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Analysis of Paris meeting redefining the "Self" of the immune system

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Abstract Some ideas because of their intuitive appeal never die by neglect and survive because they are not amenable to experimental disproof. They can only be evaluated by weighing them against competing ideas and by invoking a credibility factor when used to explain observation. Most scientists would recommend ignoring such ideas, yet there is much to be learned by engaging their proponents in debate. The immune system viewed as an idiotype network, and its tweaking by the new school of "contextualists" is an example of such an idea. As chance would have it, the supporters of this idea gathered in a meeting, thereby permitting a cumulative analysis of this conceptualization. The goal of this essay is to compare the views of each of the speakers in light of a competing theory with the hope that a better understanding of immune responsiveness will emerge.

Keywords Self–Nonself discrimination · Idiotype networks · T-suppression

On June 23, 2014, a group of scholars gathered at the Sorbonne for a conference entitled, "Redefining the Self: Biological and Philosophical Perspectives." It is because they were of essentially one mind that this analytical commentary is merited. Consensus by experts using the Delphi method does not always yield the truth. The theory that the immune system is constructed as an idiotype (Id) network was a major theme for two decades between the 1970s and 1990s. It then disappeared from the immunological lexicon largely because it had little heuristic value, not because it was or could be disproven by a decisive experiment. Surprisingly, 20 years later the concept has resurfaced in this meeting as a metaconcept bolstered by input from philosophers, historians, computer modelers and Jernerian immunologists. Their single mindedness was reflected in the ignoring of a competing, contemporary conceptualization. I, therefore, felt that an evaluation of this resurgence in light of the Associative Recognition of Antigen (ARA) or two-signal theory [1–4] would be productive. The hope is that a new synthesis might emerge [5]; consequently, it is important to engage the new generation of idiotype network contextualists in debate. A probing book dealing with the idiotype network era has been written and should be read by anyone interested in the pathway that certain ideas take in the unfolding of the understanding of that which is [6].

This conference can be accessed at these two sites: (http://iridia.ulb.ac.be/bersini/Self-NonSelf/) and (http://thomaspradeu.com/researchrecherches/conferences/conference-redefining-the-self).

Although my analysis can stand on its own, it would be helpful if the presentations of the participants were viewed before reading this commentary. I feel justified in dealing with the nitty–gritty of these two opposing theories rather than with the more attractive and lofty approach discussed by the participants. I will concentrate on the analysis of the contributions of those speakers who dealt more or less directly with the "Self" of the immune system.

The central issue revolves around the meaning of the term "Self" as viewed by its role in immune function. The reason is that the father of idiotype networks, Jerne, argued that the immune system sees only itself, a dominant position at the meeting. This was not just erroneous; it was

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irrational as it left as unsolved the central problem of what is not-to-be-ridded (NTBR) and what is to-be-ridded (TBR). The "Self" that everyone claimed to redefine was, in large measure, that which is referred to by immunologists as the "Self" defined by the Self(S)–Nonself(NS) discrimination. Admittedly, the term "Self" is a poetic and allows all manners of skillful tergiversations, but it is now so embedded in the immunological literature that to communicate demands its use and, consequently, its misuse. Langman and I [7, 8] had tried unsuccessfully to substitute the "not-to-be-ridded (NTBR)–to-be-ridded (TBR)" discrimination but that too had its limitations, not the least among them being the failure to communicate. A subject that advances almost entirely by empiricism needs ambiguity to sustain it and the description, "S–NS discrimination," fills that bill. The only way to deal with the unlicensed use of the term "Self" is to define it in a way that limits its meaning, yet is still necessitated by any attempt to understand the immune system.

In order to define the "Self" of the immune system, the ground rules for a constructive dialogue must be established. The vertebrate immune system is the product of evolutionary selection and functions in a whole organism, the boundaries of which can be designated in many ways as long as they are, at least potentially, within reach of its immune system. The only fruitful conceptualizations have been those founded on evolutionary selection. Further, any analysis of the system in a way that increases understanding must be based on scientific methodology, which, for our discussion, means the formulation of a theory of mechanism and function that is predictive and potentially disprovable. Description of an observation is a "fact" whether it be a multi-step input to output of a black box or a singular step from *a* to *b*. The observation can be described in words or mathematical language or a diagram; it remains a "description" and has limited value in increasing understanding, until interpreted in a way that can be challenged empirically. It is possible to describe everything and understand nothing. Much of the science of this meeting was descriptive and as such could not deal heuristically with the immunological "Self." The philosophical extensions giving meaning to the term "Self" are food for thought but of questionable relevance because they are based on a "Self" as viewed by logic and imagination, tools not available to the immune system. They are outside of the bounds of scientific methodology in that, being untestable or not disprovable, only debatable, they do not increase our understanding of the mechanisms controlling the response behavior of the immune system. If we are going to exchange ideas in a productively meaningful way on how the immune system works, then we must shuttle back and forth from theory to experiment and use thinking based on description or analogy only as a possible pathway to finding a testable interpretation.

While the black box approach (input antigen, output effector response) has a degree of merit and was a linchpin for many of the participants, it is too deceptive and in the end unproductive because it allows too many solutions, most untestable. There are many ways for the immune system to become specifically unresponsive but only a few ways, possibly only one, to make a S–NS discrimination. It becomes important to crack open the box and look inside where this discrimination takes place. When one does that, an adaptive immune system is revealed that is made up of at least three modules, each with its own logic and data set [9–11]. These can be linked to account for the systems behavior of the immune response.

> Module 1—the somatic generation of a combining site (paratopic) repertoire that is random with respect to the recognition of Self (S) and Nonself (NS)

All somatic processes are built on the products of germline selection, which in the end have to be related and compared. The somatic generation of and selection on the repertoire is the central and defining element of the adaptive immune system. The germline generation of and selection on a repertoire expressed earlier in evolution is the province of the innate immune system. Vertebrates express both systems. Admittedly the terms, "adaptive" and "innate" are poorly chosen but there they are, like "Self." The repertoire of the innate immune system distinguishes Nonself from the Self-of-the-species; the repertoire of the adaptive system distinguishes Nonself from the Self-of-the-individual, reflecting germline versus somatic selection. We will return to this distinction later as the "Self" under discussion at this meeting was that defined by the adaptive, not the innate immune system

> Module 2—the sorting of the somatically generated paratopic repertoire into those specificities which, if functionally expressed (anti-S), would *debilitate the host* and those which, if not expressed (anti-NS), would result in the death of the host by trauma (e.g., infection)

The effector output of the immune response is, in large measure, biodestructive and ridding. Therefore, the anti-S must be purged from the repertoire leaving as the residue, anti-NS. This defines the metaphor, S–NS discrimination, unambiguously and with precision, as the mechanism that sorts a random paratopic repertoire (Module 1) into anti-S and anti-NS. Under the ARA model, all "Self" must have been present as a functioning tolerogenic ligand during a developmental time window when the only response of the newly arising cells that recognize it is their inactivation. Further "Self" remains "Self" only as long as it persists. Whether the inactivation is by negative (deletion) or

positive (suppression) selection is another question, which we will face later. While all S is autogenously derived, not every autogenously derived component is S to the immune system. A ridding attack on autogenously derived components that is salutary (e.g., housekeeping) [12] is NS to the immune system. This is why Langman and I proposed the NTBR–TBR discrimination as a way to eliminate the worst ambiguity of the term "Self." "Auto reactivity" is *not* equivalent to "autoimmune disease" nor are they directly related. Further, it should be appreciated that the purging of anti-S occurs via binding epitope-by-epitope; the activation of antigen-responsive anti-NS cells to effectors occurs antigen-by-antigen (i.e., it is multi-epitopic). Module 2 is what this meeting claimed to be about because that is what defines immunological "Self." Treating "Self" as a purely semantic problem does not clarify the mechanism of sorting. As a mnemonic, Self is prior and persistent, whereas Nonself is posterior and transient.

> Module 3—the induction of an appropriate effector function to rid the NS, while minimizing innocent bystander pathology

The decision steps in Module 3 are germline selected, unlike Module 2 where the decision step for the adaptive immune system is somatically learned de novo by each individual. Module 3 is the least well understood because it is so highly multi-factorial and full of easily misinterpretable activities. Most of the phenomenology discussed by the participants encompassed elements of Module 3, not Module 2, and, therefore, does not relate to the S–NS discrimination as defined by immune behavior.

These three modules provide a pathway to output that allows a pragmatic and constructive way to analyze the functioning of the immune system. They have been detailed and justified [9–11] requiring no further commentary on my part here. Module 2, the subject of this symposium, has been dealt with not only by Id-network theory but also by the Associative Recognition of Antigen (ARA) model [10, 13, 14]. The concatenation of modules provides one way to approach a computer model of the immune system.

The speakers at this meeting can be divided into three categories; those directly interested in the immune system as a functioning biological entity; those interested in the methodological impacts of other fields (physics, computer sciences, neurobiology, social behavior, etc.) on the immunologist's analysis of the biology of immune responses; and those interested in the impact of history and philosophical thinking on the immunologist's faltering interpretations or usage of the term "Self" over the past century. Presumably, everybody's goal was to redefine the term "Self." Ironically, the only one not interested in redefining "Self" was the immune system. To it, "Self," as defined by Module 2, seemed clear enough.

Now let us turn to specific comments on the insights of each speaker, beginning with Category 1, to whom most of this commentary will be directed.

