

From the Clonal Selection Concept to the Hydrophobicity Theory

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Abstract

This article 1) reviews the concepts of clonal selection and deletion of self-reactive lymphocytes that, although formulated about 70 years ago, have never been experimentally validated until now, and 2) indicates hydrophobicity as the physico-chemical factor able to prevent self-reactivity, thus governing immunotolerance and immunogenicity.

Keywords: Clonal selection, Hydrophobicity, Peptide sharing, Cross-reactivity, Immunogenicity, Immunotolerance, Self/Nonself, Adjuvants

1 The Clonal Selection Concept

The concept of clonal selection proposed in 1957 by Burnet [1] is that, during lymphocyte development, the immune system selectively recognizes and deletes self-reactive lymphocytes, e.g., lymphocytes reacting with amino acid sequences (or structures) present in the human host. In this way, according to the clonal selection concept, any possibility of autoimmune cross-reactivity is eliminated and self-tolerance is established in the host.

In the 60s, the clonal selection concept was scientifically impressive, but to date, disappointingly, it has never been validated. Today, in 2022, the molecular basis and the mechanism by which the immune system recognizes, selects, and deletes self-reactive lymphocytes remain obscure [2, 3], so that vaccination protocols have been and still are based on a never validated hypothesis. Indeed, scientific-clinical research on immunotolerance, autoimmunity, and cross-reactivity continues to be centered on the vision of an immune system endowed with deliberate consciousness and factual capacity of knowing and discerning between protective lymphocytes which provide a defensive adaptive immune response

against pathogens, and self-reactive lymphocytes which can lead to autoimmune cross-reactivity against the host's proteins.

In this regard, in Cohn et al. [4], Weigert has already observed that "*the idea that self-tolerance is achieved by a process that 'eliminates' lymphocytes is a vast over-simplification and, in the end, incorrect*" and, in like manner, also Cohen [5, 6] noticed that the clonal-selection concept does not address the complicated state of affairs in tolerance and autoimmunity.

Additionally, Coutinho [7] posed the '*space*' and '*time*' issues. He observed that "*for ensuring tolerance by negative selection, it would be necessary that every developing lymphocyte encounters every self-antigen along its development. In other words, it would be necessary that all relevant antigens in the body are present in thymus and bone marrow and, in addition, that all self-antigens would be expressed by the few 'presenting cells' that each differentiating lymphocyte contacts before it completes maturation. The old 'time problem' was, perhaps, even more difficult: since tolerance is established only in the embryo while new lymphocytes are produced throughout life in the presence of both self and non-self antigens, it would seem necessary that differentiating lymphocytes in the adult had access to some kind of memory of the 'self' antigenic composition in development. This could obviously not be achieved by negative selection*".

Furthermore, if the clonal selection concept was intended as a defense from autoimmune cross-reactivity, then the postulated selection and deletion of self-reactive lymphocytes seem to be at least fallacious or defective, given the spread of autoimmune conditions worldwide [8-10].

However, although the clonal-selection concept seems to be an unsolved paradigm [4-6], despite Coutinho's observations [7], although the clonal-selection concept is epidemiologically challenged by the increase in autoimmune diseases around the world [8-10], although Diamond's laboratory [11] has shown that pathogenic autoantibodies are routinely generated during the response to foreign antigens, despite all this, the concept of *a priori* purging of the immune repertoire with consequent '*veto*' phenomena for self-reactive lymphocytes persists in current immunologic research [12-24]. Indeed, the molecular mechanism that can cause immunological '*veto*s' is unknown. Actually, *in absentia* of scientific experimental proofs, the clonal-selection concept suggests a creationist immunologic view where the immune system sees, knows, understands, discriminates, judges, decides, and destroys self-reactive lymphocytes. In short, the immune system is considered to be a thinking and sentient entity.

