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Immunological Memory from Thucydides to Burnet and beyond

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ABSTRACT

Immunological memory, from Thucydides to Burnet

Immunological memory has been observed since ancient times because those who recovered from an epidemic (infectious) disease usually did fell sick it a second time. For centuries, fanciful hypotheses were put forward on the origin of this acquired refractoriness to specific diseases, which mainly imagined the depletion in the host of some factor that normally allowed the production of the pathologies. In 1890, the antibody was discovered, and the problem became twofold. On the one hand, as the result of an infectious or antigenic stimulus in the body, how could specific antibodies appear, and how were antibodies made?
On the other hand, what does immunological memory depend on, that is, how is the specific trace of the encounter with the infectious challenge or an antigen preserved? Immunochemical research demonstrated the intrinsic or spontaneous diversity of antibodies. The specificity of recognition is not absolute or exclusive to one antibody but rather the result of a multiplicity of partial recognitions by antibodies with different affinities for the antigenic determinant(s). Furthermore, a comparison of successive immune responses showed that a second stimulus with the same antigen elicits faster and more chemically effective antibodies. At that point, diversity could be imagined and then established to pre-exist, i.e. it resulted the condition that allowed the immune response to be thought of as adaptive. In the meantime, each specific antibody was synthesised by differentiated cells undergoing clonal expansion. Therefore, the functional logic of immunological memory was based on the formation of B or T cells, which spontaneously express on their surfaces receptors with predefined specificity and undergo clonal expansion following the encounter with the antigen. Selected antibodies made by plasma cells can remain in circulation for some time. Some B and T cells evolve into memory cells ready to be activated in case of a further

stimulus from the same antigen. Explaining the functional logic of immunological memory has inspired one of the most successful neurobiological models of how the brain works as a selective system, Gerald Edelman's theory of neural Darwinism.

Keywords: Adaptive Immunity - Specificity and Diversity - Clonal Selection - Neural Group Selection

Introduction

During the first hours and days of an infection, an initial defense front, called innate immunity, is activated in a vertebrate organism, recruiting macrophages, monocytes, or NK cells, and humoral factors such as complement. These responses trigger an inflammatory response and proceed to eliminate pathogens. Suppose the first line is unsuccessful, which rarely happens. In that case, the escaped microorganisms are in the meantime engulfed and biochemically fractionated by a heterogeneous family of cells that "publicly" expose fragments (antigens) on their surface and, therefore, they are called "antigen-presenting cells" (e.g. dendritic cells). This exposure stimulates an activation of specific T and B lymphocytes, or the clonal expansion of cells expressing receptors capable of complementary recognition, i.e. with significant affinity, of the molecular profiles of the foreign element, triggering different effector mechanisms (e.g. release of cytokines, production of immunoglobulins, cytotoxic activity, etc.): this is the so-called adaptive immunity, which is specific (i.e. selective or targeted against a particular antigen/parasite) and keeps a more or less persistent memory of the encounter with the antigen/parasite. This memory will neutralise reinfection with the same pathogen more rapidly and effectively. And it is because of this trait of adaptive immunity that it is possible to induce - even artificially using vaccines and inoculations - a protective memory against specific infections[1].

For a long time, innate immunity was thought to be nonspecific and devoid of memory. Still, in recent decades this idea has been challenged following the discovery of pathogen molecular structure recognition receptors (PRRs). These are expressed in a variety of inflammatory cells and recognise the distinct components of microorganisms. The combination of PRRs expressed by an immune cell may allow for the partially specific identification of the type of microorganism encountered. For example, innate immune cells recognise the difference between a Gram-negative and a Gram-positive bacterium, although not between two closely related species or strains. In addition, innate immunity would be modulated by previous encounters with microbes or microbial products. This property has been termed "trained immunity", which is thought to constitute a form of memory. However, the immunological memory property - the one that allows vaccine prophylaxis through vaccines wherein the immune system can be taught to recognise and neutralise a specific pathogen, even if never encountered before - remains the one based on antibodies and lymphocytes[2].

What is immunological memory?

