidence for this prediction [3, 17]. Bowerman et al. [23] studying the K^b-restricted 2C TCR point out that a substitution in a peptide anchor residue "may affect TCR binding indirectly, since Tyr3 is embedded in pocket D of K^b through numerous interactions. Arg155 of K^b, one of the residues that contacts Tyr3, also interacts Tyr31 of the 2C TCR, a residue that contributes significant binding free energy to the interaction with SIY/K^b. Thus,

we propose that this network of interactions is disrupted by the Y3A substitution" in an anchor residue. In other words, recognition of the allele-specific determinant by the TCR can be affected by interactions of the anchors with the R-element. This has important consequences for the interpretation of replacement data.

A pinch of history and to what it has led

The Kappler/Marrack team [9, 24] as do many immunologists [5] like to cite Jerne [25] for the hypothesis that "TCR genes have evolved to encode proteins that are inherently MHC specific" [26]. They contrast this assumption with one in which the "initial TCR repertoire may be random, and positive selection may pick out the rare TCRs with appropriate MHC specificity and affinity [26]."

Jerne was trying to explain the origin of the adaptive repertoire of the BCR which he reasonably assumed was also the antigen receptor of the T-cell. He based his theory solely on the intuition that the germline-selected combining site specificities were anti-self, an a priori questionable assumption that he never abandoned, in fact reemphasized during the idiotype network era. These anti-self specificities would be somatically selected against by "tolerance" leaving their mutants that had lost recognition of self to function as the anti-nonself repertoire. He then searched the literature for an example and his friend Simonsen pointed him toward MHC, but this was not essential to his argument as he, himself, pointed out [27, p. 349]. Any set of self-components would have satisfied his assumption. It is not my intention to deal with this a priori untenable proposal. Rather, I wish to stress that the reasons for arriving at a conclusion are as important as the conclusion itself because the fortuitous right conclusion for a wrong or irrational reason misguides the interpretation of data and the design of experiments.

The phrases "inherently MHC specific" or "appropriate MHC specificity" do not imply allele-specific recognition, although I will treat them as doing so. The competing hypothesis as stated by Dai et al. [26] is ruled out on logical grounds. There is no way that an individual can know what the alleles of the species are. Therefore, positive selection cannot extract from a random TCR repertoire a unique subset displaying allele-specific restrictive and allo-recognition. The only way to give meaning to that competing assumption is to challenge the observation that the TCR recognizes the alleles of R. The Standard Model is based on such a challenge, which is difficult to rationalize as the alleles of R that we are discussing are defined by the TCR.

A group acting as a workshop [28] came to two conclusions based on the Standard Model that challenge this conclusion.

> …negative selection functions to eliminate T-cells that have the highest degree of MHC(R) and peptide (P) degeneracy and this biases the repertoire toward recognition of peptide side chains.
>
> …in the absence of negative selection, TCRs can react with MHC proteins in a class and allele-independent fashion.

These two conclusions are derived from an interpretation of the studies of the Kappler/Marrack group who raise the question:

> How could evolution select for TCR segments with affinity for generic features of MHC proteins if all the TCRs that illustrate this point disappear in the thymus before they could be of use for the survival of their host [12]?

As they view the system in the Standard framework, "thymocytes bearing TCRs with strongest MHC reactivity are lost by negative selection; only TCRs with attenuated ability to react with MHC appear on mature T-cells [9]."

With this in mind they answer their own question:

> The TCRs that survive positive and negative selection may display just portions of the conserved interactions, sufficient to allow them to have the ability to engage self-MHC but not enough to allow them to be negatively selected. Thus, the selected, useful repertoire of TCRs in any given animal is stamped with a faint imprint of what has been selected over time in the species [12].

While it would not be unreasonable to ask, how does germline-selection operate to recognize an allele-specific determinant that is only expressed as a "faint imprint," their question and their answer illustrate a basic ambiguity that illustratively, is derivative of the Jerne paradox, how does evolution select for either a silent or a debilitating recognition of self? The problem is with the concept of a TCR repertoire that is random with respect to the recognition of NADs (even when modulated by some degree of bias, predisposition, obsession, predilection, partiality, preoccupation, etc., for/with MHC). Given the Standard Model with its anti-NAD repertoire, logic renders it inevitable that negative selection operating on peptide-centric recognition of the NAD would affect the R-centric recognition of the allele-specific determinant because the peptide and the allele-specific determinant are visualized as a meld epitope. There are four conceptual arguments to face.

First, allele-specific and common determinants on thymic-R are both self to the individual, not distinguishable by negative selection. Allele-specific or restrictive recognition requires positive selection involving two germline-selected elements, the allele-specific determinant on R and the

anti-R site on the TCR. Allele-specific recognition derived from a random or even biased repertoire needs rationalization, if it is to be treated as "a faint imprint of what has been selected over time in the species."

Second, when thymic-R (R_T) functions in the host, it is *not* acting as a self-antigen. Rather, the R_T-element is playing a role as a component in the physiology of the host, in this case as a platform presenting intracellularly derived peptides to the TCR [29]. Negative selection does not delete allele-specific or restrictive recognition of host-R. It deletes recognition of self-P. There are no "generic features of MHC proteins" the recognition of which by the "TCR disappears in the thymus."

Third, does negative selection cull the positively selected broadly MHC reactive and peptide promiscuous population to produce a T-cell repertoire specific both for peptide and host MHC alleles [12]?

While their findings can be safely interpreted as negative selection playing a role in contributing to the determination of the "specificity" of the anti-P site, its role in determining allele-specific recognition can only be indirect (if it plays any role). The conceptual argument that allele-specific recognition is germline-selected is as strong as the present day experimental argument that it is not. Negative selection is the somatic process determining the self–nonself discrimination and operates on anti-P, whereas positive selection sorts the germline-selected anti-R repertoire to determine restrictive recognition.

Fourth, can the absence of negative selection be responsible for a population of TCRs that see "MHC proteins (R-elements) in a class and allele-independent fashion?"

As negative selection cannot distinguish allele-specific from common determinants on MHC (R) because both are self, it is not likely to be a factor that plays a role in determining the class of R recognized or allele-specific recognition. These coupled properties are determined by germline-selected sites (anti-R) not influenced by a somatic process like negative selection. Besides, negative selection is a somatic process that is peptide-specific and except for the situation discussed earlier (see "The two corollaries to this framework") is not concerned with the discrimination of determinants on R. Negative selection does not operate to delete recognition of host R by positively selected V-domains; it operates to delete recognition of self-P.

In sum, self-tolerance cannot bias the positively selected TCR repertoire away from promiscuous recognition of MHC (i.e., recognition of epitopes common to many R-alleles) to one that is allele and class specific [12].

We will consider the data that led to the conclusion that negative selection affects allele-specific recognition of R in the section "Reinterpreting illustrative data."

The nature of the anti-P site

The probing studies both by Bevan and coworkers [30, 31] and by the Kappler/Marrack [12, 32–35] team to be discussed later necessitate a more detailed look at the anti-P site. It is reasonably assumed to be generated somatically at the junctions of V–N–D and D–N–J of the β-subunit in complementation with V–N–J at the α subunit. The potential amino acid variability of this complementarity-determining region 3 (CDR3) is quite large ($>10^{15}$). The anti-P repertoire determined by this region is viewed as random with respect to the recognition of self or nonself peptide. By contrast, the P-repertoire is small. If peptides of roughly 10 amino acids are presented by R-elements, 5 of which are available for interaction with the TCR, then the P-epitopic repertoire is capped at $20^5(3.2 \times 10^6)$. The anti-P repertoire has the potential to recognize this capped P-repertoire. The anti-P site is visualized as being able to bind n random peptides. The term proposed to describe this anti-P site that can bind n random peptides is "polyspecificity" [28]. Several theoretical analyses [36, 37] have shown that the optimal value of n (n_{opt}) occurs when there is an average of one Pself bound per anti-P site. If the probability of being a self-peptide is SI (the specificity index) and each TCR binds at the optimum, n_{opt} random peptides, then the probability, that a given TCR will be negatively selected occurs at $1-e^{-1}$. At n_{opt}, 63% of TCRs will be deleted. At small values of SI, $n_{opt} = 1/SI$.

The analysis of the relationship between the P and anti-P repertoires requires a separate discussion. There are two models that deal with this question, the black and white model and the grey model. The black and white model as I originally proposed it was based on a precise testable reformulation of the classical lock and key analogy [38, 39]. The findings of Huseby et al. [12, 32, 34] rule it out for the TCR. The grey model is a precise formulation of what is now generally accepted to be the behavior of the TCR anti-P site described under the phrase "polyspecificity [28]" (Clark and Cohn, in preparation). In that paper, we will quantitate the analyses of the data of Huseby et al. with respect to the effect of negative selection on the "specificity" of the anti-P site. In general, we are in agreement with them. Here, I would like to concentrate on the claimed effect of negative selection on the allele-specific recognition of the R-element because that strikes at the heart of the Standard NAD model. While the Standard Model predicts that negative selection would affect (or even determine) allele-specific recognition of the R-element, the Tritope Model would be disproven by such a demonstration. Huseby et al. feel that they have provided such evidence and, therefore, their experiments must be reinterpreted if the Tritope Model is to have any validity (see discussion

III. "The experimental input", Reinterpreting illustrative data, "The contribution of negative selection to the recognition of the R-element").

Alloreactivity

The McCluskey team also operates in the Standard framework to analyze alloreactivity [6]. They stress that "the MHC is the most polymorphic region of the genome." A polymorphic allele is one that has been germline-selected such that its frequency in the population is much higher than would be expected from mutation alone (i.e., $> 1\%$ by convention). As the selection pressure establishing this polymorphism is the necessity for restrictive recognition of peptide, germline-selection for allele-specific recognition is an unavoidable, indeed singular postulate.

