

# **If the immune repertoire evolved to be large, random, and somatically generated, then ...**

Rodney E. Langman and Melvin Cohn<sup>1</sup>

Conceptual Immunology Group

The Salk Institute for Biological Studies

10010 North Torrey Pines Road, La Jolla, CA 92037

email: [cohn@salk.edu](mailto:cohn@salk.edu)

1. This work was supported by a grant (RR07716) from the National Center For Research Resources at the National Institutes of Health.

**Running title: The repertoire is somatically generated and sorted**

**Key words: Self-nonsel discrimination, sorting repertoire, T-helper, models of tolerance, associative recognition of antigen.**

## ABSTRACT

The evolution of a somatically generated random immune repertoire depended on the concurrent appearance of a somatic process that sorted the repertoire into anti-self and anti-nonsel. A somatic sorting process requires that antigens be classified based on their behavior, not on their physical or chemical properties. As specific recognitive combining sites (paratopes) define antigenic determinants (epitopes), the sorting of the repertoire operates epitope-by-epitope. By contrast, the coupling of the paratope to effector function must operate antigen-by-antigen because the response to each epitope on the antigen must be in the same effector class (i.e., coherent). This distinction resolves a long standing debate.

## **Orientation**

There is universal agreement that the repertoire of receptors responsible for antigen recognition is large, is generated somatically, and is random with respect to the origins of the epitopes it recognizes. It is also safe to say that there is no agreement on the kind of process that sorts this random repertoire into those specificities which would kill the host if they were permitted to function and those which are required to function in order to kill the pathogens that would otherwise kill the host. What appears quite unsafe is to give a name to this sorting process. Ehrlich(1) called it *Horror Autotoxicus*, whereas Burnet and Fenner(2) called it the self-nonsel discrimination, the term in common use today.

Historically, although Burnet identified the problem and linked it with the concept of Clonal Selection, he did not manage to find a solution (3). Next came Lederberg (4) who formulated the problem and offered a suggestion as to what an answer might look like. Then Bretscher and Cohn (5) combined the ideas of Lederberg (4) and Forsdyke (6) with the newly emerging facts of cellular and molecular immunology to provide the first conceptual foundation for the sorting problem. The Bretscher-Cohn model was important not only for the solution it proposed but also for the key question it posed; namely, what is the origin of the effector T helper (eTh)? Oddly, in the absence of either a better mechanism, or a better explanation, many in the immunological community side-stepped the issue by putting the emphasis on the inadequacy of the terms “self” and “nonsel.” It remains popular today to define self andonsel with either such narrowness or such ambiguity that even the phrase, a self-nonsel discrimination, can

be made to sound absurd (7-10) . By discrediting the terminology, the Bretscher-Cohn mechanism was made to appear correspondingly absurd.

Finally Langman and Cohn (11-14) completed the model by providing an answer to the question of the origin of the “primer” effector T-helper. This problem had been largely ignored for the better part of 30 years until interest in the topic was rekindled by Matzinger (15) who vigorously promoted a new phenomenon called “Danger” and, in doing so, attempted to bury the self-nonsel discrimination one last time. As a recent issue of Seminars in Immunology (16) on the self-nonsel discrimination revealed, there are now many voices in this hotly debated war of words.

### **A proposal to solve the naming problem**

The repertoire of random specificities is a mixture of those able to kill the host or kill the pathogen and evolution had to find a way to sort this mixture into two non-overlapping patterns of response. Only those specificities that are unable to kill the host are coupled to the biodestructive elimination mechanisms. An unofficial poll suggests that it would be sufficient to describe two categories of antigens as either “To-Be-Ridded” (TBR), or “Not-To-Be-Ridded” (NTBR).

The immune system, then, must define antigens as Not-To-Be-Ridded (NTBR) or To-Be-Ridded (TBR) because it has no way to determine what is germline-encoded or originates under the skin (i.e., the usual definition of self). The ambiguity arises

because, while all self is autogenously generated, not everything autogenously generated is self to the immune system.

### **Using the new terminology**

Talmage (17) was the first to appreciate that a large and random paratopic repertoire of specific binding sites (paratopes) divided the antigenic universe into combinatorials of linked determinants (epitopes). Paratopes do not recognize antigens; they recognize epitopes independent of the context within which the epitope is situated. In order to sort paratopes, the epitopes, even though they may comprise a single antigen, must be classified individually as either NTBR or TBR. In principle, the immune system can define NTBR-epitopes and leave all else as TBR-epitopes, or conversely the immune system can define TBR-epitopes and leave all else as NTBR-epitopes.

