Ontogeny of the Clonal Selection Theory of Antibody Formation*

Reflections on Darwin and Ehrlich

JOSHUA LEDERBERG
The Rockefeller University
New York, New York 10021

This is an idiosyncratically personal account of the origins, about 30 years ago, of the clonal selection theory, a no longer controversial integrating theme of immunological research. As an interested participant, the perspectives I can offer are those within my own ken, inevitably an egocentric one. This will unfortunately understate the independent roles played by a host of others, including several in these proceedings. Other historical accounts may give a more objective view. However, some parts of my story have not been told before. It will be of particular interest to students of the philosophy and sociology of science to analyze the processes of resistance and acceptance of clonal selection theory after 1957, until its general acceptance around 1967.

My personal mise-en-scène begins in 1955. I had been at the University of Wisconsin since 1947, having gone there directly from my work in Ed Tatum’s lab at Yale and Francis Ryan’s at Columbia. If I needed any reinforcement about the interest antigens and antibodies would have for general biological theory, I would have received this amply from M. R. Irwin. Ray Owen had left Wisconsin for Caltech just before I arrived, but his intellectual trace was everywhere. However, my own work was strictly confined to the genetics of Escherichia coli and of salmonella. The diversity of serotypes in salmonella had been one of the conceptual clues to genetic recombination in bacteria, and I had at least one experimental contact with immunology, namely, serology of flagellar and somatic antigens.

The principal antecedental threads of clonal selection, at least for this microbiologist, were: (1) physicochemical concepts of serological specificity, spanning from Paul Ehrlich to Karl Landsteiner and Linus Pauling; (2) the revalidation of Darwinian models (namely, prior spontaneous mutation and natural selection) in their application to adaptation in microorganisms, such as the development of specific resistance to antibiotics; (3) an emerging understanding of gene expression in protein synthesis, particularly in substrate-induced enzyme synthesis in bacteria; and (4) a developing conception of a genetics of somatic cells by analogy with the genetics of bacteria (Mendelian models).

Karl Landsteiner’s “The Specificity of Serological Reactions” focused attention on antigen-antibody reaction as a prototype of biological specificity. Pauling’s chapter in the 1945 edition showed how “specificity can arise in the interaction of large molecules as a result of the spatial configuration of the molecules.” The seminal value of this stereochemical axiom was unfortunately not matched by well-founded speculations on the mechanism of antibody synthesis. In the early 1950s, there was notably little serious discussion of the mechanism of antibody formation. The most prevalent

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a Dedicated to the memory of Frank Macfarlane Burnet (1899-1985) and Peter Medawar (1915-1987).
notions were those elaborated by Haurowitz, that the antigen itself acted as a template on which the antibody globulin was molded. Pauling and Campbell had even published experiments in 1942 claiming the synthesis of antibody \textit{in vitro} by the renaturation of globulin in the presence of antigen. One minor variant challenged the need for the continuous presence of the antigen and supposed that an intermediate mold was generated, perhaps in many copies, from the initial antigen conformation. Another gave homage to the central role of RNA and DNA in protein synthesis, but supposed that antigen could be attached to or modify the nucleic acid in directing the course of protein synthesis. These models, which I later classified as "instructive," reflected a miscomprehension of the most basic feature of the genetic coding theory: the linear correspondence of the nucleotide sequence in the DNA/RNA to the amino sequence of a protein. 

My own research, starting in 1946, had made extensive use of artificial selection to discover rare recombinant or mutant genotypes in large microbial populations. Francis J. Ryan introduced me to this at Columbia in an investigation on a leucine-dependent mutant of Neurospora. Placed on nutritionally deficient media, this mutant would "adapt" to that constraint on its growth. We established that this adaptation was a genetic reverse-mutation with crossing studies. We presumed that it occurred spontaneously, the deficient medium selecting for the mutants, but we could adduce no compelling evidence. Our thinking was of course influenced by Luria and Delbruck's demonstration in 1943 that the statistics of phage resistance in bacteria also agreed with the Darwinian paradigm. Shortly after the Neurospora experiments, a similar method of selection enabled the discovery of genetic recombination in \textit{E. coli} K-12, which achieved a certain reinforcement to "think selection" for a variety of experimental purposes and as a pervasive strategy in natural process.

Many of the aforementioned findings went against contemporary traditions. For example, many bacteriologists still held that drug resistance was evoked by some chemical reaction of the drug with the bacterial protoplasm—a view that continued for many years to be nourished by the authority of Sir Cyril Hinshelwood, President of the Royal Society of London. Several never unraveled the difference between genetic changes in individual cells, changes in the proportion of genotypes in populations, and the reversible regulation of enzyme synthesis by inducing substrates. To others, it was congenial as a last stronghold of Lysenkoism: a direct effect of environment on hereditary traits. Francis Ryan continued to devote much of his energy to studying adaptive mutation in bacteria.