In the immunological literature, immunological memory is defined as a change in the state of the immune system of a host following acute infection (or vaccination) that makes it prepared to respond more quickly and more accurately (affinity maturation) to a second encounter with an antigenic stimulus of the same nature. The mnestic trail that forms during the induction of immunological memory, between the first infection and a reinfection, sees the circulation of specific antibodies and the formation of memory cells, and confers on the organism that ability to respond more efficiently and effectively[3]. Memory cells can be either B-lymphocytes or T-lymphocytes (CD4+ or CD8+), operating from different tissue sites and in the context of different moments or phases of the response to repeated antigenic stimulation[4].

The immune system is endowed with a "plasticity" of response and it does not involve the physical storage of a kind of molecular template or digitalization of the antigen, but rather a continuous processing and re-processing in the presence of an antigenic experience. When we vaccinate, we induce in the immune system the false memory of having had contact with a pathogen, which instead has never been encountered before. This is possible because the immune system is not a "tabula rasa", but it learns by modulating functionally because of experiences of organized physiological activities that proceed spontaneously[5].

Experimental studies on mice and humans have revealed a very complex cellular and molecular network at the basis of immunological memory, where B cells and T cells mature in different places and ways with receptors expressed on the cell surface: B cells can respond in ways independent or dependent on T cells to antigen, T and B cells differentiate as a result of the encounter with the antigen in variable times and modes, etc[6]. It is not the purpose of this article to retrace the history of studies carried out on the complex and nested mechanisms in details that in detail or in the context of specific experimental models produce the phenomenology of the immunological memory, but only to tell how the explanatory principles to which these mechanisms refer have changed. In particular, through which theories and experimental data it has been understood that the formation of antigen-specific antibodies and memories are processes driven not by the characteristics of the antigen, i.e. by the external stimulus or instructive, but are instantiations of a selective or Darwinian logic.

The discovery of immunological memory and the first insights into its nature

It is no coincidence that we owe the first description of the immunological memory to Thucydides and his account of the so-called "plague of Athens" of 430 BC. Specific epidemiological conditions were necessary to observe the phenomenon: an acute viral or bacterial infection of high lethality, which killed the host in a short time but in some cases healed, and a concentration of a population numerically sufficient to observe

healings and new contacts of the healed with the sick. The author of the Peloponnesian War wrote that "the same man was never attacked twice - at least never fatally"[7]. Rarely did the healer become ill again, but in the event that he did get ill, he did not die. Even Procopius, in 541, in the middle of the first pandemic plague (socalled of Justinian), observed that when the disease returned in a region already intensely affected, it caused practically no deaths[8]. The Arab physician Abu Bakr Mohammad Ibn Zakariya Al-Razi (Al Rhazes), in his *Treatise on Smallpox and Measles* (ca 910), reiterated that an epidemic disease never strikes an individual twice[9].

The intuitive explanations of the phenomenon called into question either the expulsion of some humoral excess, or the exhaustion of some fermentative principle or condition of the development of pathological processes, or the retention of some component of the intoxicating factor, which was transformed into a protective principle. Louis Pasteur, who developed the first artificial vaccines, was one of the lasts to conceive the acquisition of immunity as a passive change for the organism and one of the firsts to seek a biological explanation[10]. In 1880, the inventor of the first artificially attenuated vaccines thought that immunization was a consequence of the fact that a benign form of the microbe would consume the substrate necessary for the growth of more severe forms[11]. Later on, Pasteur thought that acquired immunity was the consequence of the growth in the body of a live, attenuated variety of an agent[12]. This hypothesis was abandoned after 1880, following the discovery by Daniel Elmer Salmon and Theobald Smith that even the killed pathogen can induce immunity[13] and from the observations that the pathogenic action of diphtheria and tetanus bacilli was due to non-living components, i.e. toxins[14].

The discovery of adaptive immunity: the beginning of serology

In 1890, Emil von Behring and Kitasato Shibasaburō discovered that, in response to the inoculation of exotoxins, the organism produces "antitoxins", which are able to selectively neutralize and prevent the harmful action of poisons. The acquired resistance to infection could be transferred passively from one animal to another through the serum of an immunized donor, which manifested a specific antitoxic property[15]. Paul Ehrlich introduced in 1892 the distinction between actively and passively acquired immunity[16], which integrated the distinction between natural and acquired immunity, emerged during Pasteurian experiences of vaccination. With the discovery of antitoxin, the problem of the nature of immunity took a turn in favor of a humoral basis of the phenomenon. The humoral explanation of immunity, favored by German microbiologists, was in competition with the approaches of the Pasteurian school of the zoo pathologist Elie Metchnikoff, for whom immunity was an active response of the organism to an invasive agent, related to the normal physiology of cells, in particular to nutrition, and to the cooperative and competitive processes that

ensure the functional integrity of the organism. In 1884, he had hypothesized that immunity was due to the phagocytic activity of leukocytes (phagocytosis theory), arriving at this conclusion on the basis of experiments on the digestive power of mesodermal cells distributed among different evolutionary phyla and the observation of inflammatory reactions[17].

