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Introduction

According to UNAIDS, during the year 2016, 36.7 million people on our planet were living with HIV, while 1.8 million people were newly infected by the virus. Since the start of the epidemic in the mid-80s, 35 million people have died of AIDS-related illnesses. Although considerable progress has been made in the treatment of HIV patients with antiretroviral drugs, it is now increasingly accepted that only an effective HIV vaccine will be able to end the AIDS pandemic. A few large-scale HIV vaccine trials have been undertaken, but none, so far, has elicited an adequate level of protection against infection.

It may seem paradoxical that although HIV has become the virus we know most about, after three decades of unparalleled high-quality research funded by billions of dollars, we are not yet sure whether a preventive HIV vaccine will ever be developed. This eBook brings together 24 scientific papers published by the author in the last 20 years which propose explanations for why it has been so difficult to develop a vaccine against what is probably the worst pandemic that has confronted humanity in recent times.

The book is divided into three parts: Immunochemistry (4 chapters), Reductionism (7 chapters), and the Search for a preventive HIV vaccine (12 chapters).

Part 1 consists of five chapters that introduce basic concepts in the immunochemistry of viral proteins which are important for understanding the nature of the reactions between viral antigens and antibodies. These are the key players involved in the immunological mechanisms that protect vertebrates against infections caused by viruses, although they are not the sole actors in immune systems that help fighting off virus infections. Another important player, known as the cellular arm of the immune system, consists of different types of immune cells such as T cells (i.e., thymus-derived), T regulatory cells, and dendritic cells which together with other immunoregulatory mechanisms are important for protecting organisms against viral infections. These other players will not be described in detail because it would have required writing an introduction to the whole of immunology which seemed not to be indispensable for understanding how vaccinologists have tried to harness neutralizing antibodies for protecting humans against HIV infection. The only immune cells

that will be described in detail are the so-called B cells (i.e., bone marrow-derived) which are cells that in their membranes possess B-cell receptors (BCRs) that correspond to antibodies possessing an additional tail that anchors them in the B-cell membrane. When a host is immunized with an antigen, certain BCRs in the host immune system interact with complementary antigens and the corresponding B cells become activated by interleukin-4 cytokines released by helper T cells. The B cells are then transformed into plasma cells that subsequently release antibodies which correspond to BCRs devoid of their anchoring tail.

The following two books are recommended to readers interested in a more comprehensive introduction to immunology: *Immunity* (Paul 2015) and *Immunity: The Making of a Modern Science* (Gallagher et al. 1995).

Part 1. Immunochemistry

Chapter 1 describes the chemical structure of small regions in proteins, called epitopes, which are recognized by the binding sites of antibodies, called paratopes. So-called discontinuous epitopes in a protein are made up of 10–20 amino acid residues located on 3–5 separate segments of the peptide chain that are brought together at the protein surface by the folding of the chain. Continuous epitopes are short peptide fragments of 5–8 residues which are often part of a larger discontinuous epitope and which can bind to antibodies raised against the protein antigen. Classifying epitopes as either continuous or discontinuous may give the erroneous impression that the smallest units of immunological recognition are amino acid residues, although it is at the level of individual atoms that interactions take place. Paratopes consist of 10–25 residues assembled from short stretches of residues located on the six complementarity determining regions of antibodies which together form a discontinuous binding site. Short peptide regions of 3–5 residues present on these six hypervariable antibody loops are often able on their own to bind to continuous epitopes, and these short loop sequences could then be viewed as continuous paratopes. Such binding is usually caused by the type of hydropathic complementarity between hydrophilic and hydrophobic residues that is commonly observed between the sense and antisense peptides encoded by complementary sense and antisense messenger RNA strands. This remarkable pattern arises because RNA codons (sense) and anticodons (antisense), without any exception, always code for amino acids of opposite hydropathy, i.e., either hydrophilic or hydrophobic residues that have a strong affinity for each other. This phenomenon gave rise to the hypothesis that the genetic code initially evolved to favor the simultaneous emergence from complementary RNA strands of sense and antisense peptides that could assume the role of receptors with their complementary ligands (Biro 2005).

