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The discussion with Jacques Miller: illustrating the limitations of pure empiricism

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In a general discussion,¹ I outlined the opposing views of the mechanism of the self (S)–nonself (NS) discrimination. Under debate here is the role of developmental time in determining the S–NS discrimination. Miller's position^{2–4} is that the developmental stage of the individual is not a key factor in determining the S–NS discrimination.

To make the problem precise, I have equated the S–NS discrimination with the sorting of the repertoire.^{5–9} For this continuing discussion, I wish the reader to consider the proposition that the sorting of the repertoire is both necessary and sufficient from an evolutionary standpoint to explain the S–NS discrimination. Although not explicitly stated by him, I doubt that today Miller would question the necessity for sorting. Therefore, our debate justifiably revolves around the question—is it sufficient? I might add that Miller's pioneering of the denial of a role for developmental time in determining that S–NS discrimination has become the dominant position of today (see Discussion Forum based on Cohn⁶).

Let me begin by considering the 'necessary' component.

The sorting of the repertoire requires the earlier sorting of the antigenic universe into self and nonself. This can only be accomplished as a function of developmental time. Bretscher,¹⁰ who has pioneered a good deal of the thinking in this field, refers to this as the 'historical postulate.' There is no need to repeat that logic here.^{5–7,9,11}

What is there about developmental time that is critical?

First, the naive or initial state cells must be born during embryogenesis under conditions in which they are inactivatable-only on interaction with ligand (Signal (1)). This can be a property of the naive cell (Lederberg model) or a property of the system (sufficiency or insufficiency of effector T-helpers (eTh), the Associative Recognition of Antigen (ARA) model^{7,11}) or both (not likely).

Second, there must be a period during ontogeny when all self and no nonself are present. Further, the state of tolerance is maintained only as long as self persists. During the period in embryonic life when the system dictates 'inactivatable-only,' there is an insufficiency of eTh and in the absence of Signal (2), interaction with ligand (self) results in inactivation of anti-self cells (Signal (1)). Around birth, when a sufficiency of eTh appears, the system becomes responsive. A responsive system cannot distinguish newly arising self from nonself. Any late arising self must have been ectopically expressed as a ligand for T-helpers in the thymus or the periphery, when the developmental window was open.^{7,11,12} This is presumably the role of *Aire* regulation of the expression of the self that is ectopically expressed in the thymus and peripheral antigen presenting cells.

The sorted repertoire is a steady state of two populations of cells. The anti-self cells on the pathway to inactivation constitute the autoimmune boundary and are the source of cells mediating autoimmunity when tolerance is broken. The anti-nonsel self cells turn over slowly and constitute the immune boundary, which is the source of cells responsible for protection against infection. This is the part that is 'necessary.'

Now let us turn to the question, is the sorting of the repertoire sufficient to explain 'natural' tolerance? To pin down the discussion, two terms should be clarified,^{7,13} see page 82 in Cohn.¹⁴

'Unresponsiveness' is the observation that an experimentally manipulated animal will not respond to an antigenic challenge to which it would normally respond.

'Tolerance' is the theoretical extrapolation of the observation of unresponsiveness to a mechanism for the sorting of the repertoire (that is, the S-NS discrimination).

It is essential to appreciate that the S-NS discrimination involves epitope-specific sorting of the repertoire. Further, the antigen-driven steps following activation are not elements in the sorting of the repertoire. They involve the regulation of expression of effector class, a process, which requires germline-selected steps, not a somatically learned or historical process.

With this as our background, I would like to evaluate Miller's arguments that sorting the repertoire as a function of developmental time is insufficient to explain the origin of tolerance.

I see nowhere in Miller's response⁴ a probing argument that developmental time is ruled out or plays no role in sorting the repertoire (that is, the S-NS discrimination). This was the thrust of his two papers^{2,3} that initiated this discussion. I, therefore, will deal only with the basis for his need to nickel-and-dime or tinker a solution to the sorting problem.

