Molecular and cellular aspects of immunologic tolerance

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This review seeks to explain the most exciting recent data concerning the nature of self/non-self discrimination by the immune system in a manner accessible to a biochemical readership. The nature of recognition in the two great lymphocyte families, B cells and T cells, is described with special emphasis on the nature of the ligands recognized by each. The history of the field of immunologic tolerance is surveyed, as are the key experiments on conventional mice which provided a conceptual framework. This suggested that tolerance was essentially due to 'holes' in the recognition repertoires of both the T and B cell populations so that lymphocytes competent to react to self antigens were not part of the immunologic dictionary. There were essentially two ways to achieve this situation. On the one hand, self antigens might 'catch' developing lymphocytes early in their ontogeny and delete the cell, a process of clonal abortion. On the other hand, self antigens might signal lymphocytes (particularly immature cells) in a negative manner, reducing or abolishing their capacity for later responses, without causing death. This process is referred to as clonal anergy. Evidence for both processes exists.

Special emphasis is placed on a wave of experimentation beginning in 1988 which imaginatively uses transgenic mouse technology to study tolerance. Transgenic manipulations can produce mice which synthesize foreign antigens in a constitutive and/or inducible manner, sometimes only in specific locations; mice which possess T or B lymphocytes almost all expressing a given receptor of known specificity; and mice which are an immunologic time bomb in that the antigen is present and so too are lymphocytes all endowed with receptors for that antigen. These experiments have vindicated the possibility of both clonal abortion and clonal anergy in both T and B cell populations, the choice of which phenomenon occurs depending on a number of operational circumstances. For T cell tolerance, clonal abortion occurs if the self antigenic determinant concerned is present within the thymus; if not, clonal anergy is more likely. For B cell tolerance, the strength of the negative signal and therefore the choice between abortion and anergy depends on the molar concentration of the self antigen, the capacity for multivalent presentation to a B cell, and the affinity of the B cell's receptor for the antigen in question. Some B cells with low affinity for self antigens certainly escape censorship and remain capable of secreting low affinity anti-self antibodies, which however do no harm. Mechanisms which prevent the emergence of hypermutated, high-affinity anti-self B cells are discussed.

It is an honour and also a considerable challenge to be asked to summarize an essentially immunological topic, and an important and rapidly moving one, for a biochemical readership. The task is worthwhile because biochemistry and molecular biology have revolutionized immunology over the last quarter century, and because immunologic tolerance remains the single most important issue in immunology from both an academic and a practical standpoint. Experimental tolerance research seeks, of course, to uncover the basis of self-tolerance, namely the fact that we can mount a vigorous immune attack against foreign molecules, organisms, cells or tissues, while we do not, in health, do so against our own molecules, cells or tissues. Given that lymphocytes cannot think, how do they tell the difference between a person's own kidney, tolerated for a lifetime, versus a kidney transplant, destroyed totally within 10 days unless immunomodulatory treatments are used?

Four orienting considerations must precede analysis of the cellular and molecular basis of tolerance. First, recognition in the immune system differs radically from that in all other known macromolecular recognition systems. Virus-receptor interactions, enzyme-substrate recognition or hormone-receptor binding all represent recognition phenomena where eons of evolution have shaped the reaction and endowed it with a given affinity. In the immune system, recognition depends on two types of receptors. B lymphocytes, the cells responsible for antibody formation, possess immunoglobulin (Ig) receptors. T lymphocytes, the cells responsible for cellular immunity and immunoregulation, have a heterodimeric T cell receptor (TCR) consisting either of $\alpha \beta$ or of $\gamma \delta$ chains. In each case, the receptor is generated somatically through translocations of members of large sets of V, D and J minigenes, the combinatorial effect of which is to create large repertoires of receptors. The process, while not entirely random, has a major stochastic element. Without doubt, evolution has affected the receptor variable or V genes, but the receptors which finally emerge
constitute degenerate and redundant sets of specificities guaranteed to be able to recognize any antigen, but not specifically designed to fit it uniquely. As a result, the affinities of Ig or TCR receptors for an antigen vary widely. Whether an effective interaction ensues depends critically on the concentration of antigen and the valency of antigenic determinants or epitopes.

Secondly, each lymphocyte cell expresses only a single specificity. The $3 \times 10^4$ receptors of the T cell or the $10^4$ receptors of the B cell all possess identical combining sites. Moreover, the last translocation which completes the process of V gene assembly occurs late in the development of a lymphocyte. There is thus no great clonal expansion of each specificity within the lymphocyte-generating organs, the thymus for the T cell and the bone marrow for the B cell, and only a small number of exemplars of each specificity exit to equip the immune system.

