SECTION 2

The Immune System and Resistance to Tuberculosis

Woman, cat and bird.
I have a cat with a mouse's name –
Alicia Leal Veloz
Serigraph/paper
(62 x 82 cm)

'Mirrors are not more silent
nor the creeping dawn more secretive;
in the moonlight, you are that panther
we catch sight of from afar.'

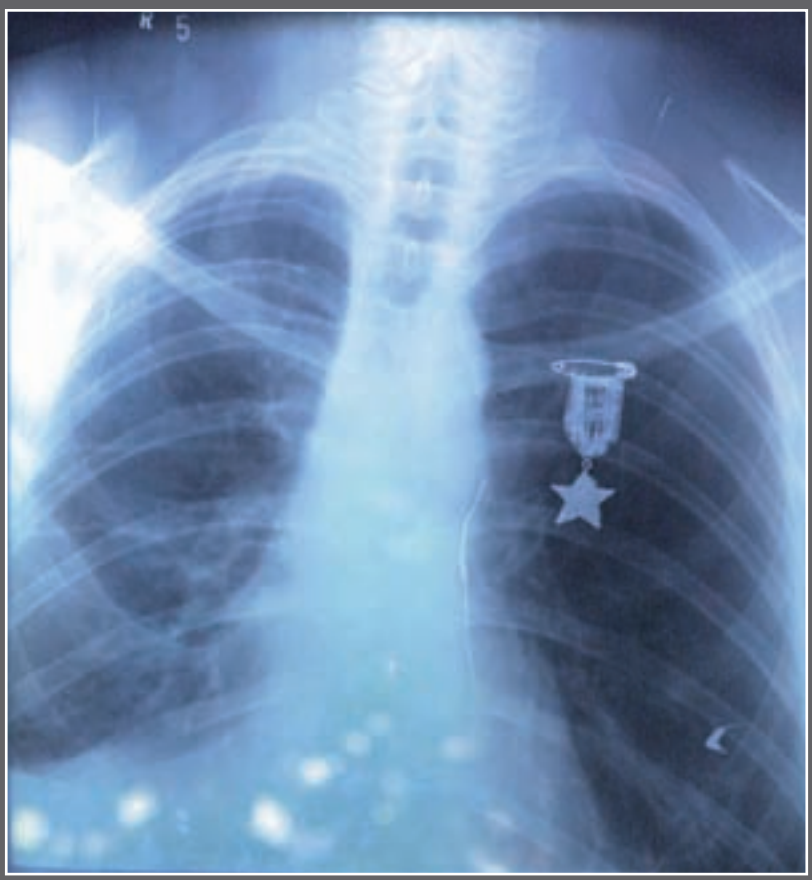
To a Cat **(Poem) – Jorge Luis Borges**

Alicia Leal Veloz

Sancti Spíritus, Cuba

Alicia Leal Veloz graduated in 1980 from Havana's San Alejandro Academy of Fine Arts. She has had one-woman shows in Havana, Matanzas, Sancti Spíritus, Kuala Lumpur, Kingston, Houston and Berlin. She has also taken part in collective exhibitions in numerous countries. Her work forms part of permanent and private collections in many countries internationally. She has illustrated a number of Cuban and foreign books, and cultural magazines.

MTB is an accomplished immunologist; we just need to listen to what she tells us about regulating the immune system



Untitled – Angel Ramírez
Mixed/X-ray film

'Because man himself is a secret....'

Conference for students. University of Princeton (USA) 10 May 1939 – Thomas Mann

Angel Ramírez
Ciudad de La Habana,
Cuba

Angel Ramírez graduated from National School of Arts and from the Higher Institute of Art, both in Havana.

He started exhibiting in the 1980s. He has shown his works in Argentina, Brazil, Chile, Germany, Holland, Japan, Norway, Poland, Spain, Venezuela, and Yugoslavia.

He has received prizes in competitions like the XIII Provincial Juvenile Salon (Havana), the II National Encounter of Engraving (Havana), the Salon of the City, the Biennial Latin American and of the Caribbean of Engraving (San Juan), the International Triennial of Engraving (Fredrikstad) and in the First Biennial of Graph (Argentina).

He is a recipient of the Order for National Culture and is a member of UNEAC.

CHAPTER 3

Infections, Biomarkers, and Vaccines

Irun R Cohen

Introduction

How we approach the design of vaccines is inspired by our views regarding the immune system and the host–parasite relationship. Let us consider two points of view: immune defense and immune maintenance.

