Published in final edited form as:

Immunol Res. 2008 ; 40(2): 128–147. doi:10.1007/s12026-007-8003-z.

An in depth analysis of the concept of “polyspecificity” assumed to characterize TCR/BCR recognition

Melvin Cohn

Instituto Gulbenkian de Ciência, Rua da Quinta Grande No. 6, 2780-156 Oeiras, Portugal

Abstract

A workshop group developed the concept of a “polyspecific” TCR/BCR in the framework of today’s consensus model. They argue that the individual TCR/BCR combining site is composed of a packet of specificities randomly plucked from the repertoire, hence it is “polyspecific.” This essay analyzes the conclusions of the workshop and suggests an alternative. “Polyspecificity” must be dissected into its two component parts, specificity and degeneracy. The TCR and the BCR must be treated differently because the TCR recognizes allele-specifically the MHC-encoded restricting element (R) that serves as the platform presenting peptide (P). Only the anti-P paratope of the TCR behaves analogously to the BCR paratope. The two paratopes are selected to recognize a shape-determinant referred to as an epitope or ligand. The paratope is functionally unispecific in recognition, not polyspecific, with respect to shape; it is degenerate in recognition with respect to chemistry. The recognized shape-determinant can be the product of many chemically different substances, peptide, carbohydrate, lipid, steroid, nucleic acid, etc. Such a degenerate set is functionally treated by the paratope as one shape/epitope/ligand and, in no sense, can a paratope recognizing such a degenerate set be described as “polyspecific.” Degeneracy and specificity are concepts that must be distinguished. The two positions are analyzed in this essay, the experiments used to support the view that the paratope of the TCR/BCR is polyspecific, are reinterpreted, and an alternative framework with its accompanying nomenclature, is presented.

Keywords

TCR; BCR; Self–nonself discrimination; Specificity; Degeneracy; Allorestriction; Alloreactivity; Restrictive recognition

The need for a discussion

The recommendations of a workshop on the recognition of ligand by the TCR and the BCR appeared recently [1]. The seventeen participants, ranging from molecular morphologists to cellular immunologists, were of a single mind operating in the framework of what I will refer to as the Standard Model of the TCR and BCR. The nomenclature proposed by them was theory-dependent to an extent that makes it gratuitous in any other context. The importance of a consideration of competing concepts is essential when making nomenclature proposals, particularly when it is based on a conceptualization that would have surfaced as questionable if it had been thoughtfully weighed against an alternative model. Besides confronting the logic of the Standard Model, I will reinterpret some of the experiments used to support it. This will illustrate why the nomenclature based on it is lacking.

The Standard Model of the TCR

While there are variations on the Standard theme, a common base is evident. The TCR is treated like the BCR in that it is viewed as having a single combining site that interacts with a ligand that is a meld between peptide (P) and its presenting platform, the MHC-encoded restricting element (R) (i.e., $[PR] \rightarrow Q$, the combining site is anti-Q). The unselected repertoire of anti-Q is viewed as large and random. This probing, early hypothesis owed to Matzinger and Bevan [2] has driven an enormous amount of crucial experimental work. Unfortunately, it has been pushed beyond its limits over the years in spite of its failure to account for allele-specific recognition when the TCR is engaging either a host restricting element (R_H) or an allo-R (R_A). The over-extended model has arbitrarily necessitated the postulate of a role of specific peptide recognition in positive selection and alloreactivity. Further, the repertoire of anti-Q is visualized without justification as being sorted by negative selection into high affinity anti-self Q and low (but functional) affinity anti-self Q, this latter being assumed *ad hoc* to be the source of the high affinity functional anti-nonsel Q repertoire by “crossreactivity (?)” or “mimicry (?)” This assumption of the anti-Q recognition of the conflated element, Q, has driven the nomenclature proposed by the workshop participants.

A potential alternative: The Tritope Model

In order to make my point I will consider only the three premises of this Model [3–7] that define the structure-function relationships of the TCR.

Premise 1

The TCR specifies two repertoires, one is germline-selected to recognize the allele-specific determinants on the MHC-encoded restriction elements (R) of the species (i.e., the anti-R repertoire), the other is random and somatically generated to recognize the peptide (P) bound to R (i.e., the anti-P repertoire).

The consensus view assumes a somatically generated random paratopic repertoire from which positive and negative selection extract allele-specific and peptide-specific recognition. This assumption is to be questioned as there is no way that a somatic process like negative and positive selection can extract from a random recognitive repertoire allele-specific recognition of R. The individual has no way of knowing what are the alleles of the restricting elements of the species. All of the determinants on R whether they are allele-specific or shared are self to the individual and indistinguishable by any somatic process. Therefore, recognition of them by the anti-R repertoire must be germline-selected and encoded.

Some immunologists have come to appreciate that the Standard Model cannot deal with allele-specific recognition. However, they try to solve it with words that glow in the dark by describing the TCR as being “intrinsically biased” or “skewed towards the recognition of MHC,” or as having a “predisposition,” “predilection,” “affinity,” “preference,” or “obsession” for MHC. Is this supposed to have meaning or is it simply a metaphorical way of saying, “germline-selected?”

Unlike the anti-R repertoire, the anti-P repertoire must be somatically generated and random, and I might add, possess a degree of specificity sufficient to make a self–nonself discrimination. The lack of a role of negative and positive selection in determining peptide-specificity will be discussed later (Section “What contribution does positive and negative selection make to specificity?”).

Two repertoires under distinctly different selection pressures require two sites on the TCR upon which these two selection pressures operate. Hence, an anti-R site and an anti-P site are

postulated. As the only segments of the TCR that are varied somatically are the junctional CDR3 regions of its α and β subunits, complementation between them must define the anti-P site. As the $V\alpha$ and $V\beta$ domains are germline-selected and do not vary somatically, they must harbor the anti-R site. Given this, the question arises as whether recognition of the allele-specific determinant is a property of complementation between $V\alpha$ and $V\beta$ or of the individual V domains. This prompts Premise 2.

Premise 2

The V-gene segments, $V\alpha$ and $V\beta$, act as a single pool recognizing the allele-specific determinants of the species. Each V-domain contains a combining site (anti-R) specific for an allele-specific determinant on R.

This premise derives from the absence of interaction alleles created by complementation of the subunits of Class II MHC-encoded restricting elements (RII). At the conceptual level, there is no way that a combining site made up of complementing subunits could track in the germline a ligand itself made up of complementing subunits because a mutation in a subunit that affected the specificity of the interaction would simultaneously change the specificity of all of the combining sites or ligands that incorporated that subunit. These would become useless either as anti-R or as R rendering many individuals concurrently immune-defective in a randomly mating population.

What about the distribution of allele-specific determinants on R-elements? This can be derived from the next premise.

Premise 3

The TCR docks on R in a fixed mode. $V\alpha$ always docks on the West (W) domain/subunit ($\alpha 2$ of RI and $\beta 1$ of RII), whereas $V\beta$ (always docks on the East (E) domain/subunit ($\alpha 1$ of RI and $\alpha 1$ of RII).

While the fixed docking mode has been authenticated by X-ray crystallographic studies [8,9], it was predictable from the biology. If $V\alpha$ and $V\beta$ docked randomly on the domains/subunits of R then those $V\alpha V\beta$ pairs that docked on the same domain/subunit of R would be unable to function. This would make half of the $V\alpha V\beta$ library functionless. As there are so many other sources of loss of thymocytes during positive selection, this factor of two acted as a selection pressure to fix the docking mode. The limited experimental findings then can be justifiably generalized as Premise 3. Given this, one need only assume one allele-specific determinant per subunit or domain of R recognized by the anti-R site of the V region defined by Premise 3.