Burnet and Fenner proposed that all NTBR-antigens carry a self-marker, whereas those not expressing the marker are TBR. Matzinger, on the other hand, proposed that all TBR-antigens carry a nonself-marker (“Danger”), and all non-dangerous antigens, that is, those not expressing the marker, are NTBR. These are examples of a germline encoding of the sorting process not epitope-by-epitope, but antigen-by-antigen.

If the repertoire is generated somatically, it has to be sorted by a somatic process, and this requires an ontogenetic component. However, before developing this point, a footnote might be helpful to explain that the specification of TBR and NTBR cannot be an example of a regulatory process that determines either the magnitude or effector

class of an immune response. Not only are there many factors governing the magnitude and class of an immune response, and not only would each have to be set differently to specify TBR and NTBR, but these regulatory processes can only function after TBR antigen has entered the system. It is the antigen acting as a coherent whole that determines the magnitude or class of the response, thereby leaving no possibility for further independent and differential regulation of the effector class by each epitope. All of these post-TBR antigen types of regulation have to act antigen-by-antigen because antigen is the unit of elimination targeted by the immune effector mechanisms. As a consequence, any epitope shared by a TBR (immunogenic) and a NTBR (non-immunogenic) antigen would have to be immunogenic and, *per force*, would be incapable of ensuring that NTBR-antigens are Not-To-Be-Ridded. Thus, the sorting of the immune repertoire has to be initiated before the arrival of TBR-antigens, and then maintained. The NTBR-components must purge the repertoire epitope-by-epitope in a way that is essentially independent of how and where the antigen interacts with responsive cells. Obviously, antigens that do not interact with the immune repertoire cannot select on that repertoire.

**Evolutionary selection for a large and random repertoire limits the possible sorting processes**

Many imaginative proposals have been made describing how it might be possible to decide whether an antigen is TBR or NTBR. However, when this decision is coupled to

a consideration of the evolutionary selection pressure that made possible the appearance of a large and random somatic repertoire, there are few solutions to the sorting problem.

The precursor of today's large and random somatic repertoire could only have been a smaller **germline** selected repertoire that recognized a maximum number of TBR-antigens with a minimal repertoire lacking recognition of any NTBR-antigens. However, this **germline** selected repertoire leaves the host exposed to mutational escape by any pathogen able to change the one epitope recognized by this germline repertoire. Although the immune repertoire will tend to be biased toward essential epitopes that the pathogen can least afford to change, the opportunity for single step mutational escape by the pathogen is still left open. In order to survive each new mutational escape the size of the repertoire must increase by randomly expressing anti-NTBR and anti-TBR specificities along with selection against the **germline** anti-NTBR specificities. This, in the end, limits the size of the germline repertoire. The host caught in this tight selectionist trap has only one escape and that is to switch from **germline** to **somatic** selection. We can assume, without regard to details, that this was the case for today's immune system (18).

It would also have been impossible for this repertoire to have arisen "big-bang" because simultaneously many anti-NTBR specificities would have been included, and **germline** selection deletes individuals expressing anti-NTBR. Only a **somatically** selected repertoire is able to test single step mutations, and by selecting against each

anti-NTBR, one cell at a time, the remaining repertoire is near optimal. The residual **somatically** derived anti-TBR repertoire is coupled to an effector output that links a random recognitive repertoire antigen-by-antigen with the pathogen that is to be coherently ridded by an effector function. Coherence requires that the activation of the anti-TBR repertoire to effector function operate antigen-by-antigen, in contrast to the purging of the anti-NTBR repertoire, which **must** operate epitope-by-epitope.

### **Sorting the anti-NTBR: examples of selection antigen-by-antigen**

First, let us consider the case where recognition of NTBR antigens is postulated to be purged by suppression. The essence of all suppressive regulation is that the recognition of one epitope on the antigen by the suppressor cell dictates the response of any cell interacting with other epitopes on that antigen. Suppression operates antigen-by-antigen to purge anti-NTBR. In principle, the anti-TBR protective response would only require interaction with one epitope for the ridding reaction. However, coherence of the effector response must operate antigen-by-antigen. This implies an obligatory role for activation of the anti-TBR response by helpers which function antigen-by-antigen. In this case, both suppression and help would be regulated antigen-by-antigen leaving no way to sort the repertoire. This leads to two conclusions:

1 – Suppression which functions antigen-by-antigen, not epitope-by-epitope, cannot be the mechanism that purges anti-NTBR.