The development of the replica-plating technique in 1952 was similarly motivated: it allowed indirect selection of resistant mutants in a fashion that assured their presence among cells that had never been exposed to the drug. As a constructive demonstration it did finally quiet that controversy. It was also a further reinforcement of "think selection."

The study of enzyme induction, and of the genetic control of \textit{B}-galactosidase, was one of the first tasks I addressed with the use of genetic recombination analysis in \textit{E. coli}. With the help of Karl Paul Link and Martin Seidman, \textit{o-nitrophenyl galactoside} became available as a chromogenic substrate for assay of the enzyme. I was soon struck by the fact that "uninduced" cells, grown in the absence of galactosides, nevertheless showed an unmistakable basal level of the enzyme. Subsequently, I found that neolactose, altrose-\textit{\beta-D}-galactoside, was a noninducing substrate that could be

\textsuperscript{16}It is curious to recall that W. Goebel and O. T. Avery had synthesized nitrophenyl glycosides in 1929 as intermediates in the synthesis of artificial conjugated haptens.
used to select constitutive mutants that produced full-blown levels of the enzyme without specific induction. These findings supported the view that enzyme specificity was inherent in the bacterial genome; the inducer was a quantitative regulator of gene expression.16

Finally, under the stimulus of conversations with G. Klein and H. Koprowski, in 1955 I started to beat the drums for a research strategy of “a genetics of somatic and tumor cells.”16,17 Bacteria had also been thought to be intractable; it seemed certain to me that mammalian cells could be made to fuse, and at least chromosome reassortment could be readily studied.

My first published thoughts about antibodies20 were a brief statement of possible analogy to induced enzyme formation. The complexity of the animal system seemed to defeat experimental analysis. Then, in November 1955, at a symposium on Enzymes in Detroit, Jacques Monod again posed the question of whether the inducer provided the information needed to mold the enzyme. In my discussion, I responded in the negative, citing the aforementioned evidence. The role of the inducer was to regulate the expression of that genetic information, as we would now all agree. In a spectacularly unprescient fashion, my impromptu discussion went on to contrast the induction of enzymes with the antibody response:

“The immune response has provoked a similar discussion. Ehrlich had proposed that specific antibodies were normal products, subject to quantitative variation under the influence of the antigen. Pauling and others believe that the antigen plays a direct role in molding the antibody protein. Enzymes are generally less specific than antibodies in their range of complex formation, but more so in their catalytic action. Furthermore, antibodies are constructed from a common gamma globulin, whereas enzymatic specificity can call on a more fundamental variety in structure. We need not assume, therefore, that both syntheses follow the same plan.”

Calling on the prevailing common wisdom, that was not my most insightful moment. The only other comment about antibody synthesis at that meeting was Pauling’s reiteration of his 1940 model.

When I returned home, I found the November issue of the PNAS and therein Niels Jerne’s paper on: “The Natural-Selection Theory of Antibody Formation.”22 I wrote him promptly to apologize for not having cited his paper, and to express my approbation of approaches that avoided an instructional role for the antigen. He responded that I was the only one to date to express any interest in his proposals. Felix Haurowitz had criticized him, on the one hand, for neglecting to mention Ehrlich’s precedent in proposing the spontaneous formation of antibodies. On the other, it was just not possible for an animal to be preadapted to form antibodies to artificial haptenes like Landsteiner’s azophenyl arsonate. Jerne responded that a million specificities randomly chosen would be far less than the “million million million” globulin molecules in the blood, the supposed targets of selection according to his model. At that point, I was sure that some Darwinian model would handle the problem of antibody formation; I was a bit skeptical of the self-replication of circulating antigen-selected globulin molecules that he was proposing. More plausible targets of selection would have been diversified protein-synthesizing units (in the cell), still bound to their antibody product. It still did not occur to me that the cell itself satisfied that criterion. In fact, not working directly in immunology, it was only at conferences that offered the stimulus of dialectic with people actively working in the field, that I would put much attention into scientific speculation. What perils meetings like this may have for the unwary!