The Kappler/Marrack [9] and McCluskey teams [6] view negative and positive selection similarly. Starting with a random TCR repertoire, selection for "modest self-reactivity" operates. This leads to the argument that the immune system's "use of self-selected T-cells to recognize foreign peptide antigens exploits the natural crossreactive potential of TCRs". As "not all T-cells are restricted to recognizing self-MHC molecules" alloreactivity is a "violation of the 'law' of MHC restriction" [6]. To view alloreactivity as a denial of restrictive reactivity, the phenomenon we are trying to explain is admittedly quixotic.

As alloreactivity and restrictive reactivity define the same alleles, it should be clear that the recognition of these alleles involves a unique germline-selected family of anti-R sites used for both reactivities. Alloreactivity cannot be accounted for by the degeneracy of recognition of a [PR]-derived NAD by a BCR-like single combining site. Alloreactivity is of high frequency compared to restrictive reactivity, a fact that McCluskey et al. view as largely unexplained in the Standard framework. Alloreactivity should not be treated as a case of "mistaken identity" or "molecular mimicry." Restrictive and allo-reactivity define the same set of allele-specific polymorphic determinants because they are recognized by the same set of V-domains, Vα or Vβ.

Restrictive recognition is dependent on the positively selected V-domain, whereas alloreactivity is dependent on the entrained V-domain. Peptide acts as a specificity element for restrictive recognition when presented on host thymic-R, whereas it acts as a structural element for allo-recognition that behaves peptide-unspecific when presented on allo-R [2, 11].

The high frequency of TCRs in an individual that recognize a given allo-allele is due to its being germline-selected and peptide-unspecific. The comparatively low frequency of restrictive recognition is due to the specificity of anti-P for peptide. In addition, a given TCR that is restricted to R^a and alloreactive to R^b is positively selected in the restricting host (R^a) and is negatively selected in the allo-host (R^b) [40–42]; see discussion in Ref. [2, p. 1434]. Under the Standard Model, this could only be a fortuity, not a general rule. Taken as a general rule, it implies that both positive selection and alloreactivity are peptide-unspecific.

The experimental input

Reinterpreting illustrative data

Single V-gene segments encode recognition of the alleles of R

The experiment of Hong et al. [43] demonstrates that single V-domains recognize the allele-specific determinants on R during either restrictive or alloreactivity. The D10 TCR is specific for a conalbumin peptide, Pcon, restricted to the Class II R, A^k. It is alloreactive to A^b (see Table 1). Line 1 illustrates normal restrictive reactivity to [$A^k_\alpha A^k_\beta$-Pcon]. Pcon is required for a response. Line 2 demonstrates that the alloreactivity is due to recognition of A^b_β by Vα2 and that it is peptide unspecific. Line 3 shows that the alloreactivity to A^b_β by Vα2 is not restricted by the recognition of A^k_α by Vβ8.2 and confirms that alloreactivity is peptide-unspecific as well as unrestricted. Lines 4,5 are controls showing that A^d_α, A^b_α and A^k_β are not recognized. A^d_α and A^b_α are not recognized as restricting elements by Vβ8.2 which is specific for A^k_α, and A^k_β is not recognized by Vα2 which is specific for A^b_β and alloreactive to it. There is a caveat in the case of restrictive recognition by Vβ8.2 as the failure to respond could be due to inability of the test R-element to present Pcon (Lines 4,5). The independent assorting of

Table 1 Response of D10 TCR to various alleleic complements of Class II element, A

Line	Allele of		Response of D10	
	Aα(Vβ 8.2)	Aβ(Vα2)	Pcon present	Pcon absent
1	k	k	+	−
2	k	b	+	+
3	b	b	+	+
4	d	k	−	−
5	b	k	−	−

Data taken from [43, 79]

Pcon, conalbumin peptide; Aα, α subunit of H-2A restricting element; Aβ, β subunit of H-2A-restricting element; (), V-domain recognizing the subunit of H-2A

responsiveness to the various complements of the alleles of the H-2A-locus establishes that single V-domains recognize the allele-specific determinants on R whether restrictive or allo-reactivity is involved and that the TCR can function in two signaling orientations.

A supporting experiment is due to Logunova et al. [44] although performed in a different context. They analyzed a mouse that was defective in processing and that expressed a single Class II molecule, A^b covalently attached to a peptide Ep. In essence, the immune system of the A^bEp mouse treats as nonself all of the peptides that the wildtype C57Bl6(H-2^b) mouse treats as self.

They compared as hybridomas the induced cells of a B6(H-2^b) anti-A^{bm12} allo-response with an A^bEp anti-B6 restrictive response. The hybridomas *per force* restricted to A^b (Class II R) in both groups were analyzed for Class I R alloreactivity. The frequency of A^b-restricted hybridomas alloreactive to Class I R was an order of magnitude higher when derived from A^bEp anti-B6 than from B6 anti-A^{bm12}. Why?

The selection for the entrained V-domain of B6 specifying alloreactivity to A^{bm12} precludes the presence in the responding-population of V-domains specifying recognition of other Class I or Class II R alloalleles. By contrast, the selection for restrictive recognition of A^b-self peptides during the response by the A^bEp mouse, entrains randomly the complementing V-domains that specify allorecognition of either Class I or Class II R. This reinterpretation of their finding requires that single V-domains specify recognition of the allele-specific determinants.

These two experiments [43, 44] support the conclusions that

1. Each restricting element, Class I or II, has the potential to express two allele-specific determinants.
2. Each functional V-domain recognizes an allele-specific determinant, Vβ on RIα1 and RIIα1 (East domains), Vα on RIα2 and RIIβ1 (West domains).
3. Alloreactivity is due to recognition by the V-domain that is unselected or entrained during positive selection.

Alloreactivity is peptide unspecific

Mullbacher et al. [15, 16] using two distinct experimental systems provide evidence that alloreactivity to Class I MHC, K^d, is peptide unspecific. We reinterpreted [2] the data of Hong et al. [43] (Table 1) to show that single V-domains recognize allele-specific determinants and restrictive and allo-reactivity are mediated in opposite signaling orientations, restrictive reactivity being peptide-specific, alloreactivity being peptide-unspecific.

"Alloreactivity" must be distinguished from "allorestriction"

I will use the term "alloresponsive" to include both "alloreactivity" and "allorestriction." *Alloreactivity* is manifested when the TCR restricted to an R-element encoded by the MHC-haplotype of the host, H-2^a, responds to an allele from another MHC-haplotype, H-2^b, as a target ligand. *Allorestriction* is normal restrictive recognition of peptide involving an allele-specific determinant that is shared by two different MHC-haplotypes.

In order to illustrate the value of this distinction, let us consider the study of Felix et al. [45] that is in design an extension of a previous analysis by Health et al. [46]. Felix et al. [45] studied 182 hybridomas from a B6·H-2^b anti-B6·H-2^k MLR. Of these, 60 responded to E^k expressed on a B-cell line, C27-E^k. These were subdivided into two groups based on reactivity to another cell line CHO-E^k; 28 responded to CHO-E^k, 32 did not. To explain this, they assumed that all alloresponses require specific recognition of peptide. Given this, the CHO-E^k line would not be expected to present relevant peptides to the 32 nonresponders whereas the C27-E^k line would present them. They demonstrated this to be the case for 9 of the 32 nonresponders to CHO-E^k by isolating defined peptides from C27-E^k and showing that these peptides conferred responsiveness to CHO-E^k. They generalized their finding by concluding that alloreactivity is peptide-specific.

The B6·H-2^b mouse is so popular that we had better take a close look at it.

First, the B6·H-2^b does not express an E-element. Eα is defective leaving E^b_β either unexpressed or possibly weakly complemented with Aα [47–50].

Second, Eα when expressed in the E-elements of the various MHC-haplotypes is minimally polymorphic [51].

Third, the responding B6·H-2^b in the allogeneic mix with B6·H-2^k is restricted to A^b, as the E-element is not expressed in B6·H-2^b.

From where do the TCRs specific for C27 peptides and specific for E^k originate? They were, after all, positively selected to be A^b restricted. One testable assumption is that A^b and E^k share an allele-specific determinant in which case what was revealed is both an allorestrictive and alloreactive response. That two MHC-haplotypes share an allele-specific determinant is not unexpected; "allorestriction" is a phenomenon seen from the point of view of the immunologist, not the TCR for which allorestriction is normal restrictive reactivity, peptide specific, in another MHC-haplotype.

This finding [45] can be translated into two families of responding TCRs. One family $V^{b/k}_\alpha V^x_\beta$ is allorestricted to A^b_β/E^k_β and alloreactive to unrelated MHC-haplotypes

(H-2^x). The other family $V^b_\beta V^k_\alpha$ is restricted to A^b_α and alloreactive to E^k_β. Restrictive recognition is peptide-specific; alloreactivity is peptide-unspecific.

Given this reinterpretation of their study of B6·H-2^b anti-B6·H-2^k, of the 182 hybridomas anti-H-2^k, 60 were alloresponsive to E^k (C27-E^k). These can be divided into 28 alloreactive to E^k, (both C27-E^k and CHO-E^k reactive) and 32 allorestricted to E^k (C27-E^k reactive, CHO-E^k unreactive), roughly 1:1. A test of this reinterpretation would be that the 28 TCR alloreactive to E^k will be negatively selected by E^k, whereas the 32 allorestricted to E^k will be positively selected by it, provided, of course, that the thymus doesn't present them with a recognized self-peptide. Even if it does, positive selection should be revealable. Further, the allorestricted clones will be alloreactive to other MHC-haplotypes, whereas the alloreactive clones will not, a result that would extend the Logunova et al. [44] observation.