Other protein epitopes of 5–10 residues, known as mimotopes, are able to react with short paratope sequences from antibodies raised against the protein even if they show little or no sequence similarity with the protein used to induce the antibody. This type of binding arises when the two peptides possess opposite hydropathic patterns of hydrophilic and hydrophobic residues in their respective sequences.

Chapter 2 analyzes the common assumption that our current knowledge of the chemical structure of epitopes and paratopes should make it possible to apply

molecular design strategies for developing synthetic vaccines. The major difficulty that has been encountered in such attempts is that the capacity of the immune system to induce a protective neutralizing antibody response depends more on numerous biological properties and regulatory mechanisms of the immune system of the host than on the elucidated chemical complementarity of single epitope–paratope pairs. Since a large number of necessary preconditions in the immune system have to be met before neutralizing antibodies can be obtained by vaccination, there are plenty of reasons why the molecular design of an HIV vaccine is not at present a realistic scientific enterprise.

Chapter 3 summarizes the numerous attempts that have been made to develop synthetic peptide vaccines by making continuous epitopes adopt the structures observed when viruses are bound to neutralizing antibodies. Although peptide vaccines would have the advantage of increased safety and stability as well as lower cost, it is remarkable that hundreds of studies using synthetic peptides had not led in 2006 to a single synthetic peptide vaccine marketed for human use (Hans et al. 2006).

A major reason for all these repeated failures is the fact that investigators relied mainly on continuous epitopes that are mostly short linear peptide fragments of more complex discontinuous epitopes which are the large majority of neutralization epitopes found in viral proteins. It must also be stressed that most peptides are immunogenic in the sense that they readily elicit antibodies that react with the peptide immunogen, although they do not cross-react with the parent protein and are not able to neutralize viral infectivity.

Chapter 4 analyzes the binding specificity of antibodies that arises from a limited number of residues in the six antigen-binding regions (ABRs) that together form the numerous paratopes present in every antibody molecule. Each ABR differs significantly in its amino acid composition and tends to bind different types of amino acids present at the surface of proteins. In spite of these differences, the combined collective preference of the six ABRs does not allow epitopes to be chemically distinguished from the remainder of the antigen surface. When the amino acid composition of the six ABRs in 200 different antibody-antigen complexes was examined, it was found that there was no noticeable difference between the amino acid composition of epitopes and that of the entire protein surface. No amino acid was significantly overrepresented in epitope regions compared to the rest of the protein surface, and since the entire surface is an antigenic continuum, most of the surface could actually be part of epitopes. These findings explain why the many algorithms that were developed over the last 20 years for predicting the location of epitopes at the surface of proteins were not very successful (Van Regenmortel 1999a, pp. 53–68).

Antibody polyspecificity refers to the fact that every antibody molecule always harbors numerous paratopes that allows it to bind to a large variety of epitopes present in different antigens. Antibody heterospecificity arises when an antibody reacts better with another antigen than with the one used to raise the antibody. Such a phenomenon is not rare and can lead to the unexpected finding that the antibody appears to have been elicited by an antigen with which it is unable to react, although

this may only reflect the fact that the binding reactivity was below the detection level of the immunoassay that was used. Antibody heterospecificity explains the common observation that early antisera obtained soon after immunization with any antigen frequently contain levels of total induced immunoglobulins that far exceed the level of antibodies able to react with the immunizing antigen. The heterospecificity of antibodies demonstrates that immunogenicity and antigenic reactivity are not always present simultaneously in the same region of a protein which is one of the reasons why the strategy of structure-based reverse vaccinology has not been successful for developing an effective HIV vaccine.

Part 2. Reductionism

Chapter 5 analyzes the appeal of reductionist thinking for trying to understand the relationship between the structure and function of antibody molecules. The structure of a protein arises from the selective attention to the visual experience of that object at a specific time. Such a static definition excludes the dimension of time and conceals the fact that pictures of biomolecules are visual time-slides of dynamic systems. The structure of a binding site, furthermore, cannot be identified independently of its interaction with a ligand since these two relational entities are defined by their mutual steric complementarity.

