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SERIES PREFACE

Immunology is a discipline just over a century old that has played a central role in medicine and, more recently, in the biomedical sciences. Immunology has often been referred to as "imperialistic" for its tendency to spread to other biomedical fields like no other discipline. A myriad of publications have continually documented the incredible series of discoveries in this field. During times when many areas of immunology have undergone a formidable revolution, antibodies have always been central to any major progress in the field. From the pioneering work of von Behring and Kisatato at the end of the last century through the seminal experiments of Bordet, Ehrlich, Landsteiner, Oudin and Kunkel, just to name a few, and the conceptualizations of Burnet and Jerne, antibodies have dominated the scene. During the last two decades such major breakthroughs as the advent of monoclonal antibodies and the development of new techniques of antibody engineering have kept antibodies in the forefront of immunology and medical science. From diagnostic tools to vehicles for modern therapy against cancer, infections and autoimmune diseases, the study of antibodies has attracted a multitude of scientists.

While the race for better molecules for diagnosis and therapy is still on, it is evident that our knowledge of antibodies – their properties and structural characteristics – is still incomplete. Antibody genes and their regulation, intracellular assembly and secretion, antigen binding properties, effector function and immunity represent just a few of the topics that continue to be investigated using the tools of molecular biology, cell biology, immunochemistry, X-ray crystallography and computer-aided three-dimensional modeling. New technological developments now afford exploration of new areas of study and medical application for antibodies.

With *The Antibodies*, it is our intent to provide the scientific community with its first platform for a comprehensive review of topics of contemporary interest for specialists in this area. At the same time, we will take the opportunity to revisit more traditional aspects of the field so that relevant information and concepts are maintained in parallel with the more modern aspects. While the work ahead can be viewed with a sense of optimism and excitement, we do not underestimate the task that it will take to cover all areas of interest.

We extend our gratitude and thanks to all our colleagues who accepted our invitation to contribute their views and work, and who have made this volume a reality. We hope this collective effort will continue, contributing to keeping the field alive and exciting, and finding a legitimate identity in the immunological literature.

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PREFACE

Volume 7

When this series began in 1995, we the editors intended to create a platform for updated information to specifically address issues relevant to the antibody molecule, Ig genes and the B-lymphocyte. We feel we have maintained our commitment with past volumes and see this continuing in Volume 7. The group of papers included in this volume cover aspects dealing more directly with (a) the genetic engineering of antibodies and their possible application; (b) the gene utilization and structure of anti-carbohydrates antibodies; and (c) some fundamental issues concerning the evolution of the V–D–J junctional region as well as the evolutionary necessity for a network of Ig V regions. We are pleased that Volume 7 covers such diverse topics and are certain that this collection of papers will once more put in perspective the field in its multi-faceted dimension.

In Chapter 1, Jacques Urbain and his colleagues revisit the concept of idiotypic networks starting with the provocative statement, "will idiotypic networks enjoy a vivid revival like suppressor T-cells?" This chapter redraws the parameters for a physiological role of the idiotypic network, focusing on its role in maintaining and shaping the repertoire of the newborn immune system. The authors argue that the major function of the idiotypic network is to induce transgenerational regulation and defenses at the level of adaptive immunity and that more generally idiotypic networks are used in building and shaping pre-memory B-cell lineages or "memories of the future." In assessing the value of neonatal idiotypic networks in ontogeny and phylogeny, the chapter restates in a forceful way that the immune system isn't merely a military machine designed to cope with the arrival of foreign invaders. Instead, through the idiotypic networks the immune system establishes not only its own survival, but also its own internal regulation and function independently of antigen. Based on selected experimental examples, the authors make a compelling argument in support of the fact that idiotype expression is governed mainly by the Igh locus and that anergic B-lymphocytes are in fact regulatory in shaping the immune repertoire through idiotypic complementarity.