These two studies [44, 45] permit us to understand the earlier finding of Schilham et al. [52] of a cytotoxic T-cell of H-2^d origin alloresponsive to D^b and E^k. It is probably allorestricted to D^b and alloreactive to E^k.

An illustrative example of the explicative value of allorestriction

In a series of papers, Allen and coworkers [53–56] characterize specificity of peptide recognition by the T-cell clone T2-102. It expresses two Vαs, and one Vβ displaying, therefore, two TCRs, Vα2 Vβ1 and Vα4 Vβ1. T2-102 is specific for a hemoglobin peptide, restricted to E^k and alloresponsive to Ep. When Vα2 Vβ1 is isolated and separately analyzed, it is not positively selected on E^k and is alloresponsive to H-2^d [53]. This latter activity surprisingly was not found in the T2-102 itself. [55].

Given these findings, in the Tritope framework there are two scenarios.

Scenario 1. Vβ1 specifies alloreactivity to an unrevealed allele, x. Vα4 is allorestricted to E^k/Ep and Vα2 is allorestricted (not alloreactive) to H-2^d. Under this interpretation, the signaling orientation is determined by the β-chain (see discussion of Ref. [57] *Dissecting the anti-P site*).

Scenario 2. Vα4 is allorestricted to E^k/Ep as in Scenario 1. The Vβ1 complemented to it is alloreactive (not allorestricted) to H-2^d. The Vα2 is allorestricted to an unrevealed allele, x. The Vβ1 complemented to it is alloreactive to H-2^d. As in Scenario 1, the signaling orientation is determined by the β-chain.

Under Scenario 1, the prediction would be that

- Vα4 Vβ1 will be positively selected on E^k/Ep, will show no alloresponsiveness to H-2^d but will be alloreactive to H-2^x. As is it allorestricted to E^k/E^p, it will be peptide-specific.
- Vα2 Vβ1 will be positively selected on H-2^d, will be peptide specific in the context of H-2^d (allorestriction), and will be alloreactive to the same haplotype (H-2^x) as Vα4 Vβ1.

Under Scenario 2, the prediction would be that

- Vα4 Vβ1 will be positively selected on E^k/E^p as in Scenario 1 but will be negatively selected on H-2^d.
- Vα2 Vβ1 will be allorestricted to H-2^x and alloreactive to H-2^d. It will, therefore, be negatively selected on H-2^d.

As the parental T2-102 does not display alloreactivity to H-2^d, which is peptide-unspecific, Scenario 2 appears to be ruled out because Vβ1 is postulated to encode alloreactivity to H-2^d. Assuming no involvement of experimental error, one possible reason under Scenario 1 that alloresponsiveness to H-2^d was not revealed is that the specific peptide needed to permit allorestricted reactivity was not appropriately presented to T2-102 when it was tested, whereas it was when Vα2 Vβ1 was assayed. Of course, the density of expression of Vα2 Vβ1 on the cell surface of T2-102 could also play a role under Scenario 1 but not under Scenario 2.

In conclusion, any interpretation of these studies requires that allorestriction be distinguished from alloreactivity.

It seems appropriate to point out that the reason for allelic exclusion being so lax at the Tα-locus is that the selection pressure for exclusion is very weak. Double TCRs, 2 Vα-1 Vβ, pose little threat because positive selection and restrictive reactivity makes the doubles function, in large measure, as singles. This interpretation under the Tritope Model is to be contrasted with that derived from the Standard Model. Morris and Allen [53] focus on double producers as the dominant factor driving Graft-versus-Host (GvH) disease. Their argument is based on the finding that dual producing T-cells are increased during a GvH response. However, this is not a telling argument. More likely, during the abnormal explosive reaction triggered by a graft-versus-host (GvH) response, the STOP condition to further rearrangement at the Tα-locus becomes leaky (a phenomenon referred to as receptor revision or receptor editing). The increased frequency of T-cells with dual TCRs during a GvH response is a consequence of the GvH response, not a cause of it.

Alloreactivity defines two signaling orientations of the TCR

The studies of Matis et al. [58] and Nakajima et al. [59] provide a commanding insight into the origin of

alloreactivity. Their experiments compare Vα- and Vβ-gene segment expression in the TCR repertoires of an E^k-restricted anti-cytochrome C (Pcyt c) response and of alloreactivity to E^k by mice that do not express E (i.e., B6/B10, H-2^b). The restricted response to E^k-Pcyt c uses predominately Vα11, which would be expected to be specific for E^k_β. The alloreactive response to E^k_β was also dominated by Vα11. The Vβ usage differed in the two situations with some overlap allowing selection of clones with identical VαVβ pairs, one restricted to E^k-Pcyt c, the other alloreactive to E^k, Pcyt c independent. These results provide further evidence that single V-gene segments encode recognition of the allele-specific determinant and that restrictive vs alloreactivity depends on whether the V-domain in the TCR is positively selected or entrained. The structural element regulating this difference in behavior would be revealed by sequencing the junctional regions of TCRs with identical VαVβ pairs that are either restricted or alloreactive to a given R-element [60]. In any case, two orientations for the delivery of Signal[1] are dramatically revealed.

An example of one such pair is described by Matis et al. [58]. Clones 3.3 and 9R.D6 express the same Vα, Jα, and Vβ gene segments differing in Nα and (NDJ)β. Clone 3.3 is E^k ($E^k_\alpha E^k_\beta$)-restricted and E^s($E^k_\alpha E^s_\beta$)-alloreactive, whereas clone 9R.D6 is E^s-restricted and E^k-alloreactive (see Table 2). As Vα recognizes E^s_β and Vβ recognizes E^k_α, when Vα provides the functioning positively selected restricting anti-R site and Vβ is entrained, the TCR is E^s_β-restricted, Pcyt c-dependent and E^k_α-alloreactive, Pcyt c-independent (clone 9R.D6). When Vβ provides the positively selected restricting anti-R site and Vα is entrained, the TCR is E^k_α-restricted, Pcyt c-dependent and E^s_β-alloreactive, Pcyt c-independent (clone 3.3).

Table 2 TCRs with identical VαVβ domains display reciprocal behaviors

APC	Pigeon cytochrome C (Pcyt c)	Clone 3.3	Clone 9R.D6
$E^k_\alpha E^k_\beta$	Absent	−	+
	Added	+	+
$E^k_\alpha E^s_\beta$	Absent	+	−
	Added	+	+
	Alloreactive to	E^s_β(Vα)	E^k_α(Vβ)
	Restricted to	E^k_α(Vβ)	E^s_β(Vα)

Data taken from Matis et al. [58]

−, no response; +, response; (), V-domain recognizing E-element

Alloreactivity reflects a functional role

We have assumed (see "Two corollaries to this framework") that alloreactivity reflects the normal process to rid T-cells that are restricted to two different host thymic R-elements (R_T). One such example can be found in a study by Eshima et al. [61]. They describe a T-cell clone QM11 that was derived from a B10.QBR mouse (K^b, A^b, D^q/L^q) that was $CD8^+$ and specific for allo-A^k. Clone QM11 is positively selected on D^q/L^q and is, therefore, a D^q/L^q restricted, $CD8^+$ cytotoxic clone. It does not recognize the H-2^b haplotype. However, it is also positively selected by D^d/L^d and is, therefore, allorestricted to it. The surprise is that on H-2^q (K^q, A^q, D^q/L^q), the transgenic TCR QM11 gives rise not only to $CD8^+$ D^q/L^q-restricted cells but also to $CD4^+A^q$ responsive cells. This clone then can be characterized as restricted to D^q/L^q, allorestricted to D^d/L^d, and alloreactive to A^k/A^q which share a determinant. As alloreactive clones are negatively selected in the allo-host [40–42], it would be expected to be negatively selected in the presence of A^q. Although the data were not interpreted to show this, it is likely. A transgenic TCR inundates the thymic selection processes with a unique TCR expressing T-cell. Using the data in Fig. 8A of reference [61], the QM11 TCR is expressed in $CD4^+$ cells at a relative level of 2.5 in a Class I negative ($\beta2$ M^-) H-2^q (A^q) mouse. However, in the wildtype H-2^q($K^qA^qD^qL^q$) Class I expressing mouse, the $CD4^+$ cells are at a relative level of 12.5. Eshima et al. describe this, with no explanation, as Class I (D^qL^q) enhancement of positive selection by Class II A^q. This, however, more likely reveals the predicted negative selection by A^q which interacts with this TCR in the allo-orientation. As all cells express Class I D^qL^q, whereas only a small proportion express Class II A^q, the D^q L^q acts as a decoy in the wildtype mouse decreasing the probability that the T-cell will encounter and, therefore, be negatively selected by A^q. In the presence of A^q and D^q L^q, the level of this TCR in $CD4^+$ T-cells is 5-fold higher than in the absence of D^q L^q. The "inhibition" of negative selection by the decoy effect of D^q L^q reveals, in addition, the concomitant positive selection by A^q that drives the $CD4^+$ pathway. The fact that the QM11 TCR is negatively selected on A^k [62] confirms this interpretation of the data because A^q and A^k share an allele-specific determinant. The reason that this informative clone QM11 was isolatable is due to its derivation from an MHC-haplotype B10.QBR that does not express A^q. Predictably, for those TCRs of the QM11 signaling orientation, alloreactivity to A^k would be absent in B10.H-2^q. What would be isolatable from H-2^q mice are clones restricted to A^q and allorestricted (not alloreactive) to A^k.

Positive selection by A^q illustrates an important corollary of the Tritope Model not sufficiently emphasized,

namely that *any negatively selectable TCR must also be positively selectable*, even if the recognitive interaction is with the unselected or entrained subunit (i.e., the alloreactive orientation). It is the recognitive interaction with the allele-specific determinant that is the trigger for positive selection.

