

DISCUSSION FORUM**The case for allele-specific recognition by the TCR**
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Abstract

There is a sharp difference in how one views TCR structure–function–behaviour dependent on whether its recognition of major histocompatibility complex-encoded restriction elements (R) is germline selected or somatically generated. The generally accepted or Standard model is built on the assumption that recognition of R is by the V regions of the $\alpha\beta$ TCR, which is not driven by allele specificity, whereas the competing model posits that recognition of R is allele-specific. The establishing of allele-specific recognition of R by the TCR would rule out the Standard model and clear the road to a consideration of a competing construct, the Tritope model. Here, the case for allele-specific recognition (germline selected) is detailed making it obvious that the Standard model is untenable.

1 | INTRODUCTION

This is a discussion that requires agreement on the meaning of the terms needed to analyse the yes or no of allele-specific recognition.

1.1 | Definitions and ground rules**1.1.1 | What is an allele?**

Given the existence of a “gene,” all distinguishable members of that gene are alleles. There are functionally distinguishable alleles and sequence distinguishable alleles. Although all functionally distinguishable alleles are, in principle, sequence distinguishable, not all sequence distinguishable alleles are functionally distinguishable. Here, we are interested in functionally distinguishable alleles. Many (most) sequence distinguishable alleles are neutral or inactivating of function. Alleles are gene-level markers.

1.1.2 | What is a haplotype?

Genes are linked on a chromosome like beads on a necklace. For our use, genes that are of similar function linked on a chromosome define a haplotype. Each individual of mammalian species is diploid; it expresses two haplotypes. Haplotypes are chromosome level markers.

1.1.3 | What is a polymorphic gene?

Genes are under evolutionary selection. Mutations that are neutral are, by definition, not under selection. Polymorphisms are maintained when the heterozygote displays a selective advantage compared to both homozygotes. The genes comprising the heterozygote are maintained by evolutionary selection at a frequency in the randomly mating population higher than that expected by mutation of the homozygote alone. Polymorphisms are defined by function upon which evolution selects, not by any chemical change in the gene

*The author MC has passed away since he wrote the major part of the manuscript. Co-authors CCA and ZD completed the text by adding and amending several paragraphs and introduced clarifying statements in a number of places (ZD made the figures and wrote their legends) to complete the text for MC, but their authorship does not imply an endorsement of the model proposed by MC.

leading to amino acid replacements in its protein product. By convention, genes that are expressed in a randomly mating population at a frequency greater than one per cent are viewed as polymorphic. Polymorphisms describe population level markers.

1.1.4 | How does this summary of basic genetics determine how we view restrictive recognition of peptide by the TCR?

The major histocompatibility complex (MHC) is a haplotype comprising a variety of linked immune-related genes. The genes in the MHC that are relevant to restrictive recognition are those that encode cell surface bound proteins that present intracellularly derived peptides (P) as ligand for the TCR. These cell surface proteins are referred to as restricting elements for reasons that will become clear and are symbolized here as R. The gene encoding R is symbolized, *r*. There are two classes of R. RI provides the ligand, P-RI, for cytotoxic T cells (Tc); RII provides the ligand, P-RII, for helper T cells (Th). The peptide is presented anchored in a groove in R (Figure 1). For discussion, we will consider a peptide of roughly 10 amino acids (AA) in length, five of which have the potential to be recognized by R and are anchored

in its groove hidden from the TCR, and five of which are exposed for recognition by the TCR.^{1,2} There are two random sequence P repertoires under consideration, one seen by R (Pr), the other seen by the TCR (P_{TCR}) when dealing with P recognition.^{3,4}

The R-element is encoded by a polymorphic gene, *r*, which has of the order of 10 alleles defined by function. The repeated statement that the MHC is a polymorphic locus known to contain thousands of alleles, is misleading, not only because it is based on the failure to distinguish chemical from functional alleles but also because, most often, the difference between gene and haplotype is ignored.