In Chapter 2, Andrew Lew and his colleagues cover a new and exciting application for Ig genes in the design of more effective vaccines. By way of experimental examples, the chapter demonstrates that Ig molecules fused with ligands such as L-selectin or CTLA4 can direct the immunogenic process *in vivo* to the site of immune induction (lymph nodes and spleen) through selective binding to CD34 in high endothelial venules and CD80/CD86 on antigen presenting cells, respectively. This is a conceptually new way to use Ig fusion genes to deliver immunity effectively. The chapter describes in a succinct and incisive way recent accomplishments using these fusion genes, placing them in the context of the wider DNA vaccine field.

In Chapter 3, Harry Schroeder and his colleagues put Ig genes (V–D–J) under the microscope of evolution to identify patterns of amino acid expression in the heavy chain CDR3 (HCDR3) in mature B-cell repertoires. The chapter discusses the role of hydropathicity in selecting HCDR3 noting the existence of a pattern of similar hydropathicity among human, mouse and shark HCDR3s in spite of the fact that these three species are highly divergent, and trace their evolution in remarkably different environments. The author makes a compelling argument that the common pattern is based on a preference for neutral or slightly hydrophylic HCDR3 sequences. The significance of this finding is discussed in the context of the evolutionary advantage for the species, its survival, and the role in shaping the mature B-cell repertoire through recombination events.

In Chapter 4, Kathryn E. Stein and her colleagues revisit eloquently the nature and genetics of antibody responses against polysaccharides. Since these are constituents of the cell wall of bacteria, capsular factors and endotoxins, anti-polysaccharide responses occupy an important place in host-defense mechanisms. In a well organized tour de force, the chapter revisits the nature of anti-polysaccharide responses, and Igh gene usage against environmental, capsular and cell surface polysaccharides. This detailed and valuable up-to-date overview of the field ends with information on the structure of prototype anti-polysaccharide antibodies obtained by X-ray crystallography analysis. The chapter provides the reader with a modern appraisal of the nature, genetics and three-dimensional structure of anti-polysaccharide antibodies.

In Chapter 5, Rainer Fischer and colleagues offer a complete and well-documented overview of the current art in antibody farming, i.e., the production of antibodies in plants that can be cultivated on an agricultural scale. While the science of antibody farming began only a decade ago, much progress has been made since due to the important pharmaceutical and economical implications.

This chapter covers in detail the basic premises to antibody engineering and phage-display technology for the generation of combinatorial library after *in vivo* immunization or *in vitro* selection. It also provides insights into approaches to select for given specificities and improve antibody affinity. As to expression systems, the chapter describes clearly the path to produce antibodies by plant farming. The reader will also find information on transgenic plants and insights on how transgenesis with antibody genes may lead to improving crops with disease resistance genes. Finally, the chapter covers initial therapeutic applications of plant-derived antibodies with special emphasis on IgA antibodies and results of clinical trials using plant-derived antibodies. The reader will no doubt see that this chapter constitutes a state-of-the-art review and analysis of antibody engineering applied to plant expression systems.

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ONE

Positive Selection of B-Cell Repertoire, Idiotype Networks and Immunological Memory

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I. INTRODUCTION

Idiotype network theories became very popular in the early 1970s. Numerous papers have been devoted to networks, but after a glory period, network theories were nearly forgotten and fell into the same disgrace as suppressor T-cells [1] (except that suppressor T-cells are now enjoying a vivid revival [2]). Even today, papers which claim that V regions of Ig receptors on naïve B-lymphocytes are essential for lymphocyte survival, ignore idiotype network ideas [3]. It has been said that idiotype networks are just a footnote in the history of immunology [4].

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As noted by M. Cohn [5]: "Today the tide of regulation via idiotype networks has receded behind an empty beach...and we have no idea what it was that produced the tidal wave or its ebb. If we find ourselves asking 'Whatever happened to...?', it is safe to say that we never learned anything from it."

The concepts of idiotype networks have been removed from recent texbooks. So it was a pleasant surprise to read the following in a recent paper of C.A. Janeway [6]: "Jerne's idiotypic network actually can act on the naïve B-cell repertoire to positively select certain heavy/light chain pairs over others." This is exactly what was proposed by A. Coutinho and ourselves, among others, many years ago [7–9]. Will idiotypic networks enjoy a vivid revival like suppressor T-cells?