Negative selection is a part of the mechanism required under the ARA model to purge the repertoire of anti-self. This process is postulated to be initiated by the interaction of the T-cell receptor/B-cell receptor with ligand (Signal (1)). Miller argues that positive selection of T cells could not occur if negative selection were due to the delivery of Signal (1) alone. All models have resolved this conundrum. Under the Standard model it is assumed that the two processes occur in different affinity windows. Under the competing Tritope model,^{8,15,16} negative selection requires specific recognition of both the allele-specific determinant on major histocompatibility complex and peptide (restrictive recognition of peptide) in order to initiate Signal (1). Positive selection only requires specific recognition of the allele-specific determinant. This means that peptide (P) must be viewed as playing two roles. For negative selection, it is acting as a specificity element recognized by the anti-P site of the T-cell receptor. For positive selection, peptide is acting as a structural element necessary for the stability and conformation of the peptide (P)-restricting element (R) complex (PR). The observation of effects of P-sequence on positive selection are because of indirect effects on the expression of the allele-specific determinant.¹⁷ In any case, Miller's claim that there exists a contradiction between the ARA model and positive and negative selection is unfounded under all extant views of T-cell receptor behavior.

Miller's response to my addendum¹ has, oddly enough, greatly strengthened my argument. To make my point, I accepted his view and optimized the conditions under which the dendritic cell (DC) might be postulated to play a specific role in sorting the repertoire. If now, as Miller points out '*...any self-antigen that can be presented by an activated DC, can also be presented by a nonactivated DC,*' then we must ask whether the same holds for

nonself. As no mechanism is envisaged by Miller for DC to distinguish self from nonself, no relevance of his discussion of DC to the sorting question is apparent.

If nonactivated and activated DC present both self and nonself, then playing a frequency game with the ratio of tolerigenic-only to immunogenic-only DC or with the ratio of self to nonself peptide presented by a given DC is destined to go nowhere, either because tolerance will be broken or because the response to a pathogen will be inadequate. In this regard, the individual is under a steady state immunogenic nonself antigenic load that engages as much as 10–20% of its immune system. It is difficult to view activated immunogenic-only DC presenting both self and nonself as a minor population.

I might add that I have tried to solve this problem in the Miller framework by suggesting a way that tolerigenic-only DC present self-only, whereas immunogenic-only DC present self plus nonself. This would make for a viable space model (see page 143 in ^{ref.18}). However, this model is still dependent on a developmental time window.

Conclusion

If we accept Miller's argument, then the DC, because it cannot distinguish self from nonself, cannot contribute to the problem of how the repertoire is sorted (that is, the S–NS discrimination).

The next point involves the extrapolation of the observation of cases of unresponsiveness to the mechanism by which the repertoire is sorted (natural/normal tolerance). Consider Miller's examples. Autoreactive T cells are envisioned to be activated by self-antigens on activated DCs. He argues that if and when that happens, the activated autoreactive T cells will be deactivated by 'dominant' mechanisms of tolerance. He gives us two examples of dominant mechanisms of unresponsiveness, exhaustive immuno-nogenic stimulation and/or the induction of T-suppression (Tregs). What he neglects to tell us is (1) how these two mechanisms learn to distinguish self from nonself, and (2) how his interpretation of these two observations can be extrapolated to a mechanism of the S–NS discrimination. Does Miller propose that the random repertoire of T cells is sorted normally by exhaustive stimulation of self-reactive T cells with no autoimmune consequences, whereas nonself reactive T cells are stimulated to become biodestructive and ridding effector cells? Clearly this would be absurd because the cells have no way of knowing whether they are self- or nonself-reactive. They must be told (Signal (2)). The same holds true for T-suppressors (Tregs). My question, to wit, are T-suppressors (Tregs) sorted to be anti-self or anti-nonself, is not rendered '*out-of-order*' by defining an affinity range for their functioning. If within that range are T-suppressors that recognize either self or nonself, then attributing a failsafe function to them is without a rationale. If they are sorted to be anti-self, they can't regulate responses to nonself (contrary to fact). If they are sorted to be anti-nonself, they can't regulate the response to self. Using his two examples, namely, a viral infection, the response to which is regulated by induced T-suppressors (Tregs), or the induction of T-suppression (Tregs) in the thymus by CD8low Sirpa+ DC, it can be concluded that Miller's position is that the repertoire of functional T-suppressors, unlike all other T/B cells is unsorted (that is, function normally as a random population of anti-self plus anti-nonself). Consequently, given his view they can play no role in the S–NS discrimination, leaving me to wonder why he introduced that discussion. Miller's comment that my question is '*out-of-order*' because of his failure to have any idea of what role Tregs were selected to play can hardly be taken as an argument for a failsafe tolerance mechanism. All this having been said, T-suppression (Tregs) was not evolutionarily selected to play a role in determining the S–NS discrimination.^{6,7,19} It likely regulates the magnitude of the effector response. Negative selection, not suppression, sorts the repertoire.