2 – In the case of TBR-antigens that share epitopes with NTBR-antigens, suppression would inhibit the induction of an effector response, a lethal situation.

The original suppressive model of Gershon (19) and the many recent formulations (20), fall into this untenable category.

Second, let us consider the case where TBR-antigens are postulated to have the universal (without exception) property of expressing a nonself-marker such as pathogenicity, danger, localization, mode of entry or for that matter, any unique, common ligand. Prior to the appearance of a somatic mechanism to generate the repertoire, the germline could have used either recognition of the nonself-marker or of an epitope to trigger the ridding reaction, but not both. In order for the germline selected recognition of the nonself-marker to become a regulatory element used to sort the somatically generated repertoire, it had to be uncoupled from the ridding device and linked via antigen to the system that used paratopes for detecting epitopes on that given antigen. The selective pressure for this could only have been that neither alone could rid the antigen. However, if recognition of the nonself-marker was sufficient to trigger ridding, as it must have been in order to be germline selected, then addition of a paratopic repertoire dependent on independent recognition of the nonself-marker in order to function would be unselectable. Put another way, any increase in the size of the germline selected immune repertoire that exceeds recognition of the nonself-marker would be unselectable. To refer back to our postulate, nonself-marker recognition is a mechanism that operates antigen-by-antigen whereas a paratopic repertoire which

recognizes epitopes must be sorted epitope-by-epitope. To require both is a contradiction.

Finally, let us consider the case of an induction-only model suggested by Zinkernagel (21, 22) who proposes that antigen is always inductive, and always leads to the expression of effector function. In this case the first cells arise during embryogenesis when there are few cells able to react with host antigens, and these, by exhaustive differentiation, produce only an ineffective level of effectors. This low, ineffective level of effector function is maintained throughout life among newly minted anti-NTBR T and B cells. The anti-TBR specificities that also arise during development are able to accumulate so that the mature immune system has an excess of anti-TBR cells ready to be induced for any antigen. Since both induction and tolerance are one and the same reaction, they must be mediated epitope-by-epitope. Zinkernagel would probably argue that the isotype specific ridding of antigen occurs after the induction of an initial IgM response and requires T cell regulation in order to make it coherent. It would require formal kinetic analysis of such a model to evaluate whether it can indeed operate as a possible mechanism of sorting. In essence, however, purging would be epitope-by-epitope and class regulation (IgM excluded) would be antigen-by-antigen.

The elimination of cells in the immune repertoire epitope-by-epitope is only consistent with the purging of the anti-NTBR repertoire, leaving by default a repertoire that is anti-non-NTBR and available for induction by non-NTBR antigens, including those that are TBR as well as innocuous antigens.

### **Multiple “fail-safe” mechanisms for sorting NTBR from TBR create a paradox**

While it is agreed that epitope-by-epitope deletion of a proportion of the anti-NTBR cells occurs in protected enclaves, like thymus and bone marrow where the cells are born, once peripheralized, it is widely assumed that a variety of sorting mechanisms operate. This assumption implies that whatever properties are attributed to the negatively selecting mechanism in the enclave cannot apply as a universal to all of the NTBR-antigens outside the enclave. This invites several comments.

1) Different mechanisms operating on different properties of NTBR-antigens pose a contradiction because the random assortment of epitopes among antigens implies that any one mechanism must be able to cope with any epitope in any context (inside or outside the enclave).

2) An inadequate sorting mechanism creates a selection pressure for an adequate sorting mechanism. However, given evolutionary time, the adequate mechanism will replace the inadequate one, and operate solo. Eventually, one mechanism is responsible for sorting.

3) All of the actually proposed “failsafe” sorting mechanisms are lacking either because they operate antigen-by-antigen (e.g., suppression, use of nonself-markers) or because they depend on sorting NTBR from TBR in space rather than in time (23).

Our conclusion is that there can be no multiple tier selection—it must be all or none, epitope-by-epitope for each anti-NTBR.