Thirdly, antigen can signal lymphocytes positively or negatively. A positive signal is one where the non-cycling small lymphocyte sufficiently specific for the antigen in question is activated to enter the mitotic cycle, and undergoes clonal expansion and differentiation towards a functional state. For example, an activated B cell will form a clone of antibody-forming cells or a T cell a clone of cytotoxic cells capable of lysing a virus-infected cell. Activation depends not only on engagement of the receptor by antigen, but also on ancillary signals, frequently involving intercellular collaboration. In particular, T cells secrete growth and differentiation factors known as lymphokines which powerfully influence both other T cells and B cells, and thus play a critical role in immunoregulation. Negative signalling is central to this review. It depends on antigen engaging the receptors in microenvironments not conducive to activation, and can involve actual death of the lymphocyte concerned or the induction of an anergic state where the capacity of the cell to respond to later activation signals is diminished or abolished. The end result of such negative signalling is the abrogation of a capacity to respond to the antigen in question.

Fourthly, the nature of what is being recognized differs sharply between T and B cells. Antibodies, and therefore B cell receptors, recognize three-dimensional configurations (chiefly portions of proteins or carbohydrates) in free solution. The antibody combining site is a rather flat area of $7 - 8 \text{ nm}^2$ in area, comprising 15 - 17 amino acids, and it 'sees' mainly conformational determinants of equal dimensions which frequently do not correspond to linear sequences of a protein. Thus overlapping short peptides covering the totality of a protein may only absorb out 5% of all the antibody made to a whole, intact protein. In contrast, T cells are designed to recognize short peptides (8 - 10 amino acids in the case of a CD8-positive T cell and peptides 8 - 15 amino acids long for a CD4-positive T cell) not in free solution but occupying space in a special groove of cell surface molecules known as major histocompatibility complex (MHC) molecules. In other words, T cells do not 'see' foreign antigens as such, rather they recognize fragmented, processed antigen in short bits, the antigen having become associated with an MHC molecule and thereby being present at the surface of a cell. Antibodies neutralize viruses and toxins, and aid the phagocytosis of micro-organisms. T cells fight invaders (possibly including mutated autologous cells) more subtly by killing (e.g. virus-infected) cells, thus impeding spread, and by promoting inflammation at sites where the foreign entity has become entrenched. Given this duality in the immune system, discussions about immunologic tolerance frequently centre on whether one or both of the cellular populations, T cells and B cells, are involved.

The nature of immunologic tolerance

While Ehrlich [1] clearly recognized the need for the body to avoid mounting an immune attack against itself, the first clear articulation of the central importance of distinguishing 'self' from 'non-self' antigens came from Burnet and Fenner [2]. They saw self-recognition as a task that the developing immune system must learn, and predicted that if foreign antigens could be introduced into the body in embryonic life, before the immune system had matured, the foreign antigen would come to be regarded as self.Billingham, Brent and Medawar [3] provided brilliant experimental validation of this prediction using living allogeneic cells as the antigen in question. They defined this actively acquired immunologic tolerance as a specific central failure of immune responsiveness. The tolerance was antigen-specific, distinguishing the phenomenon from generalized immunosuppression like that induced by cytotoxic drugs or ionizing radiation; and it was central, i.e. a property of the lymphocyte population, distinguishing it from the type of failure of responsiveness obtained by giving passive antibody (which neutralizes the antigen) or by blockading the antigen-processing pathway with large doses of colloidal carbon. Their work was followed by an avalanche of confirmatory studies, with many kinds of foreign antigens being artificially introduced in embryonic or neonatal life as surrogates for some self antigen. Tolerance research developed two main directions: one using foreign cells as the toleragen and a later skin or organ graft as the test of whether tolerance had been induced; the other using pure protein antigens to induce tolerance, with a later challenge with the same antigen together with an immune adjuvant to test antibody-forming capacity. It was only realized much later that the former measured chiefly T cell tolerance but that the latter involved B cells.

A new conceptual framework for tolerance research was provided by the development of the clonal selection theory [4, 5] and the demonstration of the 'one cell, one antibody' paradigm [6]. This made it possible to think of immunity as the activation of specific members of the lymphocyte population and tolerance as the specific silencing or elimination of the same, relevant cells as outlined above and in Fig. 1. Obviously if the lymphocyte repertoire were purged of those members or 'clonotypes' capable of responding to the self antigen X, by some means, then the animal concerned would be incapable of a later response to antigen X. As knowledge about antibody structure, immunoglobulin gene structure and organization and lymphocyte surface receptors accumulated, the clonal selection theory became more popular, although methodological constraints prevented formal proof until 1976 [7]. Before describing the molecular and cellular basis of tolerance, let us list some of the key findings which emerged in the 1953 - 1980 period.

a) Experimentally-induced tolerance lasts only while the tolerogenic antigen remains in the body. This is why living cells work so well in tolerance induction. After the toleragen has been finally eliminated, the lymphocyte-generating organs, the thymus for T cells and bone marrow for B cells, export new cells which gradually make up for the prior tolerance lesion. Of course, for authentic self antigens, continued production of the antigen by the body itself makes this consideration irrelevant.