Factually, a fundamental issue is that current immunology does not consider the massive sharing of peptides existing between microbial and human proteins [25, 26] and leaves it unexplained how the immune system can discriminate between, e.g., the hexapeptide GAGAAP which — according to Pir Peptide Match program [27] — occurs in 7 human proteins and the same identical hexapeptide GAGAAP which occurs in 31 proteins of bacteria, viruses, protozoa and other non-mammalian organisms. From a logical point of view, the massive inter- and intra-proteomic peptide sharing among pathogen and human proteins implies that any immune response against a pathogen can inevitably be associated with peptide

cross-reactivity, that is with a massive self-attack in the human host [28].

2 The Hydrophobicity Theory

In conflict with the staticity of a clinical immunological research based on the clonal selection idea, the rapid progress of proteomics over the past two decades has allowed precise comparative analyses of proteomes, proteins, and peptides between different biological entities, from viruses to *Homo sapiens*. Today, such an integrated network of data on thousands of protein sequences and related functional information is freely available on Universal Protein KnowledgeBase (UniProtKB) [27].

In parallel, epitope definition was obtained by using the Immune Epitope DataBase (IEDB) resource [29], a catalog of experimental data on B- and T-cell epitopes studied in humans, non-human primates, and other animal species in the context of infectious disease, allergy, autoimmunity and transplantation. Indeed, IEDB exploration documents that hundreds of immunoreactive epitopes are pentapeptides. The IEDB data validate the concept of epitope as an immune peptide determinant formed dimensionally by a grouping of 5 residues, so that today we can exactly locate antigenicity and immunogenicity within pentapeptides [30-32]. It was a fundamental advancement since, citing Landsteiner [33], “*one cannot safely offer an opinion concerning the specific groups of proteins (‘determinants’) as long as it is not known what the size of such a determinant can be*”.

Knowing the size of an immunoreactive peptide epitope, it was possible to apply mathematics and physical chemistry to define the immunogenicity of a peptide sequence, thus finally answering old questions about self-nonsel and immunotolerance. In mathematical terms, the epitope quality of a pentapeptide is associated with a zero/low number of occurrences in the host [30-32, 34-40]. Physico-chemically, said immunoreactive pentapeptides which are absent/rare in the host are specifically characterized by being hydrophobic [41, 42]. That is, hydrophobicity emerges as one of the main physico-chemical properties capable of defining the boundaries between immunogenicity and immunotolerance. Hydrophobicity materializes as a selectionist filter by which the terminal hydrophobic sequence of the germline B-cell receptor avoids the frequently occurring hydrophilic self-sequences and binds to rare non-self hydrophobic pentapeptide determinants [41]. Then, substances that can enhance/modify cellular hydrophobicity, such as adjuvants [43, 44], can promote indiscriminate binding to host’s peptide sequences, thus allowing potentially cross-reactive immune responses and destroying the immunotolerance status.

3 Conclusions and Prospect View

A profusion of experimentally validated data taken from public databases such as UniProtKB and IEDB [30-32, 34-40] allows us to define immunotolerance and

immunogenicity — that is, the self-nonsel self dichotomy — on the basis of mathematics and physical chemistry. Basically: pentapeptides that are rare/absent in the host represent the immunogenic molecular platform and hydrophobicity is the mechanism by which the host's immune responses are evoked, governed, and modulated [41, 42].

In immunopathology, crossing UniProtKB and IEDB data [43-53] offers the possibility to analyze and quantify the potential cross-reactivity of epitope sequences and the consequent autoimmune pathologies that can associate with host immune responses [11, 45-55].

In diagnostics, defining self and nonself sequences can eliminate the currently prevalent false positive results [56, 57].

Clinically and of outstanding relevance, usage of hydrophobic short peptides that are rare/absent in the human host can lead to safe, specific, and efficacious prophylactic/therapeutic protocols against infectious diseases, cancer, and autoimmune disorders as documented by already available scientific-clinical data [58-60]. Perhaps the time has come to give immunotherapies a winning role.

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