In August 1957, however, I found myself in Macfarlane Burnet’s laboratory in
Melbourne, on a trimester's Fulbright fellowship. I had gone there to learn about the influenza virus, and its recombinational processes, and was dismayed to hear that Mac had just closed down his research on flu, he had decided to go full blast into the mechanism of antibody synthesis. We began earnest discussions about the new wrinkle that Mac had placed on Jerne's proposal: it had to be the cells that varied and were subject to selection.* But, I expostulated, there must be far many more species of antibody than there are cells available! "Mac, how do you know that? How do you know as a matter of experimental fact that there are more than a few thousand species?" I realized instantly how I had taken for granted a spurious "fact" that had misled the entire field. (A complete history would trace the ultimate origin of that icon, of the infinity of antibodies. Today we would use information-theoretic criteria to measure specificity, and might avoid such pitfalls.)

Our discussion became intense, although somewhat clouded by Burnet's tendency to resist the "simplistic" mechanisms of DNA-based molecular genetics that are today's foundation stone. I would receive his exciting ideas, and then have to translate them into a contemporary idiom to get the full benefit of his marvelous biological intuition.

There was also an opportunity to construct some experiments to test the hypothesis, as difficult as this was in the absence of any reliable procedure to clone antibody-forming cells. Working with Burnet was a young, audacious, postdoctoral fellow, Gus Nossal. He was more than eager to attack the theory. Could we at least study the phenotype of individual cells in animals stimulated with two or more antigens. The Pauling model made no particular exclusion; on a clonal selection model, cells making two kinds of antibodies would be vanishingly rare, barring second order complexities.

I had been doing serological microassays with motile salmonella strains, in this case to study the genetics of the flagellar antigens in single-cell pedigrees of the bacteria. I suggested that we characterize the antibody released by single lymphoid cells by immobilization of the bacteria in microdroplets in paraffin oil. The feasibility of the assay was proven during the brief months I still had in Melbourne, and Nossal continued thereafter until 62 reactive cells had been tested: 33 immobilized Salmonella adelaidae, 29 S. typhi, none both. This was only one step toward proof of clonal selection. Propagable clones would be needed for that. The paper made a few mumbles of alternative possibilities, like an analogy to mutual exclusion of viruses. This was my first and last experimental involvement. I need hardly tell you about Nossal's further career. When I went to Stanford in 1959, I persuaded him to join me for an interval, but his roots in Australia ran very deep and he returned, eventually to succeed Burnet as director of the Hall Institute.

Returning to Wisconsin in November 1957, I had a number of other matters in mind besides antibody synthesis. Sputnik had opened up the exploration of space in ways that were dramatized by an encounter with J. B. S. Haldane in Calcutta, en route; and I saw little evidence that scientific objectives were to be honored in the development of the nation's space programs. It seemed an urgent task to move the National Academy of Sciences to take leadership for this objective and to include biological questions on its agenda. What was later termed "exobiology" was initiated the spring of 1958. I also became engaged in the negotiations that would lead to my going to Stanford. But during 1958, Burnet's ideas came up on a number of occasions

Burnet's memoirs have a small factual error—he had me in Melbourne November and December, after he had published his paper on clonal selection theory; in fact, it was August through October 1957. Briefly visiting Melbourne at that time was Carlton Gajdusek, just on his way to New Guinea to study kuru among the Fore—and to discover the slow viruses.
where I felt they would receive greater due after being retranslated into DNA language.

When Bernard Davis invited me to give the Howard J. Mueller memorial lecture at Harvard that November, I decided to use the occasion to frame a critical reformulation of the clonal selection theory. Burnet’s uncanny biological intuition was not matched by his resonance with molecular biology or a detailed familiarity with its chemical precepts. At one point he refers to himself as “positively schizophrenic about molecular biology”—his main grievance “the arrogance which defines biology as the chemistry of the nucleic acids.” By 1958, I had long since consolidated the philosophical position he had repudiated. Meanwhile, David Talmage, at the University of Chicago, had reached a substantially similar posture. Quite independently of Burnet’s revelation of how to read Jerne, he had published a succinct statement of the same theory of clonal selection of cells. In October, I asked him if he would meet in Madison. The upshot was an exchange of manuscripts and an agreement that we would submit papers to Science, for publication back to back. Meanwhile, I had still other diversions: a surprise invitation to revisit Stockholm once again (I had attended the International Congress of Microbiology in August), this time in December on Alfred Nobel’s birthday. I was far too busy to prepare still another paper that would do credit to the occasion; quite literally, I was packing to move my home and my lab to Stanford, targeted for end January. But I did manage to present the Mueller lecture, and was gratified by the interested, if mostly skeptical, discussion it aroused. The talk I finally did present in Stockholm, the next May, was in a similar mood. So much had happened in the 12 years since my initial work on genetics in bacteria that I decided to devote my address to the extent to which biology had become the chemistry of the nucleic acids, as coding agents for proteins.