Immune Defense

The clonal selection theory of adaptive immunity (CST), as formulated by Frank Macfarlane Burnet a half-century ago, directed immunologists to the formative role of specific antigens in generating the immune system (1). The roots of the CST go back to the germ theory of disease, the concept of specific receptors, and the early vaccines developed by Pasteur, Koch, Ehrlich, and their colleagues a century ago. The CST taught that the immune system during its development is purged of lymphocyte clones capable of recognizing self-molecules; mature, functional lymphocytes have been selected to see only foreign antigens. Moreover, the immune system evolved to defend the body against pathogens and does so by recognizing their specific antigens (2). Activation of a clone of lymphocytes by an antigen leads automatically to two outcomes: an inflammatory effector response that gets rid of the antigen and the agent that bears it, and a memory of the antigen that prepares the immune system for an accelerated response to a second encounter with the antigen. The immune

system, according to the CST, has to make only binary decisions: to respond or not to respond; any response is an attack (3). Vaccination, according to this view, is straightforward; any vaccine composed of a pathogen's specific antigens will generate clones of memory cells sufficient to recognize and reject that pathogen in a future infection. The success of vaccines against rabies, smallpox, polio, measles, hepatitis B, diphtheria, tetanus, and *Haemophilus influenzae* B seems to confirm that view.

Unfortunately, effective vaccination is not always that simple; witness our failure to develop effective vaccines against MTB, HIV, and other infectious agents despite our knowledge of their antigens. An effective immune response needs more information than just foreign antigens.

Immune Maintenance

At about the time that Pasteur, Koch, and colleagues were studying infectious agents and specific vaccination, Elie Metchnikoff was developing a different view of the immune system. Metchnikoff, as described by Tauber and Chernyak (4), discovered macrophages, phagocytosis, and immunity in the context, not of infection, but as a consequence of his studies on evolution, embryonic development, and inflammation. In contrast to the Pasteur–Koch school of thought, Metchnikoff saw host defense against pathogens as only one aspect of the physiology of the immune function; the ultimate aim of the immune system, according to Metchnikoff, was to maintain the organism by healing it when necessary.

My own research into autoimmunity and the discovery of natural autoantibodies and autoreactive T cells in healthy individuals led me to conclude that the CST had to be mistaken in proclaiming that the adaptive immune system was purged of antigen receptors that could recognize self-antigens (5). Moreover, we have since learned that cytokines, chemokines, and other molecules produced by immune cells are important agents in wound healing, connective tissue formation, bone repair, cell proliferation, cell differentiation, and cell apoptosis; the immune system, as foretold by Metchnikoff, is intimately involved in body maintenance and not only in body protection (5, 6). The immune system has to decide when it should mobilize an inflammatory response that rids the body of a pathogen and when it should deploy an inflammatory response of a type that will heal the body. Often the system has to carry out both programmes simultaneously: heal the body and rid it of a pathogen at the same time.

In the light of these observations, I proposed the theory of the immunological homunculus (7, 8): T cells and B cells with receptors capable of recognizing

self-molecules, easily detectable despite being forbidden by the CST, play a physiological role in orchestrating the regulation of inflammation, a process vital both to maintaining the body and to defending it (5, 6). Thus, immunity cannot be reduced to a simple binary attack-or-not decision (9). Beyond antigen recognition, the immune system has to decide what type of inflammatory response suits the situation—mending a broken bone, repairing an infarct, or destroying an invader. Moreover, the inflammation that heals the body and the inflammation that destroys pathogens are dynamic processes; they evolve with the changing state of the healing or the infection. In both cases, a great deal of information has to be integrated by the immune system and decisions have to be made on the fly. How can the immune system make complex, dynamic decisions about maintenance and defense in real time? The immune system, like any good physician, uses biomarkers.