This gives rise to the description, Tritope Model, three combining sites. The positively selected V-domain is anti- R_H ; the entrained complementing V-domain is anti- R_A ; and the junctional CDR3 region specifies anti-P.

The nature of the repertoire

Most antigen-responsive cells express one paratope (haplotype exclusion). The properties of this paratope are what is under debate here. There are two views:

1. The paratope is unispecific with respect to its recognition of a shape-determinant (an epitope);
2. The paratope is polyspecific recognizing a packet of random epitopes from the antigenic universe.

In order to analyze these two positions I will begin by pointing out that paratopes are:

1. selected upon to define epitopes, not vice versa;
2. of a specificity determined by two boundary conditions: the necessity (1) to make a self–nonself discrimination and (2) to respond sufficiently rapidly (see Section “What contribution does positive and negative selection make to specificity?”);
3. of a repertoire size determined by the degree of specificity.

These points have been developed in detail by Langman [10] and by myself [11–13] and need no elaboration here.

The repertoire under discussion is somatically generated and random with respect to defining “self” and “nonself.” For the TCR this is the anti-P repertoire. The anti-R repertoire is germline-selected to follow different rules. Our analysis of the anti-P repertoire is based on gems extracted from the writings of Landsteiner [14], Lederberg [15], and Talmage [16]. Landsteiner [14, p. 129], made us aware that a repertoire which was too large would leave no given antibody at a concentration that would be effective. Lederberg restated this by pointing out that it would embarrass any theory of clonal selection if the size of the repertoire were large compared to the number of antigen responsive cells in the animal [15, footnote 2]. Talmage [16] hit the nail on the head by postulating that the repertoire divided the antigen universe into combinatorials of determinants referred to, today, as epitopes (see discussion in [17]). “Epitope” is being used here in the sense of ligand.

However, the consequences of these ideas remained undeveloped until Langman and I incorporated them into Protection theory [18,19] which circumscribed the parameters of a functional immune system. We were able to estimate the size of the primary functional somatically generated TCR/BCR repertoire to be $3\text{--}5 \times 10^4$, which recognized of the order of 10 epitopes per antigenic unit of 5×10^4 MW. This gave us the size of the distinguishable antigenic universe, $3\text{--}5 \times 10^4 C_{10}$ or roughly 10^{40} , a comfortable number.

The estimate of an average of 10 epitopes per antigenic unit of 5×10^4 MW is based on the requirement that monomeric antigens must be seen in three or more ways by BCRs to be ridded effectively by antibody. At an average of 10 epitopes per antigenic unit, three per 10^3 monomers would be ineffectively ridded by the humoral system, an acceptable value. The functional humoral repertoire that sees the antigenic unit (5×10^4 MW) in three or more ways would effectively cope with $4 \times 10^4 C_3 = 10^{13}$ monomeric antigens [10,12,20].

An a priori glance at T-cell behavior

The T-cell is constantly surveilling the cells of the host looking for intracellular foreigners. Restrictive recognition of peptide by the $\alpha\beta$ TCR is the mechanism by which the T-cell is assured that it is examining intracellular antigens like viruses. In order to do this, the TCR docks on R via its anti-R site. This exposes its anti-P site to interact with the bound peptide. If the peptide is not recognized, the TCR disengages and the T-cell moves on to another potential target. If anti-P recognizes the bound P, it signals the T-cell which responds depending on its state of differentiation. The R-element is the platform presenting P. The simple recognition of an allele-specific determinant on R is not a signal to the T-cell because R is not acting as an antigen during this process. It is the P–anti-P interaction that initiates signaling.

The T-cell interacting with an APC that is displaying a cognate peptide will dock on a signaling patch in which most TCRs will be engaged uniquely via an R–anti-R interaction. A few TCRs will be engaged in a signaling interaction involving anti-P recognition of the cognate P. In the absence of the latter, the T-cell will disengage. In its presence, the signaling patch will reorganize to permit information transfer. While the understanding of the disengagement/engagement process is primitive, self-peptide cannot play a specificity role in this process

because a given TCR cannot recognize self-P and nonself-P as differentially signaling. They would both be in the same mimotopic array (see Section “Some definitions and their consequences”) as either self or nonself. The self-P (Ps) plays a structural role, meaning that the stabilizing effect of the [PsR]-complex in the signaling patch is due to the R–anti-R interaction only. There is no need to postulate a role for self-P acting as a specificity element in T-cell activation or effector function.

Some definitions and their consequences

I have reviewed the background for the below definitions [21]. Here I only wish to consider their consequences.

A “*paratopic clan*” is a family of combining sites (“paratopes”) distinguishable one from the other that functionally recognizes a given single antigenic determinant (“epitope”).

A “*mimotopic array*” is a set of epitopes distinguishable one from the other that interacts functionally with a given single paratope.

A “*crossreactive set*” of antigens (collection of linked epitopes) is a family that shares one or more mimotopes.

The term “functionally” is used to emphasize that there are *thresholds* that characterize responsiveness. The level or time of occupancy of the receptor by its ligand has a cut-off below which no signal is delivered to the cell and above which a signal is generated. The threshold is a composite of several factors that are summed to determine signal transduction. Evolutionary selection operates on the threshold.

The TCR/BCR bind epitopes, *not* antigens, which are combinatorials of linked epitopes. As concerns the TCR, the rules governing interactions at the anti-P site must be distinguished from those for the anti-R site. A model of the TCR is one that tells us how interactions at the anti-R and anti-P sites are integrated to signal the cell [6,7].

A given paratope defines as a single epitope the mimotopic array with which it functionally interacts. If any one of the mimotopes is a self-ligand then every member of the array is a self-ligand.

A given epitope defines as a single paratope the paratopic clan with which it functionally interacts. If any one of the paratopes is anti-self then every member of the clan is anti-self.

The total number of paratopic clans equals the total number of mimotopic arrays. The size of the paratopic repertoire is equal to the number of paratopic clans (equal to the number of mimotopic arrays). The functional repertoire size is *not* defined by the total number of chemically distinct paratopes (i.e., sequence different TCR/BCRs).

Paratopes define epitopes, not antigens. Antigens are combinatorials of linked epitopes associatively recognized by the responding immune system. Therefore, tolerance cannot be broken by “molecular mimicry” because all members of the mimotopic array are functionally identical, albeit chemically distinguishable. Tolerance can be broken, that is autoimmunity can be induced by “crossreactive antigens” which are, in fact, nonself-antigens that share mimotopes with self-antigens.

What is specificity?

As discussed elsewhere [10,11], the specificity of paratopes is driven by the necessity to make a self–nonself discrimination (i.e., to sort the paratopic repertoire). Consequently, it is reasonable to define specificity in those terms.

Specificity can be defined in terms of a “Specificity Index (SI),” which is the probability that a change in sequence of a BCR/TCR that results in a functionally distinct or new specificity will be anti-self. We have estimated the SI to be of the order of 0.01 [18,19]. Using this value, if the average number of epitopes per antigenic unit (5×10^4 MW) is 10 and the size of the paratopic repertoire is 4×10^4 (equal to the number of mimotopic arrays) then the probability that two randomly chosen antigenic units will share a mimotope is $10/4 \times 10^4 = 2.5 \times 10^{-4}$.

The probability that a random nonself-antigen will share a mimotope with a self-antigen is $[1 - (1 - \text{SI})^{10}]$. At $\text{SI} = 0.01$ this probability is 0.095 or roughly 10% of nonself-antigens will share mimotopes with self-antigens (i.e., crossreact).

What is degeneracy?

The members of the paratopic clan that recognize a given single epitope are referred to as being a “degenerate” set. More importantly, the recognition of a mimotopic array by a given paratope is also referred to as being “*degenerate*,” which the workshop participants have equated to “*lacking in specificity*.” This introduces an ambiguity that must be resolved.