Even if some criticisms against network theories were right–the network theory was unable to cope with several important regulatory mechanisms in the immune system, i.e., the self–non-self discrimination phenomenon and the regulation of class effectors (11)–it is unfair to conclude that nothing was learned from a study of the concept.

In fact, we see three major reasons for the importance of idiotype networks:

1 An idiotype network can help explain the maintenance of a vast array of V, D...gene segments during evolution.
2 It is difficult (or almost impossible) to interpret "idiotype imprinting à la Konrad Lorenz" without idiotype networks (see the excellent review by Lemke and Lange [12]).
3 The idiotype priming by maternal effects implies positive idiotypic B-cell selection during ontogeny, and recent data seem to establish such a positive selection (see below).

This paper deals mainly with points 2 and 3. We shall only comment briefly on point 1, which in fact brings some authors of this paper to propose idiotype network concepts. A complete variable region gene is a somatic construct of V_H, D_H, and J_H elements brought together by DNA rearrangements in B-cells. Two hypervariable regions are located within the V gene segment. The fully assembled third CDR, which spans the joint of a VDJ rearrangement, is not found in higher vertebrates, and this third hypervariable region seems to be the most important for antigen binding. How do we explain the presence of highly non-random patterns in the germline V gene DNA which can only arise by direct antigen-binding selection forces acting on the gene product and not the DNA directly? How can we maintain the Wu-Kabat signature at the level of the germline even if most antigens will not be encountered during the lifetime of one individual? Steele *et al.* [13] have proposed a Lamarckian explanation (transfer of DNA from soma to germline; i.e., transfer of DNA from mutated B-lymphocytes to germ cells) for that paradox and have written an entire book on the subject. A more conservative explanation for the Wu-Kabat structure of germline genes is to suppose that many mutations in the CDR are neutral or advantageous while most mutations in framework regions lead mainly to non-functional antibodies. This conventional evolution can probably be much faster and more efficient if there is a functional idiotype network during ontogeny providing an internal selective pressure in the absence of foreign antigens [14, 15]. This kind of evolution will reinforce connectivity and could be a partial explanation for the high connectivity of the preimmune repertoire together with a preferential loss of unconnected idiotypes.

(A detailed evolutionary scheme will be presented elsewhere.) This in turn implies idiotype maternal imprinting, which requires positive selection of B-lymphocytes by internal idiotypic cascades.

Several experimental facts, repeatedly established in several experimental models and different species, argue strongly that the immune system can behave as an idiotype network in physiological experiments [16, 17]. More precisely, the so-called idiotype mimicry phenomena – i.e., the presence of a given maternal idiotype convinces the lymphocytes of the newborn to make the same or a very similar idiotype – imply that some idiotypes are endowed with regulatory power, which is precisely the crux of the network theory. It seems difficult to imagine a more physiological experiment than exposure of a fetal or neonatal immune system to maternal effects.

It has been proven beyond doubt that the results of some of these experiments cannot be explained by antigen or cell transfers from the mother to the fetus. In a way we could say that the baby acquires the immunological knowledge of the mother, which makes good sense in evolutionary terms.

In fact, it has been shown recently that this learning can also take place for non-adaptive defense mechanisms. For example, when Agrawal *et al.* [18] exposed water fleas to kairomones from two invertebrate predators, the water fleas developed long helmets. Furthermore, offspring of kairomone-treated mothers produced longer helmets than offspring from control mothers, in whatever environment offspring were raised. The authors saw the same effects in successive broods produced later by kairomone-treated mothers in clean water. Thus, development of long helmets in embryonic stages resulted from maternal effects and did not need to be induced directly by the chemical cues called kairomones. Induced defenses can extend across generations. Similar observations were made in plant defenses. Transgenerational induction of defenses are a new level of phenotypic plasticity across generations that may be an important component of predator–prey interactions.

In this paper we shall argue that the major function of the idiotype network is to induce transgenerational defenses at the level of adaptative immunity and that more generally, idiotype networks are used in building and shaping of what we have called "memories of the future" or the "pre-memory B-cell lineage."