Matzinger gives us her steadfastly held intuitive view of the immune system namely that the sorting of the repertoire (Module 2) is of no relevance; only its output (Module 3), responsive or unresponsive, is germane. She argues that the output of the black box can be regulated by a "danger/non-danger" switch. As the theme of the meeting was "redefining self," I assume that Matzinger feels that the need to redefine Self is superseded by the danger/non-danger switch. Of course if all Nonself were dangerous and all Self were nondangerous, this would place the S–NS discrimination at the level of Module 3. Is there any way in which a Module 3 germline-selected danger–nondanger discrimination can be substituted for a Module 2 somatically selected Self–Nonself discrimination?

A somatically generated random paratopic repertoire cannot be sorted by the recognition of germline-encoded self or nonself markers ("danger" being only one of many) because they cannot explain why, what is Self for one individual in a species is Nonself for another [15].

Two points need attention:

First, the sorting of the paratopic repertoire requires the prior sorting of the epitopic universe into S and NS [11]. This means that a theory as to how this latter is accomplished is at the foundation of any model of the S–NS discrimination. Second, there is no physical or chemical property of antigens as classes that can be used by the immune system to accomplish the sorting; it must be learned de novo by each individual. The somatically selected adaptive immune system is individual-specific.

The danger postulate does not permit the conclusion that the S–NS discrimination is an aside or that the sorting of the somatically derived repertoire into anti-S and anti-NS can be accomplished by the germline-selected recognitive elements of Module 3. When I took danger theory out of the realm of Module 2 and placed it as an element in Module 3, it took on a central role as a factor in the decision functions associated with the expression of effector function [16]. My point is that, taking a black box view, it may be "possible to define an immune system based on the idea that its *primary function* is to discriminate between things that do damage and things that don't" as Matzinger claims. However, in the absence of Module 2, "anti-Self" ends up doing damage. An unsorted repertoire, in this framework, is always in the presence of a ubiquitous "danger" (the steady-state immunogenic load) and therefore would inevitably trigger autoimmune disease and innocent bystander pathology. Specificity of the immune system's receptors with respect to S- or NS-epitopes cannot be ignored as it is the necessity to make a S–NS discrimination that is the evolutionary selection pressure

driving the specificity of its recognitive elements. Lastly, no immunologist has been "working with the idea that the primary function of the immune system was to distinguish between self and nonself." The term "primary function" permits a purposeless gross distortion repeated during the conference by several of the speakers. In any case, for over 40 years now, the S–NS discrimination has been defined as Module 2, the sorting of the paratopic repertoire. Recognition of nonself markers, and many have been proposed during that time (e.g., danger, pathogenicity, localization, context, tuning, discontinuity), is germline-encoded and cannot give rise to a mechanism for the sorting of a somatically derived random paratopic repertoire any more than Burnet's "self marker" was able to do. A somatic learning or historical process based on developmental time is a default assumption. These nonself markers may have been used by the innate system during the evolution of its germline-selected paratopic repertoire, or by Module 3, but they cannot be used by the adaptive system to sort its somatically derived repertoire (Module 2). Further, the basic assumption that all NS is dangerous, whereas all S is nondangerous, has been experimentally ruled out [17]. The transcription factor *Aire* controls the ectopic expression of a subset of peripheral Self-antigens in thymus. *Aire*-negative mutants that fail to express this peripheral subset as tolerogens in thymus succumb to autoimmune disease. Using a mouse construct in which the expression of *Aire* was controlled by the experimenter, it was demonstrated that the shutdown of *Aire* perinatally resulted in an autoimmune attack on this peripheral Self subset, whereas shutdown after a few weeks and into adulthood failed to elicit an autoimmune response. Two conclusions can be drawn. First, all nonself-marker theories are ruled out (e.g., danger, pathogenicity, discontinuity). Secondly, strong support for the developmental time model for the establishment of tolerance was demonstrated. Further, a reasonable assumption would be that this subset is composed of delayed expression Self-antigens [18].

Lastly, not only as a point of pure semantics but also for understanding, the immune system can, in principle, detect tissues that are stressed, harmed, traumatized, intoxicated, etc., but it cannot, in principle, detect the potential of an agent to stress, do harm, traumatize or intoxicate (e.g., danger, pathogenicity, toxicity).

The answer then to Matzinger's artful question, "Does the immune system really care about Self vs nonself?" (Module 2) is, of course it does, just as much as it cares about "stressed vs nonstressed" tissues (Module 3). The two "cares" are not either–or, mutually exclusive, unless one takes the black box approach by leaving what is in the box as not worth "caring about." If there is a "Danger Model" that is predictive and disprovable, let us have it stated. "Danger," viewed as an element permitting one to bypass the need for a mechanism that sorts the repertoire, is all dressed up but has nowhere to go.

The "discontinuity" proposal of Pradeu, Jaeger and Vivier, is, like the one of Matzinger, untenable as a sorting mechanism for the adaptive repertoire and for the same reasons, but it does permit further discussion. As pointed out above, the repertoire of the "innate" immune system is determined by germline selection. Clearly, any mutation that resulted in the recognition of a self-of-the-species component would be eliminated by the death of the offspring in a mating between that mutant and an individual expressing that allelic species-self component. Evolution had two ways to deal with that situation, either to add a discriminatory inhibitory control mechanism if that mutant specificity is too valuable to lose or to inactivate that mutant receptor. The specificity of NK cells illustrates the former; the fact that animals expressing the innate defense system only (e.g., invertebrates or $RAG^{-/-}$ vertebrates) accept grafts from other members of the species presupposes the latter. As a consequence of its limited paratopic repertoire, the innate defense system was selected to recognize epitopes that are common to many different targets. The "Self" under discussion here is defined by the adaptive, not the innate system. Its repertoire is somatically generated and random with respect to Self and Nonself. Only a somatic learning mechanism can sort that repertoire into anti-S and anti-NS because "Self" is individual-specific. Recognition of "trauma" (danger) or "discontinuity" is germline-selected properties that might well be elements of the innate system or Module 3, but they in no way impact on a definition of Self by the adaptive system. Module 2 requires a somatic learning process.

It should be clear that Self-components have been evolutionarily selected upon to function in the physiology of the organism; they have not been selected to escape the immune system, except possibly for the special case of privileged sites (see later). It is the immune system that has been selected upon not to attack these components. Any theory (and none have been proposed) of the sorting of the adaptive repertoire by germline recognition of such signals as danger, discontinuity, pathogenicity, etc., must involve a postulate as to how the innate system communicates these signals to the adaptive system permitting the latter to sort its repertoire into anti-S and anti-NS. As a closing point, it is possible to reinterpret "discontinuity theory" as a restatement of ARA theory, namely that all Self is prior and persistent, whereas Nonself is posterior and transient because it is ridded, but I am certain that the authors would not accept this translation.

Twenty-five years ago in a truly searching essay [19], Coutinho tried to reconcile the inadequacy of idiotype (Id) network theory with what he conceived as clonal selection theory by proposing the existence of two functioning

systems operative in the individual. His presentation at this meeting involved a somewhat different view. Stewart (see later) develops the two immune system theory. Coutinho gives us a multifaceted, sometimes internally contradictory survey of immune behavior. He begins with the assumption that the paratopic repertoire is transcendental ("complete"), and therefore, every conceivable shape (epitope) is recognized by it. This leads to a generalized concept of "idiotype networks" in which he melds the immune system into the functioning of all other systems of the individual, nervous, circulatory, digestive, endocrine, etc., which it recognizes and with which it establishes a functional regulatory ensemble. This leads him to introduce a new word, "immunosomatics," the study of the role of immune activity to protect other physiological systems (i.e., what others call *homeostasis* or *integrity*). Given his downplay of negative selection, a transcendental paratopic repertoire would interact with every available epitope in the host, thus making an immunoglobulin (Ig) idiotypic network a close duplicate of what is nonidiotypically engaged by the repertoire. In the absence of a sorting of the repertoire by negative selection, recognition of the combining sites (idiotypes) of the BCR/TCR and secreted Ig would be dwarfed by the recognition of all the other epitopes on these same molecules. In principle then, Coutinho redefines "Self" as any autogenous entity that is both recognized by and recognizes the combining site elements of the immune system as part of a physiologically relevant regulatory network; what is "Nonself" is either not distinguishable from "Self" or is a question left open, as is what is anti-NS. A functional "complete" Jernerian network is clearly untenable as a generality. In any case, Coutinho argues a *non sequitor*, namely that the shutting off of the effector response to Self requires a Self-specific suppressive mechanism. This begs the question, what would be the need for an independent suppressive mechanism (Treg) anti-S given a system that both ignores a distinction between S and NS and is claimed to be auto-regulatory? Left unanswered in this framework are what regulates the normal response to NS, what is the role of T-helpers, what is the signal for negative selection and who controls what is exported to Module 3, the biodestructive and ridding output? Arguing that the perturbations of the network's steady state define "Nonself" is a crutch, not a theory. It is the mechanism implied by his conceptualization that comes into question. As Langman and I have analyzed this internally ambiguous framework [20–23], here I would only like to comment on the enormously popular subject of suppression as the mechanism for the sorting of the repertoire. We have repeatedly argued that suppressor T-cells (Ts), referred to today as Tregs, play an essential role in regulating the magnitude of the effector response (Module 3), not in the decision step that sorts the repertoire (Module 2) [24–26].

In order to avoid ambiguity, we should distinguish two sets of terms used during the conference, "degeneracy" from "multi- or poly-reactivity" and "tolerance" from "unresponsiveness."

"Degeneracy" is defined from the point of view of the epitope. A family of paratopes that are chemically distinguishable and that signal consequent to an interaction with a unique or given epitope, are defined as a degenerate set (i.e., chemically distinguishable entities that perform the same function [27]). "Multi- or poly-reactivity" is defined from the point of view of the paratope. A given paratope that can recognize as signaling a family of chemically distinguishable epitopes that are random with respect to the property, Self or Nonself, is defined as a "polyreactive paratope." This has paradoxical consequences as we will see.

