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Molecular Immunology 42 (2005) 651–655
Molecular Immunology
www.elsevier.com/locate/molimm

Discussion

Degeneracy, mimicry and crossreactivity in immune recognition

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Received 16 August 2004
Available online 6 November 2004

Abstract

Degeneracy of recognition of antigen by the immune system is being used as an argument that the self–nonself discrimination cannot be a property of the specificity of its antigen-receptors, TCR and BCR, but must rely on emergent properties derived from a set of complex interactions and pathways. This essay analyzes an alternative view by showing that degeneracy and specificity are not mutually exclusive properties. The self–nonself discrimination is the sole evolutionary selection pressure for the specificity of the TCR and BCR, which can be quantitated as a "Specificity Index." Degeneracy is a non-issue for the self–nonself discrimination largely because it is a problem of chemistry, not of biology.

Keywords: Self–nonself discrimination; Specificity; TCR; BCR; Degeneracy; Immune recognition; Associative recognition of antigen

1. Introduction

An entire issue of "*Molecular Immunology*" (Sercarz and Cohen, 2004a) was devoted to this subject from which the consensus appeared to emerge that "specificity" of the combining site (paratope) is a vanishing paradigm to be replaced by "degeneracy" of the system. Given this, the editors raised the question, "if the immune system cannot rely on the specificity of its receptors at the molecular level, then how can it behave with such marvelous specificity at the operational level?" (Sercarz and Cohen, 2004b).

This consensus is in such direct opposition to the concept of specificity which we have developed (Cohn, 1997, 2002; Cohn and Langman, 1990; Langman, 2000; Langman and Cohn, 1987) that I believe a comparison of the two positions is merited.

The best way to approach the question of "specificity" is to ask "what was the evolutionary selection pressure for the 'specificity' of the paratopes of the antigen-receptors, TCR or BCR?" Why were not these receptors selected to be universal glue? Or why were not these receptors selected to be "infinitely" specific? What is it that determined their observed intermediate discriminatory behavior?

To begin it might be noted that paratopes are under evolutionary selection to define epitopes (not vice versa). Epitopes are integral parts of antigens which are selected to function in the physiology of the organism; they are not selected to be ligands of the immune system. *The selection pressure for the specificity of paratopes is the necessity to make a self–nonself discrimination*. The resultant specificity is a compromise between two extremes.

If the paratope were a universal glue ("zero" specificity), the size of the repertoire would be one, but the host would autodestruct being unable to distinguish self from nonself. If the paratope were "infinitely" specific, the size of the repertoire would be transcendental, but nonfunctional because the time to respond to a pathogen would be too long and the host would die of infection. An optimized compromise is reached when the degree of specificity is just sufficient to make adequate self–nonself discrimination. At this point, because the size of the repertoire is sufficiently small, it can respond sufficiently rapidly. Natural selection cannot operate to perfection; it only operates to adequacy.

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doi:10.1016/j.molimm.2004.09.010

2. What property of the paratope is under selection?

The property under selection is the number of complementarity-determining (CD) interactions required to initiate signaling. This can be described as the size of the paratope. The larger the paratope, the greater the number of CD interactions required to signal, the higher the specificity. At the extreme, if the paratope were so large that it treated the entire antigen as a single epitope, it would be "infinitely" specific. As the number of antigens is transcendental, a repertoire of one paratope per antigen would also have to be transcendental and therefore, nonfunctional. The smaller the paratope, the fewer the number of CD interactions required to signal, the lower the specificity. At the extreme, if the paratope were so small that it recognized an epitope the size of a single amino acid, it would behave as a universal glue; a few paratopes would cover the entire antigenic universe, but an adequate self–nonself discrimination would be impossible.

Optimally then the size of the paratope is selected such that the repertoire divides the antigenic universe into determinants that are combinatorially distributed on antigens; this defines an antigen as a collection of linked epitopes. The average number of epitopes per unit molecular weight seen by the repertoire of paratopes is a function of the selected paratopic size. A reasonable estimate would be that the repertoire sees an average of 10 epitopes per monomer of molecular weight 5×10^4. A paratopic repertoire of size 10^5 would distinguish ${}_{10^5}C_{10} = 10^{43}$ antigens of average MW = 5×10^4.