Between 1890 and 1905, the main phenomena due to the interaction between the immune sera and components of bacterial, organic or artificial nature were described, and it was understood that in the serum there are non-specific and thermolabile factors, such as complement but also cells with phagocytic action, and specific and thermostable factors that were called with the names of the different reactions they caused (antitoxins, agglutinins, precipitins, hemolysins, etc.), and, finally, "antibodies"[18].

Therefore, a heated discussion about the chemical nature of antibodies and the type of bond that they form with antigens began at the time. However, the most relevant theoretical question was the one raised by natural and artificial immunization: now that it was known that acquired immunity was due to the appearance of a protective factor in the blood following contact with a parasite or an antigen, where did this factor come from? What physiological or biochemical process, i.e. what change could allow the organism to acquire the ability to respond in such a targeted manner? Why could such specific ability persist over time?

In search of the physiology of immunity

Starting from 1884, Pasteur moved toward a "chemical" explanation of acquired immunity, attributing to the same microorganism the secretion of a chemically defined antagonistic substance, which prevented its subsequent development[19]. After the discovery of antitoxin, the first 'class' of antibodies identified, the hypothesis that appeared more plausible to most researchers, given that the chemical nature of antitoxin was unknown, was that the same toxic substance entered materially to form the antibody.

In 1893, biochemist Hans Büchner wrote that only the common origin of both substances from bacterial plasma, the poisonous and the protective, made the specific nature of the protection and its persistence intelligible[20]. The hypothesis was shared by Metchnikoff and Max von Gruber, who considered it the only 'logical' explanation for the specificity of antibodies. However, Emile Roux, in the same year, showed that the continuous bleeding of an immunized animal did not decrease the titer of antibodies in the serum, even after a quantity of blood equivalent to the original volume had been taken[21]. Other experiments showed that, for each unit of toxin injected into the horse, it could form more than 100,000 units of circulating antitoxin and, since it was quite difficult to explain these facts in terms of the hypothesis of Büchner, this was abandoned. In 1929, Michael Heidelberger and Forrest E. Kendall will confirm that the

amount of antibody produced by the immunized animal is far greater than the amount of antigen used for immunization, also establishing that antibodies against artificial antigens do not contain traces of the immunizing compound[22].

The German physician and physiologist Paul Ehrlich suggested in 1897[23] that antibodies were nothing more than side chains or pre-existing receptors on the protoplasm of cells, whose normal function was to chemically bind the nutrients needed by the cell, but also toxic substances that happened to have the same chemical structure as the nutrients. When the second eventuality occurred, it determined a functional damage to the cell, which reacted by producing, for "overcompensation", side chains of the same type in excess, which were released into the bloodstream as antibodies. The origin of specific antibodies and the acquisition of persistent immunity over time, according to Ehrlich, were thus not shrouded in mystery, but demonstrated what he called the ancient "wisdom of protoplasm". The formation of antitoxins was, therefore, devoid of any finalistic character, being a process entirely analogous to the processes of synthesis on which cellular metabolism is based[24].

In Ehrlich's theory, antibodies directed against antigenic stimuli came neither from nothing nor from the microbe, but pre-existed as cellular receptors, and the lasting protection, the immunological memory, would be ensured by the constant excess of specific antibodies synthesized by the cells as a result of the functional impediment due to chemical interaction with the toxic material. While assuming the preexistence of side chains/antibodies, Ehrlich thought that these had absolute specificity for the antigen and denied that there were chemical cross-reactions or affinity enhancement in the course of the response. The German doctor argued that the affinity between antigen and antibody was due to covalent bonds and not to weak links that give rise to interactions of the stereocomplementary type "key-lock", where a given key always opens one and only one lock. Svante Arrhenius, on the other hand, explained immunochemical reactions in chemical-physical terms, and in the first thirty years of the twentieth century, the antigen-antibody interaction became a model for colloid chemists to study the chemistry-physics of an interaction thought to be mediated by surface interactions between colloidal complexes[25].