The literature contains many examples of TCRs with the properties of QM11. In order to discuss this point, a nomenclature problem arises. I will use the term "synreactive" to refer to a TCR that is positively selected to be restricted to one host allele and entrains a subunit that is specific for another allele expressed by the host MHC-haplotype. As pointed out earlier, roughly 10-20% of unselected TCRs will fall into that category. Under the Tritope Model, the entrained subunits will specify "alloreactivity" to the host allele (synreactivity) because of its signaling orientation. Normally, synreactive clones would be negatively selected by the synreactive host allele.

Given this, let us briefly consider two additional clones OT-1 and 2C [63] which display "synreactivity" as does the above discussed QM11.

Clone OT-1 (Vα2.3-Vβ5) is K^b-restricted and A^b-synreactive. Clone 2C (Vα3.1-Vβ8.2) is K^b/L^d-allorestricted and A^b-synreactive. As expected, OT-1 and 2C are positively selected by K^b to yield $CD8^+$ cells. The surprise in both cases is that in RAG-minus H-2^b mice, $CD4^+$ cells are also present suggesting positive selection by A^b, the only Class II R present. This was indirectly confirmed by Ge et al. [63]. In the absence of Class I R (K^b) (TAP-minus), CD4 cells are absent. This was interpreted as requiring both Class I R and Class II R for positive selection of the 2C TCR. Alternatively, concomitantly with positive selection by K^b, the 2C TCR is negatively selected by the synreactive A^b. In the presence of K^b, this latter process is inhibited as discussed earlier for the clone QM11 [61] and positively selected $CD4^+$ cells are revealed.

A distinct change in my interpretation of the 2C TCR is required. Previously I had assumed [2, 17] that 2C was K^b-restricted, L^d-alloreactive. This is no longer tenable. The 2C TCR is allorestricted to K^b/L^d and negative selection in H-2^d mice [40] is presumably self-peptide dependent. The alloreactivity is directed to A^b (synreactivity) and is predicted to be peptide-unspecific. The specificity of recognition of peptide presented by K^b or L^d deals with allorestricted recognition, not alloreactivity which engages A^b. This has consequences for the interpretation of experiments on the peptide specificity of "alloreactivity" and on the interpretation of structure–function relationships [23].

Because of the complexity of these interactions, a variety of outcomes is possible but no contradictions are revealed. By way of illustration, OT-1 behaved somewhat differently from 2C [63]. In RAG-minus mice expressing A^b, the level of $CD4^+$ OT-1 cells was tenfold higher in the absence of K^b than in its presence. This is unlike the findings with QM11 and 2C where the level of $CD4^+$ cells is higher in the presence of Class I R than in its absence. For OT-1, K^b is not measurably protective against the putative negative selection by A^b. If we take a Sherlock Holmes approach (i.e., "if we have eliminated all but one hypothesis, this latter however improbable must be the truth"), then OT-1, unlike 2C and QM11, is negatively selected by a self-peptide presented by K^b as well as by synreactive A^b. In this situation, positive selection by A^b yielding $CD4^+$ cells would be masked by the dual sources of negative selection, K^b-self-P and synreactive A^b.

It might be stressed that any interpretation of experiments on the specificity of recognition of peptide during what, under the Standard Model, is referred to generically as "alloreactivity" requires that one be dealing with a T-cell clone that is not allorestricted. The three clones QM11, OT-1, and 2C analyzed here are good examples. The studies of Allen's group [45, 53–56], as well as those of the Kappler/Marrack team [12, 32–34], as discussed here, are further illustrations. Under the Tritope Model, restrictive recognition, whether it be syn or allo, will be peptide-specific. Alloreactivity is postulated to be mediated peptide-unspecific and triggered in a signaling orientation opposite to that of restrictive reactivity. This remains to be disproven.

Dissecting the anti-P site

In order to analyze this site, two approaches have been tried based on limiting the amino acid sequence diversity and analyzing its consequences. One is to study the anti-P repertoire in the absence of N-additions; the other is to limit the contribution of rearrangements.

What happens when one limits the amino acid diversity at the TCR CDR3 junctional regions?

Several groups [64–67] have generated terminal deoxynucleotidyl transferase negative (TDT) mice that fail to make N-additions in the CDR3 regions. These mutants lack, therefore, the major source of amino acid diversity but still retain significant variability due to deletions and P additions as well as expression of the three D reading frames. Neonatal mice also lack N-additions.

Gavin and coworkers [30, 31] using such mice analyzed the range of D^b-restricted peptides recognized by independently derived polyclonal CTL lines, as well as the specificity of TCRs induced by a single D^b-restricted peptide. Using peptide libraries with fixed amino acids corresponding to the anchor residues used by D^b, they were able to analyze the effect of the absence of N-additions on the size of the D^b-restricted peptide repertoire that is recognized. An overall decrease in repertoire diversity was observed, confirming the role of the junctional region as

the anti-P site. Concomitant with the decrease in repertoire size was the appearance of D^b-restricted clones that respond to a large number of sequence distinguishable peptides from the library. These clones become detectable because their frequency in the selected population is increased due to the deletion of the highly specific TCRs by the TdT° mutation. In essence, the repertoire is being squeezed between deletion by negative selection at the highly promiscuous end and deletion by TdT° at the highly specific end to yield a population of intermediate promiscuity.

The evolutionary selection pressure driving increase in specificity of the anti-P site is the necessity to make a self–nonself discrimination. It operates on the size of the combining site (i.e., the number of complementarity-determining residues that must be engaged in order to signal the T-cell). If the site was so small that it responded to a single amino acid, the TCR could not make a self–nonself discrimination; it would be a universal glue or to use Gavin/Bevan nomenclature it would be highly "promiscuous." This means that the number of distinguishable peptides recognized by a single anti-P site (i.e., the value of *n*) would be large. As the size of the paratope increases, the average size of *n* would fall to some evolutionarily acceptable value (see Theoretical framework, "The nature of the anti-P site").

The absence of TdT catalyzed N-additions in fetal and neonate immune systems probably reflects the recapitulation of phylogeny during ontogeny. In the ancestral immune system, the size of the combining site in the absence of N-additions was minimally capable of a self–nonself discrimination. However, as the pathogenic load increased, the breaking of tolerance became a selection pressure to increase the size of the combining site (i.e., to increase specificity) by N-additions. This question of the repertoire size and value of *n* will be analyzed quantitatively elsewhere (Clark and Cohn, in preparation).

Another study [57] starts with the OT-1 TCR which, as discussed earlier, is K^b-restricted and specific for an ovalbumin peptide (K^b-Pova). TCR OT-1 is composed of Vα2.3-Jα26 and Vβ5-Jβ2.6. A mouse was engineered that limits variation in the CDR3 region to CDR3α by expressing the intact OT-1 Vβ5-Jβ2.6 as a transgene complemented to Vα2.3 that can rearrange to either Jα26 or Jα2. The OT-1 β-subunit is unique and fixed; the α-subunit is varied somatically at the junction by deletions and N-additions upon joining to either Jα26 or Jα2.

When analyzed in the presence of the H-2^b haplotype, the limited mouse would be expected to express $CD8^+$ $CD4^-K^b$-restricted TCRs. The interesting finding is that $CD4^+$ $CD8^-$ T-cells are also present suggesting positive selection by A^b, the only Class II R-element present.

Given this, in the Tritope framework, the limited mouse can be envisaged to express two categories of TCR.

1. $V_\beta^{K^b}$ $V_\alpha^{A^b}$ is $CD8^+$ K^b-restricted and synreactive to A^b.
2. $V_\alpha^{A^b}$ $V_\beta^{K^b}$, is $CD4^+$ A^b-restricted and synreactive to K^b.

The two postulated categories of TCR use the identical Vα and Vβ as discussed earlier [58] (see Table 2) but would have opposite signaling orientations. The K^b-restricted orientation would be $CD8^+$; the A^b-restricted orientation would be $CD4^+$.

Normally, a K^b-restricted TCR that expressed A^b recognition in the allo-orientation or vice versa would be negatively selected. In the absence of a characterization of the restrictive and allo-specificities expressed by the limited mouse, an interesting speculation is possible, namely that the CDR3α contributes to determining the signaling orientation. Further, some of the CDR3α sequences could, in addition, inactivate the allo-signaling potential permitting an A^b-restricted, K^b recognitive TCR or a K^b-restricted, A^b recognitive TCR to survive "allo" negative selection and be functional without harm in restrictive recognition because it is nonsignaling in the allo-orientation. To date, I have assumed that the D_β-reading frame was the signaling regulator [68] but the situation could well be more complicated or the hypothesis could be wrong. If the D_β-reading frame determines the signaling orientation then only the first category of TCR ($V_\beta^{K^b}V_a^{A^b}$) cited above could be present in the limited mouse as the β-subunit of OT-1 is invariant. Revealing the existence of an A^b restricted, K^b synreactive TCR in the limited mouse would disprove the D_β-reading frame hypothesis.

An important confirmation of these interpretations of the limited mouse, OT-1, comes from two studies, the one discussed earlier ("Alloreactivity reflects a functional role") by Ge et al. [63] and the other on homeostatic proliferation by Hao et al. [22]. Homeostatic proliferation is dependent on recognition of the MHC-allele. The peripheral survival of OT-1 was shown to be dependent on either K^b or A^b. Hao et al. conclude, using the Standard Framework, that "in contrast to T-cell positive selection in the thymus that is mainly conditioned by TCR affinity, peripheral clone size appeared to be determined by TCR promiscuity [22]." The "promiscuity" that they refer to can be translated into a K^b-restricted, A^b-synreactive OT-1 as illustrated above. As Hong et al. [43] have shown the D10 member of the Vα2 family to be A_β^b-specific (see Table 1), it is a reasonable conjecture that OT-1 is restricted to K^b via Vβ5 and is A^b-synreactive via Vα2. A direct demonstration (e.g., an MLR) that OT-1 is synreactive to A^b would substantiate these assumptions.