Immune Biomarkers

Biomarkers are measurements or patterns of information that reduce a complex phenomenon into a relatively simple surrogate marker (10). For example, the complex process of a myocardial infarction can be reduced to a biomarker composed of the presence of some enzymes in the blood and a particular electrocardiogram trace along with a characteristic set of patient symptoms; a white blood count can serve as a surrogate biomarker that, with some additional information, distinguishes between a viral and a bacterial pharyngitis; a smile or a frown can serve as a biomarker for a very complex human relationship. The reader is free to suggest his or her own examples of simple biomarkers that serve to signal or diagnose exceedingly complex processes. My point here is that the immune system too carries out complex functional response programmes by using its receptors, both innate and adaptive, that sense relatively limited sets of highly informative molecules (7, 8). For example, a pathogenic Gram-negative bacterium may contain thousands of different antigen molecules, yet the immune system focuses on a relatively few antigen molecules such as lipopolysaccharides (LPS), using both innate and adaptive receptors on lymphocytes and other cells (7). Certain of the bacterium's antigens provide an address for the inflammatory response and bacterial ligands for toll-like receptors (TLR) and other innate receptors provide information that determines the biologic character of that inflammatory response (5). Note that the nature of the inflammation produced by an immune response to an infection is influenced to a great degree by the impact of the infection on the host; the immune system needs to know about the type of damage inflicted on the body by the infection (5). This internal information is encoded by biomarker molecules produced by

the tissues of the host in response to the evolving infection. In other words, the inflammatory response to a bacterial infection is determined by a relatively few biomarker signal molecules that originate both from the pathogen and the host. Note that there is nothing intrinsic to the chemistry of a molecule that causes it to function as an antigen or biomarker signal; molecules are turned into antigens and biomarkers because they are detected by immune system receptors, innate and adaptive; the functional information is in the receptor not in the ligand (5).

Similarly, the immune maintenance response that heals wounds, broken bones, or infarcts; that activates angiogenesis, scarring, regeneration; and that rejects aged or transformed cells makes use of self-antigens encoded in the immunological homunculus and of self-ligands for innate receptors such as stress proteins, p53, and other maintenance molecules (5, 10). Here I can merely introduce the general idea of immune biomarkers in the host–parasite relationship; the details are beyond the scope of this short chapter. As we shall see below, vaccines too need to include biomarker signals; but before getting into that, we first need to apply the biomarker concept to the evolution of the host–parasite relationship.

Co-Evolution of Humans and Their Pathogens

The co-evolution of *Homo sapiens* and its pathogens has been going on for millions of years; during most of that time humans (and their predecessor primate species) lived in small, relatively isolated groups of migrant hunters and food gatherers. The discovery of agriculture and animal husbandry about ten thousand years ago eventually converted most humans from roaming hunters and gatherers to settled existence, leading to growing populations, the accelerated evolution of human culture, urbanization, the industrial and informatic revolutions, and on to the globalization we experience today. Thus, most of human biological evolution and co-evolution with infectious bacteria, viruses, and eukaryotic parasites was dictated by our lifestyle as foragers. Genetic studies indicate that MTB accompanied the first bands of humans who migrated out of Africa to populate the earth (11). This means that MTB and the other primordial infectious human pathogens had to evolve the capacity to make their living infecting a world population of humans that numbered less than 5 million individuals divided into small groups scattered across the continents (5). To survive by parasitizing such a sparse species, MTB and the other aboriginal infectious organisms had to satisfy a number of constraints.

Low virulence: An infectious agent that incapacitated or killed its human host, given the few humans in any foraging group and the wide distance

between groups, had little chance of maintaining an infectious life cycle within the human population. The successful infectious agent had a vital interest in keeping infected humans mobile and relatively vigorous, especially when the infected human was the pathogen's primary vector for dissemination, as well as the pathogen's reservoir. Low or moderate virulence was the order of the day; MTB would not have survived had it quickly killed its infected hosts. Thus, the MTB parasite and its human host co-evolved a dialogue mediated by mutual immune signalling: MTB would efficiently signal the host immune system by way of immunogenic antigens and innate immune receptor ligands (effective biomarkers furnished by MTB for the host's immune system); the infected human, in its part of the bargain, would respond immunologically to these biomarker MTB signals and mobilize an inflammatory response that would confine the MTB infection and reduce its virulence (12). But low virulence was not enough.

Persistence in the immune host (13): Unless MTB could persist despite the host immune response, a single round of infection would suffice to eradicate the pathogen. MTB had to arouse host immunity that was sufficient to keep the infection at bay, while leaving the bacterium still viable. The bacterium had to wait patiently for an opportunity to infect non-immune contacts—often children recently born into the group. MTB seems to have learned well the trick of persistence: primary TB in a well-nourished human leaves the host functionally intact, until an ageing immune system falters and allows a latent infection to become active; coughing grandfather now infects his susceptible grandchildren. In this way MTB survives horizontally and vertically by cycles of latency and reactivation.