The role of maternal protection should not be underestimated. It should be emphasized that the concept of immunological memory remains an enigma. As stated by R. Zinkernagel [19]: "Why should the host need memory? If the host survives the first infection, the host immune system has proven itself fit to deal with repeat infections; if the host is killed by the first infection there is no need of immunological memory. Under such circumstances, is immunological memory of survival value? The main role of memory is to overcome problems of infectious diseases during the time needed for maturation of the newborn immune system. This is dramatically illustrated by the fact that most calves not given colostral milk during the first 18 hours after birth – the short period when intact antibodies can be absorbed in the gut – do not possess protective maternal antibodies and die of infections within weeks."

This maternal protection is essential for species survival, and we shall argue the idiotype network can prime the newborn immune according to maternal experience. This idiotypic priming implies, of course, a positive selection of some

B-lymphocytes, and we shall propose that this positive selection plays a major role in the generation of immunological memory partly due to internal idiotype cascades.

II. FORMAL NETWORKS AND INTERNAL IMAGES

The immune system is able to respond to an enormous array of antigenic structures. But every idiotype can also induce the synthesis of anti-idiotypic antibodies. These two sets of antibodies must be largely overlapping. In other words, the diversity of the immune system is such that, as a statistical necessity, idiotypes and auto-anti-idiotypes coexist inside the repertoire of one individual. By the same token, auto-anti-idiotypes coexist with anti-anti-idiotypes. The immune system forms a web of V domains and cannot avoid the recognition of itself. This has been called the formal network which, in a simplified manner, can be represented by a cascade Ag (+) → Ab1(−) → Ab2(+) → Ab3(−) → Ab4(+) . . .

In fact, such a cascade has been studied using different rabbits for each step of the cascade [20–22]. The bulk of Ab3 antibodies is made up of molecules sharing idiotypic specificities with Ab1, but are non-antigen binding. This set (the parallel set) is denoted id+ag−. A subpopulation of Ab3 antibodies shares idiotypic specificities with Ab1, but in addition, binds antigen (id+ag+). This subset is selected and amplified after antigen immunization and gives rise to the so-called 'Ab1' subset of antibodies. In addition, part of Ab4 looks like Ab2 [23].

Inside the repertoire of one individual, the presence of complementary partners has been demonstrated repeatedly. The network hypothesis states this coexistence has regulatory consequences in the functioning of the immune system. The immune system is using its own diversity to regulate itself. As such the hypothesis is rather vague in the sense that it does not predict the regulatory consequences. The hypothesis predicts that the immune system has an inner life in the absence of foreign antigen. The immune system is not only a military machine designed to cope with the arrival of foreign invaders.

From the concept of formal networks emerges the concept of internal images. Let us consider an idiotype recognizing antigen X. This idiotype can also be recognized by several anti-idiotypes; the diversity of the system is such that some anti-idiotypes could bear a "positive image" of the antigen, recognizing on the idiotype the same area of the active site as the one recognized by antigen. This does not imply the three-dimensional structure of the internal image is a perfect match of the antigenic determinant.

The concept of internal image received its first experimental support from the studies of Sege and Peterson [24, 25]. Rabbit anti-idiotypic antibodies were raised against rat anti-insulin-antibodies. These anti-idiotypic antibodies are able to inhibit the binding of insulin to raT-cells. These anti-idiotypic antibodies also bind to the insulin receptor and can mimic insulin.

Some rabbit anti-idiotypic antibodies to rabbit anti-TMV (tobacco mosaic virus) are clear examples of internal images [26]. Such anti-idiotypes react with anti-TMV antibodies from other rabbits, chickens, goats and horses. Furthermore, some rabbit anti-idiotypic antibodies injected into mice, which have never seen the virus induce anti-TMV antibodies [27], behave like antigen. Structural similarities have been found between anti-idiotypic antibodies and antigen, notably in the GAT system [28].