"Unresponsiveness" is the description of an observation, usually experimental, whereas "tolerance" is the extrapolation of that observation to a theory of the mechanism used normally to sort the adaptive repertoire. It is the loose use of these two terms that fueled much of the discussion at this meeting.

Can suppression be the mechanism used by the S–NS discrimination to sort the repertoire (Module 2)? The answer is "totally unlikely" for reasons both experimental and conceptual. [Note that I will use Ts, not Treg, to symbolize the suppressive function. Treg is one more badly chosen term because it is not sufficiently circumscribed. For example, T-helpers (Th) are just as "regulatory" as T-suppressors (Tregs)].

Some of these reasons are:

1. For epitope-responsive or initial state(i)-cells anti-S to be inactivated by suppression, the Ts-cells, unlike all other T-cells, must be sorted to be anti-S and, of course, themselves not be suppressible. To accomplish this sorting, Coutinho postulates three thresholds based on increasing TCR avidity that operate in thymus. In order to discuss his proposal, I must ask for poetic license to give his formulation some detail. The avidity being considered is determined by the cooperative binding of the TCR to Self-P and to an allele-specific determinant expressed on an MHC-encoded restricting element (R) to which the Self-P (Ps) is bound [28]. Threshold 1 separates death-by-neglect of T-cells that fail to recognize a host allelic determinant on R from positive selection of those T-cells that do recognize one. At the same time, the restriction specificities of the survivors are established, so is function (helper or cytotoxic). The cells at this point are sorted with respect to R. Threshold 2 separates positive selection

for restriction specificity from that of the induction of Ts anti-S, a subset of RII-restricted cells. These must, within the fixed avidity window for Ps-RII, recognize at least one S-epitope per S-antigen, and this latter must be displayed in thymus. In order not to miss too many S-antigens, the Ts-population must see an average of >7 epitopes per S-antigen ($e^{-7} \sim 1$ per 10^3 antigens missed at any moment in time). These Ts provide the only mechanism for peripheral tolerance. It takes effector recognition of one peripheral S-component to debilitate an animal, making this correlate unlikely. Further, Ts (RII-restricted) must function primarily by inhibiting, ligand specifically, Th/c, which do not express RII. This requires that they function via an APC, which, if specificity is to be accounted for, the configuration of the iTh/c-APC-eTs needs spelling out, as does the state of differentiation of the interacting T-cells. At the level of Threshold 2 avidity, the other classes of restricted T-cells, helper (Th) and cytotoxic (Tc) that recognize "Self" are, in fact, negatively selected and the residue is peripheralized as the functional anti-NS repertoire. Uniquely for Ts, the selection by Ps-RII means that no Ts anti-NS exist as the suppressive phenotype is induced by interaction with thymic self-ligands, Ps-RII. Further, it would be expected that negative selection of anti-Ps-R would be initiated at avidities above Threshold 1 when restriction specificities and functions are established. If this occurred, there would be no anti-Ps-R cells upon which Threshold 2 induction of Ts anti-Ps-RII could operate. Threshold 3 separates induction of Ts anti-S from the deletion of all remaining Th/c-cells anti-S, which are those of highest avidity. Coutinho's postulated Threshold 3 is gratuitous as an assumption of deletion of Th/c anti-S with avidities above Threshold 1 would have been sufficient. There are many questions concerning this formulation based on the different selection pathway of Ts and all other T-cells that are unanswered. For example, if the Th and Tc phenotypes anti-NS were determined at avidities above Threshold 1, what would be the source of precursor cells upon which Threshold 2 induction of Ts anti-S operates? Further, his postulate that deletion (negative selection) operates on Th/c anti-S for all avidities above the Threshold 3 while positively selecting for Ts anti-S below it challenges, in part, his conclusion that "clonal deletion is not sufficient (if at all necessary) to ensure natural tolerance." Lastly, the peripheralized anti-NS repertoire of Th and Tc is of all avidities above Threshold 1 while that of Ts is in the avidity window between Thresholds 2 and 3. This needs quantitation to show that it is viable in permitting function.

2. The Ts, postulated to be thymically selected as anti-S within a fixed window of avidities, cannot regulate the response to NS and yet display any degree of specificity. In spite of this, many examples of Ts regulation of the magnitude of the response to NS have been published (e.g., [29]). Unavoidably then, there exists only two choices, either the Ts repertoire is thymically selected by purging anti-S leaving a residue anti-NS as is the case for all other T-cells or Ts are unselected with respect to the property anti-S or anti-NS. Under either assumption, Ts cannot be used to sort the repertoire (i.e., cull out anti-S leaving anti-NS specificities). Given this, what do the Ts do and how do they know when to do it based on their postulated superimposition on an autonomously regulated humoral idiotype network?
3. "Fail-safe": "tolerize one, tolerize them all" is in contradiction with fact. Unlike the innate system, which accepts grafts from individuals of the same species (i.e., the mating pool), the adaptive system rejects them. This rules out suppression as the mechanism of tolerance for the S–NS discrimination by the adaptive immune system. The cited experiments of Le Douarin and colleagues do not demonstrate dominant unresponsiveness or suppression as the mechanism for sorting the repertoire. Her findings are totally compatible with the Associative Recognition of Antigen (ARA) model of negative selection given an *Aire* controlled ectopic expression in quail thymus of a delayed expression peripheral self-antigen in quail wing resulting in the deletion of its recognition [11, 18]. Chicken thymus does not express this component; quail, as well as the chimeric chicken/quail thymi, do express it. The target of the rejection was never identified; if it had been, an experimental resolution of this interpretation would have been possible.
4. Coutinho's assumption that Ts anti-NS do not exist due to lack of thymic induction of that phenotype would leave the immune system with no feedback control of the biodestructive and ridding effector output directed against NS (Module 3). In this situation, the immune system is predicted to and, as Coutinho illustrates using the IPEX and *Scurfy* mutations, does light up like a Christmas tree resulting in both innocent bystander and autoimmune pathology. The observed bystander tissue targets would depend on their sensitivity to destruction by the spilling over of the immune effector mechanisms giving the appearance of specificity. If Ts anti-NS are generated peripherally from other classes of presorted T-cells anti-NS, then thymic selection for Ts anti-S becomes gratuitous as the consequence would be functionally indistinguishable from no selection.

5. Lastly and more subtle, the theme that sews together the contributions of others and voiced explicitly by Coutinho is the idea that the network of interactions is mediated by Ids and anti-Ids rather than by antigens and anti-antigens. As discussed elsewhere [30], the assumption upon which it rests, namely that the TCR/BCR repertoires are essentially transcendental ("complete") and therefore, *per force* are functionally and totally interactive with themselves, is a mirage. Further, an idiotype network is only possible for humoral antibody (including the BCR); it is excluded for MHC-restricted T-cells (i.e., the TCR) as we will see later. Rather than repeating my reasoning here [20–23], I will try a new tack, illustrating my argument using the TCR [31]. Its recognition of peptide is easier to describe, but the line of thought directly extrapolates to the ligands for the BCR.

The ligand for the TCR is an epitope on a peptide (P) of roughly 10 amino acids presented in the groove of an MHC-encoded restricting element (R). Of the 10 amino acids, only 5, at maximum, are available in epitopes for recognition by the TCR anti-P site. The rest are buried in the groove that anchors the peptide. Given that the unit of recognition is an amino acid side chain, this means that the maximum peptide (P) repertoire available as a ligand for the TCR anti-P site is 20^5 (3.2×10^6). Assuming that the repertoire of TCR anti-P sites has individual members that can be signaled by interaction with either 1 or 2 or 3 or 4 or 5 amino acids of the peptide, then from the point of view of the TCR, it is absolutely unispecific, not polyreactive. For example, at one extreme, consider a TCR that specifically recognizes as a signaling ligand one given amino acid in a fixed position in the peptide. From the point of view of the immune system, that given TCR has the potential to bind 20^4 different peptides, but from the point of view of the immunologist it can bind 20^9 different peptides. As the point of view of the immunologist was not evolutionarily selected, the maximum number of peptides that can interact to signal via this given TCR is 20^4. Now at the other extreme, consider a TCR that requires as a signaling ligand the recognition of 5 specific amino acids. From the point of view both of that TCR and of the immune system, it would be unispecific. Consequently, polyreactivity is defined as the potential number of recognizably distinct peptides that a given TCR can entrain. The family of TCRs, each absolutely unispecific from their own point of view, ranges in polyreactivity from maximally 20^4 to minimally 1. This has as a consequence that negative selection by S would skew the functional anti-NS repertoire toward lower levels of polyreactivity but would not eliminate it. Simply put, the probability that a TCR of high polyreactivity will see an S-peptide and be deleted is greater than it is for one of low polyreactivity. Clearly, a TCR that recognizes only one amino acid is certain to encounter it in an S-peptide and be deleted. A symmetrically opposite picture applies to the Ts anti-S that are postulated by Coutinho to undergo positive selection in thymus by S. This selection would skew their repertoire toward higher polyreactivity. The TCR of a Ts that recognizes as signaling one amino acid would have the potential to entrain and be signaled by 20^4 peptides most of them NS. If Ts-cells regulate the response to S, they must do so by shutting it off. Their more highly polyreactive TCRs would have a greater probability of seeing NS than those of low polyreactivity and will therefore turn off the response to NS, a lethal situation. Polyreactivity, because it is random with respect to the property anti-S or anti-NS, limits the regulation by Ts to responsiveness to NS. If polyreactivity obtains, then Ts cannot be selected to be anti-S. The two effector activities of Ts are antithetical. All responses to S must be completely turned off, whereas responses to NS must be finely tuned, certainly not shut off. Consequently, I conclude that the repertoire of TCRs used by Ts must be negatively selected as are all other T-cells to be anti-NS and have limits imposed on them as to how they regulate the magnitude of the response to NS (Module 3). They play no role in the learning mechanism of the S–NS discrimination (Module 2). The immunologist can manipulate the system so that effector T-suppressors (eTs) shut off the response to an S-antigen that is behaving as an NS-antigen during an autoimmune disease, but such findings cannot be extrapolated to the normal mechanism of the S–NS discrimination. The pathway for the breaking of tolerance at the level of the Ts is an interesting question that is an aside here. The conclusion is that negative selection (Signal 1) is an imperative for Module 2 for both theoretical and experimental reasons.