Restrictive recognition of peptide by the TCR means that the TCR must recognize both R and P in order to deliver a signal to the T cell. The recognition of R tells the TCR that it is looking at antigen of intracellular origin. The recognition of P tells it, which intracellular antigen is involved. The term “restrictive” means that the recognition of R is allele-specific, a conclusion that is under dispute because it challenges the way we view TCR recognitive behaviour. It is agreed that the TCR must see R to signal; under disagreement is whether the recognition is allele-specific.

In order to discuss this question, we must consider the two extant models of TCR behaviour.

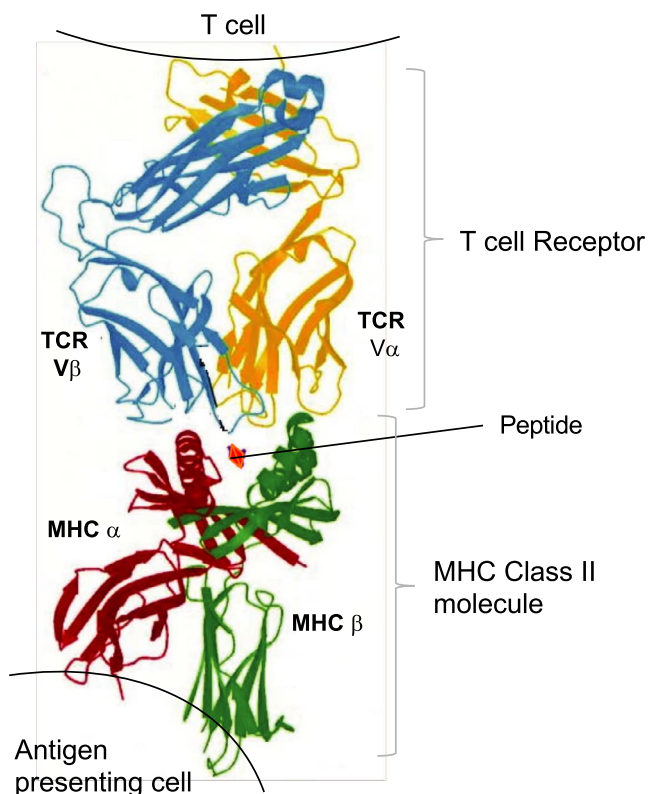


FIGURE 1 T cell Receptor—peptide/MHC Class II interaction—a side view of C-atom ribbon crystal structure. The T cell receptor—MHC Class II with a peptide interaction is schematically shown

2 | THE STANDARD VS THE TRITOPE MODEL

My referring to the two models of TCR behaviour as Standard and Tritope is somewhat misleading in that there is no coherently argued Standard model, only bits and pieces that I will hammer together to reflect the average position of the immunological community.

2.1 | The Standard model

Historically, it was assumed that there is only one antigen receptor (BCR) that mediates both B-cell recognition and T cell recognition. When this was shown to be incorrect, the similarity in organization of the TCR and BCR gene loci led to the irresistible belief that the two receptors functioned similarly. This in its various forms gave rise to what constitutes today, the Standard model, referred to here as the “BCR-like TCR.” Basically, the TCR is postulated to have a single site that sees a meld determinant between P and R. These sites characterize the population of TCRs that reads a gradient between recognition of the R, consequent to complementation of the V α and V β domains, and the recognition of peptide due to complementation of the CDR3 (N regions). This leaves us with a family of TCRs that has the potential to see as P-R ligand, at one extreme R alone, at the other extreme P alone and, in between a meld of P and R with a continuous

variation in the proportions of P and R recognized. The P + R meld is symbolized as Q . This sliding scale in the ligand (Q) composition has been given the description, P-centric or R-centric. As the recognition of R alone would be lethal and the recognition of P alone does not exist (restrictive recognition), an assumption tying recognition obligatorily to a P-R complex, Q , is required. For example, the TCR ligand-binding site sees a segment of the P-R epitope, that is, Q , which includes P and R in variable ratios. It would be fair to say that this is the Standard model. The recognition of R could well be via an invariant or “anywhere” site. In any case, recognition of R via an allele-specific determinant would be a gratuitous assumption.