As antigens are a chemically diverse collection of entities (protein, carbohydrate, lipid), in order for a limited paratopic repertoire to divide them into combinatorials of linked epitopes, the paratope must recognize shape, not chemistry. This can be illustrated by the demonstration that anti-carbohydrate or anti-steroid antibodies will recognize unique peptides from a large combinatorial library. The term "mimotope" has been used to describe chemically distinguishable epitopes that are recognized by a single paratope (Granoff et al., 2001; Manivel et al., 2002; Meloen et al., 2000; Monzavi-Karbasdsi et al., 2001; Monzavi-Karbassi et al., 2002; Shin et al., 2002; Valadon et al., 1996). However, this fact does not lead to or obviate any functional definition of "specificity."

3. Some definitions are needed

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 that family which shares mimotopes.

The term "functionally" is used to highlight that there are thresholds that characterize induction of responsiveness. The level or time of occupancy of a receptor by its ligand has a cut-off below which no signal is delivered to the cell and above which a signal is generated. This parameter is a composite of several factors that are summed to define whether the threshold is reached. Selection operates on the threshold.

The TCR and BCR bind to epitopes, not antigens. The term "epitope" is used here in the sense of "ligand." For the BCR it is a shape-patch on the surface of an antigen. For the TCR it is a peptide derived from the antigen. The other recognitive interactions of the TCR (Cohn, 2003) are not relevant here; only the specificity of the anti-peptide site is germane.

4. The consequences of this framework

Viewed in terms of evolutionary selection, the immune system treats the "paratopic clan" as a single functional paratope and the "mimotopic array" as a single functional epitope. This means that, if any member of the paratopic clan is an anti-self paratope then all members of the clan are anti-self paratopes. Similarly, if any member of the mimotopic array is a self-epitope, then all members of the array are self-epitopes.

As paratopes define epitopes, the total number of paratopic clans equals the total number of mimotopic arrays. The size of the paratopic repertoire is defined as the total number of paratopic clans; this equals the number of mimotopic arrays. Repertoire size is not defined as the total number of chemically distinguishable paratopes or sequence different antibodies. Stated differently, the number of functionally non-overlapping paratopes equals the number of functionally non-overlapping epitopes. Paratopes define epitopes, not antigens, which are linked collections of epitopes associatively recognized as such by the responding immune system.

Tolerance cannot be broken by "molecular mimicry"; all members of a mimotopic array are functionally identical. Tolerance can be broken by crossreactive antigens, that is, nonself-antigens that share mimotopes with self-antigens. Autoimmunity does not arise from molecular mimicry between epitopes; it arises from crossreactivity between antigens that share mimotopes.

5. Specificity

Since specificity of paratopes is driven by the necessity to make a self–nonself discrimination, it is reasonable to define it in those terms.

We have defined 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 value of SI to be of the order of 0.01 (Cohn, 1997, 2002; Cohn and Langman, 1990; Langman, 2000; Langman and Cohn, 1987).

For purposes of illustration, if the average number of epitopes per antigen is epi and the size of the paratopic repertoire is T (the number of paratopic clans = the number of mimotopic arrays), then the probability that two randomly chosen antigens will share a mimotope is epi/T. If epi = 10 and $T = 10^5$, then this probability is 10^{-4}.

If now we ask, what is the probability that a random nonself-antigen will share a mimotope with a self-antigen, this will be $1-(1-\mathrm{SI})^{\mathrm{epi}}$. At SI = 0.01 and epi = 10, this probability is 0.095 or roughly 10% of nonself-antigens will share mimotopes with self-antigens (i.e., crossreact).

6. Degeneracy versus specificity

I have avoided using the term "degeneracy" because its use in the literature has become degenerate. So let me restate what I have said, before commenting on the competing view.

The immune system treats as a single paratope, the family of chemically distinguishable paratopes ("paratopic clan") that functionally recognize a given single epitope. This has been referred to as "degeneracy" of paratopes.

The immune system treats as a single epitope, the family of chemically distinguishable epitopes ("mimotopic array") that are functionally recognized by a given single paratope. The members of this family are referred to as "mimotopes," the phenomenon as "molecular mimicry." Recognition of the mimotopic array by the given paratope has been referred to as being "degenerate" or lacking in specificity.

When distinguishable antigens (collections of linked epitopes) share mimotopes, they are said to be "crossreactive."