Epitopes and paratopes are thus not intrinsic constituents of proteins and antibodies that exist independently of this relational nexus. Only a small fraction of the residues that are assigned by functional assays to a binding site contribute to the binding free energy and residues that are not in contact at the interface can also affect binding affinity. The reductionist approach of analyzing the structure of epitope–paratope complexes by crystallography has therefore been of limited use for designing functional entities that will exhibit a neutralizing activity when placed in the context of a particular cellular environment in the infected host.

Chapter 6 analyzes the reductionist expectation that assumes that the biological phenomenon of protection against infection achieved with neutralizing antibodies can be reduced to the level of chemistry. It is not possible to predict the neutralizing activity of an antibody simply from its chemical structure because protective immunity only occurs in the context of whole organisms since tissues and organs cannot be vaccinated. The structures of epitopes and paratopes present in a crystallographic complex correspond to a terminal state after a dynamic process of induced fit and conformational selection, and they give little information on the epitope conformation present in the immunogen-bound BCR that triggered the appearance of a neutralizing antibody activity. This activity is not selected by the BCRs during immunization since the neutralizing capacity of the antibody will be revealed only subsequently in the context of a complex cellular environment.

Chapter 7 summarizes the debates that took place during a conference on Reduction and Emergence held in Paris in 2003. William Wimsatt explained that a reductionist starts by choosing and designating a system for analysis which partitions the world of study into that system and its environment. Assumptions will be made about which variables must be controlled or randomized and the description of the environment will be greatly simplified compared to the description of the entities

internal to the system under study. Fitness, for instance, will be described as if it were a property of phenotypes or genes and the fact that it is a relation between organism and environment will tend to be ignored. When environmental variables are not monitored, the investigator will be unaware of the context dependence of biological phenomena.

Causal explanations are reductive because one factor is singled out for attention and is given undue explanatory weight on its own. However, in biological systems any observed effect always results from a complex network of interactions and an explanation in terms of a single cause is never satisfactory. Although molecular biology has clarified the intricate mechanisms that allow genes to be translated into proteins, it has provided little insight on the innumerable causal chains that link genes to phenotypic traits. A common fallacy is to assume that the function of a gene is to produce whatever the system fails to do when that gene is absent. As a result, genetic determinism has not been able to unravel the complex series of events that link phenotypes to genes.

Chapter 8 discusses why it is so difficult to analyze the complexity of biological systems in a satisfactory manner. One reason is that complex systems always possess emergent properties that are absent when the parts are studied separately and which cannot be predicted or deduced from the properties of the parts. Interactions between the parts as well as inputs from the environment give rise to novel features such as network behavior that lead to the characteristic homeostasis of biological systems. Homeostasis allows biological systems to maintain a dynamic stability as well as an ability to self-repair which is largely independent of specific control mechanisms.

Biological systems cannot be described adequately without referring to the functional roles that their constituents play in keeping them alive. Darwinian evolution explains the existence of a present-day function by saying that it exists because it contributed to the reproductive fitness of the organism in the past. Biologists thus favor evolutionary explanations for presently observed structures and do not look for structural explanations for a presently observed function.

Design is defined as the deliberate and intelligent conceiving of an artificial thing or process. Since the human mind is the most efficient goal-directed system that exists, human intentionality is perceived as the cause of all purposive human actions. This leads to the anthropomorphic fallacy that the behavior and activities of all biological systems are explainable in terms of goals and purposes. Since any functional part that contributes to the working of the system can be interpreted as achieving a certain goal, this leads to the view that the system was designed by a thinking mind to function in a preordained way. This encourages the use of metaphors of end-directed features for describing biological processes in terms of design, purposes, and functions. It also encourages the expectation that the human mind has the capacity of intentionally designing an integrated immunological system that will protect against HIV infection. Since natural selection has not yet succeeded in achieving such a feat, there is considerable skepticism that a preventive HIV vaccine can be rationally designed by reverse engineering (Green 2013).