Coutinho points out that each TCR is highly polyreactive in that it can be signaled by interaction with a million distinct peptides. This illustrates the ambiguity pointed out in my above discussion. If the peptide anchored in the restricting element is on average 10 amino acids long, then, from Coutinho's point of view, a given TCR that recognizes as signaling one amino acid has the potential to entrain 20^9 (5×10^{11}) distinct peptides. The immune system via the anti-P site of the TCR can maximally see as signaling five amino acids of the ten total, so that from its point of view that given TCR has the potential to entrain 20^4 (1.6×10^5) distinct peptides. From the point of view of the immune system, the paratopic repertoire is capped at 20^5 (3×10^6), certainly not transcendental. As detailed elsewhere [31], the sorting of the paratopic repertoire by S-driven negative selection simplifies this picture enormously. The claim that "multi-reactivity necessarily results in a V-region network as experimentally demonstrated" is

a matter for debate, if we are considering function, because the data have been explicable in other frameworks [14, 30]. It is the assumption of transcendentalism, not poly/multi-reactivity, that becomes the problem and I will return to that question after discussing the vision of Cohen.

This first-order analysis of the TCR repertoire needs to be refined by incorporating the additional limits imposed by the requirements for anchoring. Further, the anti-NS repertoire is in a steady state requiring correction of what is observed at any moment in time by the half-life of its turnover.

Lastly, the proposals of Burnet and Lederberg should not be confused. Coutinho points correctly to a contradiction in Burnet's thinking, namely "the 'time problem': animals are tolerizable only in development, (tolerant to antigens that are present in development) but produce new lymphocytes throughout life." However, this does not apply to Lederberg [32] who explicitly stresses both that new lymphocytes are produced and that his model for a S–NS discrimination functions, throughout life. This contradiction originates with Jerne whose idea, already questionable in 1955 [33], Burnet just copied [34, 35], after adding a brilliant insight, namely the putting of Ig as a BCR on B-cells. However, I do not wish to confront Coutinho's view of history but rather to stress that this is not an argument against a "negative definition of immunological self" as defined by the ARA model [13].

Cohen treats us to a Gargantuan wealth of data showing that many autogenously derived components can be recognized and ridded by the immune system without any pathology, referred to as "autoreactivity," an extension of the Avreamas finding [36]. Some of these autogenously derived components are, in a totally different context, occasionally targets of ridding by the immune system with serious pathology, referred to as "autoimmune disease" or "autoimmunity" for short. From such data, both Cohen and Coutinho conclude that "autoreactivity is necessary in order to prevent autoimmunity," a conclusion far from likely, and in any case, has never been properly developed as a testable theory.

In terms of ARA theory, "autoreactivity" is to "autoimmune disease" what apples are to oranges. They arise by entirely different mechanisms; autoreactivity is salutary and selected for, whereas autoimmune disease is debilitating and selected against. Autoreactivity arises after the developmental time window closes, and the system is responsive; birth is a convenient but imprecise marker of closure of the developmental time window as it varies considerably in different species. The precise marker is the appearance of a priming level of effector T-helpers (eTh). All individuals express autoreactivity, which is, in essence, good housekeeping [2, 12, 37]. Autoimmunity, an infrequent condition, is a debilitating disease and requires the breaking of tolerance. So the question for ARA theorists is, under what circumstances or in what context might a normally autoreactive (housekeeping) target behave as an autoimmune target (the Cohen/Coutinho conclusion)? Network theorists have not given us an acceptable rationalization. After all, the immune systems of all individuals display autoreactivity; few display autoimmunity. Housekeeping targets are extracellular and ridded both by the innate system and by an adaptive antibody response, which cannot simultaneously mediate autoimmunity to them. Autoimmunity to housekeeping targets is expected to be cell-mediated as they are most often intracellular entities until released by the necrosis of cells. Antibody to housekeeping targets, which is selected as salutary, might occasionally establish innocent bystander pathology, not autoimmunity.

The existence of autoreactivity is not evidence for either the view that the immune system is self-oriented or for the existence of a functional idiotype network. Autoreactivity is readily explained by the ARA model. When the developmental time window is open (e.g., during embryonic life), cells die by apoptosis. The apoptotic granules are taken up by the innate system and phagocytized without their contents being exposed as a steady-state interaction with the newly arising adaptive system (i.e., one lacking in effector T-helpers (eTh), tolerizable-only). When the window closes and the immune system becomes responsive (i.e., the appearance of primer eTh anti-NS), in addition to apoptosis, cells die by necrosis spilling their contents to interact with the adaptive system as NS, resulting in a ridding response that is a steady state throughout life. This is the origin of the salutary housekeeping function that is antibody-mediated. B-cells do not normally respond effectively to antigens that are intracellular and presented on the cell surface as peptide bound to MHC-encoded restricting elements (R). For example, they do not respond to minor histocompatibility antigens like H-Y. B-cells respond to epitopes on intact macromolecules whether they are in solution or extractable by the BCR from the cell surface, because normal induction of B-cells to effectors requires BCR-mediated uptake and processing of the antigen for recognition by effector T-help (eTh). B-cells acting BCR-independently as APCs, along with professional APCs, can take up and process these effete housekeeping products for presentation by Class I and Class II MHC-encoded restricting elements (RI and RII) required for the induction and effector function of cytotoxic, suppressor and helper T-cells. So the question becomes, how can we visualize and experimentally reveal the conditions under which a normally intracellular housekeeping target might become a cell-mediated target for autoimmunity?

All cells display their intracellular proteins as self-peptides (Ps) presented as Ps-RI complexes, whereas only a

small population, mostly APCs and B-cells, express them as Ps-RII complexes. Ps-RI complexes are potential targets for cytotoxic T-cells (Tc). However, normally the initial state iTc-cells that recognize Ps-RI complexes are inactivated (Signal 1) because they arise in the presence of Ps-RI on a cell that lacks the RII required for eTh delivery of Signal 2. In addition, there is an insufficiency of eTh anti-Ps-RII (source of Signal 2) required to induce them to effectors (eTc). The eTh have undergone the sorting process and are therefore anti-NS. Induction to an eTc anti-Ps-RI requires the presentation of the given Ps-RI on an APC that is activated by an RII-restricted eTh. As all cells display RI, whereas few express RII, in the competition between activation and inactivation of iTc, tolerance of S dominates.

In order for a housekeeping target (h), normally dealt with by humoral antibody (Ig), to become a target for autoimmunity due to cytotoxic T-cells (Tc), several conditions must be met. First and foremost, functional RI-restricted iTc anti-h cells must be present in, or be amplified to, a sufficient concentration. This is not expected because intracellular proteins are processed and presented by RI elements on all cells. Consequently, Signal 1-driven tolerance to them in the Tc-category is quite thorough, unless the given housekeeping protein is expressed only in a rare cell-type that has the potential to become the target of the autoimmunity. The housekeeping target (h), in addition to its being ridded by antibody, must be taken up by an APC or be endogenous to it and presented as P_h-RI with which the initial state Tc-cell (iTc) interacts (Signal 1). The APC must be activated by an effector eTh (Signal 2) that, in turn, drives the iTc to become eTc. The eTc must find the viable cell expressing P_h-RI and attack it. This means that the rare target cell and the APC must process *h* to the same peptides (P_h). If the target cell is poorly or nonrenewing (e.g., hormonal or neuronal), then a response, autoimmune or innocent bystander pathology, is likely to be apparent. If the target cell is actively renewing (e.g., epithelial or fibroblast), then the deleterious response may be masked. A similar scenario can be envisaged for executive eTh1 cells that generate a destructive inflammatory environment but here the attack would, in large measure, be on cells as innocent bystanders because most do not express RII. B-cells produce antibody to autoimmune targets that are secreted or surface cell-bound as such. Intracellular material is, in general, not a target of attack by antibody. Housekeeping targets are extracellularized and ridded by antibody.

All of these autoimmune scenarios involving cell-mediated activity imply very infrequent events. Even if one could envisage a unique situation that results in high frequency autoimmunity, this would not permit the conclusion that normal autoreactivity, a ridding function mediated by antibody, is a prerequisite for the prevention of autoimmune disease. Autoreactivity and autoimmunity cannot simultaneously be mediated by the same antibody, because if it were, everybody would suffer from autoimmune disease. Autoimmune disease reflects a limitation in the functioning of the immune system via an independent pathway. Further, it is essential that autoimmunity be distinguished from innocent bystander pathology [38]. An antibody to a housekeeping target might establish innocent bystander pathology by a variety of mechanisms, one being, if the target is sticky for a limited family of healthy cells making those cells susceptible to antibody-driven complement lysis or ADCC or phagocytosis or inflammation.