Degeneracy is the term applicable to the members of a given paratopic clan or mimotopic array when describing a set of chemically distinguishable entities that behave similarly in functional recognitive interactions.

Specificity is a property distinguishing paratopic clans or mimotopic arrays one from the other as entities that behave distinctly differently in recognitive interactions.

Given these definitions, the distinction between specificity and degeneracy based on the statement, “*T-cells are specific because they recognize a small fraction of all possible ligands but are degenerate because the number of potential ligands is very large*,” [1,11] is in contradiction with the concept of polyspecificity which describes a paratope that recognizes ligands randomly with respect to self and nonself.

What is being totally overlooked is that degeneracy is ordered, *not* random with respect to self and nonself. By contrast, specificity is random with respect to self and nonself. Consider a paratopic clan said to be degenerate. If any member of the paratopic clan is antiself as defined by the individual’s immune system, then every member of that clan is antiself. Similarly, if any member of a mimotopic array (said to be degenerate) is a self-epitope to the individual’s immune system, then every member of that array is a self-epitope. This is why tolerance cannot be broken by molecular mimicry. Mimotopes are equivalent epitopes from the point of view of specificity. Tolerance can be broken by crossreactive nonself-antigens that share mimotopes with self-antigens, a direct consequence of the Theory of Associative Recognition of Antigen [22].

Specificity depends on recognition of shape. Degeneracy arises because many chemically distinguishable entities present the same shape which is defined by the paratope. Degeneracy is not a problem for the immune system; it is a problem for the immunologist. The paratope is unispecific with respect to recognition of shape (see discussion, Section “Receptor editing in its biological context”).

Specificity is quantitated by the SI. The SI is based on a distinction between paratopic clans or mimotopic arrays; it does not refer to the individual paratopes or epitopes comprising a degenerate clan or array.

Specificity and degeneracy are inversely related in determining the size of the functional repertoire. At one extreme, if the total epitopic universe were a single mimotopic array

(degeneracy were total), then the size of the paratopic repertoire would be one, anti-self. At the other extreme, if there were only one member in each mimotopic array (degeneracy were zero), that is, there were only one TCR/BCR per antigen, then the size of the paratopic repertoire would equal the size of the antigenic universe and specificity would be maximized. The SI of a universal glue would be one and of the maximized specificity would be infinitesimal. Neither repertoire would be functional.

We estimate that the evolutionarily selected paratope has an SI~0.01 and a repertoire size of $\sim 4 \times 10^4$. This repertoire divides the antigenic universe into combinatorials of epitopes taken 10 at a time.

What is Self?

As classes, self cannot be distinguished from nonself by any physical or chemical property. In addition the immune system has no way to know if the antigen it encounters originates from inside or outside, or is encoded in the genome.

Self is defined by the immune system, not the immunologist, during a somatic learning process [23,24]. Although self is usually autogenously derived, not all autogenously derived components are self to the immune system. It might be stressed that the self-ligands recognized by the cytotoxic, helper, and B-cell are entirely distinct. Further, an immune attack on a self-component must have a debilitating consequence to be evolutionarily selectable. An immune attack on an autogenously generated component that is salutary or without a debilitating consequence (e.g., housekeeping or waste disposal) is not the self defined by the immune system. With this as background, it is not possible to select in the germline for recognition of self. When the host's MHC-encoded restricting element (R_H) is acting as a presenter of peptide to the TCR, it is not functioning as a self-component. The R-element has a special relationship to the immune system [25].

The definitions of the terminology suggested by the workshop

The workshop was dominated by T-cell biology (see [1], Table 1). Hence the term “polyspecificity” emphasized two points:

1. “the ability of the TCR to recognize multiple distinct peptide/MHC ligands” (referred to here as [PR]); and
2. “the specificity with which each of these ligands is recognized.”

This introduces an ambiguity based on the Standard Model postulate of a conflated ligand, Q. The germline-selected recognition of the allele-specific determinants on R follows a different set of specificity rules than the somatically selected recognition of the peptide. Point 1, which, in essence, is reducible to a blurring of restrictive recognition, the phenomenon we are trying to explain, will be analyzed under “alloreactivity versus allorestriction” (Section “Allorestriction must be distinguished from alloreactivity”). Point 2 is best described by the term “degeneracy” as discussed above. Consequently, “polyspecificity” is both theory-dependent and of a debatable existence.

The recommended use of the term “degeneracy” is added by the comment that “*the term degeneracy is better fit for peptide binding to MHC molecules than for TCR recognition* [1].” The meaning of this term in any framework is *not* dependent either on the “degree of degeneracy” or on the recognitive elements engaged.

The term “molecular mimicry” is misdirectedly cast in the framework of autoimmune disease. “Molecular mimicry” describes the relationship between members of a “mimotopic array.”

The breaking of tolerance by a “*nonself microbial antigen*” [1] that shares a mimotope with self is due to “crossreactivity” not “molecular mimicry.” Only the shared mimotope deserves that description, the mere recognition of which could not break tolerance. It would maintain it.

Lastly, to ignore the classical use of the term “crossreactivity” by describing it as similar to “polyspecificity” illustrates the failure of the workshop participants to understand that antigen-receptors recognize epitopes, not antigens. Antigens are collections of linked epitopes. Crossreactivity of antigens means that they share mimotopes as discussed above. To state that the term “crossreactivity” should be supplanted by “polyreactivity” because the former is not “*explicit in emphasizing the existence of multiple peptide/MHC ligands*” [1] is like arguing that Notre Dame would make a better office building than a church.

The major conclusions from the workshop ([1], Table 2)

The term “polyspecificity” should be used because it is a “*well-defined term*” that emphasizes two key aspects, “*the ability of TCRs to recognize multiple peptide/MHC ligands and the specificity with which each ligand is recognized.*” The use of the term “polyspecificity” should be questioned if it requires that one bury restrictive recognition, alloreactivity and allorestriction under the rubric “*recognize multiple peptide/MHC ligands*” and degeneracy under the phrase “*specificity with which each ligand is recognized.*” To defend my argument let us now look at some of the observations on which the concept of polyspecificity is based.

Allorestriction must be distinguished from alloreactivity

When analyzing degeneracy and specificity at the level of the TCR, we must distinguish the behavior of the anti-peptide (P) site which is somatically generated from that of the anti-R site which is germline-selected. The restrictive recognition of the host R_H is determined in the individual by positive selection operating on a set of germline-selected anti-R sites. The repertoire of anti-P is sorted into anti-self P and anti-nonself P by negative selection operating on a set of somatically generated random specificities.

Under the Standard Model alloreactivity is simply due to degeneracy of recognition by restrictively recognitive TCRs. In essence the Standard Model defines alloreactivity as allorestriction. This was first revealed by Bevan [26,27] who put a strong selection pressure on an allo-mix and isolated T-cells that were described at the time as “self + X = allo + Y.” Under the Tritope Model, Bevan was not studying alloreactivity; he was revealing allorestriction. When Bevan [28] proposed the density model of alloreactivity, which was dependent on the germline-selected recognition of R-alleles, he implicitly redefined the role of peptide exactly as it would be later interpreted in the Tritope framework. *It is essential to distinguish between peptide acting as a structural element in the [PR]-complex necessary for its expression and conformation, and peptide acting as a specificity element recognized by the anti-P site of the TCR to initiate a signal.* If one substitutes “germline-selected” for “*intrinsic predisposition*” [29] in describing [TCR-R] interactions, then the high frequency of alloreactive cells is expected and the density of R becomes a less important consideration. Further an anti-R dependent, anti-P independent interaction defines alloreactivity as distinct from allorestriction which is anti-R and anti-P dependent. What Felix et al. [30] have investigated is allorestriction, not alloreactivity (discussed below). What Müllbacher et al. [31] have investigated is alloreactivity, not allorestriction.