### **Sorting the anti-NTBR: the principles of selection epitope-by-epitope**

The basis for deciding if an antigen is NTBR or not can only be made on historical and behavioral grounds. We recall that the NTBR antigens of one individual are the TBR antigens for another implying that the sorting cannot be based on a physical or chemical property of antigens as classes (e.g., self- or nonself-markers). The behavior of an antigen, or the way it changes over time, is only detectable by antigen-specific receptors that respond differentially to the presence or absence of antigen over time. This presence or absence cannot be realistically broken down into differing locations within an individual because the partitioning of antigens between these spaces cannot be based on epitope-paratope interactions, and no matter how the partitions are maintained these would have to become the basis for deciding which antigens are NTBR, not the epitope-paratope interactions.

One of the properties of NTBR antigens is their continued presence (prior and persistent) whereas TBR antigens are usually absent and appear for what is hopefully a short period (posterior and transient). In the simplest case, NTBR antigens remain present throughout the life-time of the individual and this constancy of behavior is then coupled to the elimination of NTBR-reactive cells. Conversely, cells that do not react with antigen for an appropriately defined period are retained and rendered capable of being induced to express their various ridding activities. The intuitive guess that host NTBR antigens have to change during the life-time of the individual, especially at

puberty and during pregnancy in placental mammals, attracts many supporters. However, there is, in fact, no example of a normally expressed post-pubescent NTBR-antigen; consequently there is no necessity to postulate a special specific sorting mechanism for it. If novel NTBR-antigens were to interact with the immune system after its developmental maturity, then such NTBR-antigens could only be distinguished from TBR-antigens on the basis of their persistence, given today's large and random repertoire. While the Zinkernagel model(21, 22) would treat, by definition, any persistent antigen as NTBR, the alternative Minimal Model(11) allows for an only-once-in-a-life-time decision that is based solely on a unique time-window early in life when all NTBR-antigens appear and the embryo is shielded from TBR-antigens by maternal mechanisms.

A footnote is needed to explain that this once in a life-time window of decision for NTBR-antigens clearly implies that no normal host antigen that belongs to the NTBR-class can appear after this unique time window closes. However, this does not preclude the evolution of new host components, only that they have to appear when the time-window is open, and be maintained thereafter at levels consistent with the epitope-by-epitope elimination of anti-NTBR specificities. It should also be pointed out that this single time-window is also susceptible to mimicry by infectious agents. However, it would be rare for a pathogen to both avoid maternal immunity, and establish infection without killing the fetus. In other words, these infectious agents would not be classified as strictly TBR antigens because there is no clear evidence that such antigens are lethal

and “need” to be ridged. One example of such an infectious agent is the vertically transmitted mouse mammary tumor virus which uses this time-window in mice that stays open until around the time of birth. The duration of the time-window is expected to be determined by the requirements of the species, not some universal.

### **The Minimal Model**

The original 1970 Bretscher-Cohn model agreed with Lederberg that immune cells could not begin life expressing their biodestructive effector functions, if paratope-epitope interactions are sufficient to elicit a cellular response. Because Lederberg was unaware of any necessary role for T cells in the induction of B cells, he had to assume that interaction with antigen was the only signal. Lederberg proposed that, throughout life, B cells are born in a state that we can characterize as tolerizable-only. This would permit the elimination of B cells responsive to host NTBR components. Then, in an antigen-independent step that was sufficiently slow to ensure that no anti-NTBR B cell would have survived inactivation, the window of tolerance closed and the cell differentiated into an inducible-only state that allowed antigen to signal the expression of effector function (i.e., tolerance and induction both occur epitope-by-epitope). Forsdyke who also focused on the notion of persistent self antigens and transient nonself antigens, introduced the notion of two signals, though how one for NTBR (self) and two for TBR (nonself) were generated and translated was difficult to map onto any of the then-known immune structures.

The Bretscher-Cohn model recycled the principle of two signals to distinguish the two response options of each cell. One response was signaled directly via the cell's antigen-receptors and was common to NTBR and TBR antigens (Signal[1]). Signal[1] results in the cell dying. The second response, in the case of normal TBR antigens, came when Signal[2] from another cell was delivered by associative recognition of antigen and resulted in the cell becoming an effector. We now refer to this cell, first ready to react with antigen as an i-state cell (where "i" stands for initial). The cell that has then received Signal[1], delivered epitope-by-epitope is today called an a-state cell (where "a" stands for anticipatory because the cell has two pathways open to it, death or differentiation to an effector). A second signal, Signal[2], is delivered by a special regulatory cell, known today as the effector helper or eTh cell. Signal[2] can only be delivered across an antigen bridge, referred to as associative recognition of antigen. This inductive Signal[1]+Signal[2] is, of course mediated antigen-by-antigen as required for the eventual coherent regulation of the ridding reaction. While this theory solved how to maintain the sorting mechanism, it left open the question of the origin of the effector T-helper, or Signal[2], that operated for the induction of anti-TBR, not anti-NTBR.