Although the limited mouse has a unique potential to permit analysis of both the capacity and specificity elements that characterize the anti-P site as well as the structural elements determining the signaling orientation, it

was not investigated with such questions in mind. It is only to illustrate its potential, when guided by a theory that it is discussed here.

The contribution of negative selection to the recognition of the R-element

Huseby et al. [12] investigated two families of TCRs reactive with the ligand A^b-P_{3K}, one from wildtype H-2^b mice, the other from H-2^b mice deficient in negative selection. They observed that the family of TCRs anti-A^b-P_{3K} from wildtype H-2^b mice were, on average, quite specific for A^b-P_{3k}, whereas the TCRs from H-2^b mice with inoperative negative selection appeared to be less specific for A^b-P_{3k} The hybridomas specific for A^b-P_{3k} derived from wtH-2^b mice rarely show alloresponsiveness to the test panel of H-2 haplotypes compared to the A^b-P_{3k} specific hybridomas derived from H-2^b mice deficient in negative selection. In their language, the latter "have a florid propensity for allo- and self-MHC reactivity."

The immature T-cell population that does not recognize host-R, in this case A^b, dies-by-neglect. The positively selected population has both allorestricted and alloreactive clones. The positively selected V-domain may recognize an allele-specific determinant unique to the host MHC-haplotype or to one that is shared by one or more allo-MHC-haplotypes. This allorestricted V-domain will be complemented with one that, if functional, will have specificity for an allo-allele that may be unique or shared by several allo-MHC-haplotypes. The question then is how negative selection might affect the distribution of these recognitions. In the Tritope framework, an individual that cannot process and present peptides, self and nonself would be expected to express a TCR anti-R repertoire that is normal with respect to both alloreactivity and allorestriction. These recognitions are, after all, germline-selected. This raises the question of how are the observations of Huseby et al. [12, 32–35] to be explained.

The "florid propensity for self-MHC reactivity" is understandable. The mutant deficient in negative selection is not tolerant to wildtype self-peptide (Ps). It is therefore restrictively responsive to them. There is no effect on "self-MHC reactivity." The "florid" response is to self-peptide, not to "self-MHC" (defined as expressing the allele-specific determinant of host thymic-R), which is acting as a presenting platform for peptide, not as an antigen subject to a self–nonself discrimination.

What about the "florid propensity for alloreactivity?"

There are two sets of TCR that recognize A^b-P_{3k}:

Set 1—V^b_α is positively selected by A^b_β and entrains a family of V^n_β that encode various alloreactivities. This the $V^b_\alpha V^n_\beta$ set.

Set 2—V^b_β is positively selected by A^b_α and entrains a family of V^n_α that encode various alloreactivities. This is the $V^b_\beta V^n_\alpha$ set.

If in Set 1, V^b_α sees A^b_β/E^k_β, then the TCR would be allorestricted to E^k_β and alloreactive via its V^n_β to some member of the allelic family of Class I or II MHC. This would include Eα which is defective in H-2^b and not expressed. Being minimally polymorphic, some Set 1 TCRs could respond to almost any MHC-haplotype expressing an Eα-element.

If in Set 2, V^b_β see A^b_α/E^k_α, then the TCR would be allorestricted to E^k_α and alloreactive via its $V^n_\alpha$to some member of the allelic family of Class I or II MHC. This includes E^b_β which, although not defective, is not expressed in H-2^b due to the mutant E^b_α partner.

If TCRs anti-A^b-P_{3k} were deleted by cross-reactivity with self-peptides from either Set, then the effect of amino acid replacements in A^b_α or A^b_β would depend on which Set was being studied. Further, the patterns of alloreactivity of TCRs anti-A^b-P_{3k} from each Set could be quite different. If Vβ encodes alloreactivity, its response pattern could be broad, whereas that of Vα could be narrow.

In other words, the system studied is too complex and too statistically limited to make the generalization that negative selection, which sorts the anti-P repertoire, also sorts the anti-R (allele-specific) repertoire.

Another experiment of Huseby et al. [32] was designed to test the effect of negative selection on the anti-P repertoire. While I would agree on a priori grounds with their conclusion that negative selection has a direct affect on the anti-Pnonself repertoire, the experiments purporting to show this are subject to an illustrative competing interpretation. Their experiment was to compare given TCRs derived from wildtype mice and from mice defective in negative selection.

The ligands in this study were a family of peptides covalently linked to E^k. The TCRs that they analyzed as hybridomas came from B6(A^bE^-) anti-B10.BR(A^kE^k) or B10.D2(A^dE^d) anti-B10.BR(A^kE^k). The alloresponse to A^k was blocked so that only the response to E^k was analyzed. Using the panel of E^k-SP ligands, they could divide the family of E^k responsive TCRs into three groups, Group 1 responsive to E^k bound to many different peptides (31%), Group 2 responsive to E^k bound to "self-peptides" and some test ligands (14%), Group 3 responsive only to E^k bound to the immunizing "self-peptides" (55%). As in the analysis of the study of Allen's group [45] with an analogous system, roughly 50% of the responding TCRs were alloreactive and 50% allorestricted. In the Tritope framework, Groups 1 and 2 would be pooled with the result that

45% would be viewed as alloreactive and predictably peptide unspecific, and 55% allorestricted and predictably peptide specific.

Huseby et al. point out that "none of the E^k reactive T cells…..were completely ignorant of bound peptide… [32]." When peptide is functioning as a specificity element this is expected; when functioning as a structural element such effects can only be indirect and difficultly analyzable in this system. The general conclusion from these studies [12, 32–34] is that Mullbacher et al. were correct in concluding that alloreactivity is peptide unspecific [15, 16], a prediction of the Tritope Model. In both cases, TCR interaction with its ligand is "peptide-dependent," whether it is peptide-specific or peptide-unspecific.

The immunization of wt B6 mice with (E^k-SP)-DC favors alloreactivity (peptide unspecific) as those cells are more frequent than peptide-specific allorestricted cells. Therefore, as expected they behave peptide-unspecific. More challenging are the results with mice expressing a single E^k-SP1 ligand in which positive selection would be expected to be close to normal and negative selection essentially nonexistent. The T-cells responsive to the immunizing ligand E^k-SP2 were in general specific for this ligand showing as expected some crossreactivity with self-peptides to which the animal was not tolerant. In this case, we are most likely dealing with E^k-restricted T-cells.

Consider the results with two chimeras, BM H-2^b → H-2^b thymus (Chimera 1) and BM H-2^{bxk} → H-2^b thymus (Chimera 2). In both cases, positive selection operates on A^b but in Chimera I the E-element is absent, whereas in Chimera 2 E^k-Pself is negatively selecting.

Immunization of BM H-2^b → H-2^b chimeras with E^k-SP results primarily in an alloreactive response as discussed above for the immunization of wt B6. In the case of the F1(bxk) → b chimeras, the animal is tolerant to E^k-Pself. As it is restricted to A^b, but responds to E^k-SP specifically, the response is allorestricted, not alloreactive, in agreement with the study of Felix et al. [45] as reinterpreted here.

Why does a chimera positively selected thymically to be restricted to A^b but tolerant to E^k-Pself behave both restricted to E^k and peptide-specific? This is not a violation of restrictive recognition, rather it illustrates allorestriction. The TCRs see an allele-specific determinant common to A^b and E^k. Allorestriction is normal restrictive recognition in another MHC-haplotype.

Huseby et al. [32] summarize their findings as follows: "….mice lacking E^k-specific negative selection have E^k peptide-dependent, as well as peptide-independent T-cells in relatively equivalent numbers…….. When the T-cell repertoire undergoes negative selection on E^k-single peptide, the E^k peptide-independent T-cells are eliminated [32]." How might this be explained?

The "E^k peptide-independent" (peptide-unspecific) T-cells are alloreactive and are negatively selected on E^k. That they would be deleted is predictable in the Tritope framework (see section "The two corollaries to this framework").

In the Standard Framework, recognition of the R-element alone cannot be negatively selecting and still have positive selection determining the restriction specificity. The A^b-restricted T-cell population sees E^k in one of two ways, either as an alloreactive target or as an allorestricted target. Those T-cells that see it as alloreactive are peptide-unspecific and those that see it as allorestricted are peptide-specific. The T-cells allorestricted to E^k are predictably alloreactive to one or another of the allo-alleles of the species. The T-cells alloreactive to E^k are specific for it. Thus, the findings of Felix et al. [45] and Logunova et al. [44] and Huseby et al. [32] are aspects of the same observation when analyzed in the framework of the Tritope Model.

In sum, while their conclusion is likely correct, namely that negative selection based on Pself recognition affects the degree of polyspecificity of the recognitive repertoire for Pnonself, the above-discussed experiments do not deal directly with that question.

Structure confirms predictions from biology

The translation of "allele-specific determinant" (*a*) and the site recognizing it (anti-*a*) into defined structures on the R-element and on the TCR requires introducing another factor. If one V-subunit is positively selected by docking on *a*, with what does the entrained V-subunit interact? Our postulate has been that the entrained V-subunit interacts with a public determinant common to, shared by, or invariant on each domain of R. Simply put, if Vα is positively selected because it recognizes the host *a*-determinant on the West domain of R, then the entrained V*β* will dock on a largely invariant determinant, *i*, on the East domain of R and vice versa. Consequently, in examining the structural data, we must ask, are we dealing with an *a*-anti-*a* interaction or an *i*-anti-*i* interaction. Thus far, I have treated the anti-R site as an *a*-anti-*a*-site; now anti-R has two pockets or subsites, anti-*a* and anti-*i*, which are engaged depending on the V-subunit that was positively selected. It is unlikely that the TCR docks on the two *a*-determinants of R. If it did the TCR recognitive of a given host R would be a unique Vα*V*β pair (or close to it), and many analyses show this not to be the case (discussion in Refs. [2, 17]).