Our papers appeared in Science, June 1959. Talmage focused on experimental data, including his own important contributions, on the overlapping diversity of antibodies—an essential point in the argument that antibodies are normal globulins. Mine focused on the theoretical framework of the cell selection theory. It is reprinted here (at the end of this article), the more substantial part of this presentation. It generally followed Burnet’s reasoning. One deviation was my proposal that clonal diversification was a life-long process; he would have confined that to the perinatal period as part of his model of induced tolerance.

The sharp delineation of “instructive” from “elective models” is now a matter of common understanding. Nevertheless, a reminder is needed to distinguish “elective” from “selective.” Purification of a globulin preparation on an affinity column is an elective process. If it permitted replication of the elected units, it would also be selective. Likewise, inducers play an elective role in enzyme synthesis in bacteria, by derepressing the expression of preexisting genes. They are not ipso facto selective: substrates may be so when they encourage the differential reproduction of specified genotypes. Thus, the hypothesis analogizing immunogenesis to enzyme induction was an elective one; it did not yet embrace genotypic diversification and selection therefrom. These distinctions are important in efforts to apply these concepts to further domains such as neurobiology.

For some time, many immunologists’ reaction was that they could not see what experimental basis there was to support the selection theory. This was entirely legitimate, but the alternatives to be sorted out were not always logically coherent, such as efforts to distinguish our selection theory from one based on “cellular differentiation.” Even today, to describe a phenomenon as epigenetic rather than genetic is hardly to explain it. The restriction of antibody potentialities that Nossal and I had reported (no more than one antibody species per cell) came under sharp experimental attack, especially by Attardi et al. At one point, Nossal and Makela
themselves found a few cells that, depending on the assay method used, seemed to be bipotent. This was not a mortal wound to selection theory: we were, after all, working with diploid cells; but I was acutely uncomfortable with the kinetics of the model needed to accommodate two sequential mutations, one on each chromosome. Of course, other compromises were available and one has emerged as fact: substantial reduplication of genes for immunoglobulins. Without experimental necessity, I was loathe to multiply entities. But it appears as if immunobiology falls outside the domain of Occam's razor. After 1959, I did not lose interest in immunogenetics, but my medium was an administrative one: the new department of genetics at Stanford. Gus Nossal, Avitchison, Walter Bodmer, and Leonard Herzenberg having occupied chairs there, I could confidently direct my own experimental interests elsewhere.

Meanwhile, chemistry was marching ahead. Brenner, Jacob, and Meselson had given us the messenger RNA, and the role of DNA in protein coding began to be shaped in its contemporary form. And in 1962–1964, a number of studies made it clear that the specificity of antibodies was related to their primary structure, an amino acid sequence whose determination could hardly have any other provenience than the DNA. Ollie Makela also stuck to his guns and clarified some of the methodological problems that may have given bipotent cells as artefacts; Benacerraf's group also gave a strong affirmation of unipotency of cells. It appears that Nossal and Lederberg were probably correct in 1958, but in view of the methodological problems, that has to be put down to sheer luck. The experiment had the undeniable virtue of providing a target of skeptical investigation more pointed than the generalities of the theory that was its background.

By the 1967 Cold Spring Harbor Symposium, the clonal selection theory was an undeniable fundament for almost every investigation of the chemistry of antibodies or the biology of immunocytes. It was also clear that further progress would depend on the propagation of antibody-forming cells as clones. We do not have a detailed intellectual biography of the precursors to Kohler and Milstein's famous experiment. Some of the precedent ideas about fusing immunocytes with neoplastic cells to produce such clones have been reviewed by Bodmer. In a popular piece I wrote in 1972: "Many products of differentiated cells, such as specific enzymes and antibodies, could become important in medicine if we could produce them in larger, predictable quantities. Cell fusion should enable scientists to increase the rate at which these substances are produced by cells in culture." This remark was inspired by Henry Harris's observation that the dormant nucleus of the chick erythrocyte could be reactivated by fusion with mouse cells. Into the ears of babes?

The immune response stands today as the first epigenetic phenomenon for which a chemical structural interpretation can be given. Nature often returns to the same handbook of tricks; it surely will not be the last to violate the dogma of somatic cell constancy of DNA, the apparent reversibility of cell differentiation notwithstanding.

RETROSPECTION: THIRTY YEARS LATER

1. The greatest weakness in reference 32 is its economy of cell types. What sane person would have postulated today's menagerie in 1959?
2. The interpretation of immunological tolerance needs be far more complex, although within the same general conceptual framework as offered there.
3. We would have gotten to a modern theoretical perspective as a direct yield of structural chemical studies of immunoglobulins. Doubtless, these labors got some motivational push and focus from the theoretical context. For example, I would rather
see intensive comparison of DNA sequences of selected sites in samples from
differentiated tissues: muscle, neurones, fibroblasts versus gonia, than a mindless
traverse of one complete genome. The latter would have told us nothing about
immunogenesis.