The Standard model appeared as lacking the necessity for allele-specific recognition of R, so for a variety of reasons a totally different approach was proposed, the Tritope model. These reasons have been discussed in detail in several papers,⁵⁻⁷ so here only aspects important to the allele-specific recognition of R will be dealt with. The MHC haplotypes in genetic crosses segregate obeying Mendel's laws. This, in and of itself, tells us that the recognition of R by the TCR is allele-specific (ie germline encoded). It does not tell us what are the structural elements mediating this recognition, but it does limit the choices to two (see later).

2.2 | The tritope model⁵⁻⁷

The TCR is a heterodimer made up of an α - and a β -subunit. Each subunit sees an allele-specific site on R. RI is made

up of two domains, and RII is composed of two subunits. The TCR docks on R in a fixed geometry (Figure 1). The α subunit when it recognizes an R always looks at one and the same domain/subunit, and the β subunit always looks at the other domain/subunit (Figure 2). This tells us that each R-element has the potential to express two allele-specific sites and that single TCR subunits see allele-specific sites on R. In essence, hybrid allele-specific determinants do not exist. Each subunit of RII, for example, in any combination of complements retains its allele-specific determinant unchanged.

Using mouse for illustration, there are three RI (K, D, L) and two RII (A, E) per haplotype. The TCR family is encoded by ~ 50 V α and ~ 20 V β . The TCR family as it is born in thymus is made up of roughly 1000 V α V β pairs. These undergo positive selection in thymus for those V-domains that recognize host R-elements. Given a maximum of 10 allele-specific determinants per haplotype, 10 Vs will be positively selected in the homozygote and 20 in the heterozygote. The proportion of positively selected Vs that are V α or V β is not calculable because the allele specificity of each V-subunit is unknown. Each positively selected V of the TCR will entrain a random complementing subunit. The net result is a positively selected family of TCRs that see all host Rs using the selected V subunits, and all species Rs using the entrained V subunits. It is the entrained V family that mediates alloreactivity.

In sum, the TCR possesses three combining sites, an anti-P site, an anti-host-R site and an anti-allo-R site, hence Tritope (Figure 2C).