Chapter 9 discusses the negative impact that reductionist thinking has had on HIV vaccine development. Although methodological reductionism which dissects

biological systems into their constituent parts was initially very successful in molecular biology, it was later found to possess many limitations for studying complex biological systems. For instance in the search for an effective HIV vaccine, the use of Mabs for dissecting HIV immune responses made investigators focus on individual epitopes as elicitors of neutralizing antibodies. This made them neglect the synergistic effects that are observed in polyclonal immune responses directed against individual epitopes of a major HIV antigenic site when a variety of neutralizing antibodies elicited by different epitopes are acting together.

Reductionist thinking also blurs the distinction between the chemical nature of antigenicity and the biological nature of immunogenicity. This leads to the assumption that when an HIV epitope is reconstructed by rational design to better fit a neutralizing antibody, it produces an immunogen able to elicit polyclonal antibodies with the same neutralizing capacity as the neutralizing Mab. Reductionism also fosters the belief that protection against HIV infection can be fully analyzed at the level of molecular interactions between an epitope and a paratope which in most cases is the only parameter that vaccinologists take into account when they design a vaccine. Numerous features of the host immune system are crucial for the induction of neutralizing antibodies, and because they ignored these contributory factors, investigators became engaged in hundreds of abortive attempts to transform HIV epitopes of known structure into effective vaccines. Using a convergence argument, it has been argued that so many negative outcomes justify the conclusion that the reverse vaccinology approach that was used is not capable of developing a preventive HIV vaccine.

Chapter 10 responds to a commentary (King 2016) which rejected the convergence argument that reverse vaccinology is actually unable to develop an effective HIV vaccine. It was argued that such a convergence argument is based on inductive reasoning from a limited number of cases that can never lead to a logically certain conclusion. It is indeed true that no amount of experimental evidence can ever lead to the absolute certainty achievable by deductive reasoning and the convergence argument is therefore only a backup argument. The fundamental reason why reverse vaccinology failed in the case of HIV is that it did not have a sound theoretical basis derived from our current knowledge of immunological specificity and of anti-HIV immune responses (see also Chaps. 4 and 21). King (2016), however, was right to point out that it may be considered unethical to pursue attempts to develop an HIV vaccine by a consistently unsuccessful approach if scarce resources could be used more effectively to combat the AIDS epidemic by other approaches.

Chapter 11 discusses the nature and consequences of biological reductionism in the immunological study of infectious diseases. The major message of this review is that useful information does not depend only on data input but requires procedures that include both reductionist and non-reductionist steps in order to detect distinct patterns that subsequently need to be examined experimentally.

Part 3. The Search for a Preventive HIV Vaccine

Chapter 12 discusses the limitations of structure-based design of HIV vaccine immunogens which arise from (1) misconceptions regarding the nature of epitopes,

(2) disregarding the analytical bias that arises when Mabs are used to dissect the structure of antigens, and (3) assuming that effective HIV vaccine immunogens can be identified by analyzing the structure of epitope–paratope complexes by X-ray crystallography. Epitopes and paratopes are relational entities defined by their mutual complementarity, and they depend on each other for acquiring a recognizable identity. Once a neutralizing Mab has been isolated from the serum of an infected individual, it usually becomes conceptually associated with a single discrete epitope. However, since an antibody always contains numerous paratopes, there is no reason why a vaccine containing that one particular epitope should also be able to induce a polyclonal, neutralizing antibody response. A large number of paratopes binding to the major antigenic sites of the HIV envelope (Env) have been thoroughly investigated, but this has not helped the design of an HIV vaccine.

Chapter 13 discusses the two different meanings of the term reverse vaccinology used for developing bacterial and viral vaccines. Rino Rappuoli (2001) developed a strategy, based on bioinformatic analyses of entire bacterial genomes, for identifying all the antigens that a bacterial pathogen is able to express. He called it reverse vaccinology because he started from the genome instead of from the organism for establishing which surface-exposed proteins should be investigated as potential vaccine immunogens. Whereas classical approaches investigate only the small number of purified antigens that can be obtained by fractionating bacterial extracts, reverse vaccinology made it possible to evaluate hundreds of expressed bacterial proteins and to develop successful bacterial vaccines.