4. Don't let conflicting and awkward "facts" stand in the way of an esthetically
satisfying theory whose fundamentals are consistent with the world model and with one
another! And be suspicious of "facts" that seem in the way of any coherent theory. In
some measure, the uniformity of the genome among somatic cells may be one of
these.

Note added in proof: The last word on the clonal selection mechanism is: TONEGAWA,

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GENES AND ANTIBODIES

Do antigens bear instructions for antibody specificity or do they select cell lines that arise by mutation?

Joshua Lederberg

An antibody is a specific globulin which appears in the serum of an animal after the introduction of a foreign substance, an antigen (I). Each of the many globulins is specified by its reaction with a particular antigen (2). Our present concern is to formulate a plausible mechanism for the role of the antigen in evoking large amounts of a specific de, the induction of a foreign synthcsircd under its governace. Electric instructive thmrics which suppose the organism differentiates its own constituents for the mele of the antibodie response. The immune system requires to syrdhesisc a given anti-

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cussed in detail in the following section.

Antibody Globulins

A. The stereospecific segment of each antibody globulin is determined by a unique sequence of amino acids.

This assertion contradicts the more popular notion, and the usual basis of instructive broodhness, of a uniform se-
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CURRENT PROBLEMS IN RESEARCH

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Table I. Nine propositions.

A1. The stereospecific segment of each antibody globulin is determined by a unique sequence of nucleotides in a segment of its chromosomal DNA: a "gene for globulin synthesis.

A2. The cell making a given antibody has a correspondingly unique sequence of nucleotides in a segment of its chromosomal DNA: a "gene for globulin synthesis.

A3. The genetic diversity of the precursors of antibody-forming cells arises from a high rate of spontaneous mutations during their lifelong proliferation.

A4. This hypermutability consists of the random assembly of the DNA of the globulin gene during certain stages of cellular proliferation.

A5. Each cell, as it begins to mature, spontaneously produces small amounts of the antibody corresponding to its own genotype.

A6. The immature antibody-forming cell is hypersensitive to an antigen-antibody combination; it will be suppressed if it encounters the homologous antigen at this time.

A7. The mature antibody-forming cell is reactive to an antigen-antibody combination: it will be stimulated if it encounters the homologous antigen at this time. The stimulation comprises the acceleration of protein synthesis and the cytological maturation which mark a "plasma cell."

A8. Mature cells proliferate extensively under antigenic stimulation but are genetically stable and therefore generate large clones genetically preadapted to produce the homologous antibody.

A9. These clones tend to persist after the disappearance of the antigen, retaining their capacity to react promptly on its next reintroduction.

Further immunological and chemical analysis will be of extraordinary interest. In contrast to the uniformity of rabbit globulins, normal and antibody globulins of horse serum proved to be greatly heterogeneous but equally as, a wide variety of N-terminal groups being found in all preparations (20). This confirms the concept of the plurality of antibodies evoked by a given antigen, which have in common only the general properties of normal gamma globulins and the capacity of reacting with the evoking antigen. The globulin of man, and in particular the characteristic globulins produced by different patients suffering from multiple myeloma, are likewise recognizably different, inter se, in amino acid composition (21).

Gene for Globulin Synthesis

A2. The cell making a given antibody has a correspondingly unique sequence of nucleotides in a segment of its chromosomal DNA: its "gene for globulin synthesis.

This postulate follows plausibly from proposition A1, and would trace antibody-forming specificity to the same source as it is imputed to other specific proteins. As the deterministic of genetic hypotheses, it should be the most vulnerable to experimental test. For example, a single diploid cell should be capable of at most two potentialities for antibody formation, one for each chromosome.

In case of single antibody-forming cells from rats simultaneously immunized against two Salmonella serotypes, Novak and I (22) could find only mono-specific cells producing one or the other antitoxin. Coons (23) and White (24) have reached a similar conclusion in applications of fluorescent labeling technique. However, Cohen and Lennox (25) have convincing evidence for some bispecific antibody-forming cells in rabbits serially immunized against two bacteria. Experiments pertinent to the possibility of a single cell's carrying more than two antibody-forming specificities remain to be done (26).

The chromosomal localization of antibody-forming specificity is uncoupled from its elective origin in proposals (7, 9, 27) that an antigen induces a mutation in a gene for globulin synthesis, though not necessarily involving a new nucleotide sequence.