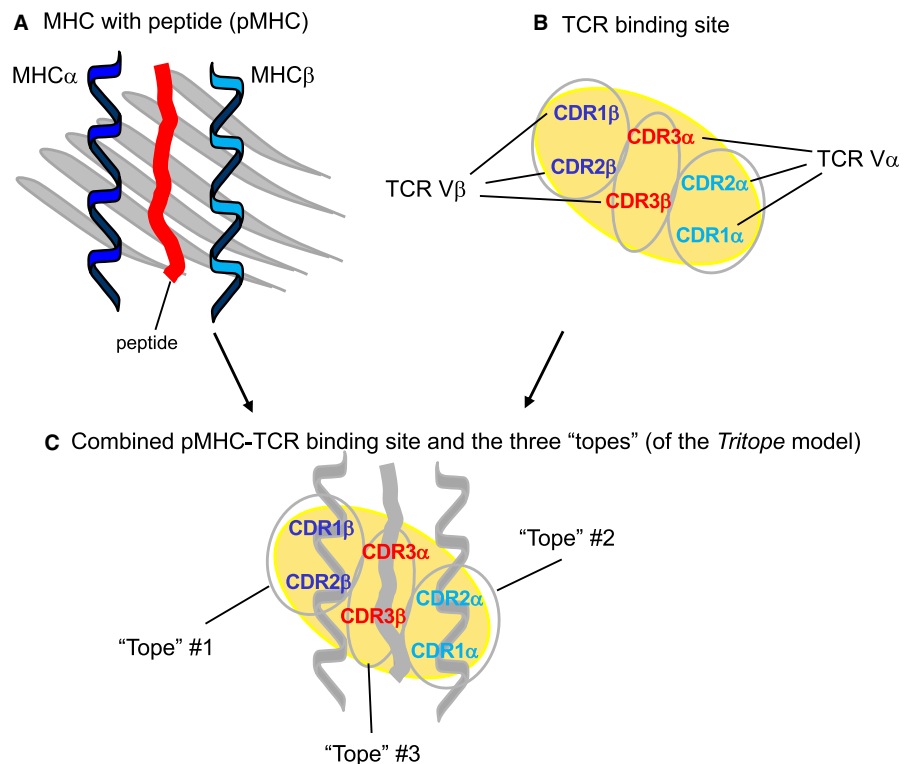


FIGURE 2 TCR V region sites in contact with peptide-MHC—a schematic view from the top. A, TCR-pMHC view from the top. B, The TCR binding site comprising CDR1, CDR2 and CDR3 regions of TCR V α and V β chains. C, Combined pMHC-TCR binding site and the three “topes” of the Tritope model: The “anti-P site” resides in the Tope #3. The “anti-host-R” site could be either Tope #1 or Tope #2, and the “anti-allo-R site” would then be either the Tope #2 or the Tope #1, respectively

2.3 | Alloreactivity

It might be clarifying to introduce here this aside about alloreactivity. This response has always been a mystery because it made no sense for evolution to select for a high-frequency activity directed against a target, allo-R, that the individual rarely, if ever, normally encounters. The above explains it. The entrained or unselected Vs recognize the total family of allele-specific determinants of the species. Included in this entrained repertoire is the recognition of host-R itself, which would be lethal. These anti-host-R specificities must be purged by negative selection. Consequently, both the α - and the β -TCR subunits must be able to transmit a signal to the TCR (Signal 1). The positively selected V recognizing host-R can only deliver Signal 1, if P is also specifically engaged. The entrained V can deliver Signal 1, P unspecifically. An alloreactive response is high frequency because Signal 1 is peptide-unspecific and Signal 2 delivered by the T-helper is not limiting. Alloreactivity is the consequence or concomitant of the necessity to be able to rid a potentially lethal autoreactivity to host-R. When host-R is acting as a ligand, it delivers deletional Signal 1 to the T cell. When host-R is acting as a presenter of P, recognition of both R and P are required to deliver deletional Signal 1 to the T cell. It is important to appreciate that P can act either as a specificity element or as a structural component necessary for the stability and conformation of R. For restrictive reactivity, P is acting as a specificity element; for alloreactivity, P is acting as a structural component. The experimental evidence for this picture has been detailed in several papers.^{5,7,8}

2.4 | Comments on the two models

Under the Standard model, the ligand, Q, is recognized by a somatically generated repertoire, anti-Q. This is why allele-specific recognition of R is denied.⁹ In its place are a set of metaphors, the TCR has a “bias,” “predilection,” “obsession,” etc for MHC. In sum, a somatically generated repertoire cannot tell which epitopes on R are allele-specific and which are shared.¹⁰

Under the Tritope model (Figure 2C), the recognition of R is germline encoded (allele-specific) in the V-gene segments and the recognition of P is somatically encoded in the NDN regions (CDR 3). The alleles of R are polymorphic and their recognition is under constant germline selection.

There is a sophisticated argument that arises from the above, namely that the actual assays for restrictive recognition are inadequate to distinguish allele-specific recognition of R from allele-specific recognition of P.