Cohen views his findings with T-cells in terms of an Id-network. This is surprising as an Id-network is not possible at the level of T-cells, which are restricted. Direct $CD4^+$ Th–Th or Ts–Ts interactions are ruled out because Th/Ts are RII-restricted and murine T-cells do not express RII. $CD8^+$ Tc–Tc interactions are ruled out even though T-cells express RI. If eTc–eTc interactions were involved, they would kill each other. If iTc–iTc (initial state (i) without effector function) interactions were involved, then they would both be deleted by Signal 1-driven inactivation. Similarly, all combinations of the above are ruled out. In general, signaling interactions between T-cells require a third-party APC. This raises questions, which I will leave to the Id-experts, concerning how T-cells, in particular Th and Ts, interact functionally either with themselves or via an Id-network or with the putative humoral Id-network to regulate anything. After all, T/B-cells exist as either iT/B or eT/B and nobody has told us how the Id-network deals with them, nor how the *i* to *e* (naïve to effector) transition is accomplished.

As an example of this problem, Cohen describes the studies of his group with a cloned $CD4^+$ effector T-cell, C9, presumably RII-restricted, that enhances an attack on the β-cells of the pancreas resulting in diabetes [39, 40]. The consensus is that β-cells do not present functional levels of P-RII [41]. If that be the case, C9 must recognize its ligand, the peptide P_{277} derived from the *hsp60* of β-cells when presented by an RII element on resident APCs. This requires that the β-cell releases *hsp60* by some route in order to be captured by resident APCs. The attack of C9 on β-cells must be indirect, for example, via inflammation; the β-cell must be an innocent bystander. This interpretation is supported by the observation that a minor set of islet resident dendritic cells, CD8α/CD103, are absolutely essential for the development of diabetes in NOD mice [42]. The authors postulate that these dendritic cells capture secretory granules from the β-cells, process their contents to peptide and present them to relevant T-cells. As this would be expected to be the source of C9-type T-cells,

then the destruction of β-cells that lack expression of P_{277}-RII must be indirect as innocent bystanders, not as autoimmune targets. In this framework, there seems to be no reason that a peptide (P_{277}) from hsp60 should be a unique target. C9 would be an experimentally selected clone from the family of responding Th-cells of different specificities.

Immunization of NOD mice with the C9 clone enabled the isolation of a cell line referred to as anti-C9 "Id", which protects against the more rapid onset of diabetes induced by C9. They show that the idiotope in question is the CDR3β peptide of the C9 TCR. Whereas the C9 clone is made up uniquely of $CD4^+$ T-cells, the anti-C9 "Id" T-cell line is made up of 1:1 $CD4^+$ and $CD8^+$ cells. Further, C9 and the anti-C9 "Id" cells can interact in vitro, measured as a stimulation index, in the absence of APCs [39]. Both cell lines, C9 and anti-C9 "Id", are likely in the effector phase of differentiation. This suggests two scenarios:

1. If the observed in vivo inhibition of C9-induced autoimmunity by anti-C9 "Id" were mediated by the $CD4^+$ cells in the anti-C9 "Id" line, an APC intermediary would be required. An APC-independent interaction of the $CD4^+$ anti-C9 "Id" with C9, both RII-restricted, is not possible in vivo or in vitro recalling that murine T-cells do not express RII. If in vivo an APC were involved, then the $CD4^+$ cells in the anti-C9 "Id" line must see $P_{CDR3\beta}$-RII and C9 must see P_{277}-RII on that APC. Considering the derivation of these two peptides (see Scenario 2), it is unlikely that both would be simultaneously expressed on an APC essentially ruling out Scenario 1. In any case, the $CD4^+$ anti-C9 "Id" would have to be a T-suppressor acting via an APC on a $CD4^+$ C9 T-helper, which is the source of the innocent bystander pathology.
2. The observed in vivo inhibition is due to a cytotoxic T-cell (Tc) direct attack on C9 mediated by the effector $CD8^+$ cells engaged in a $P_{CDR3\beta}$-RI-restricted interaction, APC-independently. In vitro, in the absence of APC, only this interaction is possible. A chromium-release assay for cytotoxicity would settle this interpretation.

Scenario 2 requires C9 processing of its unbound TCR in order for the CDR3β peptide of C9 to be presented by its RI. This makes it very unlikely that an APC is involved in vivo as it has no way to capture that peptide. Further, it is not expected that the CDR3β peptide from C9 would meet the docking requirements for both RI and RII making any postulated role for the APC unlikely. The in vitro anti-C9 "Id" response to C9 measured by thymidine uptake (i.e., the stimulation index) must reflect direct stimulation by the C9-presented $P_{CDR3\beta}$-RI- to the RI-restricted $CD8^+$ Tc-cells to divide in the absence of APC. No Id–anti-Id interaction leading to a network is implied here, nor is one possible. Lastly as an aside, even given the framework of an Id-network, it would remain unexplained how the TCR, which is processed to multiple peptides in situ, induces a response solely to the CDR3β as an Id. Under the ARA model where negative selection sorts the repertoire, this is expected. The individual would be tolerant of all peptides derived from the framework/invariant regions of the V/Cα and V/Cβ of the TCR. If T-suppression determined tolerance to them, no response to CDR3β and no T-cell Id-network would be possible.

As a closing point, the rationale used experimentally by Cohen, as illustrated in the above study, has been mirrored over the years in the humoral system in which Id–anti-Id and anti-Id——anti-anti-Id were studied. No resolution was achieved as to the existence of a functional role for Id-networks largely because the experimental findings were compatible with both theories. However, humoral Id-networks were unable to deal with any aspect of Module 3 and were incompatible with the experimental evidence for a negative sorting of the repertoire (Module 2). The real interest of the $CD4^+$ C9 T-cell clone is that being RII-restricted, it initiates such a dramatic destructive effect on the β-cell, to which it appears to be blind as that cell is not believed to express functional levels of P_{277}-RII; the so called autoimmunity would, in reality, be innocent bystander pathology making a direct relationship between autoreactivity and autoimmunity totally obscure.

Now let us consider the Cohen/Coutinho vision that the functional paratopic repertoire is transcendental ("complete"). Using as a criterion for counting the total potential amino acid diversity of the CDR3 regions of $CD4^+$ T-cells, one can calculate for CDR3β 20^{10} (10^{13}) sequences times CDR3α 20^5 (3×10^6) equals 3×10^{19}. Cohen/Coutinho uses 10^{15} as their estimate, an acceptable illustrative number for this discussion. Equating the number of different potential amino acid sequences in CDR3β to the number of functionally distinct combining sites would be an absurdity [43], and therefore, it cannot be used as an argument for the inevitability of a functional Id-network, which in any case is ruled out at the level of T-cells. Based upon the frequency of repeats in given CDR3β amino acid sequences present in different individuals, the data presented by Cohen suggest, by my rough estimate, that the expressed CDR3β pool is limited to roughly 10^6. The minor class of very high frequency repeats ("public sequences") in CDR3β implies that they are germline, not somatically encoded, and, therefore, are part of the innate system. However, this obvious explanation is ruled out by the finding that unique public CDR3β amino acid sequences are encoded by many distinct nucleotide sequences. What might be a sufficiently strong somatic

selection pressure on random CDR3β amino acid sequences to derive a family of such high frequency (public) is ripe for speculation, given that the anti-P combining site is made up of complementation with a random family of CDR3α. The strongest selection pressure would be exerted by housekeeping antigens that are in a steady state of being ridded throughout life. This selection pressure would operate on $CD4^+$ Th required for the induction of the ridding antibody.

Putting aside the public CDR3β amino acid sequences, if a repertoire of CDR3β amino acid sequences of 20^{10} (10^{13}) were expressed, then, at any moment in time, no overlaps in CDR3β amino acid sequence would have been found when comparing individual mice of identical genetic background or between humans, mice and monkeys. If the amino acid diversity were to equal the functionally distinct combining site diversity, no immune system would be functional. Even an elephant would see <0.01 of the repertoire, and what little it did recognize would be nonfunctional [43, 44]. If now one introduces polyreactivity, this is equivalent to reducing the potential combining site diversity toward the observed level. Polyreactivity could help to explain Cohen's findings because a given TCR that recognized one or two amino acids would entrain the recognition of 20^4 and 20^3 peptides, respectively. Polyreactivity could contribute, in part, as a factor that limits the potential CDR3β amino acid sequence diversity of 10^{13} to the observed $\sim 10^6$. The other part might be that most CDR3β sequences are functionless and rapidly ridded, or that the biosynthesis of CDR3β is nonrandom and limited, or that degeneracy in codon usage is a factor.

Lastly, the lilt of such statements as: "The immune system looks at HSP60/p277 to detect and regulate cell state (a cell looking at itself (?))," or "The immune system is very busy looking at the self body, and looking at itself looking at the self body (and looking at the cells looking at themselves)," does have a certain Shakespearian cadence full of sound and fury, but what does it signify? Is it a description or a theory and of what? What specific role does the immune system play that permits it to "detect and regulate cell state"? And what experimental challenge of this insight would anyone be willing to accept? As a closing comment, everyone agrees using widely different descriptions that the output of the immune system is designed to "heal the body in the face of trauma" (Module 3). The difference of viewpoint is in the answer to the question, can it do it in the absence of a mechanism to sort the repertoire, the S–NS discrimination (Module 2)? It is unconvincing to argue that by limiting the discussion to cells and molecules, the concept of a self–nonself discrimination becomes irrelevant without telling us how these molecules and cells "heal the body in the face of trauma" without themselves being traumatizing. Reducing the term "Self" to a semantic nightmare is too easy a way of not solving the problem. Give us another term or another definition or, preferably, a competing model for Module 2. Of course, the germline-encoded decisions of Module 3 (i.e., "maintain and heal") can be made without recourse to the somatically learned decisions of Module 2, but they cannot be made without a concomitant need for an evolutionarily selected mechanism against high frequency autoimmunity. Recalling that "autoimmunity," as contrasted with "autoreactivity," is a debilitating disease, to argue that "the immune system constantly reacts to self constituents, hence autoimmunity is not a dysfunction, but the basis of normal immunity" has to be either a matter of semantics or of fustian. If it were a question of semantics, that is, autoimmunity means autoreactivity, then its postulated role as "the basis of normal immunity" would become a triviality, because autoreactivity is normal immunity to NS under the ARA model.