The crystal structure of an idiotype recognizing lyzozyme anti-idiotope complex has been determined. The anti-lyzozyme antibody uses essentially the same combining site to bind the lyzozyme or the anti-idiotope. Particularly striking is the fact that of the 18 residues used by the antibody to contact the anti-idiotope, 13 are also in contact with lyzozyme. In this particular case, the hypervariable regions of Ab2 which are not helical can "mimic" the structure of an epitope which is partly alpha helical [29]. As stated by the authors, the mimicking is functional, involving similar binding interactions rather than exact topological replicas, which in most cases would be almost impossible to achieve, especially for anti-idiotypic antibodies acting as surrogates of antigen which are not proteins (e.g., carbohydrates). Finally, these Ab2 antibodies have been shown to induce anti-lyzozyme antibodies.

Another interesting anti-idiotopic system is a large glycoprotein antigen, E2, from the feline infections peritonitis virus [30]. The complex formed by Fab fragments of Ab1 and Ab2 has been crystallized and the three-dimensional structure at 2.9 Å resolution has been determined. A comparison of sequences revealed a strong homology between two hexapeptides located in the CDR of Ab2 and the E2 antigen. In this case, the authors suggest a transmission of information through the anti-idiotopic by linear sequence preservation.

Also some recent examples using abzymes are particularly striking [31]. BALB/C mice were immunized with Bacillus β-lactamase. From 150 monoclonal antibodies, one was chosen to immunize, an MAb that significantly inhibited the enzyme. All antibodies induced were accompanied by a significant increase of β-lactamase activity. One isolated hybridoma from Ab2 was able to hydrolyze penicillinic and cephalosporinic substrates with Michaelis-Menten kinetics. So starting with an enzyme antigen, it is possible to obtain anti-idiotypic antibodies, which exhibit enzymatic properties.

As stated above, the idiotype network hypothesis rests on the assumption of the coexistence of complementary partners in the repertoire of one individual.

It was shown long ago that rabbits can make anti-idiotypic antibodies when injected with the idiotype previously synthesized by the same animal [32, 33].

Kluskens and Köhler [34] were the first to demonstrate the appearance of spontaneous auto-anti-idiotypic antibodies in BALB/C mice immunized with *Streptococcus pneumoniae*. Furthermore, this response is characterized by multiple waves of idiotypes and auto-anti-idiotypes. Spontaneous auto-anti-idiotypes have also been described in the response of rabbits to TMV [35].

Some auto-anti-idiotypic or syngeneic antibodies exhibit unusual properties. In mice, syngeneic auto-anti-idiotypic antibodies are notoriously difficult to induce. Lange and Lemke [36] used the 2-phenyloxazolone system (phOx) where the primary response is characterized by a dominant idiotype (idOx1) and is replaced by other idiotypes in the secondary and tertiary responses. As compared to the response to conventional antigen (including oxazolone), the anti-idiotypic response in syngeneic mice exhibits a lag phase of three weeks.

An anti-idiotypic antibody secondary response could be induced even with the soluble idiotype, but the level of the anti-idiotypic antibodies after boosting was not higher than in the primary response. During this secondary response, idiotypically non-related IgM anti-hapten antibodies appear. There is thus an intriguing dissymmetry between the idiotypic and anti-idiotypic response.

The frequency of auto-anti-idiotypic B-cells was investigated using the arsonate system in A/J mice by limiting dilution analysis after polyclonal activation with lipopolysaccharide (LPS) [37]. While the frequency of B-cells able to produce the idiotype is detectable in every naïve A/J mouse (the frequency is around 10^{-4}), the precursor frequencies of auto-Ab2 B-cells were below the limit of sensitivity of the technique in the majority of A/J mice. Auto-Ab2 B-cells could be detected in only 20% of naïve A/J mice. Upon immunization with Ars-KLH, however, a large increase in auto-Ab2 precursor frequency was found (10^3-fold). This effect was seen very early after immunization (six days). These results suggest the frequency of B-lymphocytes potentially able to produce auto-anti-idiotypic antibodies is much higher than the measured frequency. Significantly, this shift is not observed when A/J mice are injected with KLH or when BALB/C mice that do not produce the CRI_A idiotype are injected with Ars-KLH. As in the previous case, the auto-Ab2 lymphocytes are somewhat impaired in their activation properties.