Multiple specificity would stand against a simple chromosomal basis for antibody formation (28), leaving two alternative possibilities: (i) replicate chromosomal genes or (ii) extrachromosomal particles such as microsomes. These might best be disentangled by some technique of genetic recombination.

The differentiation of microsomes must be implicit in any current statement of a theory of antibody formation that recognizes their central role of protein synthesis. The main issue is whether or not their specificity is dependent on that of the chromosomal DNA. Autonomy of microsomes, in contradiction to proposition A2, is implicit in most interactive theories; the microsome carries either the original or a copy of the antigenic message. On the other hand, a powerful electrophoretic theory is generated by substituting the term microtropic DNA for the term chromosomal DNA and gene in the various propositions. Since a single cell may have millions of microsomes, this would theory allow for any imaginable multiplicity of antibody-forming information in a single cell. If the potential variety of this information approaches that of the total antibody response, further instructions in an antigenic input would become moot. In addition, the complexities of selection of cellular populations would be compounded by those of microsomal population within each cell. Degrees of freedom which blur the distinction between microsomal instruction and selection favor the utility of the chromosomal hypothesis as a more accessible target for experimental attack.

Genetic Diversity of Precursor Cells

A3. The genetic diversity of the precursors of antibody-forming cells arises from a high rate of spontaneous mutation during their lifelong proliferation.

Three instances of this statement should be emphasized: (i) that antibody-forming cells are specialized, (ii) that their diversity arises from some random process, and (iii) that the diversification of these cells continues, in company with their proliferation, throughout the life of the animal.

Item (i) and its justification by various experiments have already been discussed as an aspect of proposition A2. Talmage (2) also stresses the specialization of antibody-forming cells by referring to their progressive differentiation. This is entirely consistent with propositions A3 and A4, which then postulate a specific mechanism of cell for differentiation, in this case gene mutations. If, on Talmage's model, fully differentiated cells are ultimately left with no more than one antibody-forming specificity per chromosome, the general consequences will be the same whether this final state represents the unique activation of one among innumerable chromosomal loci (27) or the evolution of one among innumerable specific alleles at a given locus. Once again, the final resort for decision may have to be a recombinational technique.

If the discrepancy between the experiments of Novak and Lederberg (22) and those of Coon and Lennox (25), as di
cated under proposition A2, is real and depends on the timing of immunization, it may furnish strong support for (ii), the random origin of antibody-forming specificity. If antibody-forming cells can have two (or any small number of) specificities randomly derived, only a negligible proportion will have just the two being tested for. This would correspond to the case of simultaneous immunization with the two test antigens. If, however, a population of cells carrying one specificity is selected for, followed by selection for a second specificity among all available cells, this is the case of serial immunization and is precisely the method one would predict to obtain a close “heterogeneity” for two mutant allics. Simultaneous versus serial immunization would be analogous to the suppression versus selection of bacterial mutants resistant to two antibiotics (29). Further experiments are needed to exclude some trivial reasons for the scarcity of bisplicific antilgbulin-forming cells.

Item (iii) diverges from Burnet’s proposal that the “randomization” of antibody-forming cells is confined to perinatal life, thereby generating a set of 30 stable clones corresponding to the antibody-forming potentiality of the animal. These clones would then be irreproducible if lost either by random drift or as a consequence of premature exposure to the corresponding antigens. The arguments against Burnet’s proposal are by no means decisive; however, the correspondence between cells and antibodies is made more difficult by having to maintain each clone at a sufficient population size to compensate for loss by random drift. Further, the occurrence of antibody-forming specificity is supported by experiments showing the decay of immune tolerance in the absence of the corresponding antigen (30, 31), contrary to proposition A6). Since immune reactivity in these experiments may return during adult life, susceptibility to the induction and maintenance of tolerance by the timely introduction of the antigen may have only a coincidental relationship to the immunological incompetence of the newborn animal.

Hypermutability

A6. This hypermutability consists of the random assembly of the DNA of the “globalon gene” during certain stages of cellular proliferation.

This ad hoc proposal is directed at the least defensible of the propositions, and certainly the furthest removed from experimental observation. It is stated to illustrate that extensive replication rather than mutability is the more remarkable phenomenon, whatever the detailed mechanism for the variation. If, as has been suggested, many nucleotide triplets are nonfunctional (21), the triplets rather than single nucleotides would have to be posted as the unit of assembly in this case.