In 1906, the possibility to provoke a specific response against artificial antigens was discovered and, therefore, the number of side chains or specific antibodies became theoretically incommensurable that would have to admit the pre-existence: this made the Ehrlichian hypothesis about the mechanism of formation of the antibody unsustainable. In the following decades, the hypothesis would prevail that the specificity of the antibody should depend on the existence of some biochemical mechanism capable of printing the stereocomplementary form of the antibody using the surface chemical conformation of the antigen[26]. This type of explanation was taking shape in the context of chemical research on biological macromolecules and the molecular basis of the biochemical interactions on which enzymatic and immunological specificity

depend[27]. Several models imagined how the antigen, somehow present at the site of antibody formation, could function as a template for continuous synthesis of immunoglobulins that would be found in circulation even after the antigen disappeared. The most famous and influential theory of the antigenic template was conceived by Linus Pauling in 1940[28]. This theory, as well as other instructive theories, claimed that the diversity of antibodies produced against an antigenic stimulus was the consequence of the imperfection of the mechanism of synthesis and it was not able to explain the phenomenon of recall and immunological memory. Even less so was immune tolerance, observed and experimentally reproduced in the 1940s, whereby an animal could become tolerant to a normally immunogenic stimulus if it was exposed to it in the early stages of embryonic development[29].

Immunity as a model of adaptive response

The adaptive nature of specific immunity, that is, the fact that the organism proved capable of keeping track of an infectious experience or an experimental antigenic stimulus, was considered one of the most characteristic examples, along with psychic (and/or nervous) phenomenology, of the functional plasticity of the organism. The immunopathologist Ilya Metchnikoff interpreted the phagocytic activity of leukocytes as an adaptive trait that can be modified through experience[30]. Paul Ehrlich, who contributed fundamentally to the construction of the experimental methodology for the study of immune sera, considered antibody formation - as we have mentioned - a manifestation of the "wisdom of protoplasm"[31]. However, non-immunologists were also affected. For the greatest exponent of modern vitalism, the embryologist Hans Driesch, it was precisely from the field of immunity studies that the fact that the organism cannot be compared to a machine emerged most clearly, since it is not possible to imagine a mechanism "whose chemical constituents are such as to correspond adaptively to almost every need"[32]. For the psychologist Edward L. Thorndike, immunity belonged to the same category as learning and growth in what it represents a modified condition of the organism that predisposes it to respond differently to identical situations[33].

Specificity from diversity

Immunochemical studies carried out 'directly' on antibodies at the end of the 1930s showed that removing their essential nature as proteins they stood out above all for their heterogeneity, rather than for uniformity. Antibodies directed against the same antigen, as against different antigens, 'varied' in many ways. First, from a physical-chemical point of view, as evidenced by electrophoretic investigations, and then with respect to the ability to give rise to secondary reactions, such as complement fixation, precipitation, agglutination, or sensitization reactions, and differed in avidity for

homologous antigen and cross-reactivity with related antigens as well as for other characteristics. In 1938-39, Arne Tiselius and Elvin A. Kabat, applying the technique of free-phase electrophoresis, conceived by Tiselius himself a few years earlier, localized antibodies in the gamma fraction of serum and showed in a definitive way their heterogeneity[34]. At the same time, there was the problem of establishing the spectrum of chemical affinity within which the interaction between the antibody and the antigen takes place, that is to characterize in quantitative and functional terms, directly measuring the state functions and the chemical-physical heterogeneity. Toward the end of the 1940s, with the method of equilibrium dialysis, it was found that the values of the association constant can vary between 10^4 l/M, for weakly related antibodies, to 10^9 or 10^{12} l/M, in the case of antibodies with high affinity[35].