Once again there is a striking dissymmetry between Ab1 and Ab2. Several explanations can be considered, either structural or physiological. One of them posits that in some idiotypic systems Ab1 and Ab2 precursors belong to distinct lymphocyte compartments or subsets and that Ab2 precursors belong to the subset of anergic or tolerant B-lymphocytes [35]. Such a state of anergy could be transiently broken by immunization, e.g., Ars-KLH in A/J mice by bringing auto-Ab2 precursors in the vicinity of helper T-cells. Structural explanations (such as rare V–D–J combination or rare N regions) cannot account for the observations.

III. THE NETWORK THEORY AND IDIOTYPE REGULATION

In his remarkable book, "A History of Immunology," A. Silverstein [38] explains how network theories go back to the side chain theory of Ehrlich. *"Perhaps the best known instance of a premature theory was Ehrlich side chain theory of 1897."* This model was for all practical purposes, the first natural selection theory of antibody formation. It dominated the field for perhaps a decade, only to fall in disrepute and nearly be forgotten for half a century until Jerne and Burnet gave the selective theory of antibody formation its modern form and appeal. But Ehrlich's theory was more than the concept of antibody formation: it speculated broadly also (within the obvious limitations of contemporary scientific knowledge) about the structure and function of antibodies.

Implicit in the side chain theory was an even more startling conceptual anticipation of the future: the binding site of an antibody is a unique structure and might be immunogenic, an anti-antibody might be formed against the specific site, the shape of an anti-antibody combining site would be that of the corresponding antigen for which the original antibody was specific.

In 1967, Gell and Kelus [39] reviewed the literature about autoantibodies and idiotypes. With impressive foresight they wrote: *"It is hard to believe that the body can regularly react to private determinants on its own antibodies not so much because the process would be self-destructive as that it would lead to an infinite regress of anti-antibody production."* Furthermore, they proposed that self-tolerance to idiotypic determinants, in the sense of clonal deletion, does not provide a likely explanation since, "it would entail the elimination of a number of clones equal to the number of possible

antibodies." So they first realized the universe of potential anti-idiotypes might equal in size the universe of possible antibodies. This concept is the basis of Jerne's network theory summarized below [40, 41].

If we consider only the idiotypic properties of an antibody, an immunoglobulin can be described as p1-i1 where p1 is the active side (paratope) recognizing the epitope E and i1 is the set of idiotopes outside the paratope. Now p1 can also recognize the idiotopes i2 of a molecule p2-i2. I2 can therefore be called an internal image. On the molecule p1-i1, the set of idiotopes i1 recognize the paratope p3 of the anti-idiotopic set p3-i3 and so on. These p2-i2 and p3-i3 are thus anti-idiotypic molecules. As a crude approximation, Jerne proposed that recognition of paratopes on lymphocytes led to stimulation while recognition of idiotopes led to suppression. Therefore, any immune response is a perturbation of an internal equilibrium reached through activating and suppressive idiotypic interactions. Using this framework, Jerne tried to explain the low zone tolerance phenomenon, the 7S inhibition effect, the breaking of tolerance by crossreactive antigens, the antigenic competition phenomena, and the existence of parallel sets. Network concepts were also proposed on the basis of quite distinct premises. For example, Urbain [42] proposed a network concept to solve the problem of maintaining a large number of germline genes with their Wu-Kabat structure, i.e., germline genes in which the CDR can be easily identified without the need of somatic mutations and the selection of available repertoire.

Is the immune system a functional idiotype network? Is the network selfish with no obvious regulatory advantage? The mere fact an immune response can be obtained without idiotypic interactions is not proof of the absence of idiotypic regulation. The data obtained from idiotype manipulations (suppression or activation by anti-idiotypic antibodies, idiotype vaccines) do not need the existence of a functional network but can be explained in terms of clonal selection alone.

