On the definition of a criterion of immunogenicity

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The main objective of immunology is to establish why and when an immune response occurs, that is, to determine a criterion of immunogenicity. According to the consensus view, the proper criterion of immunogenicity lies in the discrimination between self and nonself. Here we challenge this consensus by suggesting a simpler and more comprehensive criterion, the criterion of continuity. Moreover, we show that this criterion may be considered as an interpretation of the immune "self." We conclude that immunologists can continue to speak of the self, provided that they admit that the self/nonself discrimination is not an adequate criterion of immunogenicity.

continuity | self | nonself | tolerance

The main objective of immunology is to establish why and when an immune response occurs, that is, to determine a criterion of immunogenicity. Since the 1950s a consensus has formed on the acceptance and the adjustment of Burnet's seminal ideas (1, 2). According to this consensus, the proper criterion of immunogenicity is the discrimination between self and nonself (3). The central mechanism of any immune system is allegedly the recognition of what is foreign: every element that distinctively belongs to the organism does not trigger an immune response, whereas every foreign element triggers an immune response (4).

Yet this consensus is illusory, because experimental data accumulated in the last two decades have put the self/nonself criterion (SNC) into question. Unsurprisingly, this criterion has been criticized both conceptually (5–7) and experimentally (8–10). However, no consensus has emerged on a new, more convincing criterion. Here, after a presentation of the experimental data that question the SNC, we suggest the adoption of another, both simpler and more comprehensive, criterion: the criterion of continuity (CC). According to our hypothesis, an immune response is triggered not by every foreign entity, but by every strong discontinuity of the antigenic patterns (whether endogenous or exogenous) with which immune receptors interact. In other words, the immune system does not respond to nonself, but rather to abrupt modifications of the antigenic patterns with which it is in contact.

We conclude that the self can still be considered as the object of immunology, but no longer as a proper criterion of immunogenicity.

Experimental Challenge of the SNC

Although the concepts of self and nonself are widely considered as central in immunology, no unique and clear-cut definition of these concepts has ever been suggested (6, 7). Three definitions have coexisted since Burnet: (*i*) the self conceived as the genome of the organism, (*ii*) the self as the sum of all phenotypic constituents originating from the genome of the organism, and (*iii*) the self as the sum of the peptides that were not recognized during selection, typically thymic selection. As we shall see, the SNC cannot be grounded in any of these definitions. Contrary to what the SNC asserts, self constituents commonly trigger immune responses, and many foreign (nonself) entities do not trigger immune responses.

Self Constituents Commonly Trigger Immune Responses. During the selection of lymphocytes in primary lymphoid organs (thymus for T lymphocytes and bone marrow for B lymphocytes), cells that react strongly with antigenic patterns displayed by antigen-presenting cells and those that do not react at all with these patterns are deleted. Hence, weak reaction to self constituents is a necessary condition of lymphocytic survival in primary lymphoid organs (11). Furthermore, this selection continues throughout the lifetime of the organism: in peripheral organs (spleen, lymph nodes, etc.), circulating lymphocytes that do not react with self antigens die (12). Collectively, these data show that reactions with self constituents are not merely a possibility, but a necessary condition for an efficient immune system. In normal conditions, therefore, the immune system reacts constantly (and weakly) with the constituents of the body. Nonetheless, there is a difference between immune reaction and immune response: an immune "reaction" refers to the interaction between immune receptors and antigenic patterns, whereas there is an immune "response" when the interaction (reaction) leads to the activation of immune cells, that is, to the triggering of effector mechanisms. It might therefore be tempting to say that a good definition of the self has been reached: the self would be conceived of as the set of molecular patterns that trigger weak immune reactions, but no response. Self-reactions, however, are not limited to continuous interactions between immune cells and normal, endogenous components of the body. The actual activation, by self components, of immune cells and molecules commonly occurs, as illustrated by two examples. The first example is the phagocytosis of cells that undergo changes in their patterns, particularly dying cells: they are genetically and phenotypically self cells, but they are recognized as entities to be destroyed and trigger immune effector functions (13). The second example concerns regulatory T cells (TReg), which respond to other, normal lymphocytes by down-regulating their activation. TReg, which are involved in the balance of autoimmunity, in tolerance of tumors, etc. (14), are self cells (in all of the possible senses of the term) that respond to other self cells. Collectively, these data illustrate the fact that the immune system is a set of homeostatic processes in which reactions with self components are indispensable and involve most of the time effector mechanisms similar to those responsible for responses to pathogens.