If there were one *i-site*, then the entrained subunit could dock on either Class I or Class II R-elements as the study by Logunova et al. [44] indicates. It is unlikely that all R-elements and all functional V-domains interact at an invariant *i–anti-i-site*. The existence of several *i-sites* could

have a dramatic effect on the choice of entrained V-domains expressed with each MHC-haplotype. This is an important consideration because it bears on the structural basis for the asymmetry in signaling orientation between restrictive and alloreactivity.

The *i-site* is postulated to stabilize the [PR-TCR] complex, not to contribute directly as a signaling interaction. The *a-site* is an obligatory component initiating the signal via the TCR to the cell whether the interaction is one of restrictive or alloreactivity. The problem that arises is whether the structural studies reveal interaction with an *a-* or an *i*-site. Additional information is needed to decide this. It has not escaped my attention that the postulate of more than one i-site raises questions as to the precise criteria that distinguish a-sites from i-sites (private versus public), but for this discussion let's keep it simple, as described.

The very existence of allele-specific recognition as a prerequisite for a specific response to peptide [1] required the postulate of dual recognition by a single receptor [69, 70]. While these two cited dual recognitive, single receptor models were disproven, the principle of a single receptor with two sites, anti-R and anti-P, remains inviolate. Although the principle has been long buried, it is beginning to resurface as it must and this is reflected in the recent structural studies. While I will discuss only two examples, what I wish to emphasize here is not simply the requirement for dual recognition but rather that the logical conclusions from the structural studies are being overlooked or contradicted.

The revealing of an "interaction codon" on R seen by individual V-domains

As discussed earlier, the structural studies revealed a docking orientation; Vα always docks on the West domain and Vβ on the East domain of the R-element. The peptide is anchored in the groove between these domains. Garcia's team [71, 72] has revealed the existence of what they refer to as a "structurally encoded recognition motif" seen by "**each** TCR variable region gene product." Thus, we now seem to be in agreement that single V-domains of the TCR recognize the allele-specific determinants on R-elements.

According to Garcia et al. [72], "…the germline bias of the TCR for MHC (i.e., restrictive recognition of peptide) was…predictable by the first principles of physical chemistry." It is not my intention here to confront this intriguing claim. Rather, I wish to point out that it was predicted from the biology [3, 10, 11].

They describe the site on R recognized by the V-domain as an "interaction codon." Specifically they show that Vβ8.2 binds to a unique site on the A-element whether the Vβ8.2 is in a TCR specific for A^k-Pcon or A^u-Pmbp. This finding (discussed in detail in Ref. [73]) of a docking site common to A^k_α and A^u_α favors the assumption that the "interaction codon" is the structure defining the allele-specific determinant shared by A^k_α and A^u_α. The cited study of Hong et al. [43] (Table 1) supports this assumption that the interaction codon is an *a-site*, not an *i-site*, on A^u_α/A^k_α

While the realization that "**each** TCR V-gene product" engages "recognition motifs" on R-elements represents a major change in the thinking of Garcia et al. [72], the consequences of their conclusion that single V-genes encode recognition of the allele-specific determinants on R were not faced (see "A structuralist looks at alloreactivity").

The experimental dissection of the anti-R site

The studies of the Kappler/Marrack team [9] give us an insight into the contribution of the germline-selected V-domains to the recognition of the allele-specific determinants. Their study is important because they use a functional criterion to determine signaling, namely triggering of IL-2 production by a [TCR-PR]-interaction. Clearly, not all interactions of the V-domain with R as defined by morphology were germline-selected to play a role in the signaling process Eventually, function must be assayed. These studies complement those of the Garcia team [72] who define the structure that appears to be the allele-specific determinant (i.e., "the interaction codon").

After carefully weighing whether TCRs were evolutionarily selected to recognize allele-specific determinants on R-elements, Marrack et al. [9] provide us with a very clear analysis of the potential residues in the V-domains that determine this recognition. Not unexpectedly, the recognition by the V-domain of these allelic determinants can range from highly to loosely restricted. Further, bulky peptides can affect the [TCR-PR] interaction but there is no evidence that this results in the restrictive recognition of an allele that was not positively selected. The central question of a relationship between the specificity of binding of anchor residues and the expression of the allele-specific determinant is only beginning to be appreciated [23]. However, the conclusion that Vα- and Vβ-gene segments were evolutionarily selected to see allele-specific determinants on R-elements seems to be settled by the findings of these two groups. The two conformational changes in the TCR consequent to an R–anti-R interaction followed by a P–anti-P interaction that deliver Signal[1] to the T-cell is now ready for experimental attack.

A structuralist looks at alloreactivity

Garcia et al. [72] view alloreactivity as follows: "The TCR…is specific for the MHC but is also 'crossreactive'

with many MHC molecules. That is, each αβTCR can in principle recognize any MHC-allele."

This is once again no more than a denial of restrictive reactivity. As the same alleles are defined by restrictive and alloreactivity, this statement needs examination. If docking occurs in a fixed orientation (i.e., Vα docks on RIα2 or RIIβ1; Vβ docks on RIα1 or RIIα1), then each of the cited domains of R has the potential to express an allele-specific determinant. If Vα is positively selected in thymus because it recognizes an allele-specific determinant on the host RIα2 or RIIβ1, then it entrains by complementation a family of Vβs that can recognize allo-alleles on RIα1 or RIIα1. Conversely, if Vβ is positively selected because it recognizes an allele-specific determinant on the host RIα1 or RIIα1, then it entrains a family of Vαs that can recognize allo-alleles on RIα2 or RIIβ1. Consequently, alloreactivity must be a property of the unselected or entrained V-domain. A T-cell restricted to R^a and alloreactive to R^b will be positively selected in thymus by R^a and negatively selected by R^b (discussed in [2, p. 1434]). What would be the characteristic of a crossreactive system that would predict this finding as a general property? In any case, these findings necessitate two signaling orientations of the TCR.

The TCR, when functioning in an individual, docks on the allele-specific determinant. This exposes the anti-P site to recognition of P. If P is not recognized, the TCR disengages and looks elsewhere. If recognized, Signal[1] is delivered to the cell. There is, therefore, no rationale for the conclusion that "this crossreactivity is essential in that it enables the TCR to briefly dock and 'scan' the peptide context of many different MHC molecules." [72].

What happened to the "structurally coded recognition motifs" seen specifically by "**each** TCR V-gene product?" A given TCR only encounters the R elements of its host. An individual never encounters allo-R. What would be the structurally based rationale for postulating that a given TCR can "'scan' the peptide context of many different MHC molecules (allo-R)" but can only scan the peptide context of a single R molecule in its host? An H-2^a animal treats H-2^b as allogeneic and vice versa. According to Garcia et al., this is due to a crossreactivity in which "practically any αβ combination can…recognize most MHC molecules." Curiously enough, in the (H-2^a × H-2^b)F1, those TCRs using the same V-domains treat the same R-elements as unique, without crossreactivity (restrictive recognition). How does a recognitive anti-R site on a given TCR distinguish the products of the gene-loci (K vs. D, A vs. E), not to mention their alleles, during restrictive recognition, yet fail to do so during allorecognition? Clearly, their concept of alloreactivity contradicts their observation of germline-selected "interaction codons" seen by "**each** V-gene product."

Feng et al. [71] tell us that "….in a way similar to antibodies, germline-encoded TCR CDR loops may have evolved a chemical and conformational optimum to satisfy the opposing requirements of specific but cross-reactive recognition of a diverse spectrum of MHC surfaces."

The Standard Model of the TCR-PR interaction is driven by a role model, the BCR. With some previously cited metaphorical qualifications ("bias" for MHC being the one preferred by Garcia et al. [72]), the recognitive repertoire of the unselected αβTCR is viewed as essentially random recognizing various shape-patches (epitopes) on the surface of the [PR]-complex. The abbreviation "MHC" is now clearly ambiguous. Does it symbolize R-element alone or a [PR]-complex? Assuming the latter, the logic of this picture requires that TCR recognition be treated as being on a sliding scale between peptide-centric and restricting element-centric (previously referred to and discussed as 'slip-and-slide' [3]). Garcia et al. [72] are unable to decide whether the role of their "interaction codon" (allele-specific determinant) or of the slip-and-slide tweak should guide their thinking. Clearly, either the recognition of the allele-specific determinant (interaction codon) or the "crossreactivity with many MHC molecules" (slip-and-slide) should dominate our thinking about the interaction. They are, after all, incompatible!

The Standard Model is internally contradictory because the allele-specific and common (shared) determinants on thymic-R are not distinguishable by an individual's immune system with respect to the property, self, or non-self. Therefore, they cannot be distinguished by the somatic process of negative selection. Similarly, positive selection can only be functional in establishing restrictive recognition, if the TCR has a germline-selected recognitive site for the allele-specific determinant. No requirement for specific recognition of peptide is implied or need be assumed. The peptide is functioning as a structural, not a specificity element. Lastly, the properties of the R–anti-R interaction are not evolutionarily driven by the necessity to make a self–nonself discrimination. Only those of the P–anti-P interaction are driven by this discrimination. Therefore, the question of a self–nonself discrimination does not apply to the R–anti-R interaction; it only applies to the P–anti-P interaction.

The take home lesson

The TCR possesses three combining sites (Tritope). One site is positively selected to specify restrictive recognition. The second site is entrained by complementation and specifies allorecognition. The third site recognizes peptide. As the anti-R site, whether it functions in restrictive recognition or allorecognition in a given TCR, is the same,

treating alloreactivity as crossreactivity of R-alleles is inappropriate.