In virology, the reverse vaccinology approach of Burton (2002) refers to a completely different strategy. Instead of the usual approach of generating antiviral antibodies by vaccination, it uses a so-called reverse approach for trying to generate a vaccine from the known 3D crystallographic structure of one paratope identified in neutralizing anti-HIV antibodies. From the structure of epitope–paratope complexes, attempts are made to elicit neutralizing antibodies by reverse engineering epitopes in order to endow them with the capacity to induce neutralizing antibodies.

Calling these two completely different strategies “reverse vaccinology” makes little sense, and it would have been preferable to call them genome-based and structure-based reverse vaccinology, respectively. Calling them reverse vaccinology 1 and reverse vaccinology 2 is also not particularly instructive because it obscures the fact that the genome-based approach has been successful, whereas the structure-based approach has systematically failed for more than 20 years (see Chap. 22).

Chapter 14 describes the structure of many continuous and discontinuous HIV epitopes identified in Env spikes and of their complementary paratopes as well as the many unsuccessful attempts that were made to modify the epitope structures in order to improve their vaccine potential.

Chapter 15 summarizes the discussions that took place during a workshop held in Baltimore in 2013. The participants proposed several new research paradigms that better fit our increasing knowledge of HIV immunopathology and which could possibly be more helpful for guiding future HIV vaccine research than past unsuccessful approaches. It was stressed that the development of a successful HIV vaccine required new prescriptive knowledge in the form of a practical invention rather than

increased propositional and fundamental knowledge about the inner workings of the immune system.

Chapter 16 is the Introductory Editorial to a Research Topic entitled "Paradigm changes are required in HIV vaccine research" published in 2014. In his influential book *The Structure of Scientific Revolutions*, Kuhn (1962) argued that researchers in every field of scientific enquiry are always guided by theoretical assumptions, presuppositions, and hypotheses that constitute the prevailing scientific paradigm under which they operate at a given time. This paradigm commits them to using certain strategies and experimental approaches considered to be essential for trying to solve the problem at hand. The implicit presuppositions that gave rise to a particular paradigm are rarely stated, and when investigators obtain results that are not compatible with the hypothesis underlying the paradigm, they may fail to appreciate that the paradigm has been refuted and should be abandoned or revised. Scientists, however, tend not to abandon their guiding paradigm when they obtain contradictory results because their main goal is not to try to confirm the validity of the assumptions underlying the paradigm they follow. Instead, they will invent new *ad hoc* hypotheses for resolving contradictions between theory and experimental observations which allow them to pursue their investigations within the framework of their chosen paradigm, even at the risk of pursuing unfruitful lines of investigation. The following five paradigms had a detrimental effect on HIV vaccine research because they were based on erroneous assumptions: (1) Vaccine immunogenicity can be predicted from viral antigenicity, (2) there is a primary and intrinsic epitope for each BCR and its corresponding antibody, (3) unraveling large numbers of antibody maturation pathways will necessarily allow the identification of numerous HIV-1 immunogens suitable for repeatedly vaccinating large populations of genetically variable individuals, (4) rational design of HIV-1 immunogens is more effective than classical empirical screening of immunogens, and (5) reactions of viral antigens with protective Mabs are more specific than the combined reactivity of antibodies in a polyclonal antiserum.

Chapter 17 describes in detail one of the main reasons for the failure of structure-based reverse vaccinology (SBRV) to develop a preventive HIV vaccine. Adepts of SBRV tend to focus their attention mainly on residues in HIV epitopes and paratopes that make contact with each other, and they ignore the fact that the binding activity of these sites is often influenced by structural features distant from the sites themselves. In addition, the structure of the epitopes and paratopes observed in the complex is the result of the mutual adaptation and induced fit that occur when the partners interact during the immunization process and they are unlikely to correspond to the structure of the free sites. Another common assumption is that all antibodies and Mabs are monospecific for a single epitope, although they always contain numerous paratopes able to bind a variety of epitopes as well as autoantigens with various degrees of fit (Mouquet and Nussenzweig 2012). Once it is accepted that the epitope structure observed in an nMab-HIV1 Env complex is only one of the many epitopes that can be accommodated by a polyspecific Mab, there is no reason to assume that the epitope of known structure is the one that elicited the nMab. The central assumption

of SBRV is therefore invalid since the one epitope whose structure was established is not necessarily able to elicit antibodies that neutralize HIV-1.