Stewart adds an idea to the above, also voiced by Coutinho [19]. He proposes two modes of functioning in the adaptive immune system, CIS and PIS. The central immune system (CIS) is a highly connected idiotype network that tolerates all antigens that it encounters. Therefore, it defines an "*≪immune self ≫ because an idiotypic network given its autonomous dynamics….constitutes itself as a ≪ self≫*." I presume here that by "autonomous" Stewart means "autoreactive"; if CIS functioned independently of the rest of the individual, it would be useless. The peripheral immune system (PIS) "populated only by recently produced lymphocytes that are waiting to die (not network connected) respond to antigen (S or NS?) with an uncontrolled immune response which will increase until the antigen is eliminated." This leaves as unanswered and likely unanswerable: (1) how the system knows whether to respond in the CIS or PIS mode when it encounters an antigen; (2) how do CIS and PIS arise; (3) where in the animal each functions; (4) what is the function of CIS that cannot be accomplished by PIS; and (5) how did the PIS arrive at being uniquely anti-NS and the CIS as anti-S? No amount of tweaking (e.g., not even the wave of Merlin's wand, "proprioception") will save this model of a Self–Nonself discrimination. As an aside both Stewart and Coutinho by endorsing this formulation deny the existence of a mechanism for peripheral tolerance, which admittedly is an opinion shared by many immunologists, myself excluded. The existence of receptor editing of the TCR and the BCR, as well as somatic hypermutation of the BCR, both of which generate T/B anti-S in the periphery, makes a mechanism for peripheral tolerance unavoidable. And lastly, it is difficult to ascertain how this framework permits the "immunological self" to be put into a "biological context," the title of his presentation.

Kourilsky gives us a more realistic view of the "Self" but leaves unclear what he wishes to substitute. He

appropriately considers the "discrimination" between Self and Nonself, not the ambiguities of each isolated term. However, I find it unclear where he is heading. The describing of the various ways that autogenous material is presented to the immune system is a useful reminder that T-cells recognize as epitopes, peptide presented by RI or RII, whereas B-cells see epitopes on intact antigen, soluble or cell surface bound. Therefore, the immune system sees as epitopes three categories of somatic self, but this does not mean that three different mechanisms are required to sort the repertoire of paratopes specific for each class, as ARA theory demonstrates [11].

Kourilsky tries to describe the immunological self.

1. What are its borders"?

This question preoccupying several of the speakers has been a subject of debate for many years. On the borders are what have been described as "privileged sites," the prototypes being the eye and the brain. However, with time, the perspective has changed as pointed out by Stein-Streilein and Caspi [45]. We have come to realize that many of the various tissues contribute unique regulatory inputs during an immune response. Further, the domain of "privileged sites" has been greatly broadened to include testes, reproductive tract, tumors and fetus, but the one occupying the participants of this meeting was the gut and skin microbiomes. So let us face the question, how does the existence of borders that include "privileged sites" affect our definition of "Self." This is best answered after considering Kourilsky's second question.

2. Over what time frame does "self" remain unchanged?

From the point of view of the immune system, "self," once learned, does not change. However, defending such a counter intuitive position for the participants of this meeting requires attention to the mechanism defining the immune self. The immune system is born during ontogeny in the presence of what is all Self and as tolerizable-only (Signal 1) because of the absence of eTh (Signal 2). The antigen-responsive cells (i.e., initial state iT and iB) are inactivated both centrally and peripherally (Signal 1). When this developmental time window closes, and the system becomes responsive (appearance of eTh anti-NS), the persistence of Self maintains the state of tolerance to Self. Any antigen that appears de novo after closure is treated as Nonself, and any Self that no longer persists becomes Nonself to the immune system. Any Self-antigen that is delayed in expression, for physiological reasons, to after the system is responsive would require that it be expressed ectopically as a tolerogen for iTh in thymus while the window was open in order to escape immune attack [18]. Many of the examples of autogenous antigens that are in privileged sites are likely Nonself to the immune system because they were not encountered when the window was open and therefore would ordinarily be attacked. These antigens require special mechanisms unique to each privileged tissue to prevent such attack. Physical barriers and inhibitory mechanisms are invoked as part of Module 3, but they are not elements involved in the sorting of the repertoire (Module 2).

Kourilsky now gives us ground rules for considering immune responsiveness.

1. "The self–nonself discrimination cannot be described as a digital event (0 or 1)."

This challenge to ARA theory is totally unclear to me. The virgin or initial state cell, iT or iB, cannot know whether its TCR/BCR receptor is interacting with epitope on a Self or Nonself antigen. It must be told. The two-signal model is based on a digital event. Interaction with an epitope (Signal 1) puts the cell on an inactivation pathway. If it receives a second signal (Signal 2) delivered by an eTh anti-NS, it is diverted to an activation pathway. The sorting of the repertoire (i.e., the S–NS discrimination) is an entirely digital event.

2. Immunogenicity must be evaluated as a function of "concentration."

This is a truism, but the relationship to function is often best analyzed as a threshold event. Left without discussion is how this impinges on the definition of self and how does the answer deal with a putative functional humoral idiotype network in the absence of a cell-mediated idiotype network, the dominant model of the meeting?

Lastly, Kourilsky's "dialectic self model" for an S–NS discrimination is not well enough defined to be heuristic, and his conclusions are too unspecific but they do give us food for thought. To describe the "Self" defined by the S–NS discrimination as a "set of self-described norms" could be interpreted as distinguishing the Id-network from the ARA theory were it to be experimentally testable.

Eberl deals with Module 3, the determination of effector class, essentially ignoring the problem defined by the meeting, the definition of "self." He concentrates on a privileged site, namely the gut and its microbiome. Given the developmental time model, any antigen not encountered as a tolerogen when the window is open (i.e., when there is an absence of eTh) will be treated as NS when the window closes (i.e., when eTh anti-NS appear). Therefore, the gut microbiome is NS to the immune system. This seems to be a general property of the contents of all privileged sites. The inhabitants of these sites are protected from the host immune system in part by physical barriers, blood–brain, blood–eye, blood–placenta, blood–gut, etc. Besides the barrier, further factors unique to each site must be in play. When compromised by infection, the privileged

site must be defended by the immune system without provoking either autoimmunity or innocent bystander pathology. This might well be one role of Ts (Tregs). Eberl does not confront this question, leaving the problem as one of semantics, namely that the role of the immune system is to maintain "homeostasis." Others have used the term "integrity." Whether or not its role is to maintain homeostasis/integrity, the need to make a S–NS discrimination cannot be bypassed and consequently, in the event that their contents are NS, there is a requirement for special mechanisms in privileged sites. The "superorganism," an individual, must comprise all of these privileged sites. We agree! But it does not change the definition of Self as proposed here.

The use of computer modeling to analyze immune behavior needs an introduction of principle. Computers are witless; they must be told how to think before they can tell you what to think. This means that the modeler must spell out the postulates of the theory that is being modeled [11]. The model should encompass more than one observation and, further, be predictive, testable and, at least in principle, disprovable.

Now let us look at Bersini's view of immune function. He accepts the views of the Id-network proponents but uses a nomenclature based on metaphor and mathematical symbolism. He does not weigh competing theories, and his discussion is essentially a description, not a conceptualization. One always hopes that the rigors of mathematics would reveal an original critical position, or, if not, would at least give us a decisive decision between competing theories.

Bersini reminds us (1) that the immune system displays "nonlinearity of interactions," (2) that "some of its biological 'nodes' are much more connected than others," and (3) that its regulation involves positive and negative feedback loops ("circular causality"). He refers to "these" as "classical physical facts" but, alas! "these" did not come from physics, engineering or computer sciences providing a theoretical framework. They were derived from the output of dedicated experimentalists who revealed discrete interacting entities and pieced the crossword puzzle together empirically in a variety of situations (generation of repertoires, cell types, cell trafficking, cell–cell interactions, messenger interleukins, effector mechanisms, regulatory positive and negative signaling pathways, ontogenetic expression, etc.). It can only be hoped that someday "systems biology" or summed "modularization" would put it all together as a unified whole permitting predictable outcomes. Only obliquely does he deal with the "redefinition of self" assuming that second-generation idiotype networks provide a sufficient definition, whatever that is.

If we accept these "classical physical facts," Bersini claims that his synthesis leads to the view that the internal input (i.e., the "behavioral autonomy" of the immune system) is as "important" as any "external impacts" (i.e., pathogens, housekeeping waste, toxins). Given this, all that remains to do is to translate "behavioral autonomy" into discrete known physical processes, demonstrate that immune behavior is autonomous and that this results in an evolutionarily selectable function, and define "importance." Of course, I appreciate that Bersini understands all this; now I would like to also.