The investigations of Feng et al. [71] and Hong et al. [43] showed that each R-domain expresses an allele-specific determinant seen by a given single V-domain. This has several consequences.

- There cannot be more polymorphic allele-specific determinants in the species than there are V-gene segments to recognize them, in all likelihood significantly less. How they might be distributed is discussed in [17], page 641. The idea that the polymorphism is vast [5, 12] now has a boundary condition.
- There must be two signaling orientations for each TCR, one for restrictive reactivity, the other for alloreactivity [2, 17]. A minimum postulate would be that peptide is acting as a specificity element for restrictive reactivity and negative selection. However, for alloreactivity and positive selection, the assumption that peptide is acting as a specificity element is gratuitous. Rather, it is functioning as a structural element. Here, X-ray crystallography as pointed out earlier is beginning to be helpful [23] in revealing how changes in the anchor residues of the peptide affect the expression of the interaction codon (allele-specific determinant) (see also discussion in Ref. [17], Page 642.)

It is thanks to the fact that "the molecular basis of TCR germline bias for MHC" is "so surprisingly simple [72]," that we were able to derive the conclusion that single V-domains recognize allele-specific determinants [2, 3, 10, 17]. This means that "the outstanding question" [72] is no longer what is the structural morphology of the interaction of R with anti-R, but rather how is the recognition of ligand by each of the ligand-recognitive sites, anti-R and anti-P, integrated to signal the cell [2, 3].

It is quite clear that a conformational Signal[1] delivered via the constant domains, Cα and Cβ, is mandated by the biology. Most observations of conformational changes are concerned with recognition of [PR] by a malleable ("induced fit") site that does not trigger a signaling conformational change (reviewed by Armstrong et al. [74]). This has now changed thanks to a revealing recent study by Beddoe et al. [75] who demonstrate the existence of a [PR]-ligand-driven conformational change in the constant domain of the $\alpha\beta$TCR. This change was observed in Cα only. As a general rule for all ligands, signaling via Cα only, not Cβ, would be surprising, given the evidence for two signaling orientations, one functional in restrictive reactivity and the other functional in alloreactivity, and for the central signaling role played by the asymmetrically complexed cofactor, CD3. However, the story is far from complete and one can expect the role for Cβ in signaling to emerge when TCRs restricted via Vα or Vβ are compared and when TCRs with restrictive and alloreactivities are analyzed. After all the TCRs unlike the C_H of the BCR have two distinct transmembrane components, Cα and Cβ. These points along with well-devised models have been ably discussed [76–78] and the reader is referred to them.

Lastly, any given functional TCR can undergo three different interactions resulting in a signal via the TCR to the T-cell:

1. An R–anti-R interaction during positive selection, peripheral survival and homeostatic proliferation;
2. An R allo–anti-R allo interaction of alloreactivity that results in delivery of Signal[1] to the T-cell;
3. An R–anti-R plus a P–anti-P interaction of restrictive recognition of peptide resulting in Signal[1] to the T-cell.

How these three signals are mediated such that they are distinguished is an open and pressing question.

Acknowledgments This work was supported by a grant (RR07716) from the National Center For Research Resources (NCRR), a component of the National Institutes of Health (NIH) and its contents are solely the responsibility of the author and does not represent the official view of NCRR or NIH.

Conflict of interest The author has no financial conflict of interest.

References

1. Zinkernagel RM, Doherty PC. Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytic choriomeningitis. Nature. 1974;251:547–8.
2. Cohn M. The tritope model for restrictive recognition of antigen by T-cells: I. What assumptions about structure are needed to explain function? Mol Immunol. 2005;42:1419–43.
3. Langman RE, Cohn M. The standard model of T-cell receptor function: a critical reassessment. Scand J Immunol. 1999;49:570–7.
4. Matzinger P, Bevan MJ. Hypothesis: why do so many lymphocytes respond to major histocompatibility antigens? Cell Immunol. 1977;29:1–5.
5. Collins EJ, Riddle DS. TCR-MHC docking orientation: natural selection, or thymic selection? Immuol Res. 2008;41:267–94.
6. Archbold JK, Macdonald WA, Burrows SR, Rossjohn J, McCluskey J. T-cell allorecognition: a case of mistaken identity or deja vu? Trends Immunol. 2008;29:220–6.
7. Cohn M. The immune system: a weapon of mass destruction invented by evolution to even the odds during the war of the DNAs. Immunol Rev. 2002;185:24–38.
8. Cohn M. What are the commonalities governing the behavior of humoral immune recognitive repertoires? Dev Comp Immunol. 2006;30:19–42.
9. Marrack P, Scott-Browne JP, Dai S, Gapin L, Kappler JW. Evolutionarily conserved amino acids that control TCR-MHC interaction. Annu Rev Immunol. 2008;26:171–203.
10. Cohn M. On a key postulate of TCR restrictive function: the V-gene loci act as a single pool encoding recognition of the polymorphic alleles of the species MHC. Immunology. 2007;120:140–2.

11. Cohn M. The Tritope model of restrictive recognition by the TCR. Trends Immunol. 2003;24:127–31.
12. Huseby ES, White J, Crawford F, Vass T, Becker D, Pinilla C, Marrack P, Kappler JW. How the T cell repertoire becomes peptide and MHC specific. Cell. 2005;122:247–60.
13. Cohn M. An alternative to current thinking about positive selection, negative selection and activation of T-cells. Immunology. 2004;111:1–6.
14. Cohn M. On the logic of positive selection. Immunology. 2006; 117:452–3.
15. Müllbacher A, Hill AB, Blanden RV, Cowden WB, King NJC. Alloreactive cytotoxic T cells recognize MHC Class I antigen without peptide specificity. J Immunol. 1991;147:1765–71.
16. Müllbacher A, Lobigs M, Kos FJ, Langman RE. Alloreactive cytotoxic T-cell function, peptide nonspecific. Scand J Immunol. 1999;49:563–9.
17. Cohn M. The tritope model for restrictive recognition of antigen by T-cells: II. Implications for ontogeny, evolution and physiology. Mol Immunol. 2008;45:632–52.
18. Naeher D, Daniels MA, Hausmann B, Guillaume P, Luescher I, Palmer E. A constant affinity threshold for T cell tolerance. J Exp Med. 2007;11:2553–9.
19. Palmer E, Naeher D. Affinity threshold for thymic selection through a T-cell receptor—co-receptor zipper. Nat Rev Immunol. 2009;9:207–13.
20. Park J-H, Adoro S, Guinter T, Erman B, Alag AS, Catalfamo M, Kimura MY, Cui Y, Lucas PJ, Gress RE, Kubo M, Hennighausen L, Feigenbaum L, Singer A. Signaling by intrathymic cytokines, not T cell antigen receptors, specifies CD8 lineage choice and promotes the differentiation of cytotoxic-lineage T cells. Nat Immunol. 2010;11:257–64.
21. Alves NL, Huntington ND, Mention J-J, Richard-Le Goff O, Di Santo JP. A thymocyte-thymic epithelial cell cross-talk dynamically regulates intrathymic IL-7 expression in vivo. J Immunol. 2010;184:5949–53.
22. Hao Y, Legrand N, Freitas AA. The clone size of peripheral CD8 T cells is regulated by TCR promiscuity. J Exp Med. 2006;203:1643–9.
23. Bowerman NA, Colf LA, Garcia KC, Kranz DM. Different strategies adopted by K^b and L^d to generate T cell specificity directed against their respective bound peptides. J Biol Chem. 2009;284:32551–61.
24. Huseby ES, Kappler JW, Marrack P. TCR-MHC/peptide interactions: kissing-cousins or a shotgun wedding? Eur J Immunol. 2004;34:1243–50.
25. Jerne NK. The somatic generation of immune recognition. Eur J Immunol. 1971;1:1–9.
26. Dai S, Huseby ES, Rubtsova K, Scott-Browne J, Crawford F, Macdonald WA, Marrack P, Kappler JW. Crossreactive T cells spotlight the germline rules for $\alpha\beta$ T cell-receptor interactions with MHC molecules. Immunity. 2008;28:324–34.
27. Jerne NK. Generation of antibody diversity and self tolerance. In: Smith RT, Landy M, editors. Immune surveillance. Augusta: Academic Press; 1970. p. 343–436.
28. Wucherpfennig KW, Allen PM, Celada F, Cohen IR, De Boer RJ, Garcia KC, Goldstein B, Greenspan R, Hafler D, Hodgkin PD, Huseby ES, Krakauer DC, Nemazee D, Perelson AS, Pinilla C, Strong RK, Sercarz EE. Polyspecificity of T cell and B cell receptor recognition. Semin Immunol. 2007;19: 216–24.
29. Cohn M. The self-nonself discrimination: reconstructing a cabbage from sauerkraut. Res Immunol. 1992;143:323–34.
30. Gavin MA, Dere B, Grandea AG, Hogquist KA, Bevan MJ. Major histocompatibility complex class I allele-specific peptide libraries: identification of peptides that mimic an H-Y T cell epitope. Eur J Immunol. 1994;24:2124–33.
31. Gavin MA, Bevan JJ. Increased peptide promiscuity provides a rationale for the lack of N regions in the neonatal T cell repertoire. Immunity. 1995;3:793–800.
32. Huseby ES, Crawford F, White J, Kappler JW, Marrack P. Negative selection imparts peptide specificity to the mature T cell repertoire. Proc Natl Acad Sci USA. 2003;100:11565–70.
33. Huseby ES, Crawford F, White AJ, Marrack P, Kappler JW. Interface-disrupting amino acids establish specificity between T cell receptors and complexes of major histocompatibility complex and peptide. Nat Immunol. 2006;7:1191–9.
34. Huseby ES, Kappler JW, Marrack P. Thymic selection stifles TCR reactivity with the main chain structure of MHC and forces interactions with the peptide side chains. Mol Immunol. 2008; 45:599–606.
35. Kosmrlj A, Abhishek KJ, Huseby ES, Kardar M, Chakraborty AK. How the thymus designs antigen-specific and self-tolerant T cell receptor sequences. Proc Natl Acad Sci USA. 2008;105: 16671–6.
36. Percus JK, Percus OE, Perelson AS. Predicting the size of the T cell receptor and antibody combining region from consideration of efficient self-nonself discrimination. Proc Natl Acad Sci USA. 1993;90:1691–5.
37. Nemazee D. Antigen receptor "capacity" and the sensitivity of self-tolerance. Immunol Today. 1996;17:25–9.
38. Cohn M. Degeneracy, mimicry and crossreactivity in immune recognition. Mol Immunol. 2005;42:651–5.
39. Cohn M. An in depth analysis of the concept of "polyspecificity" assumed to characterize TCR/BCR recognition. Immunol Res. 2008;40:128–47.
40. Sha WC, Nelson CA, Newberry RD, Kranz DM, Russell JH, Loh DY. Positive and negative selection of an antigen receptor on T cells in transgenic mice. Nature. 1988;336:73–6.
41. Capone M, Curnow J, Bouvier G, Ferrier P, Horvat B. T cell development in TCR-ab transgenic mice. J Immunol. 1995;154: 5165–72.
42. Dao T, Blander JM, Sant'Angelo DB. Recognition of a specific self-peptide: Self-MHC Class II complex is critical for positive selection of thymocytes expressing the D10-TCR. J Immunol. 2003;170:48–54.
43. Hong S-C, Chelouche A, Lin R-h, Shaywitz D, Braunstein NS, Glimcher L, Janeway J, Charles A. An MHC interaction site maps to the amino-terminal half of the T cell receptor α chain variable domain. Cell. 1992;69:999–1009.
44. Logunova NN, Viret C, Pobezinksy LA, Miller SA, Kazansky DB, Sundberg JP, Chervonsky AV. Restricted MHC-peptide repertoire predisposes to autoimmunity. J Exp Med. 2005;202: 73–84.
45. Felix NJ, Donermeyer DL, Horvath S, Walters JJ, Gross MI, Suri A, Allen PM. Alloreactive T cells respond specifically to multiple distinct peptide-MHC complexes. Nat Immunol. 2007;8:388–97.
46. Heath WR, Hurd ME, Carbone FR, Sherman LA. Peptide-dependent recognition of H-1 K^b by alloreactive cytotoxic T lymphocytes. Nature. 1989;341:749–52.
47. Viret C, Janeway CA. Functional and phenotypic evidence for presentation of E alpha 52–68 structurally related self-peptide(s) in I-E alpha-deficient mice. J Immunol. 2000;164:4627–34.
48. Ewijk WV, Ron Y, Monaco J, et al. Compartmentalization of MHC Class II gene expression in transgenic mice. Cell. 1988;53: 357–70.
49. Rubin B, Gouaillard C, Weideranders G, Kuklmann J. The IE allogeneic response of T cells from C57B1/6 mice is associated with genes of the TCRa locus. Scand J Immunol. 1993;37: 388–97.
50. LaFuse WP, Savariravan S, McCormick JF, David CS. Identification of 1-E alpha genes in H-2 recombinant mouse strains by F1 complementation. Transplantation. 1987;43:297–301.