For example, anti-H-2^b monoclonal Abs against its b-haplotype mutants (bm1-12) cannot distinguish the roles of each mutant in the restrictive recognition. Similarly, assays using

pentameric (or tetrameric) MHC molecules cannot separate the roles of each subunit of TCR V (CDRs 1-3) in allele-specific recognition of R or allele-restrictive recognition of P. One could imagine that it is currently feasible to test these ideas, but it would be very challenging. One could use a defined TCR that has known peptide agonists and altered peptide ligands (APLs). The Tritope model predicts that for mutations that change an allele-specific amino acid of R, there will be a high loss of recognition by the TCR, whether or not R contains the agonist or an APL. This is because in Cohn's view these amino acids are critical for recognition of R by the TCR (Figure 2).⁵ The Standard model^{11,12} instead predicts that changing each allele-specific amino acid will often result in a loss of response to either the agonist or APL, that is the response to one will be maintained. This is because in the Standard model the allele-specific amino acids affect recognition of the peptide more so than recognition of R; recognition of R is just as likely to be via regions that are not allele-specific as via allele-specific regions.

2.5 | Allorestriction

An allorestrictive response is different from alloreactivity and is a part of the high frequency of alloreactivity.⁸ The difference lies in allorestrictive Signal 1, which is peptide-specific, while the primary component of alloreactivity is by definition P-unspecific; and Signal 2 delivered by the T-helper is not limiting for either.

The conceptual difference between the two recognition specificities can be understood as follows: alloreactivity can be viewed as an integration of all allo restricted P specificities plus allo-R specificities with unspecific P recognition of an individual (when tested against other individuals of the same species). As stressed above, for allorestrictive reactivity, P is acting as a specificity element; for alloreactivity, P is acting simply as a structural component. As depicted in the Figure 2C, the “anti-P site” is always the Tope #3. The anti-host-R site is either Tope #1 or Tope #2, and the “anti-allo-R site” is either the Tope #2 or the Tope #1, respectively. Namely, in the Tritope model, positive selection in the thymus never selects both Topes (1 & 2) for “anti-host-R” reactivity. Thus, if someone's thymus positively selects Tope #2, it is the “anti-host-R” site. The other Tope (Tope #1 in this case) is then being carried by the TCR *per force* (“entrained”), and it turns out to be the “anti-allo-R” site, which should have (according to the Tritope model) an intrinsic alloreactivity and sometimes allorestrictive capacity (see below).

Further, the assays for alloreactivity cannot distinguish alloreactivity from allorestriction. For example, because the tri-topes are interconnected, one would need anti-single-tope labelling ability in terms of monoclonal antibodies (less likely) or chemicals that could distinguish each private (single) anti-P or allo-R subsites on the TCR in order

to analyse them. Hence, such tools (ie monotope distinction by biochemical or biophysical methods) would be a means to disprove this model and thus give the Tritope model its deniability.

3 | THE LOOSE ENDS

It has been argued that the functional assays cannot tell us whether the allele-specific recognition of the P-R ligand is a property of a determinant on R or is a consequence of the different peptide libraries that each allele of R presents. The failure to respond to P-H-2^b by a TCR positively selected on H-2^a could be due either to a failure to recognize P (of the P-H-2^b), as predicted in the Standard model, or a failure to recognize R of the b haplotype, as predicted by Tritope (ie the entrained TCR chain for this TCR selected on H-2^a does not bind R that is H-2^b).

3.1 | Alleles defined by R or by P

As stated previously⁸ it is theoretically correct to define alleles by their function. The problem here is that the functional assays used cannot tell us exactly what we see. For example, allele specificity in restrictive recognition may be explained by seeing differences in the MHC alleles directly or by differences in peptide binding, that is, the response read out to p+MHC^a cannot occur in MHC^b because it does not present the same peptide. Alloreactivity is even more problematic because there is no biological assay that could distinguish between alloreactivity and allorestriction, in most assays we see a mix of these two in unknown ratios. That is why it is considered in the reference⁸ that the differences in amino acid sequences between a and b are the most reliable sign for alleles. Answers to the questions raised herein would provide an understanding of whether alloresponses are primarily mediated by restrictive recognition or P independent alloreactivity and may influence the design of strategies to prevent allograft rejection.

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How to cite this article: Cohn M, Anderson CC, Dembic Z. The case for allele-specific recognition by the TCR. *Scand J Immunol*. 2019;00:e12790. <https://doi.org/10.1111/sji.12790>