The Bretscher-Cohn model made answering the question of the origin of the first effector T-helper cell (eTh) a glaring necessity, though a solution as to its origin was lacking at the time. Later, Langman and Cohn were able to pick up a thread from the Lederberg model and made the proposal that the first effector T-helper cell was derived

by the antigen-independent differentiation of an iT<sub>H</sub> to an eT<sub>H</sub> whereas all other cells, iT<sub>C</sub>, iT<sub>H</sub>, and iB had to be driven by antigen (i.e., Signal[1]) to become aT<sub>C</sub>, aT<sub>H</sub>, and aB respectively before their rapid conversion to the e-state by Signal[2] delivered by eT<sub>H</sub> via associative recognition of antigen.

### **Epitope-by-epitope sorting requires a conformational change for Signal[1]**

It is important to appreciate that the Minimal Model requires that for B-cells, single epitope-paratope interactions be able to generate Signal[1]. Our proposal has been that the epitope-paratope interaction induces a conformational change in the B-cell antigen-receptor that translates into self-complementation to deliver Signal[1](18, 24-26). The prevailing view has always been, and still is, that polymers or aggregates of monomers are required in order to initiate signaling by direct aggregation. Of course, if the B cell receptor has to be aggregated by polymers, then signaling would depend on a mechanism to aggregate monomeric antigen and this, *per force*, would function antigen-by-antigen, not epitope-by-epitope. The standard view of B cell signaling via cooperative binding to polymers or aggregated monomers would generally result in secreted antibody of too low an affinity to function in solution. A resolution of this paradox has been suggested previously(24-26) and we note here only that selection for a large and random antibody repertoire requires one epitope to select on one paratope, and this is inconsistent with today's standard view of signaling via the B-cell antigen

receptor, which requires B-cells to be blind to monomer (i.e., to interactions with epitopes).

## **Conclusions**

After an embarrassing number of tries over the years we believe that Ehrlich's chemical view of *Horror Autotoxicus* can finally be recast as a problem in evolution. A large, random, and somatically generated repertoire became necessary for vertebrate hosts that evolve vastly more slowly than the invading pathogens. The variety of pathogens experienced during the lifetime of a vertebrate is simply too large for all that information to be maintained in a small, fixed repertoire that is germline-selected for both recognition of new pathogens, and failure to recognize the host. Evolution started with a small and **germline** selected repertoire that evolved one step at a time towards an increasingly large, random, repertoire. It was the selection against each individual anti-NTBR epitope that allowed this large and random repertoire to evolve. If the existence of a large and random repertoire is accepted without worrying about its origin, then there have been many seemingly viable views as to how the distinction between TBR and NTBR antigens might be accomplished. However, by adding that this somatically generated repertoire had to have evolved from some smaller germline repertoire that itself was selected one mutation at a time, the emphasis shifts from the large and random repertoire, to how anti-NTBR is eliminated one paratope at a time.

Purging the repertoire of anti-NTBR one paratope at a time, and hence by recognition of one epitope at a time, is very different from how the immune system rids pathogens antigen-by-antigen. The class and magnitude of an immune response are determined after TBR-antigen has arrived, and crafted to track the overall ridding reaction. After all the varied discussions, we conclude that the sorting of anti-NTBR from anti-TBR is defined only once, early in life, and is, in essence, not open for review during the life time of the individual. When viewed in this light there is only one model that describes the necessary steps and that is the Minimal Model(11). But, since many of the components of the Minimal Model were borrowed from elsewhere, we do not claim ownership of the model; we are, perhaps, robbers with taste.