Chapter 18 is a commentary on the paper published by Andrieu et al. (2014) in the Research Topic mentioned in Chap. 16. These authors reported results obtained in Chinese macaque experiments that explored a new vaccine concept aimed at inducing tolerance to the simian immunodeficiency virus (SIV). They administered inactivated SIV intragastrically together with living bacterial adjuvants such as *Lactobacillus* bacteria with the goal of inducing tolerance to SIV antigens. Although this approach did not elicit SIV-specific antibodies nor cytotoxic T-lymphocytes, it protected 23 out of 24 experimental animals from mucosal and parenteral challenges. A previously unrecognized population of non-cytolytic MHV Ib/E-restricted CD8+ regulatory T cells was identified which suppressed the activation of SIV-positive CD4+ lymphocytes (Lu et al 2016). In view of the dearth of novel approaches in HIV vaccine research, such intriguing and unexpected results clearly need to be confirmed or refuted by further investigations with Chinese macaques (Carnathan et al. 2018).

Chapter 19 analyzes the common assumption that Mabs derived from HIV-infected individuals are the most effective reagents for dissecting antibody responses to HIV infection. It is often not appreciated that the apparent specificity of a Mab very much depends on the selection process that was used to obtain it. For instance, when a Mab is selected for its ability to bind a linear peptide in a peptide library that mimics a viral epitope, it is not astonishing that it will bind more strongly to the selecting peptide than to the virions that initially induced the Mab production in the infected host. Since most Env epitopes are discontinuous, their binding activity cannot be assessed by extracting all the constituent epitope residues from the protein to show that they are able to bind on their own when they are not embedded in the native protein structure. A short linear peptide that is part of such a discontinuous epitope may of course retain some binding activity, and this may lead to the peptide being called a continuous epitope.

It is often assumed that Mabs are better reagents for studying immune responses to HIV infection than polyclonal antibodies in spite of the fact that most effective antibody responses are polyclonal and directed to the multiple neutralizing epitopes that constitute the antigenic sites of HIV Env. Although the paratope structures in many nMabs have been elucidated, this knowledge has not helped the design of effective vaccine immunogens. Although it is possible by rational design to improve the ability of one epitope to bind to one paratope in an nMab (i.e., improving the antigenicity of a protein), the claim that what is being designed is an HIV vaccine immunogen able to trigger a protective immune response is the basic flaw of the SBRV approach because it assumes that when an HIV epitope reacts with an nMab, it should also be able to induce similar neutralizing antibodies in an immunized host.

Chapter 20 summarizes the discussions that took place during a workshop on new strategies in HIV vaccinology held in Rome in 2016. Eleven participants were asked to respond to the following five questions: (1) What new approaches should be followed in HIV-1 vaccinology? (2) Should HIV-1 inactivation be reconsidered? (3) Can therapeutic vaccines help the development of a preventive HIV vaccine?

(4) Can therapeutic vaccines lead to a functional cure? (5) If you had the authority to do it, what vaccine concepts would you support for testing? The variety of answers that these questions elicited clearly indicates that novel research strategies for developing a possible HV vaccine are urgently needed.

Chapter 21 explains the many reasons why the rational design of a preventive HIV-1 vaccine by SBRV failed to deliver an effective vaccine. The review describes in considerable detail the structure and dynamics of epitopes and paratopes as well as the considerable improvement of our understanding of antibody specificity in recent years. It has for instance been clearly demonstrated that the amino acid compositions of epitopes and of the entire surface of proteins are very much the same, which finally explained why it had not been possible for more than 25 years to predict accurately the location of epitopes in proteins on the basis of amino acid propensities such as hydrophilicity or accessibility (see Chap. 4).