Degeneracy is the term used to describe a paratopic clan or mimotopic array as a set of chemically distinguishable entities that behave similarly in recognitive interactions.

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

7. The alternative view

The alternative view as stated by Parnes is "that promiscuous receptors and degenerate processes are leading principles." Given this he feels that simply asking the question "can the immune system discriminate between self and nonself" is "misleading." This leads him to conclude, "if we assume that the immune system is preoccupied with the integration of neoantigens to the body, the distinction between Self and Nonself becomes meaningless" (Parnes, 2004). Parnes echoes Dembic who asks "Does the immune system 'want' to discriminate self from nonself" (Dembic, 2004)? Cohen et al. argue that "without strict specificity of recognition there would be no self–nonself recognition…" "Specificity is not given to the immune system but is created by the immune system despite the degeneracy of its component clones" (Cohen et al., 2004). From this Cohen et al. conclude, "the immune system is concerned mostly with the physiology of body maintenance."

If one ignores that the effector output of the immune system is biodestructive and ridding, and postulates that it has been evolutionarily selected to regulate the physiology of the host (integrative, maintenance, healing) then, true enough, the logic would be that a self–nonself discrimination is irrelevant. However, that latter assumption does not obviate the existence of a biodestructive and ridding role that does require a self–nonself discrimination and that is what we are analyzing. Parenthetically, I question that the immune system was selected to regulate the physiology of the host.

Since a self–nonself discrimination requires a definable level of "specificity," does "degeneracy" impact as a consideration? The answer is NO because degeneracy is ordered, not random, with respect to self and nonself. Specificity, on the other hand, is random with respect to self and nonself. What is meant by this?

Consider a paratopic clan, said to be "degenerate." If any member of that clan of paratopes is anti-self defined by the individual's immune system, then every member is anti-self. Similarly, if any member of a mimotopic array (said to be "degenerate") is a self-epitope to the individuals' 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 specificity-wise. Tolerance can be broken by crossreactivity of nonself-antigens that share mimotopes with self-antigens, a direct consequence of the Theory of Associative Recognition of antigen (ARA) (Cohn, 1992, 1998, 2002; Langman and Cohn, 2002). The breaking of tolerance requires that a nonself-epitope from the nonself-antigen crossreactive with self be recognized by an effector T-helper ($\mathrm{eT_h}$) anti-nonself and the shared self-mimotope be recognized by an antigen-responsive anti-self cell. The associative recognition of the two epitopes results in activation of the latter and initiation of autoimmunity. Specificity is quantitated by the Specificity Index (SI), loosely defined as the probability of being anti-self. SI sums paratopic clans and mimotopic arrays; it does not sum the individual paratopes or epitopes comprising the degenerate clan or array.

Cohen et al. define "poly-clonality" as the response to a single epitope of a population of lymphocytes expressing the receptors of a paratopic clan, and "poly-recognition" as the response of a single lymphocyte to a mimotopic array (Cohen et al., 2004). From this they argue "that immune specificity cannot be reduced to receptor specificity" and, "without strict specificity of recognition there could be no self–nonself discrimination." Further, given that poly-recognition exists, immune specificity cannot be the property of the receptor, TCR/BCR, but rather must be an emergent property, one that is "not reducible to the discrete properties of individual components."

Emergent properties might be envisioned to define the effector output, magnitude and class of response. However, the self–nonself discrimination is determined by the sorting of the T-cell paratopic repertoire into those specificities (anti-

self), which, if expressed, would debilitate the host by autoimmunity and those specificities (anti-nonself), which, if not expressed, would result in the death of the host by infection. This purging of anti-self from the somatically generated large random repertoire leaving the residue to function as anti-nonself is crucial to a self–nonself discrimination and is a function of receptor specificity as defined by the "Specificity Index." "Degeneracy" describes the functionally equivalent members of a paratopic clan. This has no bearing on the receptor specificity required for an adequate self–nonself discrimination because specificity deals with the sorting of clans, not paratopic members of a clan. As poly-recognition or paratopic recognition of a mimotopic array is not random with respect to self or nonself, it presents no challenge to the concept of specificity that we have developed. If degeneracy (poly-recognition) were random with respect to recognition of self and nonself, no emergent property could make a self–nonself discrimination. After all, what is self for one individual of a species is nonself for another.