Allorestriction arises when two distinct gene loci within different MHC-haplotypes share an allele-specific determinant, for example, in mouse, H-2 A and E or K and D. This situation can arise in several ways, one being by gene duplication and divergence that keeps the “allele-specific determinant.” This is to be distinguished from the MHC-haplotypes that share gene

loci, usually derived by recombination. In this sense the term, “allorestriction,” is a misnomer as the given TCR is actually responding as restrictively as it would in a syngeneic situation. It is the experimenter not the TCR that is defining the interaction as allogeneic. The Tritope Model [6,7] faces the signaling consequences of a distinction between alloreactivity and restrictive reactivity (“allorestriction”). This model predicts that allorestricted T-cells will be positively selected, while alloreactive T-cells will be negatively selected in the allo MHC-haplotype.

A recent study [30] and its accompanying commentary [29] show us why this distinction is important, as once made, the Standard Model is in jeopardy.

Felix et al. [30] studied 182 hybridomas from a B6.H-2^b anti-B6.H-2^k mix that responded to B6.H-2^k. Of these, 60 responded to E^k present on a B-cell line, C27 (H-2^a, E^k). These were divided into two groups based on reactivity to another cell line, CHO.E^k; 28 responded to it, 32 did not. Operating in the framework of the Standard Model, Felix et al. assumed that all alloresponses require specific recognition of peptide and that the CHO.E^k cell line did not present the relevant peptides for the 32 non-responders whereas C27.E^k did. They demonstrated this to be the case for 9 of the 32 non-responders by characterizing defined peptides from C27.E^k and showing that they conferred responsiveness to CHO.E^k.

In order to analyze these findings some background is needed.

1. The B6.H-2^b does not express an E element. E _{α} is defective leaving E _{β} ^b unexpressed [32–35].
2. E _{α} when it is expressed in the various MHC-haplotypes is essentially monomorphic, referred to here as E _{α} ⁰ [36].
3. The responding B6.H-2^b in the allogeneic mix with B6.H-2^k is restricted to A^b only, the E element being absent.

The question arises, “from where do the TCRs specific for a given peptide and restricted to E originate?”

As they could not have been positively selected in the responding B6.H-2^b, we are left with the default assumption that the V region that was positively selected because it recognized A^b also recognizes a determinant on E^k required for the restrictive recognition of peptide bound to E^k. Given this, Felix et al. were studying allorestriction, not alloreactivity. The term allorestriction can be misleading because, in fact, the response to P-E^k is normal restrictive recognition due to the sharing of an allele-specific determinant by A^b and E^k. “Allorestriction” is a phenomenon described from the point of view of the immunologist, not the TCR.

It might be instructive to carry the analysis one step further. The R-element, A^b, to which these hybridomas are restricted, can be seen by the TCR in two ways (Premises 2 and 3):

1. If V _{α} ^b recognizes A _{β} ^b, then it would be positively selected entraining a family of V _{β} ⁿ to yield TCRs with V _{α} ^b V _{β} ⁿ composition.
2. If V _{β} ^b recognizes A _{α} ^b, then it would be positively selected entraining a family of V _{α} ⁿ to yield TCRs with V _{β} ^b V _{α} ⁿ composition.

Allorestriction arises when the allele-specific determinant from the allo-MHC is recognized as being shared or as a mimotope by the positively selected V _{α} ^b or V _{β} ^b of the responding

population resulting in findings such as those of Felix et al. showing that the restrictive response to peptide is both anti-R and anti-P dependent.

Given the two potential A^b-restricted TCR populations ($V_{\alpha}^b V_{\beta}^n$, $V_{\beta}^b V_{\alpha}^n$), which one is responsible for the recognition of E^k via a mimotope shared by A^b and E^k?

If $V_{\alpha}^b V_{\beta}^n$ is recognitive of E^k, then V_{α}^b would have to recognize the allele-specific determinant on E^k. If the $V_{\beta}^b V_{\alpha}^n$ is recognitive of E^k, then V_{β}^b would have to recognize the “allele-specific” determinant on E_α which is essentially monomorphic (symbolized E_α⁰).

To close the discussion there are in the Tritope framework predictable ramifications that should be investigated in this system.

1. Allorestricted TCRs will be positively selected, whereas alloreactive TCRs will be negatively selected in the allo-MHC haplotype.
2. If the alloresponse is directed toward E_α⁰, then allorestriction will be seen in many MHC-haplotypes which express an E-element.
3. Alloreactive cells will be a large proportion of those that respond to both C27.E^k and CHO.E^k.
4. The allorestricted cells will be shown to be also alloreactive by screening their response to a variety of MHC-haplotypes lacking expression of E or with which an interaction with E is blocked.

The Standard Model is in jeopardy because two recognitive sites, anti-R and anti-P are now experimentally found to be mandatory.

What about alloreactivity?

One good example discussed at the workshop is to be found in the studies on the 2C TCR which is a receptor for a CD8⁺ cytotoxic cell that is K^b-restricted and “alloreactive” to L^d [37]. This TCR is positively selected by K^b and negatively selected by L^d as would be explained by the Tritope Model [6] as a general case [38–40]. Under the Standard Model, this would be a fortuity, not a general case, because there is no a priori reason that P_s–K^b should be low affinity (positive selection) whereas P_s–L^d should be high affinity (negative selection). The interesting question regards the role of peptide. Is it a specificity element recognized as a signaling agonist ligand (allorestriction) or is it behaving as a structural element necessary for the stable expression and conformation of the L^d allele-specific determinants (alloreactivity)? The anchor amino acids of P could have an indirect effect on the expression of allele-specific determinants on R giving the impression that P is playing a direct role as a specificity-element interacting with the anti-P site of the TCR [37] when it is not [7]. It is not clear to me what a decisive experiment would entail. In general, the active peptides presented by allo-L^d have a broader sequence dispersion than those presented by syn-K^b suggesting that the peptide is playing a structural role, not specific role ([37], Table 1). This is where molecular morphology would be helpful. Can the altering of amino acid anchors interacting with the “pockets” on R have an effect that is transmitted via R to alter the expression of its allele-specific determinant?

With the development of the Tritope Model, I have changed my position, which has been that alloreactivity is an unselected byproduct of restrictive reactivity. Clearly it would have been just as simple for evolution to select for restrictive reactivity in one orientation leaving the opposite orientation non-signaling in any given TCR. In the Tritope framework, the positively unselected orientation that mediates alloreactivity plays an important role in negatively

selecting or ridding any TCR that would be restricted in both orientations to two host restricting elements. Such TCRs could have quite deleterious effects [6,7].

There is a general comment to make at this point. The evolutionary selection pressure is on a family of V-gene segments present in an individual, the products of which must track the polymorphic allele-specific determinants of the species R-elements. Whatever the mechanism of the interactive selection [7], the recognition of allele-specific determinants must, in large measure, be gene-locus specific. Exceptions revealed by allorestriction are to be expected. However, alloreactivity that involves a Class I restricted T-cell that responds to allo Class II R or vice versa, would be expected to be the consequence of glitches in TCR structure or coreceptor function [6], not mimotopes of allele-specific determinants expressed on disparate Class of R-elements [41,42].

Lastly, there is no rationalization for the assumption that P is acting as a specificity element during positive selection and alloreactivity. A minimal model treats P as a structural element in the [PR]-complex necessary for its functional conformation and stability. All observed effects on positive selection and alloreactivity resulting from amino acid replacements in P are predictably indirect. P acts as a specificity element during inactivation (negative selection), activation and triggering of effector function where engagement of anti-P is obligatory to signaling (restrictive recognition of P).