To carry this speculation one step further, heterochromatin has been proposed to be, on the one hand, a random sequence, and, on the other hand, a dis-synchronously assembled segment of the genome (22). If both views are correct, proposition A4 might be restated: “the globulin gene is heterochromatic during certain stages of cellular proliferation” (becoming by implication, euchromatic in the mature stages of propositions A8 and A9).

For the theory of micromutational election it might be postulated that globulino-genic micronuclei are initially fabricated as virtual replicas of the globulin gene, but are then capable of exact, autonomous replication.

Pending more exact knowledge and agreement of opinion on the morphogenetic relationships of antibody-forming cells, the term certain stages cannot be imposed upon. On the other hand, as is shown under proposition A8, a model might be constructed even on the basis of a constant but high mutation rate of all antibody-forming cells.

Further insight into the mechanism of cellular diversity in antibody formation may be won by studies on the genetic control of reactivity to various antigens in inbred animals (33); two cautions, however, must be stated: (i) for effects on the transport of particles of different size, and (ii) for effects from cross-reactions with gene-controlled constituents evoking autotolerance.

Spontaneous Production of Antibody

A5. Each cell, as it begins to mature, spontaneously produces small amounts of the antibody corresponding to its own genotype.

Note the implication that antibody is formed prior to the introduction of the antigen into the antibody-forming cell.

The function of spontaneous antibody is to mark those cells preadapted to react with a given antigen, either to suppress these cells for the induction of immune tolerance (proposition A7) or to excite them to massive antibody formation (proposition A7). Therefore, the antigen need not participate in no type of specific reaction with cell constituents other than antibody itself, the one type of reaction available to chemically diverse antigens that requires no further special pleading. There is no agreement whether the reactive globulins found in the serum of untreated animals are produced spontaneously or by casual exposure to cross-reacting antigens (see 2). Accordingly, the spontaneous antibody postulated in proposition A5 may or may not be produced in the quantity and form needed for it to be liberated and detected in the serum. The non-specific fragment of antibody-globulin described by Porter raises the possibility that the same determinant segment may be coupled either to a diffusible or to a cell-bound residue, the latter corresponding to various aspects of cellular immunity, including the suppression or excitation of antibody-forming cells by reactions with the corresponding antigens.

Induction of Immune Tolerance

A6. The immature antibody forming cell is hypersensitive to an antibody-antigen combination. It will be suppressed if it encounters the homologous antigen at this time.

This is the first of four propositions which bear less on the source of antibody-forming specificity than on its subsequent expression in terms of cellular behavior. These propositions are therefore equally applicable to immune theories.

The duality of reactions of antigens with antibody-forming cells is simply a restatement of the experimental observations of tolerance versus immunity (34). It seems plain that every cell of the antibody-forming system must be marked to inhibit its reactivity both to the autologous antigens of the same animal and extraneous antigens introduced and maintained from a suitably early time of development. In the light of current evidence for the persistence of antigenic molecules (2, 6) and for the loss of tolerance when a given antigen has disappeared (30) there are no more plausible candidates for the self-markers than the antigens themselves. The distinction between the function of an antigen as inhibitor (self-markers) or as inducer of antibody formation is then the time when the antigen is introduced into the potential antibody forming cell. We may predictably define maturity in terms of
the progression of the cell from sensitiv-
ity to reactivity.

The suppression of this process of ma-
turation is a sufficient attribute to ac-
count for tolerance, and this need not in-
volve so drastic an event as the de-
struction of the cell. However, the elect.
ive hypersensitivity program that only a lim-
ited number of cells will spontaneously reac-
t with a given antigen, so that their de-
trocity by premature reaction can
safely be invoked as the means of their
suppression. It may be hoped that pres-
ently documented phenomena of cellular
hypersensitivity may furnish a means of
tolerance, and this need not be
invoked as the means of their

The destruction of invading lymphocytes
in the course of rejection of a
sensitizing homologous antigen (36) supports the
speculation of some role of cellular de-
struction in immune antibody-forming
in the induction of tolerance.

The nature of immaturity remains
open to question. It might reflect the
sensitizing nature of the antibody-
forming cell—for example, sensitive lymphocytes
→ reactive plasma cell
(39), some particular composition of immu-
nodependent sensitizing antibody, or merely a
very low level of antibody so that com-
plexes are formed in which antigen is in excess.

Finally, one additional hint of an im-
lication of hypersensitivity in the early
stages of the antibody response: the transient skin sensitivity of delayed type
and transferable by cells) appearing in
the course of immunization, as observed by
several workers (38). If these skin
reactions reflect the destruction of some
antibody-forming cells, it would speak for
some overlapping or reversibility of
the two stages of maturation.