Cytomegalovirus (CMV), another primordial infectious agent of humans, has also learned to persist and cycle in the immunized host. Primary CMV infection usually takes place in childhood; the childhood infection is cleared by the host who suffers only minimal symptoms. However, despite the host's immune response, CMV remains latent in the salivary glands into adulthood and is disseminated to the next generation of children by the parents' oral secretions (14). Thus, CMV cycles in humans from generation to generation without seriously affecting human health. CMV infection creates a clinical problem when a woman has missed natural infection with CMV during her own childhood years and undergoes primary infection when she is pregnant; since she is not immune, the CMV infection can reach the developing fetus and cause serious damage (15). CMV also becomes a problem when the virus is reactivated in an immunosuppressed patient (16). In either case, persistence of the pathogen is based on biomarker signals produced by the pathogen that modulate the host's immune response.

Symptoms Serve Transmission

Infectious agents like MTB, that have co-evolved with their human hosts before urbanization, need only bother the host enough to provoke clinical signs and symptoms that serve its transmission. Pulmonary TB, for example, is disseminated by coughing, so infectious MTB organisms evolved to induce the host to cough. Coughing is another example of the co-evolution of immune inflammation and parasite signalling. Co-evolution is a trade-off resulting from the principle of live and let live.

In this brief chapter we cannot elaborate on the molecular mechanisms deployed by MTB to create its ecological niche in human biology; indeed, they are not adequately known. But it should be obvious that MTB survives, not by evading the host immune system, but rather by signalling the immune system through its biomarkers to supply the conditions necessary for the style of life evolved by MTB (12). In principle, MTB and other infectious agents that have co-evolved with humans carry out their survival programmes by carefully manipulating the inflammatory response of the host to fit the evolving state of the host–pathogen relationship, from primary infection, to immune confinement, to latency, to secondary reactivation, and dissemination.

Death caused by virulent pathogens, paradoxically, is also mediated by the immune system; immune over-reaction can produce toxic or septic shock (17). Thus, it is the host immune system and its interaction with the infectious agent that determines if both organisms survive, the host eradicates the parasite, or the parasite kills the host. Host and parasite both accommodate and kill through immune mediation. Immunologists have much to learn about immune-system regulation from our infectious agents—MTB is an accomplished immunologist; we just need to listen to what she tells us about regulating the immune system.

Incidental Pathogens

Note, however, that co-evolution results only when an infectious agent has cast its lot with us. There exist many virulent pathogens who kill humans with impunity: rabies virus, the plague bacterium (*Yersenia pestis*), Clostridia, and the cholera bacillus, for example, infect humans only incidentally; these pathogens have no evolutionary investment in human survival because their own survival reservoir is not in humans, but in bats, wild rodents, soil, and water, respectively. The evolutionary niches of these pathogens are not diminished if they happen to kill an infected human. Such incidental pathogens are inherently dangerous to humans because they do not need to parasitize us for their own survival.

We evolved in moderation with infectious agents, like MTB, that grew up with us—our welfare was their welfare. But times have changed.

Changing Times

The co-evolution of MTB with humans has undergone significant alteration since the evolution of human culture, which has now generated an explosive growth of human populations, widespread malnutrition, global travel, infectious transmission in the closed environments of refugee camps and prisons, improper use of antibiotics, and other factors that have now selected MTB for the expression of higher virulence, more labile latency, and antibiotic resistance (18). Immunosuppressive treatments for transplantation and autoimmune disease and the natural immune suppression induced by HIV and other emerging pathogens have reduced the ability of infected persons to confine MTB to a harmless persistence. In today's world, it is no longer necessary for MTB to behave with moderation; the rampant post-biologic cultural evolution of the human species is now selecting for more virulent variant organisms. The world houses so many humans crowded together that MTB no longer needs a functional human host to maintain its chain of infection. On the contrary, a sick, immobilized human host is an effective way for MTB to spread to other humans in the environments of cities, hospitals, prisons, and so forth. But if human culture has caused the TB problem by evolving globalization, the evolution of human science could provide the answer: an effective vaccine against MTB.

Tailoring Biomarkers for an MTB Vaccine

The aim of preventive vaccination is to generate within the immune system a memory that anticipates the pathogen and prepares the system for an effective response to a future contact with the organism or with its pathogenic effector molecules. The aim of a therapeutic vaccine is to transform an absent or ineffective response into a response that effectively eliminates the pathogen. (The pathogen could be a tumour cell as well as an infectious agent.)