51. Zy-Tmg E, Chu C, Carswell C, Cole BC, Jones PP. The minimal polymorphism of Class II Ealpha chains is not due to the functional neutrality of mutations. Genetics. 1994;40:9–20.
52. Schilham MW, Lang R, Benner R, Zinkernagel RM, Hengartner H. Characterization of an Lyt-2^+ alloreactive cytotoxic T cell clone specific for H-$2D^b$ that crossreacts with I-E^k. J Immunol. 1986;137:2748–54.
53. Morris GP, Allen PM. Cutting edge: highly alloreactive dual TCR T cells play a dominant role in graft-versus-host disease. J Immunol. 2009;182:6639–43.
54. Felix NJ, Suri A, Walters JJ, Horvath S, Gross ML, Allen PA. I-E^p-bound self-peptides: identification, characterization, and role in alloreactivity. J Immunol. 2006;176:1062–71.
55. Daniel C, Grakoui A, Allen PA. Inhibition of an in vitro $CD4^+$ T cell alloresponse using altered peptide ligands. J Immunol. 1998; 160:3244–50.
56. Daniel C, Horvath S, Allen PM. A basis for alloreactivity: MHC helical residues broaden peptide recognition by the TCR. Immunity. 1998;8:543–52.
57. Correia-Neves M, Waltzinger C, Mathis D, Benoist C. The shaping of the T cell repertoire. Immunity. 2001;14:21–32.
58. Matis LA, Sorger SB, McElligott DL, Fink PJ, Hedrick SM. The molecular basis of alloreactivity in antigen-specific, major histocompatibility complex-restricted T cell clones. Cell. 1987;51: 59–69.
59. Nakajima PB, Betz CJ, Hansburg D. Expression of identical VαVβ gene pairs by IE-alloreactive and IE-restricted, antigen-specific T cells from MHC disparate mice. J Immunol. 1990; 144:348–57.
60. Cohn M. Distinguishing the tritope from the interaction antigen models. Trends Immunol. 2004;25:8–9.
61. Eshima K, Suzuki H, Shinohara N. Cross-positive selection of thymocytes expressing a single TCR by multiple major histocompatibility complex molecules of both classes: implications for $CD4^+$ versus $CD8^+$ lineage commitment. J Immunol. 2006;176: 1628–36.
62. Suzuki H, Eshima K, Takagaki Y, Hanaoka S, Katsuki M, Yokoyama M, Hasegawa T, Yamazaki S, Shinohara N. Origin of a T cell clone with a mismatched combination of MHC restriction and coreceptor expression. J Immunol. 1994;153:4496–507.
63. Ge Q, Holler PD, Mahajan VS, Nuygen T, Eisen HN, Chen J. Development of $CD4^+$ T cells expressing a nominally MHC class I-restricted T cell receptor by two different mechanisms. Proc Natl Acad Sci USA. 2006;103:1822–7.
64. Gilfillan S, Dierich A, Lemeur M, Benoist C, Mathis D. Mice lacking TdT: mature animals with an immature lymphocyte repertoire. Science. 1993;261:1175–8.
65. Komori T, Okada A, Stewart V, Alt FW. Lack of N regions in antigen receptor variable region genes of TdT-deficient lymphocytes. Science. 1993;261:1171–5.
66. Gilfillan S, Bachmann M, Trembleau S, Adorini L, Kalinke U, Zinkernagel R, Benoist C, Mathis D. Efficient immune responses in mice lacking N-region diversity. Eur J Immunol. 1995;25: 3115–22.
67. Gilfillan S, Waltzinger C, Benoist C, Mathis D. More efficient positive selection of thymocytes in mice lacking terminal deoxynucleotidyl transferase. Int Immunol. 1994;6:1681–6.
68. Cohn M. A hypothesis accounting for the paradoxical expression of the D gene segment in the BCR and the TCR. Eur J Immunol. 2008;38:1779–87.
69. Langman RE. Cell-mediated immunity and the major histocompatibility complex. Rev Physiol Biochem Pharmacol. 1978;81: 1–37.
70. Cohn M, Epstein R. T-cell inhibition of humoral responsiveness. II. Theory on the role of restrictive recognition in immune regulation. Cellular Immunol. 1978;39:125–53.
71. Feng D, Bond CJ, Ely LI, Maynard J, Garcia KC. Structural evidence for a germline-encoded T cell receptor - major histocompatibility complex interaction 'codon'. Nat. Immunol. 2007; 8:975–83.
72. Garcia KC, Adams JJ, Feng D, Ely LK. The molecular basis of TCR germline bias for MHC is surprisingly simple. Nat Immunol. 2009;10:143–7.
73. Cohn M. What does the T-cell receptor recognize when it docks on an MHC-encoded restricting element? Mol Immunol. 2008; 45:3264–7.
74. Armstrong KM, Piepenbrink KH, Baker BM. Conformational changes and flexibility in T-cell receptor recognition of peptide-MHC complexes. Biochem J. 2008;415:183–96.
75. Beddoe T, Chen Z, Clements CS, Ely LK, Bushell SR, Vivian JP, Kjer-Nielsen L, Pang SS, Dunstone MA, Liu YC, Macdonald WA, Perugini MA, Wilce MCJ, Burrows SR, Purcell AW, Tiganis T, Bottomley SP, McCluskey J, Rossjohn J. Antigen ligation triggers a conformational change within the constant domain of the $\alpha\beta$ T cell receptor. Immunity. 2009;30:777–88.
76. Rojo JM, Bello R, Portoles P. T-cell receptor. In: Sigalov AB, editor. Multichain immune recognition receptor signaling: from spatiotemporal organization to human disease, Heidelberg: Springer; 2008. p. 1–11.
77. Risueno RM, Ortiz AR, Alarcon B. Conformational model.; In Sigalov AB, editor. Multichain immune recognition receptor signaling: from spatiotemporal organization to human disease, Heidelberg: Spinger; 2008. P. 103–120.
78. Minguet S, Schamel WWA. Permissive geometry model. In: Sigalov AB, editor. Multichain immune recognition receptor signaling: from spatiotemporal organization to human disease. Heidelberg: Springer; 2008. P. 113–120.
79. Portoles P, Rojo JJ, Janeway CA Jr. Asymmetry in the recognition of antigen: self class II MHC and non-self class II MHC molecules by the same T-cell receptor. J Mol Cell Immunol. 1989;4:129–37.