Many Foreign (Nonself) Entities Do Not Trigger Immune Responses. Immune tolerance is certainly not a new idea, but what has been admitted increasingly within the last decade is the diversity and the ubiquity of tolerance mechanisms. All multicellular organisms contain massive quantities of foreign elements that do not trigger immune responses. These include the following:

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Abbreviations: CC, criterion of continuity; SNC, self/nonself criterion; TReg, regulatory T cell.

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1. Bacteria. Many bacteria live in multicellular organisms without inducing immune responses, and, in some cases, they are even beneficial to the host, especially on the mucosal surfaces (lungs, gut, sensory organs, and organs of reproduction). The gut is thought to be colonized by 10^{14} commensal microorganisms, which contribute to the defense of the host and to its digestive capacity (15, 16). In many organisms, commensal bacteria are even indispensable for the development of an efficient immune system (17, 18).
2. Protozoan parasites and parasitic worms (helminths). Parasites display, most of the time, large quantities of antigens at their surface, and yet in many cases they induce no immune response at all (e.g., *Trypanosoma cruzi*) (19).
3. Fetus. In the great majority of cases, although its genome is semidifferent from that of the mother, the fetus does not trigger any maternal immune response or is protected against such a response. Induction of tolerance mechanisms, such as those due to HLA-G (20) and TReg (21), have been proved to play a critical role in this tolerance.
4. Chimerism. Chimerism is the process by which some cells are exchanged between two organisms and maintained in at least one of them, even though they are foreign to the host. The most striking example is fetomaternal chimerism: in humans, components originating from the child have been found in the mother up to 27 years after delivery (22).

In all of these cases, the immune system does not simply ignore the antigens, but rather it actively tolerates them. In other words, immune receptors interact with the antigens involved, but this interaction does not lead to a destructive response.

Thus, whatever definition of self one may favor, the criterion of immunogenicity sought by immunologists cannot be the SNC: many self components induce immune responses, whereas many nonself components do not. Of course, every scientific hypothesis, especially in life sciences, faces some exceptions; but it is worth asking the question, how many exceptions are needed to start to question the hypothesis itself? Our view is that the SNC is currently challenged by too many exceptions to remain unaltered. We believe that the immune system simply does not discriminate between self and nonself, although it may contribute to define the self (discussed in *Conclusion: Should We Abandon the Self/Nonself Language in Immunology?*).

Situation of Contemporary Immunology

With regard to these data, contemporary immunologists may adopt several different attitudes. The first attitude is to maintain the SNC. This option demands a new consensus, both on the definition of the concepts of self and nonself and on the mechanism of self/nonself discrimination, a new consensus that is clearly missing today (23). A second attitude is to say that immunology should not seek a criterion of immunogenicity or a theory anymore and should focus only on experimental work (24). Yet this option is unsatisfying, for experimental data make sense only within a theoretical framework. A third attitude, which, although rarely made explicit, is quite common among immunologists, is to define the self as that which does not trigger an immune response and the nonself as that which triggers an immune response (25). Yet this option is a vicious circle, because it does not offer a criterion of immunogenicity, but only a name for an observed phenomenon. Indeed, this option offers no prediction and no explanation.

The difficulty comes from the fact that no scientific hypothesis can be called into question unless another, more convincing view emerges. Here we suggest a very simple and comprehensive criterion around which immunologists may rally.

Our Suggestion: The CC

Principle of the CC. The SNC is closely interlinked with the view that an organism results from the unfolding of its inner characteristics, i.e., the idea that an organism is the set of the constituents (cells, molecules) originating from its genome. The SNC sees the immune system as defending the integrity of these endogenous constituents, which compose the organism. The criterion for the definition of the organism is in this case the opposition between the endogenous (what comes from the inside of the organism, that is, the products of its genome) and the exogenous (what comes from the outside of the organism and cannot be part of it). Therefore, we understand how the immunological SNC continues this general conception of the organism: the immune system enables the organism to exclude and destroy everything that is different from itself, that is, everything that does not come from the "inside."