The regulation of the induction of the ridding reactions has to be based on selection antigen-by-antigen, not epitope-by-epitope. The magnitude and class of the immune response are decisions made after the developmental time window closes and TBR-antigens are presented to the immune system. As a consequence regulation of the effector class can be expected to be susceptible to manipulation in dealing with human disease. When viewed in an evolutionary context it would be surprising to find no somatic regulatory control over the class of the immune response. In order to treat human disease, manipulations designed to affect the TBR-NTBR decision are less likely to succeed than manipulations at the level of class regulation. It would not be far fetched given that there is antigen-by-antigen regulation of responsiveness, to imagine that the immune response could be manipulated somatically so that in some cases

ineffective responses become effective, and in other cases effective responses become ineffective, In fact, the regulatory roles of Danger and suppressor T cells are best understood in this framework.

## References and Notes

1. Ehrlich, P. and Morganroth, J., Ueber Haemolysine: Fuenfte Mittheilung. *Berl. Klin. Wochenschr.* **38**, 1901.
2. Burnet, F. M. and Fenner, F., Genetics and Immunology. *Heredity* **2**, 289-325, 1948.
3. Cohn, M., in "Dialogues with Selves. Historical Issues and Contemporary Debates in Immunology," Editions Elsevier, France, 2001.
4. Lederberg, J., Genes and antibodies. *Science* **129**, 1649-1653, 1959.
5. Bretscher, P. and Cohn, M., A theory of self-nonsel self discrimination. *Science* **169**, 1042-1049, 1970.
6. Forsdyke, D. R., The liquid scintillation counter as an analogy for the distinction between "Self" and "Not-self" in immunological systems. *The Lancet* **1**, 281-283, 1968.
7. Cohen, I. R., "Tending Adam's Garden: Evolving The Cognitive Immune Self," Academic Press, London, 2000.
8. Tauber, A. I., "The immune self: Theory or metaphor," Cambridge, 1994.

9. Bernard, J., Bessis, M. and Debru, C., "Soi et non-soi," Éditions Du Sueil, Paris, 1990.
10. Matzinger, P., Flajnik, M., Nemazee, D., Rammensee, H.-G., Rolink, T. and Stockinger, G., in "The Tolerance Workshop," Editiones (Roche), Basle, Switzerland, 1986.
11. Langman, R. E. and Cohn, M., A minimal model for the Self-Nonself discrimination: A return to the basics. *Seminars in Immunology* **13**, 189-195, 2000.
12. Langman, R. E., "The Immune System," Academic Press, San Diego, 1989.
13. Cohn, M., The self-nonsel self discrimination: Reconstructing a cabbage from sauerkraut. *Res. Immunol.* **143**, 323-334, 1992.
14. Cohn, M., in "Progress in Immunology V," Academic Press, Orlando, Florida, 1983.
15. Matzinger, P., Tolerance, danger and the extended family. *Annu. Rev. Immunol.* **12**, 991-1045, 1994.
16. Langman, R. E., editor, Self-Nonself Discrimination Revisited. *Seminars in Immunology* **12**, pgs. 344, 2000.
17. Talmage, D. W., Immunological specificity: An alternative to the classical concept. *Science* **129**, 1643-1648, 1959.
18. Cohn, M. and Langman, R. E., The Protecton: the evolutionarily selected unit of humoral immunity. *Immunol. Reviews* **115**, 1-131, 1990.

19. Gershon, R. K., T-cell control of antibody production. *Contemporary Topics in Immunobiology* **3**, 1-49, 1974.
20. Parham, P., Editor, Regulatory T cells. *Immunol. Revs.* **182**, 1-227, 2001.
21. Zinkernagel, R. M., Pircher, H. P., Ohashi, P., Oehen, S., Odermatt, B., Mak, T., Arnheiter, H., Burki, K. and Hengartner, H., T and B cell tolerance and responses to viral antigens in transgenic mice: Implications for the pathogenesis of autoimmune versus immunopathological disease. *Immunol. Revs.* **122**, 133-171, 1991.
22. Moskophidis, D., Lechner, F., Pircher, H. and Zinkernagel, R. M., Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. *Nature* **362**, 758-760, 1993.
23. Langman, R. E. and Cohn, M., A short history of time and space in immune discrimination. *Scand. J. Immunol.* **44**, 544-548, 1996.
24. Langman, R. E. and Cohn, M., Has Immunoglobulin Come to a Sticky End? *Scand. J. Immunol.* **33**, 99-109, 1991.
25. Cohn, M., Some thoughts on the response to antigens that are effector T-helper independent ("thymus-independence"). *Scand. J. Immunol.* **46**, 565-571, 1997.
26. Langman, R. E., The specificity of immunological reactions. *Molecular Immunology* **37**, 555-561, 2001.