Nicholson and Wraith express the express the view that the sorting of the T-cell repertoire involves the elimination of high and low to zero affinity anti-self cells; the residual intermediate affinity anti-self cells are somatically selected to function as the high affinity anti-nonself repertoire (Nicholson and Wraith, 2004). Given this, they ask, why doesn't the response to nonself mediate catastrophic autoimmunity?

They refer to this random high affinity recognition of nonself by intermediate affinity anti-self cells as TCR degeneracy and suggest that the potential for catastrophic autoimmunity is dealt with at two levels:

1. activation thresholds are tuned to be optimized in a way that limits "the biological cost of degenerate activation," and
2. the context of activation can adapt a given T-cell to be either a suppressor or an aggressor effector cell.

Consider two individuals of a species, A and B, and two antigens, AgA and AgB, that are encoded in their germlines as alleles. AgA is self for A and nonself for B, whereas AgB is nonself for A and self for B. How do the germline-selected properties of "tuning" and "context" allow the somatically selected immune repertoires of the two individuals, A and B, to define AgA and AgB reciprocally as self or nonself? The proposed semantic solution (Nicholson and Wraith, 2004) invoking "tuning" and "context" is neither necessary nor sufficient as "tuning" and "context" are identical in the two individuals, A and B.

Sercarz and Maverakis argue that autoimmunity can arise by molecular mimicry when a dominant determinant on a nonself-antigen induces T-cells which crossrecognize a subdominant/cryptic self-determinant (Sercarz and Maverakis, 2004). As the two determinants in question are mimotopes, they are either both self or both nonself. Being mimotopes, they cannot be one self and the other nonself to the immune system. They can only be one self and the other nonself to the immunologist. A cryptic determinant, even though it is encoded by the host germline is one that has never been encountered by the immune system and, therefore, is nonself to the immune system which has no way of knowing what is germline-encoded. The response, then, referred to by Sercarz and Maverakis is to nonself; no breaking of tolerance is involved, even if immuno-pathology were to be the consequence.

The structural problem of how the TCR anti-peptide site manages to signal upon binding what looks like such a diverse collection of peptides occupies, and justifiably so, a significant effort (Bankovich et al., 2004; Ford and Evavold, 2004; Holler and Kranz, 2004; Shih and Allen, 2004; Wilson et al., 2004; Wucherpfennig, 2004). However, it is evident that no matter how diverse or random the mimotopic array may seem to be to the immunologist, it is a singularity to the given anti-peptide paratope. There must be a set of rules or boundary conditions governing the diversity of a so-called "degenerate" mimotopic array or paratopic clan. This point was made previously (Shih and Allen, 2004).

There is widespread belief (Wilson et al., 2004) that cytotoxic T-cells (T_c) and helper T-cells (T_h) differ in that the degree of degeneracy of recognition by T_h is significantly greater than by T_c (i.e., the mimotopic array seen by the anti-peptide paratope of the average T_h is larger than that seen by the anti-peptide paratope of the average T_c). This belief is in need of rationalization as T_c and T_h use the same gene loci to encode their TCRs and require positive selection to tell them what is their effector function. Given that T_c require effector T_h to be activated, it is likely that the anti-peptide paratopic repertoire of T_c and T_h is the same in size and in the value of the Specificity Index.

Assuming greater degeneracy of T_h recognition, it is argued (Wilson et al., 2004) that autoimmunity is limited by two key factors: (1) compensation by fastidious activation requirements and (2) the rarity of presentation of peptide-class II MHC. As these two germline-selected factors limit equally the response to nonself, no discrimination between self and nonself is involved. Quite clearly, in order to make a self–nonself discrimination, the T-cell repertoire must be sorted based on specificity (defined by SI) not on degeneracy.

The collecting of distinguishable paratopes, epitopes and antigens respectively into paratopic clans, mimotopic arrays and crossreactive sets permits a heuristic analysis of specificity and its relationship to the self–nonself discrimination and regulation of class. Degeneracy does not challenge, nor is it a substitute for the concept of a Specificity Index upon which the self–nonself discrimination depends.

Acknowledgements

This work was supported by a grant (RR07716) from the National Center for Research Resources at the National Institutes of Health. This paper was written while Melvin Cohn was a visiting scholar at the Gulbenkian Institute, Portugal.

The hospitality and lively debates particularly with the Director, Dr. Antonio Coutinho, are gratefully acknowledged.

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