As an advent of immune receptor diversification and the almost limitless heterogeneity of the repertoire, a new problem arose in evolution. The immune system had to deal with connectivity creating a noisy background due to idiotypic interactions between V genes within the repertoire of a single individual. At a more basic level, such a problem had already been encountered and solved in the evolution of organisms. At first, genetic networks (interactions between the products of different genes or between the products and the genes) must lead to noise but also and mainly, to coherent regulatory circuits resistant to unavoidable noise.

It is highly unlikely that selection alone can extract order from chaos; rather self-organizing properties of networks must provide a partial solution towards meaningful regulation. Genetic redundancy, repression, and mainly hierarchical control of genes offer partial clues to reduce the noise of undesirable interactions.

Leaving aside T-cells whose receptors are mainly concerned with MHC molecules, we have proposed a model of the ontogeny of idiotype networks for B-lymphocytes [9]. The model predicts the presence of maternal or early Ab1 will promote the induction of Ab1 in the offspring. The new immune system can learn the historical experience of the mothers. It should be emphasized that the model was not designed to explain the effects of maternal immunity but was prompted by the relationships between immunological tolerance and idiotype networks.

We first must distinguish between the somatic self and the immunological self: the somatic self is the whole self minus V regions. The diversity of the somatic self and the immunological self differ by orders of magnitude. The immune system cannot become tolerant to all idiotypes because this will lead to an empty immune system.

As precursors of B-lymphocytes develop, some of them will be confronted with somatic self-antigen. There is considerable strong evidence from old and new data using transgenic mice that such B-lymphocytes will be rendered anergic (tolerant).

We then suppose such anergic B-lymphocytes are not simply condemned to death but will perform a regulatory function through idiotype interactions. More precisely, such regulatory B-lymphocytes will be responsible for positive selection of their complementary partners (i.e., auto Ab2 lymphocytes). As usual, positive selection means acquisition of a longer life span. B-lymphocytes with a long life span will tend to become dominant in immune responses against external antigens, i.e., some recurrent idiotypes are anti-idiotypes of antiself idiotypes. Idiotype dominance is then predicted by broken symmetries in the preimmune repertoire. Such anti-antiself idiotypes constitute the repertoire of the "prememory B-cell pool," which could correspond to some extent to the HSA^{low} subset [43].

As unselected B-lymphocytes enter the primary B-cell pool (the HSA^{high} subset), there is nothing that can distinguish a self-epitope from a self-idiotope. Therefore, self-idiotopes present on maternal idiotypes can reach sufficient concentrations to be treated as self-epitopes. Therefore, maternal idiotype will tolerize anti-idiotypic B-cells which will positively select complementary partners (including Ab1). In this context, maternal Ab1 idiotypes can prime for offspring Ab1 idiotypes. By the same token, the presence of Ab2 can lead to long-term suppression of Ab1. At the level of T-cells, it has been shown that anergic T-cells can become regulatory T-cells [44].

The first example of idiotype mimicry was reported in a private idiotypic system using rabbits immunized against TMV. Irradiated recipients of a3 allotype (a marker of V regions) were repopulated with hyperimmune spleen or bone marrow cells from donors bearing the a1 allotype. Recipient rabbits, when immunized with TMV, produce antibodies idiotypically cross-reactive with donor antibodies despite the fact that different rabbits immunized against TMV produce antibodies idiotypically non-crossreactive. The use of the a allotype marker establishes the fact that recipient antibodies were indeed synthesized by recipienT-cells and not by donor cells. This was interpreted to suggest that the presence of donor cells had idiotypically imprinted the emerging immune system from the irradiated recipients [45].

The same conclusions can be reached by considering the results of maternal effects in the *Micrococcus lysodeikticus* antigenic system – for example, the case of female rabbits learning to synthesize a silent idiotype by the idiotypic cascade [46]. These female rabbits produce Ab3 antibodies. Only a very small subset of these Ab3 antibodies are able to bind antigen. These female rabbits, that have never been exposed to antigen, are crossed with naïve males. After two months, the mothers, fathers and offspring are injected with antigen. All synthesize large amounts of anti-carbohydrate antibodies but now *40% of the offspring make the same idiotype as the mothers*. It is evident in this system of idiotypes "à la Oudin" that the