What contribution does positive and negative selection make to specificity?

How should we deal with experimental findings that appear to challenge general conceptualizations? When should we change our mind?

The “degree of specificity” of the TCR/BCR is a germline-selected property not subject to determination by any somatic process. It is selectively determined by the necessity to make a Self–Nonself discrimination.

Consider a random somatically generated TCR anti-P repertoire that divides the antigenic universe into combinatorials of epitopes (peptides). Evolution is selecting on the size of the recognitive site (i.e., the number of complementarity-determining interactions with peptide). If the site were so small that it recognized a peptidic bond then a Self–Nonself discrimination would be impossible. If the site were so large that it recognized an entire polypeptide chain (i.e., one TCR per antigen) then no T-cell (one TCR per T-cell) would be in sufficient density to respond effectively. Between minimum and maximum specificity there is an average degree of specificity, germline-selected, that permits an adequate Self–Nonself discrimination, on the one hand, and a sufficient rate of responsiveness, on the other hand. This average degree of specificity is defined by the SI. This cannot be altered by any somatic process. The somatic process that permits sorting of the repertoire into antiself and anti-nonspecific is dependent on the germline-selected value of SI but cannot change it [10,19].

The studies of Huseby et al. [41,42] on T-cells are interpreted as a challenge to this conceptualization, and have formed a basis for many of the conclusions of the workshop. Their findings face the same difficulties of interpretation as those discussed above (Section “Allorestriction must be distinguished from alloreactivity”).

I will give one illustration of how their data might be reinterpreted.

Two families of TCRs reactive with the antigen A^b.P3K were isolated, one from wildtype H-2^b mice and the other from H-2^b mice deficient in negative selection. The two families had statistically different behaviors.

The family of TCRs anti-A^b-P3K from wild-type H-2^b mice were, on average, quite specific for A^b and P3K, that is, they restrictively recognized A^b-P3K.

The family of TCRs anti-A^b-P3K from mice with inoperative negative selection, on average, appeared to be less specific in recognizing A^b and P3K.

The workshop participants concluded first that “*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.*” [1]

If degeneracy of recognition of ligand by the TCR/BCR were random with respect to self and nonself, then it would also be random with respect to nonself antigens because what is self for one individual is nonself for another. In this case the immune system could not regulate the expression of effector class. As degeneracy is non-random with respect to self and nonself (i.e., all members of a mimotopic array are either self or nonself), the elimination of the largest size mimotopic self array is irrelevant and, in any case, cannot bias the repertoire away from the recognition of R and toward recognition of peptide.

Second, it was concluded that “*in the absence of proper negative selection TCRs can react with MHC proteins in a class and allele-independent fashion* [1].” While I cannot imagine how such a conclusion could be arrived at by the workshop participants, it illustrates the poverty of the pure empiricism. The one way that experiment could reveal such a result would be if the mutant defective in negative selection by chance made it experimentally easier to reveal limitations in the accuracy of positive selection. To conclude that there is a causative relationship between defective negative selection and a violated “function-class of R” relationship would require a lot more than its mere observation in the mutant.

These are good examples where a theoretical challenge to the interpretation of experiment is as well-grounded as an experimental challenge to the validity of the theory.

Let us try to explain in the Tritope framework, the example cited [41,42] by the workshop upon which these two above conclusions were based.

Using the nomenclature of Section “Allorestriction must be distinguished from alloreactivity” there are two sets of TCR recognitive of A^b (Premises 2 and 3).

Set 1 – V_{α}^b is positively selected by recognition of A_{β}^b and entrains a number of V_{β}^n that encode various alloreactivities. This is the $V_{\alpha}^b V_{\beta}^n$ TCR set.

Set 2 – V_{β}^b is positively selected by recognition of A_{α}^b and entrains a number of V_{α}^n that encode various alloreactivities. This is the $V_{\beta}^b V_{\alpha}^n$ TCR set.

These two sets recognize the peptide, P3K, in two opposite orientations, in essence treating P3K as two different peptides. For example, in the orientation used by $V_{\beta}^b V_{\alpha}^n$, the P3K might be seen in a self mimotopic array that includes a self-peptide (Ps). In the opposite orientation used by $V_{\alpha}^b V_{\beta}^n$, P3K might be seen in a nonself mimotopic array because it does not include any self-peptide.

In the mouse deficient in negative selection (lacking A^b-Ps) both TCR sets, $V_{\alpha}^b V_{\beta}^n$ and $V_{\beta}^b V_{\alpha}^n$ will be positively selected. In the wild-type mouse the same would be true, but the $V_{\beta}^b V_{\alpha}^n$ will be negatively selected by Ps leaving only the $V_{\alpha}^b V_{\beta}^n$ set recognizing P3K.

The observed findings [41,42] characterize the negatively selected $V_\beta^b V_\alpha^n$ set, which is absent in wild-type but present in the mutant. As discussed in Section “Allorestriction must be distinguished from alloreactivity,” $V_\beta^b V_\alpha^n$ could well be A^b and E_α^0 restricted. As Ps is common to many strains of mice and E_α^0 is essentially monomorphic, allorestriction to many MHC-haplotypes that express E, is expected. The alloreactivities encoded by V_α^n will be normal. While the specificity of recognition (SI) of the P3K in the opposite orientations is the same, the effect of amino acid replacements is expected to have a different consequence for each TCR set.

It should be pointed out in passing that the effect of P3K itself on the TCR-R interaction in the two orientations should also be considered [6,7].

In conclusion then, negative selection (itself restricted) is a somatic process functioning to sort the random anti-P repertoire into anti-Ps and anti-Pns. It cannot change the specificity of the allele-specific anti-R interactions required for positive selection, alloreactivity, and restrictive recognition nor can it change the intrinsic value of the SI.

Receptor editing in its biological context

Receptor editing is the consequence of a limitation in the effectiveness of the STOP signal to further rearrangement as required for haplotype exclusion. All models agree on this point. They differ sharply in the biological framework within which they are cast. Two such frameworks emerge as dominant.

1. It is argued that the proportion of anti-self cells in an unselected population is so high that receptor editing was evolutionarily selected to reduce the massive loss of T/B cells that would result from a unitary mechanism of negative selection.
2. The efficacy of the STOP condition required by the mechanism of haplotype exclusion has a limit when the further reduction of the proportion of double producing cells is without selectable functional consequence.

Framework 1 casts receptor editing as a major mechanism for the sorting of the paratopic repertoire (i.e., the Self–Nonself discrimination).

Framework 2 casts receptor editing as a second order phenomenon in that, if it didn't exist, that is to say, haplotype exclusion were perfect, the animal would not be the worse off for it. While the total absence of double receptor producing cells is itself unselectable, the existence of such a perfect state would not be debilitating.

The defenders of Framework 1 base their position on experimental and theoretical arguments purporting to demonstrate that a large proportion (>50%) of naïve (unselected) T/B cells are anti-self.

The theoretical argument [43–45] is derived from the question “what is the optimal size of the paratopic repertoire that one can stuff onto a fixed number (N) of Ig molecules?” The mathematical treatment of the question demonstrates that the optimum number of paratopes per Ig is determined by the probability that one of them is anti-self. The number of functionally distinct anti-nonsel self paratopes that can be expressed by N Igs is maximized when they harbor an average of one anti-self per Ig.

Only theoreticians and mathematicians can ask this question; evolution never had a chance to consider it, as the physical molecule (one Ig—many paratopes) is unselectable. Langman [10] in his “must read” essay “The Specificity of Immunological Reactions” made this point

with arguments at the structural and physiological level. I will approach it in the context of the workshop discussion [1].