The experimental study of the chemical-physical basis of antigen-antibody interaction overturned the concept of Ehrlich about the relationship between immunological specificity and chemical affinity, showing that, in the definition of these relationships, the structural aspect of stereospecific recognition should be supported with a dynamic concept, that is, a biological immune response. In 1959, Davit Talmage published an article entitled "Immunological specificity", in which he reinterpreted the heterogeneity of antibodies, defined through immunochemical studies, in light of a selective model of antibody formation[36]. The concept of immunological specificity connoted not so much the steric complementarity of a single antibody or binding site for the antigenic determinant, but rather a "unique combination of natural globulins", with different degrees of affinity and that together performed the recognition and produced the response. Talmadge neutralized the argument given by immunochemists against the pre-existence of receptors, precisely based on the concept of heterogeneity of the antibody response arising from the study of antibody diversity. Admitting, that is, that the different specificities manifested by an antiserum are the result of changes in the relative concentrations of a limited number of different specific antibodies, the need to assume an unlimited repertoire of pre-existing antibodies able to recognize an unlimited number of possible antigens was eliminated. This concept becomes known as *degeneracy*, a trait that occurs when within biological systems, structurally different components/modules/pathways can perform similar or identical functions (i.e. are in fact interchangeable) under given conditions while performing distinct functions under other conditions. Degeneracy is thus a relational property that always requires comparing the behaviour of two or more components. In particular, if degeneracy is present in a pair of components, then there will exist conditions in which the pair will appear functionally redundant but other conditions in which they will appear functionally distinct. In various biological contexts, ranging from the genetic code to the immune system and the nervous system, it is the condition that allows biological systems to increase in complexity and evolve[37].

The Danish immunologist Niels Kay Jerne, meanwhile, showed that the avidity of antibodies for antigen depended only on the value of the association constants of a particular toxin-antitoxin system. He found that the value of the association constant K1, as a measure of toxin avidity, varied in the case of diphtheria antitoxins from 0.02 to 4.0 and increased with time after immunization[38]. However, he observed that, in the first phase, lasting about a week, the avidity remained low, so it could be assumed that the cells secreting the antibodies produced toxins of different avidity, with which they would combine the antigen still present in the circulation. This idea led him to a different orientation in the study of the mechanism of the immune response compared to the immunochemical tradition. Informed by the theoretical developments in the field of bacterial genetics, where a selectionist or Darwinian explanation had supplanted the instructivist explanation of enzymatic adaptation with the experiments of Max Delbruck and Salvatore Luria in 1943[39], Jerne elaborated in 1955 a similar reasoning regarding the physiological bases of the immune response[40]. Instead of considering the antigen as a template to shape the configuration of the specific antibody, he hypothesized the pre-existence of antibodies with different affinities, interpreting the encounter with the antigen as a (natural) selection, operated within a spectrum of heterogeneous antibody structures, actively produced by the organism, which was followed by the reproduction of the most suitable antibodies in recognizing the antigen.

The experimental investigation of immunological memory

Jerne's observation of increased avidity of antibodies synthesized in response to an antigenic stimulus was the norm by the 1930s, as immunochemical experiments were conducted with animals receiving multiple injections of the antigen. As a result, most experiments translated immunological memory into a graph recording a significant difference between the amount and affinity of antibody produced in response to the first and second or subsequent administrations. It was the Australian virologists and immunologists Frank Macfarlane Burnet and Frank Fenner who first noted the differences between the primary responses induced by antigenic stimulation and the secondary responses resulting from the fact that a memory of the first experience had been forme[41]. Rabbits injected intravenously or subcutaneously with Staphylococcus antigen provided the model for testing antibody activity. Antibody response results from individual animals injected intravenously or subcutaneously demonstrated that antibodies could be detected more rapidly after secondary challenge (in 2 days) than after primary injection (in 8-13 days). In addition, the amount of antibodies produced after a second antigen injection was increased.

Burnet and Fenner wondered what the mechanism was that led to the rapid increase in antibody titers after a second injection, hypothesizing that it resulted from a phase of the response in which antibody-forming units multiply at a relatively constant rate

somewhere in the body. Although they did not know the identity of these "antibody-forming units", they thought they were cells. Burnet and Fenner also hypothesized that a primary antigenic challenge would induce an increase in the number of cells and that subsequent contact of the antigen with these cells would cause further cell proliferation followed by increased and more rapid synthesis of antibodies. When Burnet and Fenner published *"*The Production of Antibodies", immunologists agreed that second exposure to an antigen led to more rapid production of a greater number of antibodies, but some questions were still open: which cell was responsible for antibody synthesis? What role does the antigen play in determining the specificity of the antibody? How long does the memory and, therefore, the protection last?