B-cells cemented together in an Id-network by secreted Ig constitute a web so circular that it has no effector output when challenged. But that is the least of its problems. On the one hand, any antibody in sufficient concentration to interact productively with an Id is in sufficient concentration to activate an effector function, not to mention, a tolerogenic Signal 1. Under any formulation, this is either a recipe for autoimmunity or a generally disrupted regulation of output. On the other hand, all of the ridding effector functions of the humoral system require aggregation of the antibody by antigen. This requires that the antigen be seen in three or more ways. As the Id-interactions tie up all of the combining sites, the effector response to the source of trauma (the input) would be considerably reduced. To this might be added that there are several classes of Ig with varying functions that would be scrambled in the Id-network. We need solutions to such contradictory problems that manifest themselves in this framework. To be told that "the topology of the network can change the way an external impact is treated by the network" is in need of an example, a testable theory and a way of distinguishing it from other conceptualizations. If what is meant by "circular causality" is none other than feedback effects, I would point out that "circular causality" was understood a very long time ago. Without feedback regulation, the immune system would be more of a hazard than a guardian angel. However, if one feels that an example of "circular causality" is that "regulatory T-*cells* (suppressors) seem to be crucial for tolerance," then a lot more than the metaphor, "circular causality," would be needed as discussed above.

As a T-cell network does not exist, given Bersini's framework, T-cells have no way to connect with the humoral network of B-cells such that a functional effector output would result. Effector Th-cells interact with individual B-cells that have processed the antigen via BCR uptake (i.e., have received Signal 1). In experimentally constructed situations, a Th anti-Id-peptide can activate a B-cell expressing that Id-peptide via the processing of its BCR when that BCR is ligand-bound (Signal 1). In general, however, antigen must be the major link, not the processed

idiotype and, if so, then postulating a functional humoral Id-network is without a raison d'etre.

While there is no difficulty in inventing conceptually two "shape spaces," one a tolerance zone and the other an immune zone, when translated into the workings of an immune system, it is lacking in heuristic value as I pointed out in discussing Stewart/Coutinho's CIS and PIS.

No immunologist has been working under the assumption of "linear causality" (meaning absence of feedback regulation), not even Langman and Cohn. For over 30 years now, feedback regulation has been accepted and under analysis. However, why linear causality as distinct from circular causality implies "no past, no history, no dynamics" is mystery to me. It becomes even less clear when Bersini describes the distinction between linear and circular causality as being that for the latter, "the endogenous regulatory mechanisms are as important as the exogenous impacts." I know of no immunologist, and they are of many different persuasions, who would not accept that statement as the term "important" is sufficiently ambiguous. While idiotypic networks can be viewed as an example of "circular causality," it is certainly not the first example. Feedback by suppression was envisioned by Gershon in the 1970s to be "crucial for tolerance." As Langman and I argued even at that time, this is untenable [24, 46]; suppression can only and does contribute to a form of unresponsiveness.

Now to some of Bersini's conclusions from all this that are too good to be true:

1. The immune system maintains each encounter with antigen ("impact") in a "data base" associated with a classification, "good, bad, or curing."

There is no way to do that. The effector output is largely biodestructive and ridding, target ("impact")-independent. Whether the consequence is "good, bad or curing" is a property of the target (the "impact"), not the effector response. Ridding of housekeeping waste is "good," of a pancreatic β-cell is "bad," and of a pathogen or wound debris is "curing." The immune system optimizes the choice of effector mechanism simply for ridding.

2. "An impact is never good or bad…. It is learned to be so. Nothing is known a priori."

As this is a statement of Module 3, the term *"learned"* needs clarification. Learning is a historical process; the response depends on the previous experience of the system with respect to that impact (antigen). Good or bad is determined by the target (*impact*), not by the immune system (see Point 1). I made this point earlier when discussing Matzinger. The immune system can detect and assay trauma, but it cannot learn to detect and assay the *potential* of an "*impact*" to traumatize.

3. "Learning by experience is a key ingredient."

True enough! But it only applies to Module 2, the S–NS discrimination, which is somatically learned. Module 3, the mechanism for the determination of effector class, is germline selected (i.e., learned, to be more precise, during evolutionary, not somatic time).

Bersini's discussion is largely descriptive. He presents us with no model that can be empirically challenged. From the point of view of this conference, he accepts an Id-network as the cloaked framework of his discussion implying that no distinction between S and NS is meaningful. Lastly, the conclusions of Bersini should have been, but were not, the postulates used to construct a model, the translation of it into a computer program, which reveals relationships that can guide experimentation and potential disproof.

The two systems operating somatically that are capable of learning are the nervous and the adaptive immune systems. It has been irresistible to compare them [27, 47–49], as Bersini and Hershberg also illustrate. The immunologist has pirated terms used by neurobiologists in order to describe the immune response (e.g., learning, memory, recall, associative recognition, tolerance, synapse). Unfortunately, as an assay of the sophistication in thinking, the pirating has been unidirectional. The two systems operate via such distinct mechanisms that the comparisons thus far have not been useful in the search for a unifying specific learning theory predictive for both. The analysis of the immune "Self" from the point of view of neural cognition is of limited value largely because the proponents attempting that analysis assumed the validity of the assumptions of the immunologists who treat immune self and cognitive self as having a never stated common higher-order set of rules. The philosophers and historians impose their conceptualizations of cognitive self on the analysis of immune self. This is a useful exercise only as long as the perception of immune self has validity. There is little chance that a disproof of one or the other view will be achieved.

Hershberg guides us through the principles governing information flow in cognitive systems, and this has the potential to be heuristic. However, it is difficult to get past the "defining trait of cognitive systems."

> In a cognitive system, its capabilities are not preordained by the plan of the system. Only after interaction with its environment will the system's capabilities be defined.

If one defines as the "system's capabilities," only that which has been learned, then clearly at any moment in time the definition of its capabilities will be different, but there are cognitive systems that do not learn in somatic time (e.g., the innate immune system), only in evolutionary

time. The somatic cognitive system is built on an evolutionarily selected base. In this sense, there is a preordained limit placed on the adaptive system's capabilities, which are built on the base given it by the innate system.

The basic problem with the descriptions of the immune system presented by Bersini and Hershberg is their lack of pragmatic value for the bench immunologist who is dealing with discrete interacting entities that are ordered empirically. The description of a set of rules and pathways is not equivalent to a theory that is testable.

What Hershberg's analysis evokes, admittedly an aside here, is the importance of directionality in the signaling. When a receptor interacts with its ligand, the signal is delivered via the receptor not via the ligand. For example, consider two cells, an eTh anti-P-RII and an iTc anti-P-RI, that interact on an APC so that the eTh can deliver Signal 2 to the iTc. The eTh upon interaction with a P-RII ligand on the APC receives a signal via its TCR that translates into the expression of a ligand that is recognized by a receptor on the APC. The APC transmits this signal to the bound iTc that has received Signal 1 via its TCR converting it to an intermediate symbolized as aTc. The activated APC must express a ligand that the aTc recognizes as Signal 2 via a receptor that is not its TCR. There is one-way traffic in signaling. The eTh talks, it does not listen; the iTh/c, as well as iB listen, they do not talk. This one-way signaling is important because it provides the reason why inductive Signal 2 must originate from the eTh and not the APC. The reason that a two-way signal was not used must be structural and is unknown to me. In the interaction between a T-cell and an APC, one can imagine simultaneity between the P-R complex acting as a ligand for the TCR, and the TCR acting as a ligand for the P-R complex, the former signaling the eT-cell and the latter signaling the APC. It possibly was not used because, in this case, signaling an effector T-helper (eTh) would be without function and therefore unselectable.

Swiatczak, a historian, forces us to once again comment on the meaning of the terminology used to characterize Module 2. He accepts that a model of the S–NS discrimination is of importance in the practical world of infectious diseases and autoimmunity. He then latches onto the perspectives provided by immunology's founders. This permits him to cite ecclesiastical authority and define as the "self/nonself model" one in which "The immune system of a healthy individual reacts only to foreign antigens while ignoring self-antigens." Without some definitions, as discussed above, this description, as viewed by Swiatczak, is not heuristic [50]. The definitions of Self and Nonself as discussed here and in many publications have been blatantly ignored by all of the speakers. However, keeping within Swiatczak's innocent challenge to the concept of the S–NS discrimination as defined by the ARA model, I might point out that while Ehrlich, a dedicated reductionist, could not have contributed to the dynamics of Module 3, he did envisage that a relationship must exist that links recognition of an epitope by an antibody combining site (paratope) to its coupling with effector function. Burnet never gave us a model for the S–NS discrimination [3, 51], but he did insist correctly, but without rationalization, that a sorting of the repertoire was via negative selection ("paralysis").

My introduction of precision in distinguishing "tolerance" from "unresponsiveness" pays off here. Successful treatment of autoimmunity and organ transplantation can be accomplished by establishing anyone of several pathways to unresponsiveness, but only one of them can be extrapolated to tolerance (i.e., the somatic mechanism that sorts the paratopic repertoire). For example, one can manipulate an organ graft to be accepted because (1) the class of the effector response is switched from effective to ineffective, or (2) an *entente cordiale* is established in the war between the two immune systems, one from the host to reject and the other from graft to protect against rejection, or (3) the induction of eT-suppressors, or (4) negative selection deleting all host cells recognizing the targets of rejection on the transplant. However, of these examples of unresponsiveness, only deletion of the recognition of the target epitopes involved in autoimmunity or transplant rejection can be extrapolated to tolerance. All of the examples cited by Swiatczak do not involve "tolerance"; they are examples of "unresponsiveness." One cannot but laud the search for "more useful metaphors in immunology" that will aid "therapeutically" but Alas! *metaphors* will not get us any closer to a clinical solution to autoimmunity or graft rejection. The metaphors, S and NS, as defined here, are as good as it gets.

Tauber revives an analogy that has, over the years, remained just that. As he is quite knowledgeable about the immune system, one would expect that his revival of the "Ecologies of Selfhood" would reveal a new and heuristic perspective on the immune definition of Self.