Consider the two mutually exclusive postulates:

1. The paratopic repertoire is selected upon to divide the antigenic universe into combinatorials of linked epitopes (i.e., an antigen is a combinatorial of linked epitopes) [16–19].
2. The paratopic repertoire is divided into random packages that are combinatorials of specificities per Ig molecule [43–45]. This converse position is that the paratopic repertoire is divided into combinatorials or packets of random specificities unrelated to their relationship in antigens. Postulate 2, unlike Postulate 1, raises the additional question, namely what is the origin of the repertoire that is partitioned into packets of random specificities. Under Postulate 1 the repertoire is selected by the need for a paratope to define an epitope [20].

As both the self–nonself discrimination, and the coherent and independent regulation of effector class depend on the immune system’s ability to read linkage of epitopes (i.e., an antigen), Postulate 2 is untenable. A paratope that recognizes collections of random epitopes is unregulatable with respect to antigens. Associative recognition of antigen is a central principle. A “polyspecific” paratope is ruled out. To restate this, the paratope (anti-P of the TCR, combining site of the BCR) recognizes a “degenerate” non-random mimotopic array, not a random “polyspecific” scattershot of packets of epitopes. I am aware of the studies on a category of antibodies referred to as “polyreactive” [46] that have the characteristics of being “polyspecific” but, to date, they have not been mapped to a definable function, and, in any case, are a small proportion of the Ig population.

No matter how one views Postulate 2, to argue that receptor editing is necessitated by the large proportion of T/B- cells that would be purged (63%) based on this theoretical argument is derouting as evolution never had a chance to exercise that option. We have estimated the proportion of anti-self cells in the naïve population of T/B to be 0.01 [18,19] based on selectable parameters.

The experimental demonstration of secondary rearrangements is not in question. The confronting of an immature B-cell with a membrane-bound ligand of significant density initiates deletion as well as secondary rearrangements [47,48]. This latter is not surprising given an (LH)₂-STOP signal. If the surface BCR is erased as it appears by interaction with an aggregating ligand, the (LH)₂-STOP signal could well be disrupted allowing continued rearrangement in a proportion of the B-cells. The cooperative interaction between receptor and ligand favors such an outcome. A similar interpretation can be applied to the interaction between a “polymimotopic” ligand like DNA and the BCR [49,50]. What is in question is the biological role of such findings [51].

The self–nonself discrimination is a decision process [22–25]. The interaction of the TCR/BCR with ligand, whether it is self or nonself, results in an inactivating signal (Signal [1]), in this case, postulated to be receptor editing (a secondary rearrangement). Given the argument that receptor editing is an evolutionarily selected major contributor to the sorting of the repertoire, then the T-helper delivered second signal (Signal ([1] + [2]) that determines activation must include a cessation of rearrangement imposed on top of the (LH)₂-STOP which operates in the absence of an interaction with ligand. Signal [2] saves the cell from receptor editing.

For such an hypothesis to be useful (or even credible) an argument, theoretical or experimental, that it solves a functional problem not solved or solvable by the known deletion mechanism, would be helpful.

It should not be forgotten that many V-gene segments are nonfunctional even though they can rearrange [52,53]. For example, of the total of $\sim 10^2$ V_L or V_H gene segments only about 40 V_L and V_H are functional. About 50 are nonfunctional but can rearrange. If they do not produce an (LH)₂-STOP signal, they can receptor edit their L chain. However, if an in-frame rearrangement has a 0.5 probability of involving a nonfunctional V-domain and a 0.63 probability of a functional V-domain being anti-self, little is gained [54]. Further the large loss of T/B cells in thymus and bone marrow, respectively, cannot be ascribed to negative (Signal [1]) selection and, in any case, death-by-neglect is not retrievable by receptor editing.

In the absence of a rationalization for editing [54], framework 2 appears by default as the more likely.

Enter! molecular morphology

The observed conformational changes in the TCR when comparing its morphology when free and ligand bound have been interpreted in the framework of the Standard Model as a requirement for plasticity in recognizing a degenerate mimotopic array. This has been stated as follows: “*These large conformational adjustments to the pMHC especially for the contacting CDRs, is an additional mechanism of enhancing the TCR recognition repertoire...* [55].” A conformational change required for signaling has been left open because the Standard Model only predicts an aggregational signal as necessary and sufficient. By contrast the Tritope Model requires that the initiation of signaling be conformational change-dependent. For restrictive reactivity both anti-R and anti-P must be engaged; for alloreactivity only R also need be engaged. The consequence of the conformational transition might well be a self-complementing signaling structure.

The basic problem is in the limitation of the methodology which seeks to translate relationships in space into events in time. Further, many of the structures involved in signaling are absent in the crystals under analysis. The biology of the interactions makes it highly unlikely that the V-gene segments were germline-selected to recognize peptide. They have been germline-selected to recognize R-alleles (restrictive recognition).

A comparison of an unbound TCR (lacking CD3) with a bound TCR interacting with [PR] as a cognate ligand (lacking CD4/8) leaves all the intermediate transitions as unknowns. What would be the TCR conform if P were not recognized (i.e., the most common situation)? The TCR bound to R via an R-anti-R interaction is an important intermediate.

It can be reasonably assumed that the complemented junctional regions (i.e., the only part of the TCR that is somatically varied) play a sole role as anti-P initiating the signal resulting from the recognition of P. When P is recognized by anti-P (Signal[1]), the V-domains might disengage from their recognition of R taking on another configuration and that might be what one sees in the bound structure, not the recognition of P by V-domains as implied above and repeatedly claimed. This disengagement might have a function (Section “An a priori glance at T-cell behavior”).

For example, a clear statement of the Standard Model as seen in the eyes of a molecular morphologist is “...*the TCR binding-site is likely not made up of “subsites” that operate independently to recognize either MHC residues or MHC-bound peptides. For instance, some CDR 1 loops can also contribute to recognition of the bound peptide and some CDR 3β loops have a bifunctional role, filling notches between the peptide and the MHC α helices.*” [56]

This direct criticism of any two site, anti-R and anti-P model must be faced. There are several points to consider:

1. While all signaling interactions require recognition of the ligand, not all physically defined interactions contribute to signaling. Further, what might be the rationalization for an extrapolation from this observed bound TCR morphology to an unobserved intermediate morphology as discussed above?
2. A model of TCR function requires that interactions at the two postulated sites be integrated to signal the cell. The “*subsites*” acting as **receptacles** may “*operate independently to recognize*” a ligand, but the TCR acting as a **receptor** must operate dependently to signal the cell by integrating cognitive interactions at the two “*subsites*.”
3. The arguments presented by Langman ([10], Sections 2.4 and 2.8) that cognitive sites, anti-R and anti-P, are essential to a TCR structure/function relationship should be faced.

Under the Standard view, recognition of R alone or P alone or Q (i.e., [PR]) are signaling events. Under the Tritope Model, both anti-R and anti-P must be engaged to restrictively signal. When the interaction of the TCR with host R (R_H) is involved, R_H is not functioning as an antigen (the interaction is not signaling); it is a platform presenting peptide. When the interaction is with allo-R (R_A), the R_A is acting as an antigen (the interaction is signaling), and this has consequences [6,7]. Under the Standard Model, alloreactivity is due to the lack of specificity (or degeneracy) of restrictive reactivity; in essence, alloreactivity is a denial of restrictive reactivity leaving both allele-specific recognition and the fact that the same alleles of R are defined by restrictive or alloreactivity, basically unexplained. This engenders the conclusion that “... $\alpha\beta$ T cells are weakly autoreactive by design and operate on the edge of autoimmunity.” [56] In the Tritope framework this would be a non-sequitur; in the Standard framework this is a necessary conclusion. It is the anti-P site, not the anti-R site, that is relevant to autoimmunity because it alone undergoes the somatic sorting process known as the self–nonself discrimination.