According to the CC, an effector immune response is indeed due to an antigenic difference, but not a difference between the endogenous (self) and the exogenous (nonself). The CC goes back to the antigenic difference itself, without interpreting it *a priori* as a difference between self and nonself. Therefore, if the CC is correct, immunologists have been perfectly right to account for the triggering of the immune response with antigenic difference, but they have improperly interpreted it as a difference in origins (endogenous vs. exogenous), whereas it is only the structural (molecular) difference that is important. Here is indeed the principle of the CC: every effector immune response is due to a strong antigenic discontinuity, that is, to the appearance in the organism of antigenic patterns (epitopes) that are different from those with which immune receptors continuously interact. The immune receptors are those of lymphocytes, dendritic cells, macrophages, etc. The antigenic patterns are the epitopes to which immune receptors bind. They can be either exogenous (pathogenic patterns, alloantigens expressed on a transplanted organ, etc.) or endogenous (tumor markers, patterns expressed on apoptotic cells, patterns recognized by TReg, etc.) Finally, an immune response is not simply an immune biochemical interaction between immune receptors and antigenic patterns, it is the actual triggering of effector mechanisms (destruction or neutralization of the antigen, up-regulation or down-regulation of other immune components). If the CC is correct, an immune response occurs when the antigens with which immune receptors interact are very different from those with which they usually interact (antigenic discontinuity). In other words, an immune response is induced by the sudden appearance of epitopes that are unusual, that is, strongly different from those with which immune receptors repeatedly interact and which remain the same or change very slowly. Immune receptors interact strongly with these abnormal epitopes, that is, with a strong affinity and/or specificity.

According to the CC, for a given organism, epitopes are immunogenic if they are unusual, but this does not imply that they are necessarily new: in the case of immune memory (for B and T cells), some immune receptors selected previously respond strongly to epitopes that have already been encountered but with which immune receptors have not continuously interacted ever since.

By speaking of strong discontinuity, the CC takes into account (*i*) the quantities of the antigen. Very small quantities of antigens do not induce an immune response. (*ii*) The degree of molecular difference between the new antigen and the antigens with which immune receptors constantly interact. There is no immune response when the antigens with which immune receptors currently interact are the same or almost the same as those with which immune receptors repeatedly interact. They are exactly the same in the case of the normal functioning of an immune system or when there is a perfect molecular resemblance be-

tween the two antigens (e.g., acceptance of grafts between two identical twins, or acceptance of autografts). They are almost the same, for instance, in the case of efficient molecular mimicry (e.g., *T. cruzi*) (26). (*iii*) The speed of the appearance of the unusual antigenic patterns. In some cases, an antigen introduced very progressively in an organism induces tolerance, not a destructive response. Similarly, when an antigen (whether endogenous or exogenous) present in an organism undergoes very slow mutations and hence slight phenotypic modifications of its surface epitopes, no immune response is triggered. On the other hand, extremely rapid changes of epitopes (as happens with some pathogens) induce no immune response either, because no specific response can be mounted by the immune system. Thus, there is a window of activation as far as the speed of antigenic discontinuity is concerned: very slow changes do not induce an immune response, and very fast ones do not either; only changes of intermediate speed induce an immune response.

Thus, the CC does not say that every modification, every antigenic discontinuity, induces an immune response. To get an immune response, a strong discontinuity (estimated according to three criteria: quantities of antigens, molecular difference with usual antigens, and speed of antigenic modification) is required.

Induction of Tolerance by Induction of Continuity. The CC says that the adequate criterion of immunogenicity lies in the break of antigenic continuity. But it also allows us to understand in which conditions a new antigenic continuity is established, that is, how a new antigen (i.e., molecularly different from those with which the immune system continuously interacts) is integrated among the normal, usual antigens of the organism without inducing an effective immune response. This integration process relies on induction of continuity. There is induction of tolerance by induction of continuity when repeated contacts with a new antigen under nonimmunogenic (weak quantities of antigen and progressive antigenic modifications) and nondestructive (no damage to the tissues of the organism) conditions occur. In this case, the probability of triggering an immune response decreases progressively; in other words, the antigen is progressively tolerated by the immune system (which, by a "habituation" process, gets "used" to the presence of this antigen). Induction of continuity implies the tolerogenic activity of regulatory components, the role of which is now well established, particularly for dendritic cells (27, 28), TReg (29), and especially those induced at the periphery (30), the HLA-G molecule of histocompatibility (31), or even several of them collaborating (32). We believe that better tolerance of a graft if antigens from the donor are injected before the transplantation (33), the efficiency of negative vaccination (i.e., the stimulation of TReg to progressively obtain a reduced immune response), temporary tolerance to paternal alloantigens during pregnancy (34), fetomaternal tolerance, fetomaternal chimerism, induction of tolerance in tumor cells, some kinds of tolerance to pathogens (especially to some parasites), and more generally the creation of tolerogenic microenvironments (30) could all be examples of induction of continuity. Induction of continuity is also consistent with immune exhaustion: T cells, for instance, can be exhausted after a long contact with the antigen, particularly with persistent pathogen, like in LCMV infection (35, 36). In such a case, the immune system progressively ceases to respond to persisting antigens.