To begin, let me recall how the immune system defines Self (NTBR). Given the fact that what is Self for one individual is Nonself for another, a *somatic learning process* defining the demarcation between Self and Nonself is obligatory. This process has been detailed as the developmental time model [10, 11, 14]. The ecology analogy deals with a variety of germline selection processes ranging from symbiosis to pathogenicity. It is simply inappropriate as the basis for a theory that must account for the somatic sorting of the randomly generated paratopic repertoire into anti-S and anti-NS. It will be argued that this is too narrow a view and that we should try soaring on the wings of our imagination, so let us do just that.

Tauber points out correctly that a Jernerian network operates as a wobbly steady state of interactions between

all epitopes created by the diversity of the combining sites of immunoglobulin (idiotypes). Any antigen introduced into the system perturbs the network and "activation occurs." There is no meaning to "activation" here because the system is either already activated by its internal connectivity and/or is unable to connect with Module 3, the effector output. It has no way to determine what is to-be-ridded (Nonself) from what is not-to-be-ridded (Self) and to argue that it has no need to, would be debatably worth a reply. Concluding that "the predicate structure of cognition has been revamped" is smoke and mirrors as it does not give us an insight into the evolutionarily selected immune system. A Jernerian network was just theater a priori and clearly unselectable [23]. As a consequence, the network concept had to be abandoned and was replaced by second-generation "contextual theories." These, too, are outside of the realm of scientific discourse as they are not disprovable. They fade away by virtue of their inability to provide a guiding principle for the experimentalist.

One might imagine that some of this led Tauber to propose an ecological description of immune function, in fact, three ecologies, that of the host, that of the microbiome and that of the environment. Central to his reasoning is the existence of symbiosis with microorganisms living in the gut and on the skin. So let us concentrate on that.

The microbiome is clearly Nonself to the adaptive immune system because it was not present when the learning period of the developmental time window was open. As pointed out above, the microbiome occupies a "privileged site," the immune response to which is regulated, in part, by mechanisms controlled by that site. The immune system has no way of knowing whether the microorganism is a symbiont, a commensal or a pathogen. If they breach the blood–skin or blood–gut barriers and interact with the immune system, they are treated as NS provoking a ridding response. The distinction between them is that the pathogen puts up a fight. The microbiome is the easiest privileged site to analyze because the ridding of any microbes that breach the barrier does not entail immunopathology for the host. By contrast, the eye and the fetus are privileged sites that house NS constituents, which would be targets of rejection if the immune system encountered them. If the blood–eye or blood–placental barrier were breached, trauma would ensue were it not for the intervention of specific inhibitory mechanisms like T-suppression. To the immune system, the bacteria of the gut and a subset of the cells of the eye or placenta are NS to be ridded. Protecting the ecological domain of a privileged site in no way challenges the immune system's definition of Self. In the case of the fetus and the microbiome, the need for isolation in privileged sites is understandable. Less clear is why the brain and the eye had to be put in privileged sites, rather than subjecting their antigens to a learned S–NS discrimination. If, for any reason, the contents of a privileged site are NS to the immune system and if an immune attack on them is debilitating, then additional mechanisms to protect them would be required. The first line of defense would be to exclude the immune system from encountering the contents of privileged sites by a blood-site barrier. If it is breached, then various inhibitory mechanisms and resistance to effector functions must be brought into play. This is tricky as an infecting pathogen must be ridded without an attack on the host elements. All this having been said, the existence of privileged sites (or if you wish, ecological domains) does not challenge the immune system's definition of Self.

Tauber's analysis illustrates the importance of distinguishing "tolerance" from "unresponsiveness." He tells us that "from the third person perspective, the immunologist reads the immune signal and assigns a category value, i.e., a reaction 'means' that something foreign has been identified; silence, on the other hand, denotes selfness. However, within the system itself there is no reader…there is only the system itself."

"Jerne's key insight was to show the distinction of characterizing the immune system from two perspectives, the networks and our own. We see the system responding and that which excites a response we interpret as a cognitive event to an external stimulus."

As discussed above and elsewhere, the "reader within the system" is the learning process postulated to be initiated during developmental time and maintained. Unresponsiveness ("silence") does *not* define "selfness." There are many ways to be unresponsive, only one pragmatic way to define "Self" (NTBR). The potential to respond is a prerequisite to a definition of Nonself, but Nonself is not necessarily "foreign." To contrast, the "perspective" of the Id-network with that of the immunologist is close to not being worth discussing. If a Jernerian Id-network theory has no heuristic value, cannot be justified as evolutionarily selectable, makes no predictions, cannot account for (in fact denies) the necessity to make an S–NS (NTBR-TBR) discrimination, has no way of being coupled coherently to an effector output and takes refuge in CIS and PIS, why would anyone view it as capable of having a meaningful "perspective"? There are no testable second-generation contextual theories, only descriptions and analogies. Translating "privileged sites" into the "ecology of the holobiont" may be an improvement in description, but it is not a theory or even a competing concept. It is simply a description. Burying the defined concept of a S–NS discrimination under the mystique, "from an ecological perspective, there can be no circumscribed, self-defined entity that is designated 'the self.' Responses are consequently based…. on how the immune system sees an 'alien' or 'domestic' antigen in the larger context of the body's economy," is hardly insightful for the misguided

immunologist like myself who spent years weeding out the ambiguities of S–NS by producing a testable theory [14]. The adaptive immune system has no way to separate "alien" (NS) from "domestic" (S) antigens by extracting information from "the larger context of the body's economy." If there is an example, let us have it; if none exists, we have been soaring on the wings of our imagination too close to the sun.

As an aside, I have found the concept of an ecosystem helpful in considering Module 3 where each effector function can be usefully viewed as a part of a seesawing evolutionary struggle between a biodestructive, ridding function and a countering defensive activity of the target [14].

Now let us try to deal with the contribution of the philosopher to the scientist's effort to understand what evolution has given us as an immune system. There is a certain unity in the analyses of three of the four philosophers at the meeting, Wilks, Moulin and Vecchi. They accept as given that the immune system is self-oriented in its resting or virgin state. They do not see Module 2 as a factor in its functioning based on analyses derived from a higher-order level of organization, namely the individual. However, cognition by the brain and by the immune system is mediated by quite different systems. Evolution did not select upon the immune system to think or upon the brain to protect against infection. So! What is the nature of the cognitive apparatus of the adaptive immune system?

In order to be effective, the immune system must be able to anticipate and deal safely with a vast number of entities (antigens) that it has never encountered, and experimentally, even many that are nonexistent in nature. In this aspect, it is like the brain, a Promethean system able to foretell the future [48, 49, 52]. To accomplish this, a random paratopic repertoire that recognizes epitopes is somatically generated. As Talmage [53] first suggested, this repertoire divides the antigenic universe into combinatorials of epitopes thus permitting a limited repertoire to distinguish a vast number of antigens. This is the role of Module 1. However, this construct requires Module 2, namely that recognition of "Self" (NTBR) be distinguished from "Nonself" (TBR) if the system is to be functional as discussed above.

Ever since the disproof of his first foray into this problem [54], Jerne became preoccupied with the recognition of Self initially as an immune repertoire germline-selected to recognize "Self," in which recognition of "Nonself" was derived by somatic mutation and negative selection. This model of the origin of the immune repertoire was a priori untenable, before it met experimental disproof because selection in the germline for Self recognition (as defined here) is not possible as the consequence would be debilitating. Later, he went to the extreme by envisioning an immune system totally preoccupied with "Self." This vision of the immune system went unchallenged by the participants at this meeting, in spite of its being irrational (not just erroneous) when viewed in the framework of the ARA model.

Now let us ask whether the reasoning of the philosopher can distinguish which of the two models, a self-oriented network or ARA, provides an accurate interpretation of immune function. The philosophers at this meeting clearly favored the "Self" defined by Network theory deriving their arguments from such giants as Hume, Kant, Descartes and Wittgenstein, etc. However, there is a distinction to be made between the source of one's ideas and the validity of the idea. To a scientist, like myself, it is disturbing that probing minds could find no reason to reject an absurdity and many reasons to accept it. Competing theories are precious only when an experiment or a decisive argument can tell us which one is disproven.

As an example, let us examine the conclusion of Wilks that "the crucial lesson that…..can be learned from Kantian epistemology in the attempt to construct a model for understanding the nature of immunological function is: 'Know thyself.'" There is no immunologist who would disagree with that conclusion because that is not where the problem lies. Recognition of "thyself" is in the hands of a random paratopic repertoire (Module 1), part of which "Knows thyself" and a remainder which does not. These two parts must be distinguished and separated as discussed above. The "construct of a model" must be based on a decision as to whether recognition of "thyself" is purged by negative or positive selection, or is unselected. In all models of Module 2, "Know thyself" is respected. As Kantian philosophy is not going to permit a decision between the sorting mechanisms, it remains a dead end approach "to understanding the nature of immune function." The ARA model is based on negative selection of the recognition of "thyself," whereas all of the other models presented at the meeting were either based on positive selection (suppression) or no selection. I have tried to show why these latter models are untenable.

From the point of view of the working immunologist, the redefinitions of "Self" at this meeting were triumphs of erudition. The attempts to redefine the immunological "Self" based on any framework discussed at the meeting were simply not explicative in a way that could be developed to explain that which is.

I have presented here and elsewhere a rationalization that denies any significant role of idiotype networks in regulating immune responsiveness [13]. As it is unlikely that any of my arguments will result in anyone changing their mind, I would like to know what it would take to do

just that. If any speaker at that meeting answers that question, I will make an effort to reveal my response with respect to the ARA model also.

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