How might molecular morphology help us sort out different interpretations of the biology?

For the morphology of a molecule (and its interactions) to be a datum that is informational, it must be integrated into a conceptual framework that is based on an evolutionarily selectable function. To date the structural information has been interpreted in the framework of the Standard Model meaning that the $\alpha\beta$ TCR and the BCR are assumed to be similarly molded. This has been of little comfort to those of us who would like the molecular morphologists to tell us why such structural look-a-likes behave so differently and why the difference is ignored or denied. Further, we should expect that structure would allow us to distinguish competing models of function by establishing rules or generalizations that answer such questions as:

1. What are the structural elements of the TCR that determine allele-specific recognition by the $\alpha\beta$ TCR? Symmetrically, what structural elements on R define the allele-specific determinant?
2. What are the structural elements of the TCR that determine recognition of peptide?
3. How are these two cognitive events integrated to signal the cell? How is the interaction between the TCR and [PR] translated into Signal [1]?
4. What determines the concordant relationship between the alleles of R defined by interaction of the amino acid anchors with the “pockets” in R-elements and by the interaction of R with the TCR?
5. What are the structural sites that allow a given TCR to recognize the allele of R_H (restrictive recognition) and an allo-allele (alloreactivity) R_A ?

6. What is the structural basis for TCRs being positively selected by the syngeneic allele (R_H) and negatively selected by R_A , the allo-allele (alloreactivity)?
7. How does structure permit us to understand why the polymorphic alleles of R defined by restrictive and alloreactivity are the same?

These are examples of questions that the Tritope Model has tried to answer by logical deduction from function. They can only be answered reciprocally by logical deduction from structure if the intermediate TCR conforms between the free and bound structures are studied.

Lastly, molecular morphology gives us at best only a glancing view of specificity and degeneracy, which are evolutionarily selected by their biological requirements. The ignoring of this point led to questionable conclusions at the workshop. For example:

What is the basis for the assumption that autoimmune TCRs are, as a class, structurally aberrant or even distinctively different from normally protective TCRs? Can the TCR/BCR be structurally altered somatically to deliver inducible-only signals?

The workshop participants put emphasis on what appeared to be the unusual binding morphology of TCRs involved in autoimmunity ([1], Section 4.2). The implication of the unusual topology was that it permitted escape from negative selection but allowed induction to effectors resulting in autoimmunity. This generalization should have raised eyebrows as there is ample reason to challenge it. No antigen-responsive cell can be induced that is unable to be tolerized because its defective TCR/BCR cannot transmit a signal upon interaction with an agonist ligand. The existence of antigen-receptors that cannot transmit a tolerogenic Signal [1] yet are normally inducible would be a lethal situation for a system of receptors that generate their structures by recombination, complementation, and random mutation. Signal [1] is included in the activation signal (Signal ([1] + [2])) in order to deal with this lethal possibility.

The bottom line

Under the Standard Model, “polyspecificity” describes an antigen-receptor, TCR or BCR, that possesses reactivity with a random packet of ligands plucked from the antigenic universe. This is untenable because such a receptor would not be regulatable with respect to the effector class.

Under the competing view, “polyspecificity” must be considered somewhat differently for the TCR and the BCR. For the TCR, as described by the Tritope Model, all functional signaling events require an allele-specific R–anti-R interaction but only inactivation (negative selection), activation and triggering of effector function require, in addition, specific recognition of P by anti-P (restrictive recognition of peptide). Positive selection and alloreactivity are initiated via an R–anti-R interaction, anti-P independently.

The repertoires of the BCR and of the anti-P site of the TCR divide the antigenic universe into combinatorials of epitopes (an antigen is a combinatorial of linked epitopes).

The TCR/BCR under the Standard Model is viewed as “polyspecific” in that it is believed to interact with a random packet of unrelated ligands. Under the alternative view, the TCR/BCR is functionally “unispecific” in that it recognizes a given shape. This requires that a distinction between specificity and degeneracy be made, as developed in the text.

Only by dissecting so-called polyspecificity into its component parts, specificity and degeneracy, can sense be made of the recognitive interactions of antigen-receptors with their ligands. Unfortunately when this is done the existence of the phenomenon referred to as “polyspecificity” comes into question.

A personal comment

The output of the workshop illustrates a growing problem in immunology, probably also true in many other fields. We are becoming fragmented by such extreme specialization that no one feels comfortable outside of a small circumscribed domain.

This has two consequences.

First, a counterproductive situation is created in which a questionable conceptualization that challenges as little as possible any of the units of the mosaic often dominates thinking and creates inertia to change even when it becomes dead-end.

Second, a given domain can be driven by a theory that is incompatible with the other areas of the subject. While this too becomes self-limiting, it is often derouting and wasteful.

Lewis Thomas was right when he wrote that science is in need of the equivalent of the literary critic or the renaissance scientist. While waiting for that need to be satisfied, we are going to have to face the question, “how do we deal with complexity?” We should be circumspect of the most articulate spokesmen for each given microcosm telling us “what to think.” Would it not be truly creative if we were to consider instead the question of “how to think” and come up with meaningful answers. If that happened, we could program a computer so that it knows “how-to-think,” let it search for the meaningful data, and then tell us “what-to-think.” In that event we could blissfully spend our time in recreational activities.

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 authors and do not represent the official view of NCRR or NIH. I wish to thank the FLAD Computational Biology Collaboratorium at the Gulbenkian Institute in Oeiras, Portugal for hosting and providing facilities used to conduct part of this research as a FLAD visiting scholar at the Institute. I appreciate the criticism, enthusiasm and support from the Director, Dr. Antonio Coutinho.

References

1. Wucherpfennig KW, Allen PM, Celada F, et al. Polyspecificity of T cell and B cell receptor recognition. *Sem Immunol* 2007;19:216–224.
2. Matzinger P, Bevan MJ. Hypothesis. Why do so many lymphocytes respond to major histocompatibility antigens? *Cellular Immunol* 1977;29:1–5. [PubMed: 300293]
3. Langman RE, Cohn M. The Standard Model of T-cell receptor function: a critical reassessment. *Scand J Immunol* 1999;49:570–577. [PubMed: 10354368]
4. Cohn M. The Tritope model of restrictive recognition by the TCR. *Trends Immunol* 2003;24:127–131. [PubMed: 12615207]
5. Cohn M. Distinguishing the Tritope from the interaction antigen models. *Trends Immunol* 2004;25:8–9. [PubMed: 14698277]
6. 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;42:1419–1443. [PubMed: 15950737]
7. Cohn M. The Tritope Model for restrictive recognition of antigen by T-cells: II. Implications for ontogeny, evolution and physiology. *Mol Immunol*. 2007 in press.
8. Garboczi DN, Ghosh P, Utz U, et al. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. *Nature* 1996;384:134–141. [PubMed: 8906788]
9. Garcia KD, Degano M, Stanfield RL. An alpha-beta T cell receptor structure at 2.5 Å and its orientation in the TCR-MHC complex. *Science* 1996;274:209–219. [PubMed: 8824178]