Why Should We Adopt the CC? Several data tend to prove that the CC is a better criterion than the SNC.

1. Regulation of immunity: the functioning of the immune system as an homeostasis. The CC subsumes under a unique explanation the phagocytosis of dead or abnormal cells and immune reactions to pathogens: in each case, it is the discontinuity in the molecular patterns displayed on the cell surface that triggers an immune response. Evidence for this discontinuity in dead cells is particularly clear (37). Normal autoreactivity, that is, the necessity for immune cells to be stimulated constantly by endogenous components, and the role of regulatory cells (especially TReg) are equally explained. This last case is particularly striking: whereas the SNC has great difficulty in explaining the triggering of TReg (which can respond to self as well as to nonself), there is no such difficulty with the CC. TReg respond to a strong discontinuity in the interactions between their receptors and the epitopes with which they react (whether self or nonself), exactly like the other immune cells do.
2. Tumor cells. Tumor cells, except perhaps those due to oncogenic viruses, are self cells, insofar as they come from the genome of the individual and are components of the organism. According to the CC, in many circumstances tumor cells trigger an immune response because the molecular patterns they display change considerably and this change thus constitutes an antigenic discontinuity. Changes in tumor cells are indeed very different from changes in normal cells: the genome of normal cells is most of the time stable whereas cancer cells undergo multiple genetic alterations; the transcriptome in normal cells is stable, whereas cancer cells are characterized by a major epigenetic instability; no tissue invasion occurs with normal cells, whereas there is invasion and metastasis with cancer cells; normal cells have a stable pattern of cytokine and growth factor expression, in contrast to cancer cells, which have an abnormal expression of cytokines and growth factors (38).
3. Immunogenic mutations. Genetic changes can, by themselves, elicit autoimmunity and tumor immunity when expressed in inflammatory environments (39).
4. Tolerance of pathogens, such as commensal bacteria or some parasites. These pathogens, especially when they do not harm the organism and even play a useful role (example: bacteria in the gut facilitate digestion), induce, by progressive induction of continuity, a state of tolerance (40). *Leishmania major* actively induces IL-10-producing TReg, and these TReg prevent the clearance of the parasite by other immune cells (41).
5. Fetomaternal tolerance and chimerism. As suggested previously, in this case induction of tolerance (42) may be realized by induction of continuity.

Thus, the CC attempts to give an account of phenomena that the SNC does not explain, or explains only by using ad hoc hypotheses. The CC is simpler than the SNC because continuity is measurable. Moreover, it is more comprehensive: it includes autoreactivity, particularly regulatory components such as TReg, and processes that imply immune constituents and yet do not come within defense of integrity, but rather within homeostasis (phagocytosis of dying cells, regulation of inflammation, etc.).

Conclusion: Should We Abandon the Self/Nonself Language in Immunology?

Recent self-proclaimed "revolutions" in immunology have not compelled recognition among immunologists, partly because of overstatements (25) and partly because of experimental inadequacies (24). By contrast, we propose to take a moderate line, that is, to keep the language of self in immunology, but to try to clarify its meaning.

Immunology is undoubtedly about the self, insofar as it focuses on the persistence and cohesiveness of organisms. Indeed, immunology's main question is: what entities are accepted by an organism, and therefore may be considered as constituents of this organism, and what entities are rejected? Thus, if the self is regarded as a synonym of "organism," then the immunological self

language will be maintained. Yet two clarifications are to be kept in mind: (*i*) The self is best seen as the product of immune interactions, not as the basis and the cause of immunogenicity. The immune system does not discriminate between endogenous and exogenous entities, and consequently the SNC cannot be maintained. Our suggestion is that the CC offers a proper mechanism of immunogenicity, which results in a definition of the immune self. (*ii*) If the concepts of self and nonself are maintained as a criterion of immunogenicity, they may well be misleading. In the last decade it took a long time for the great majority of immunologists to recognize the importance of some data, e.g., normal autoreactivity, the role of regulatory cells ("rediscovered" in the 2000s) (43), or new tolerance mechanisms (20). Even today, some perspectives might be concealed by the self language, if unclarified. Words do matter in experimental science, and if self and nonself are still considered as the basis of immunogenicity, this may impede improvements in immunology.

If seen as a proper interpretation of the immune self, the CC may be a good way of maintaining the language of self without being prone to the problems of the SNC.

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