Immunological memory is a complex trait

Between 1957 and 1959, Niels Jerne's theory of antibody formation based on natural selection was translated by Frank MacFarlane Burnet into a biologically consistent model called the theory of clonal selection[42]. The theory postulated that cells expressed their antibody as a surface receptor and could, therefore, be selected by the antigen. Early in development, encountering the antigen (self) leads to cell death, establishing self-tolerance. Subsequently, upon encountering external antigens, cells respond by clonal expansion and differentiation into antibody-secreting cells (later called B lymphocytes). This theory explained the formation of immunologic "memory" by selective expansion of specialized cells; and immunologic "learning", or improvement in antibody quality as the response progresses ("affinity maturation") by the selection of cells expressing antibodies with high affinity for the antigen.

The cellular architecture of the theory emerged slowly as well as the experiments aimed at falsifying it. These experiments led to the demonstration that the idea had solid physiological foundations. In 1948, Astrid Frageaus demonstrated that antibodies are produced by plasma cells, and twelve years later, Peter Nowell discovered that lymphocytes were not end-stage differentiated cells, but rather was able to proliferate rapidly and extensively in response to mitogens and antigens[43]. Therefore, plasma cells turned out to be differentiated lymphocytes. In 1963, Neils Kaj Jerne and Al Nordin developed the plaque assay that allowed the detection of individual spleen cells as antibody producers, contributing to the empirical validation of Burnet's clonal selection hypothesis of antibody formation[44]. While two populations of lymphocytes, thymus-derived and non-thymus-derived, emerged in the 1960s to cooperate in the antibody response, in 1970, two research groups independently identified B lymphocytes as precursors to plasma cells that were characterized as cells carrying immunoglobulins (Ig) on their surface[45]. The following year, T lymphocytes were characterized as lymphocytes lacking surface Ig but with cell surface molecules distinct from B cells[46]. During the 1970s and early 1980s, immunologists devoted themselves to

phenotyping, using monoclonal antibodies and immunofluorescence microscopy, immune cells on the basis of clusters of differentiation and creating an articulated taxonomy of subgroups of T-lymphocytes and B-lymphocytes that differently specialized in producing the articulated phenomenologies of immune responses[47].

In 1962, Gustav Nossal and Olaf Makela inoculated groups of rats once with an antigen against Salmonella flagella[48]. After 2 to 40 weeks had elapsed, they administered radioactive thymidine to the rats, 2 hours before inoculating a second dose of the flagellar antigen. Radioactive thymidine can function as a DNA precursor so that cells that proliferated in response to the second antigenic challenge incorporated the radioisotope and could be detected by autoradiography. If antibody-forming cells remained from the primary reaction, these cells would not have been radiolabeled. Nossal and Makela removed the spleens from rats injected a second time with Salmonella flagella and determined the number of cells incorporating the radioisotope. Virtually all plasma cells formed during the first 5-6 days of the secondary response were radiolabeled, that is, they had proliferated from a small number of cells rather than from the differentiation of preexisting nonproliferating cells. Nossal and Makela concluded that the antibody-forming cells "remembered" the initial antigenic exposure and divided upon subsequent stimulation with the antigen. This was consistent with the hypothesis that "immunological memory" depended on the persistence, after primary stimulation, of a continuously dividing line of primitive lymphocytes, responsive at all times to further antigenic stimulation.

The assumptions of the theory of clonal selection and biochemical discoveries concerning antibody structure raised the question of the genetic origin of the preexisting antibody repertoire and the molecular mechanism by which polypeptide chains with constant and variable regions could be formed. According to Landsteiners' immunochemical studies the body could produce antibodies to almost any foreign substance[49]. This amazing ability to generate diversity is one of the hallmarks of acquired immunity. At the same time, the specificity of serum to a substance can increase over time as it was rigorously tested in 1965, when Gregory Siskind and Herman Eisen who that, after injecting small amounts of antigen in rabbits, there was a gradual increase in the intrinsic affinity of serum antibodies to the antigen[50].