10. Langman RE. The specificity of immunological reactions. *Mol Immunol* 2000;37:555–561. [PubMed: 11163391]
11. Cohn M. A new concept of immune specificity emerges from a consideration of the self-nonself discrimination. *Cell Immunol* 1997;181:103–108. [PubMed: 9398397]
12. Cohn M. At the feet of the Master: the search for universalities. Divining the evolutionary selection pressures that resulted in an immune system. *Cytogenet Cell Genet* 1998;80:54–60. [PubMed: 9678335]
13. Cohn M. The immune system: a weapon of mass destruction invented by evolution to even the odds during the war of the DNAs. *Immunol Revs* 2002;185:24–38. [PubMed: 12190919]
14. Landsteiner, K. The specificity of serological reactions. Cambridge: Harvard University Press; 1945.
15. Lederberg J. Genes and antibodies. *Science* 1959;129:1649–1653. [PubMed: 13668512]
16. Talmage DW. Immunological specificity: an alternative to the classical concept. *Science* 1959;129:1643–1648. [PubMed: 13668511]
17. Cohn, M. Logic of the self-nonself discrimination: principles and history. In: Cambrosio, A.; Moulin, A., editors. *Dialogues with selves historical issues and contemporary debates in immunology*. France: Editions Elsevier; 2001. p. 53-85.
18. Langman RE, Cohn M. The E-T (elephant-tadpole) paradox necessitates the concept of a unit of B-cell function: the Protecton. *Mol Immunol* 1987;24:675–697. [PubMed: 3498884]
19. Cohn M, Langman RE. The Protecton: the evolutionarily selected unit of humoral immunity. *Immunol Rev* 1990;115:1–131.
20. Cohn M. What are the commonalities governing the behavior of humoral immune recognitive repertoires? *Dev Comp Immunol* 2006;30:19–42. [PubMed: 16139887]
21. Cohn M. Degeneracy, mimicry and crossreactivity in immune recognition. *Mol Immunol* 2005;42:651–655. [PubMed: 15607824]
22. Cohn, M. Conceptualizing the self-nonself discrimination by the vertebrate immune system. In: Timmis, J.; Flower, D., editors. *silico immunology*. New York: Springer; 2007. p. 375-398.
23. Cohn M. A biological context for the self-nonself discrimination and the regulation of effector class by the immune system. *Immunol Res* 2005;31:133–150. [PubMed: 15778511]
24. Cohn M. The common sense of the self-nonself discrimination. *Springer Semin Immunopathol* 2005;27:3–17. [PubMed: 15711952]
25. Cohn M. The self-nonself discrimination: reconstructing a cabbage from sauerkraut. *Res Immunol* 1992;143:323–334. [PubMed: 1631416]
26. Bevan M. Killer cells reactive to altered-self antigens can also be alloreactive. *Proc Natl Acad Sci USA* 1977;74:2094–2098. [PubMed: 141046]
27. Hunig TR, Bevan MJ. Antigen recognition by cloned cytotoxic T lymphocytes follows rules predicted by the altered-self hypothesis. *J Exp Med* 1982;155:111–125. [PubMed: 6976410]
28. Bevan MJ. High determinant density may explain the phenomenon of alloreactivity. *Imunol Today* 1984;5:128–130.
29. Nikolich-Zugich J. High specificity, not degeneracy, allows T cell alloresponses. *Nat Immunol* 2007;8:335–337. [PubMed: 17375093]
30. Felix NJ, Donermeyer DL, Horvath S, et al. Alloreactive T cells respond specifically to multiple distinct peptide-MHC complexes. *Nat Immunol* 2007;8:388–397. [PubMed: 17322886]
31. Müllbacher A, Lobigs M, Kos FJ, et al. Alloreactive cytotoxic T-cell function, peptide nonspecific. *Scand J Immunol* 1999;49:563–569. [PubMed: 10354367]
32. Rubin B, Gouaillard C, Weideranders G, et al. The IE allogeneic response of T cells from C57B1/6 mice is associated with genes of the TCRA locus. *Scand J Immunol* 1993;37:388–397.
33. Ewijk WV, Ron Y, Monaco J, et al. Compartmentalization of MHC Class II gene expression in transgenic mice. *Cell* 1988;53:357–370. [PubMed: 3259162]
34. 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;164:4627–4634. [PubMed: 10779766]
35. LaFuse WP, Savariravan S, McCormick JF, et al. Identification of I-E alpha genes in H-2 recombinant mouse strains by F1 complementation. *Transplantation* 1987;43:297–301. [PubMed: 3101250]

36. Zy-Tmg E, Chu C, Carswell C, et al. The minimal polymorphism of Class II E alpha chains is not due to the functional neutrality of mutations. *Genetics* 1994;40:9–20.
37. Chen C, Eisen HN, Kranz DM. A model T-cell receptor system for studying memory T-cell development. *Microbes Infect* 2003;5:233–240. [PubMed: 12681413]
38. Sha WC, Nelson CA, Newberry RD, et al. Positive and negative selection of an antigen receptor on T cells in transgenic mice. *Nature* 1988;336:73–76. [PubMed: 3263574]
39. Capone M, Curnow J, Bouvier G, et al. T cell development in TCR- $\alpha\beta$ transgenic mice. *J Immunol* 1995;154:5165–5172. [PubMed: 7730622]
40. 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. [PubMed: 12496382]
41. Huseby ES, White J, Crawford F, et al. How the T cell repertoire becomes peptide and MHC specific. *Cell* 2005;122:247–260. [PubMed: 16051149]
42. Huseby ES, Crawford F, White J, et al. Negative selection imparts peptide specificity to the mature T cell repertoire. *PNAS* 2003;100:11565–11570. [PubMed: 14504410]
43. 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;90:1691–1695. [PubMed: 7680474]
44. De Boer RJ, Perelson AS. How diverse should the immune system be? *Proc R Soc Scan* 1993;B252:171–175.
45. Nemazee D. Antigen receptor “capacity” and the sensitivity of self-tolerance. *Immunol Today* 1996;17:25–29. [PubMed: 8652047]
46. Notkins AL. Polyreactivity of antibody molecules. *Trends Immunol* 2004;25:174–179. [PubMed: 15039043]
47. Tiegs SL, Russell DM, Nemazee D. Receptor editing in self-reactive bone marrow B cells. *J Exp Med* 1993;177:1009–1020. [PubMed: 8459201]
48. Lang J, Jackson M, Teyton L, et al. B cells are exquisitely sensitive to central tolerance and receptor editing induced by ultralow affinity, membrane-bound antigen. *J Exp Med* 1996;184:1685–1697. [PubMed: 8920858]
49. Radic MZ, Erikson J, Litwin S, et al. B lymphocytes may escape tolerance by revising their antigen receptors. *J Exp Med* 1993;177:1165–1173. [PubMed: 8459210]
50. Gay D, Saunders T, Camper S, et al. Receptor editing: an approach by autoreactive B cells to escape tolerance. *J Exp Med* 1993;177:999–1008. [PubMed: 8459227]
51. Nemazee D, Weigert M. Revising B cell receptors. *J Exp Med* 2000;191:1881–1894. [PubMed: 10839804]
52. Matsuda F, Ishii K, Bourvagnet P, et al. The complete nucleotide sequence of the human heavy chain variable region locus. *J Exp Med* 1998;188:2151–2162. [PubMed: 9841928]
53. Zachau HG. The immunoglobulin kappa gene families of human and mouse: a cottage industry approach. *Biol Chem* 2000;381:951–954. [PubMed: 11076026]
54. Cohn, M.; Langman, RE., editors. *Semin Immunol*. Vol. 14. 2002. Haplotype and isotype exclusion: How and Why?; p. 247
55. Garcia KC, Degano M, Pease LR, et al. Structural basis of plasticity in T cell receptor recognition of a Self Peptide-MHC antigen. *Science* 1998;279:1166–1172. [PubMed: 9469799]
56. Mazza C, Malissen B. What guides MHC-restricted TCR recognition? *Sem Immunol* 2007;19(4): 225–235.