Although it is frequently stated that rational design is the best strategy for developing new vaccines, this does not seem to be the case for HIV vaccine development. Rational design applies mainly to drug design when candidate molecules are designed to fit the 3D structure of a biological target in order to bind to it with high affinity and inhibit its biological function. Such a computer-assisted approach based on molecular docking succeeds because the complementarity between a drug and its target molecules is fairly unique. In contrast, molecular recognition processes between an antigen and the many antibodies that are able to bind to it are much less specific than drug–receptor interactions mainly because of the conformational flexibility of epitopes and paratopes, the degeneracy of the immune system, and the polyspecificity of antibodies. It also seems inappropriate to use design terminology for describing the SBRV attempts made by investigators when they try to have an antigen produce protective antibodies since they only improve the antigenic binding capacity of viral epitopes and not their immunogenic capacity to trigger the immune system to elicit protective antibodies. SBRV disregards accepted immunological theory pertaining to the degeneracy of the immune system and the polyspecificity of antibodies and also underestimates the extreme plasticity of the HIV Env protein as well as the extensive conformational flexibility of epitope and paratope binding sites.

Chapter 22 points out that when HIV epitopes are called vaccine immunogens, this could be interpreted to mean that they are able to generate immune responses in the immunized host, although it should be evident that they only trigger in the host a series of reactions with BCRs that eventually may lead to the immune system producing antibodies, some of which may be neutralizing. The type of antibody that is produced depends on numerous properties of the immune system such as its antibody gene repertoire, the presence of helper and suppressor T cells, the secretion of cytokines, self-tolerance, and a variety of other immunoregulatory mechanisms. Vaccinologists who claim to be designing a vaccine immunogen by SBRV tend to ignore these contributions from the immune system because they focus on the recognition processes between single epitope–paratope pairs instead of investigating by trial and error which components of the immune system must be controlled to ensure that neutralizing antibodies are elicited. Only when it was found that HIV Env

epitopes recognized by affinity-matured antibodies obtained from HIV chronically infected individuals did not bind the germline predecessors of these antibodies was it realized that potential vaccine immunogens may only be discovered if one took into account the slow but extensive antibody maturation that is needed to obtain neutralizing antibodies. Additional immune correlates of protection are needed to guide HIV vaccine development, but these can be inferred only retrospectively when an efficacious vaccine has been developed empirically. Since we know very little about which features of the immune system regulate the production of protective antibodies, it seems unavoidable that empirical vaccination trials will continue to be required when trying to develop an effective HIV vaccine.

Chapter 23 describes the nature of the inverse problems that vaccinologists need to solve when trying to develop an HIV vaccine. Inverse problems differ from the usual direct problems in science that are solved by determining experimentally what are the effects that follow certain causes. Biological systems are made up of successive levels of increasing complexity from genes to RNAs, proteins, organelles, cells, tissues, and organs. At all these different levels, innumerable interactions occur involving genetic, epigenetic, biochemical, and physiological factors which nowadays are mainly analyzed using system biology approaches. Numerous bottom-up and top-down causal links occur across the different constituents and levels of the immune system, and as a result, our theoretical understanding of the mechanisms responsible for the appearance of neutralizing antibodies against HIV is practically nonexisting.

We are unable to imagine what are the multiple causes that could lead to protection against HIV infection because we lack an integrated theoretical model of the entire immune system. Such a model is actually required for conceiving plausible solutions to inverse problems which then need to be verified experimentally.

Solving an inverse problem may for instance require developing a model that could explain the multiple causes that produce a desired effect such as the absence of a deleterious HIV infection in elite controllers, and then subsequently demonstrating that by adjusting certain parameters in the immune system, the desired outcome can be obtained in a genetically heterogeneous population.

Whereas many direct problems can be solved because of our knowledge of the biological mechanisms that bring about certain effects, this is not feasible in the case of inverse problems that cannot be solved in this manner. Because of the complexity of the immune system, vaccinologists mostly do not have access to all the information that would be required in order to make entirely rational decisions based on a complete knowledge of all the relevant parameters. Since several inverse problems may first need to be solved at different levels of the immune system, it may in fact turn out not to be possible to rationally develop an HIV vaccine in this manner. In the last 20 years, investigators have mainly studied the rather ineffective antibody responses that occur several years after the initial acute phase of HIV infection, although these antibodies are usually unable to control the infection in the individuals from whom they had been isolated.

Since the initial acute phase of HIV infection is caused by so-called transmitter/founder (T/F) viruses that are not predominant in the individual donors, it could be