During the 1960s, two alternative explanations inspired theoretical and experimental investigations aimed at solving the so-called antibody enigma: the germline hypothesis, or the idea that the genes for antibodies were all contained in the genome, and the somatic mutation hypothesis, or the hypothesis seen that some somatic mutation processes contribute to the genes for the antibody repertoire[51]. In 1976, Susumo Tonegawa and co-workers discovered the mechanism that allows the generation of diversity at the level of antibody molecules: it was discovered that genes rearrange and recombine as well as undergo hypermutation during the proliferation of B cells to allow through selection the adaptive process of affinity maturation. After nearly a century of experimen-

tal research, the solution to the antibody enigma finally arrived, signifying the origin of the enormous diversity but exquisite specificity of antibody molecules.
In 1983 and 1984 the TCR was identified on T cell hybridoma and on normal T cells clones, and it resulted that its molecular structure contained both variable and constant portions[52]. The clonation of TCR genes cDNA encoding the first of the TCR chains revealed that the TCR genes and molecules belong to the immunoglobulin super family[53]. B and T cell precursors generate antigen recognition diversity by assembling the exons that encode Ig or TCR variable regions from individual variable (V), diversity (D), and joining (J) gene segments through recombination-activating gene-1 (RAG-1) and RAG-2 proteins[54].
Over the past half-century, experimental research has described the existence of a complex distributed immunological memory system, hinged on B and T cells that differentiate in the face of antigenic stimulus, resulting in a memory that is somewhat unique to different experiences of pathogenic antigens and is ultimately a reorganization of past experiences to keep track of novelty[55]. In 2010, Susan Swain, among the leading experts on the cellular basis of immunological memory, wrote that "we know much less about the formation, maintenance, and regulation of memory cells than we do about the primary response of naïve lymphocytes"[56].

Memory and selection in the immune and nervous systems

The experimental confirmation of the clonal selection theory inspired several researchers to imagine that the functional logic underlying memory and learning in immunity could be exported to other areas of biology. Mcfarlane Burnet emphasized that his theory implemented the Darwinian idea of adaptive response by natural selection, and in 1964, he wrote other systems endowed with memory, that is, capable of learning, such as the brain or the automated machines, perhaps used or could use Darwinian ways of operating their performance[57]. The idea that learning in the brain was based on Darwinian mechanisms was not new.
Restricting our examination of the subject to the ideas that arose from and in the context of the process that saw the theory of clonal selection established on an experimental basis, the neuroanatomist John Z. Young, who quoted and was quoted by Frank McFarlane Burnet, expanded on his cybernetic approach to the neurophysiology of behavior, elaborating a selective theory of memory, the "mnemon theory", according to which the recording of mnestic traces occurs by the elimination of unwanted channels, so that learning consists in the reduction of a large initial redundancy[58]. In the wake of Young's approaches and with reference to Donald Hebb's studies on "cellular assemblies", in 1973 Jean Pierre Changeux, Antoine Danchin, and Philippe Courrege published a synaptic theory of the formation of 'imprints' in the brain in which the 'instructive' effects produced by events were traced to selection processes at the level

of contacts between neurons, that is, as "selection of pre-programmed circuits. It was called the selective synapse stabilization theory[59].

In 1966, the immunologist Melvin Cohn, a close collaborator of Jacques Monod, after a meeting with Karl Popper in La Jolla (California), published a series of papers on the functional logic of what he called "the anticipatory systems of the individual", elaborating a "molecular biology of expectation" to speculate how the organization of the brain during learning could change through selective processes[60]. The most explicit attempt, before Edelman's theory of Neural Darwinism, to apply the model of clonal selection to explain the neurobiological basis of learning, was proposed by Niels Jerne. In 1967 he published a paper entitled "Antibody and learning. Selection vs. instruction", which contained a neo-innatist theory of knowledge and uncommonly endorsed, in fact, an essentialist conception of the functioning of adaptive events, starting from a Darwinian-inspired model[61].

Among the neurobiological theories of learning, memory, consciousness, etc. explicitly inspired by the principles of immunological Darwinism one of the most scientifically successful was proposed by the biochemist ad immunologist Gerald Maurice Edelman.