Thus, the goal of vaccination to MTB is not to induce an unspecified immune response to MTB antigens that could be exploited by the bacterium, but rather to programme the immune system such that the resulting inflammatory response to MTB will destroy it. Programming a destructive inflammatory response to MTB is difficult, if not unnatural because, as we discussed above, MTB has evolved biomarkers that signal the immune system to preserve it rather than to

destroy it. The biomarker signals deployed by MTB in its strategic dialogue with the host immune system need to be identified in detail but probably include adjuvant molecules—ligands for innate immune receptors—along with antigens that activate lymphocytes. Antigens provide an address for receptor-bearing lymphocytes; adjuvants direct the biological outcome of the response—the type and degree of the resulting inflammation. Indeed, for decades immunologists have used killed MTB organisms in formulating complete Freund's adjuvant (CFA)—the classical adjuvant for inducing the inflammation required for experimental autoimmune diseases and other functional immune reactions (19). But the immune responses activated by killed adjuvant MTB, while sufficient for the experiments of immunologists, do not induce the type of inflammation needed to eradicate living MTB. Therefore, the MTB organism alone will not provide us with the antigens and adjuvants needed for the ideal MTB vaccine. An effective vaccine needs to be formulated to include biomarkers that will modulate the natural response to MTB. We have yet to discover which molecules will provide biomarkers for an effective MTB vaccine. The immune response to an infection is influenced by host biomarker molecules in addition to pathogen molecules; could useful vaccine biomarkers be obtained from host molecules?

An HSP60 Biomarker Vaccine

Consider the human 60 kDa heat shock protein (HSP60) self-molecule. My colleagues and I have developed conjugate vaccines based on combining a specific pathogen epitope with a peptide epitope of the mammalian HSP60 molecule. HSP60 functions as a chaperone inside the cell, but outside the cell self-HSP60 functions as a biomarker molecule to the immune system (20). Since HSP60 is up-regulated by any form of stress, HSP60 is a reliable biomarker signal of serious trouble: infection, trauma, metabolic insult, genomic aberration, and other factors that might require immune intervention. It is no wonder then that HSP60 is recognized by a collective of different receptors: HSP60 is an antigen for T cells (21) and B cells (22) and an innate ligand for TLR4 or TLR2 on T cells (23), B cells (24), macrophages and dendritic cells (25). HSP60 seems to act as an internal adjuvant that can both up-regulate and down-regulate the nature and strength of immune inflammation depending on the particular HSP60 epitope, the concentration of HSP60, and the responding immune cell (20). HSP60 is thus an important biomarker component in the immunological homunculus, both as a natural self-antigen and as a self-ligand for innate receptors (6).

For these reasons, we have used a peptide of self-HSP60, termed p458, to formulate subunit vaccines by conjugating p458 to various pathogen virulence molecules such as the capsular polysaccharides (CPS) of Salmonella (26), Pneumococcus (27), or Meningococcus types C and B (28). We have also linked HSP60 peptide p458 to peptide epitopes of the West Nile Virus (in preparation) or murine CMV (29). The p458 component of the conjugate provides T cell help and the p458-CPS conjugate can also activate innate TLR4 signalling in APCs (30). The detailed results can be seen in the publications. The lessons relevant to our present discussion can be summarized as below:

1 The self-HSP60 peptide conjugate can be more effective in generating T-dependant antibodies and memory than foreign carrier molecules and can increase resistance to lethal challenge by a million fold—exemplified in a pneumococcal vaccine (27);
2 The self-HSP60 peptide conjugate can convert non-immunogenic or very poorly immunogenic molecules into strong immunogens—exemplified in a vaccine to Meningococcus B (29);
3 The self-HSP60 peptide conjugate can induce immune memory and cytotoxic T cells that abrogate the persistence of a pathogen in a naturally protected site—exemplified by the eradication of murine CMV from its hideout in the salivary glands (28). Immunization with the virus itself could not induce the mouse to clear CMV from its salivary glands.

Thus, at least some vaccines can be formed by combining pathogen antigens with host molecules such as HSP60. Indeed, preliminary results indicate that the p458 peptide of HSP60 can serve to vaccinate mice when bound to MTB molecules in a recombinant vaccine (A Acosta, ME Sarmiento, personal communication). Thus, an effective MTB vaccine might be formulated by combining biomarkers from both the parasite and its host.

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