When we consider the S–NS discrimination, we are dealing with epitope-specific events. The epitopes are defined as being self or nonself by the immune system, *not* by the immunologist. The immune system learns this discrimination consequent to a somatic process dependent on developmental time that sorts the antigenic universe into self and nonself. My point is that this process is necessary (presumably agreed upon) and sufficient (in dispute) to account for the sorting of the repertoire (that is, the S–NS discrimination). Germline-selected events or those that do not engage an epitope-specific distinction between self and nonself are not germane to this discussion. If, as Miller argues, all of my attempts to envisage a discriminatory mechanism for DC are ruled out, then his introduction of DC into the problem of the S–NS discrimination is an irrelevancy. The discrimination must rest with the eTh as postulated under the ARA model. Given an insufficiency of eTh anti-self, any T or B antigen-responsive cell anti-self interacting with its ligand anywhere or at anytime in the animal would be inactivated (Signal (1)), that is, negative selection. Auto-immunity is initiated by breaking tolerance at the level of the T-helper and the maintenance of that state. Autoimmunity is an eT-helper dependent phenomenon.

The constant problem in any attempt to confront complexity is the cry of oversimplification, and each immunologist has a pet example. Miller's example is my postulate^{20,21} that the presence or absence of the effector T-helper is the pivotal element in determining T- and B-cell tolerance and immunity. Why? Because *'there are multiple checkpoints in tolerization.'* Rather than telling us what the relevance of this is to our discussion, a review by Goodnow²² is cited in which the emphasis is on a variety of antigen-unspecific germline gene controls on growth and differentiation of antigen-responsive cells, T and B. Of course, these are important but not in the context of this discussion, as the so-called checkpoints regulate aspects of the magnitude of response being unable to distinguish self from nonself. There is, however, one aspect of the Goodnow review that is relevant, namely, his impassioned discussion of somatic hypermutation in B cells as being at the origin of autoimmunity. Although this deserves a balanced analysis in which his cited references are critically reinterpreted, here only this point need be made.

For a B cell interacting with a self-epitope (Signal (1)) to be activated, it must receive Signal (2) from an eTh that recognizes a peptide processed from that same antigen (Associative (linked) Recognition of Antigen, ARA). As there is a steady state of insufficiency of eTh anti-self P (the autoimmune boundary), the somatic mutants to anti-S would simply be inactivated (Signal (1)). It is because both Signals (1) and (2) are required for activation, that no B cell can be induced to effectors that, in principle, could not have been inactivated. Mutations to inducible-only, were they to exist, would then be quite informative. The one case of interest is the breaking of tolerance by a nonself antigen that shares one or more epitopes with self. This has a quite low (but not zero) probability of ever resulting in clinically manifest autoimmune disease for a variety of reasons.^{5,7}

If I may close on a personal note, it is quite intimidating to be told by our senior guru that I failed to consider recent work and that my contributions are unlikely to clarify an already complex subject. Even more intimidating is the galaxy of stars acknowledged to have provided helpful discussions. So, I close with this thought in my defense. If, by any chance, this community of helpful discussants were of one mind on this subject, then it would be safe to say that no one of them has been doing any thinking.

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