From clonal selection to neural group selection and Neural Darwinism

Edelman was awarded the Nobel Prize in Medicine and Physiology in 1972. In 1969, he provided the first complete structural description of an antibody molecule, from which all the functional characteristics of this protein were confirmed, especially the fact that it is composed of two heavy and two light chains which are constituted of variable and constant regions[62]. Through his studies on the biochemical basis of antibody diversity and antigen-antibody recognition, Edelman realized that the process of antigen recognition and its adaptive functional consequences, such as the immunological memory, involves several hierarchical levels, which contribute to the selective nature of immune responses, that range from the molecular mechanism that produces genetic and somatic variability, to the dynamic ways of 'storing' at the cellular and multicellular network an adaptive response[63]. He thus initiated a theoretical reflection that led him, in the early 1970s, to suggest a model of clonal selection in which the Darwinian connotations of the physiological immune system were made more explicit. In short, Edelman based his description of the selective functioning of the immune system on the presence of a) a mechanism capable of spontaneously generating a repertoire of different recognition structures (genetic and somatic generation of antibody diversity), b) ways of favoring the encounter between these receptors and the aspects of the environment that the system is capable of recognizing (the various systems of antigen capture and presentation), c) ways of selectively amplifying some of the system's activities after the encounters (differential clonal proliferation as a result of the interaction with the antigen and the communicative

interactions between the cells of the immune system, accompanied by changes in the state of the cell surface)[64].

The possibility that epigenetic alterations at the level of molecules on the cell surface could function as a signaling system regulating interactions between cells was also essential for describing the Darwinian organization of an adaptive system such as the nervous system, which lacked a precise molecular reference for variability, and in which, above all, unlike the immune system, cells no longer divide once they are arranged in their anatomical location. According to Edelman cell adhesion molecules and other molecules involved in the regulation of epigenetic processes proved to be decisive in the construction of brain anatomy or in the formation of repertoires that allowed neuronal group selection[65].

Fifteen years after receiving the Nobel Prize, Edelman published an influential book in 1987 entitled *Neural Darwinism, The Theory of Neuronal Group Selection,* in which he used findings on cell adhesion molecules and epigenetic mechanisms to apply key concepts of clonal selection theory to neurobiological dynamics[66]. The theory of neural Darwinism predicted repertoires of variability at the level of neural populations during development and in terms of synaptic populations. Selection due to experience could eliminate or conserve neurons on the basis of activation and change the likelihood of synaptic networks responding to environmental stimuli. From these dynamics emerge, according to Edelman, cognitive functions, such as memory, were defined in terms of re-categorizations, i.e. reuse of adaptive perceptual categorizations, language, consciousness, etc.

According to Edelman neurological memory is a process, like the immunological one, or "the ability to categorize or generalize associatively" (p. 241). Categorizations occur at the level of a global map and it is degenerate. "Memory is a form of recategorization based upon current input; as such it is transformational whether than replicative (p. 285). It is an iterative process of classification leading to recategorization and thus a portioning of a world that is presented "without label". Memory is the complex of capacities to carry out a particular set of procedures leading to recategorization that is recollected (p. 287), and it does not reside in the data or in storage, to the extent that it exists but memory is an integral part of the process that employs it.

Neural Darwinism represents the most comprehensive attempt to explain the adaptive/cognitive performance of the brain on the basis of the fundamental concepts and empirical knowledge acquired by evolutionary and functional biology since the 1950s, i.e. after the discovery of DNA, the mechanisms controlling the expression of genetic information, the dynamic and regulatory processes of embryonic development, and a vast neuroanatomical and neurophysiological phenomenology that highlights the dynamic and highly variable nature of the physiological processes underlying brain functioning. The distinguishing features of Neural Darwinism from all previously conceived selectionist hypotheses about brain functioning is that it is a theory that

views nervous phenomenology in terms of population dynamics, i.e., as the result of selective events affecting populations of neuronal groups and synapses through pre-existing differential correspondences between neural connections or patterns of activity and specific adaptive functions or environmental stimuli.

Conclusion

Living systems that are capable of adaptively responding to unexpected situations are endowed with memory. This is the case for biological species, which transmit hereditary memories assembled by natural selection in genes, but also for the brain and the immune system. The ability of these systems to store traces of unforeseen experiences depends on the spontaneous production of variations at the level of structural elements, among which are selectively preserved or amplified those that constitute functional responses. In the field of immunological research, the hypothesis has been conceived and confirmed that an adaptive physiological system capable of learning from experience, i.e. equipped with memory, such as the immune system, incorporates selective mechanisms that are somewhat analogous to those theorized by Darwin and Neo-Darwinism to explain adaptive changes on an evolutionary scale.

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