The implications of proposition A6 in the
electrolyte theory may be summarized as follows: If an antigen is introduced
prior to the maturation of any antibody-
forming cell, the hypoactivity of such
cells, while still immature, to an antigen-
antibody reaction will eliminate specific
cell types as they arise by mutation,
thereby inducing apparent tolerance to
that antigen. After the disipation of the
antigen, reactivity should return as soon as
new mutant cell has arisen and matured.
As a further hopeful predic-
tion, it should be possible to induce
tolerance in clones of antibody-forming
cells from adult animals by exposing a
sufficiently small number of initials to a
given antigen.

Excitation of Massive
Antibody Formation

A7. The mature antibody-forming cell
reactive to an antigen-antibody com-
position: it will be stimulated if it first
encounters the homologous antigen at
time. The stimulation constitutes an
acceleration of protein synthesis and the
cytological maturation which mark a
"plasma cell."

A8. Mature cells proliferate exten-
sively under antigenic stimulation but
are genetically stable and therefore gen-
erate large clones genetically pre-
determined to produce the homologous
antibody.

These premises at the initial re-
response to secondary antigenic stimu-
lation are widely accepted and are readily
transposable to the primary response on
the elective hypothesis whereby some
cells have spontaneously initiated anti-
body formation according to proposi-
tion A5.

Proliferation of Mature Cells

A9. Mature cells divide, increasing in
number at a given clone. It* ultimate
size is determined by one or both of
the following: (i) the increase in num-
ber of cells with reactive potentialities,
and (ii) the production of new
antigen-reactive cells in response to
the antigen.

Persistence of Clones

A10. These clones tend to persist after
the disappearance of the antigen, retain-
ing their capability to react promptly to
anterior reexposure.

This is a restatement of the possibly
controversial phenomenon of lifelong
immunity to viruses (9, 31). A substi-
tutional reservoir of immunological memory
should be inherent from one cycle of
expansion of a given clone. In ultimate
cells might be eliminated either by conti-
nued selection (that is, persistence of the
antigen) stabilization of genotypes,
or clonally (to cell division or remuta-
tion, or both) on the part of a fraction
of the clone.

Discussion

Each element of the theory just pre-
posed has some precedent in biological
fact, but this is testimony of plausibil-
ity, not reality. As has already been
pointed out, the most questionable prop-
osition is A6, and it may be needlessly
cautious to forward a too explicit hy-
pothesis of reversibility for antibody for-
mation when so little is known of its
material basis anywhere.

Theories of antibody formation have,
in the past, been deeply influenced by
the physiology of inducible enzyme syn-
thetis in bacteria. In particular, instruc-
tive polypeptides (39) from the cell-surface
substrates in enzyme induction have encouraged
the same speculation about antibody for-
mation. The interpretation of enzyme
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may be more explicitly canalized under genotypic control. Nucleotide substitution might still play a role here by modifying the level of activity rather than the specificity of neighboring loci, and selective recognition of transient states apparently determined remains as a formal, if farfetched, possibility for other morphogenetic inductions.

Reference and Notes

1. This definition excludes antibody-like substances such as the IgM antibodies found in normal human sera. These reactants do not, however, pose the problem of mechanisms of specific immune response which is the burden of this discussion.

2. Telencephalin, in the sense of science, discusses various aspects of antibody specificity, including those not discussed in current immunological thought. For in present research, however, the number is left open for experimental determination, for it would encompass a diversity of cellular selective only if it is large compared with the number of potential antibody-forming cells initially present. Selection of the most sensitive (7) has not attempted to count the antibody-forming cells in primary response, but its measures are compatible with an incidence of $10^4$ to $10^5$ of cells having antibodytitans in normal human sera after immunization. Normal $B$ cells in the spleen that bear the proper antigens in the interval after immunization. Two per cent of resting cells in a primary response are antigen reactive. These figures are derived from a number of different studies of the frequency of antibody-forming cells in the interval after immunization. Two per cent may mean that the idea that all of the resting cells are independent of the lymph node, or whether circulating cells of the same type in which readily administered antigenic specificity can be observed.

27. J. Schachner, Science 197, 907 (1953). Schachner's studies of antibodies between antibody formation and antigen recognition in Rabone, including the role of complements in this sexual transformation, were carried out in an environment which was limited by an unrealizable injection of antibodies A and B by the use of a single cellular model such as Burnett and Schachner.

28. A normal cell should be heterogeneous for at

most two alleles or one locus, but antibody production occurs as a mechanism of population, not phenotype. A cell whose progeny are considered to include a range of different states might carry a phenotypic control of information no longer represented in its environment. Its environment is not that of a single cell. The concept of antibody in \"anatomical\" sense is subject to the qualifications.


