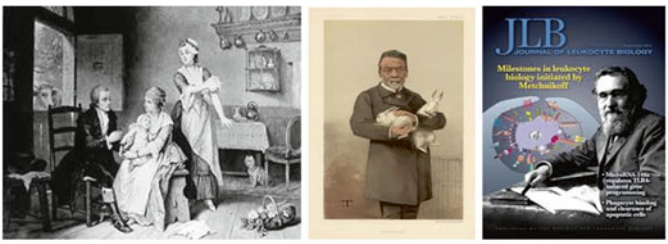
Chapter 8
Communication III (Immunological Control)

Once you die it only takes a few weeks for these organisms to completely dismantle your body and carry it away, until all that's left is a skeleton. Obviously your immune system is doing something amazing to keep all of that dismantling from happening when you are alive.

Marshall Brain (1951–)

One of controlling systems in the body which the body also uses for communication (externally and internally) is the Immune system, based upon existence of MHC molecules fundamental for recognition, and other molecules responsible for antigen-presentation and immediate or postponed reaction to it. The fundamental unique feature of immune system cells is the capability of distinguishing "self" from "non-self" cells and proteins. Communication between different cell types of the immune system is critical in the recognition of self, surveillance, defense, and clearance of foreign invaders. These signaling mechanisms involve direct cell–cell signaling as well as autocrine and paracrine signaling. The essential feature of particular cells of immunological system is memory and although still known at the level of phenomenology, presents the basis for vaccines.



Breakthroughs in Immunology and background for vaccines (small pox and rabies) and later, Rational Vaccine Design (RVD): Edward Jenner (1749–1823), Louis Pasteur (1822-1895), Elie Metchnikoff (1845–1816), NP 1908 for discovery of phagocytosis.

M. Pavlovic, *Bioengineering*, DOI 10.1007/978-3-319-10798-1_8

Communication III: Immune System and Regulation of Communication

The Adaptive Immune System: Signaling Mechanism

The unique feature of immune system cells is their capability to distinguish "self" from "non-self" (cells and proteins). Communication between different cell types of the immune system is critical in the recognition of self, surveillance, defense, and clearance of foreign invaders [1, 2]. These signaling mechanisms involve direct cell–cell signaling as well as autocrine and paracrine signaling. Direct cell to cell signaling is the best presented through antigen presentation of antigen presenting cells [macrophages, dendritic cells (DC) and B-cells] to T-naïve cells which will process the information on antigen epitopal features through T-Cell Receptor (TCR) and become educated, memory T-cells. This principle is used in rational designed vaccines (RVD) against bacteria and viruses, especially. B-cells also have a memory after acquiring antigen, but that process in the B-cells is less understood, although interesting novel discoveries are emerging [2–5]. The molecular base of immune system memory cells is still elusive and although we are **using the term**, **we still do not understand** the fundamental processes leading to that memory. Yet, with existing knowledge in mind we can design successful vaccines sometimes. There is still a lot to be understood about this very subtitle type of communication.

The communication between antigen-presenting cells and T-cells is based on the existence of **MHC** molecules (Major Histocompatibility Class) I and II that could be found on APC, reflecting two different types of molecules that are differently processed through different APC and then presented to T-cells. Almost all cells in the organism can be infected and they possess MHC molecules, but only immune system, Antigen Presenting Cells can efficiently communicate to T-cells through their MHC molecule and TCR of T-cells in order to prime them and teach them about antigen epitope features. In response, T-cells (T CD4+ and/or TCD8+) will either produce spectrum of cytokines in response in order to induce the production of specific antibodies from B-cells, or express cell-mediated cytotoxicity and kill infected cells, thus eliminating antigen [6].

T-Cell Receptor Signaling

During antigen recognition, cell–cell communication is mediated by a ligand on the surface of APC (it is MHC complex), binding to a receptor on the T cell (TCR complex). The body contains an inventory or repertoire of T cells bearing a large variety of a very different TCRs; each version of TCR is capable of recognizing a single antigen, so that population contains T-cells, that will recognize virtually any antigen. Each T-cell expresses only one type of TCR, specific for a particular antigen. Thus, millions of T-cells each with an antigen specific receptor continually sample

the surface of Apes to determine whether the presented peptide matches the binding site for the receptor they carry. This scanning process takes place in the lymph draining nodes where circulating APCs and T cells most meet. T cell briefly binds to MHC-peptide complex on the surface of APC. It is not a simple matching process but requires multiple signals: the involvement of co-stimulatory molecules, for full T-cell activation (B7 has to match to CD28). When a match occurs, signal transduction pathways are activated. This, multiple receptor-legend interactions must simultaneously occur before the specialized signal of antigen recognition can be transduced into T-cell. After recognition, T-cells will be activated and undergo clonal expansion caused by autocrine signaling of a cytokines called interleukin 2 (IL-2) which is a growth factor. Thus, many copies of the antigen-specific T-cells are produced.

Cytotoxic T-cells (Tc), activated through its binding to peptide-MHC Class I ligand, will kill the target cells by releasing granules with degrading enzymes for target, effector cells (granzymes, perforins, etc.) and destroy virus-infected cell, freeing other host cells from further infection.

Second category of T-cells, T-helper–Th cells with matching peptide MHC class II will employ paracrine signaling with secretion of cytokines which bind either macrophages or B–0 cells and activate them. So, activated macrophages will engulf and kill antigen, while B-cells will secrete antibodies and neutralize antigen (Fig. 8.1).

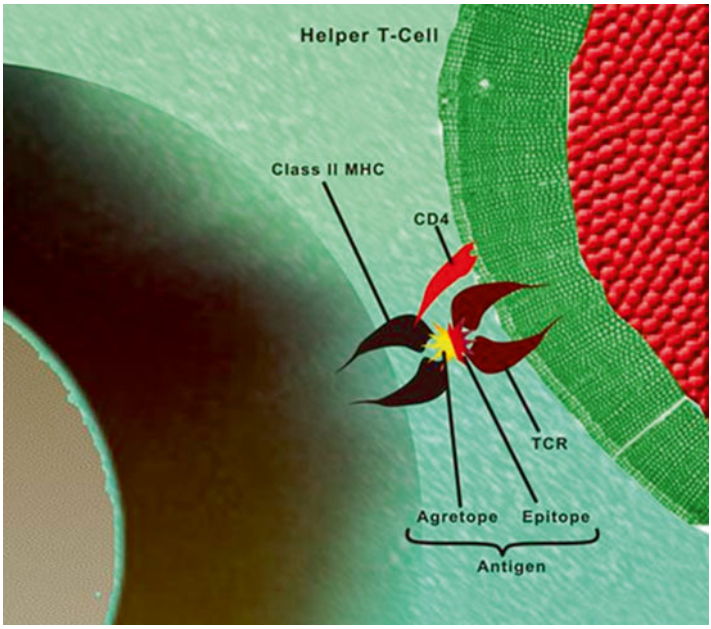


Fig. 8.1 Antigen presentation

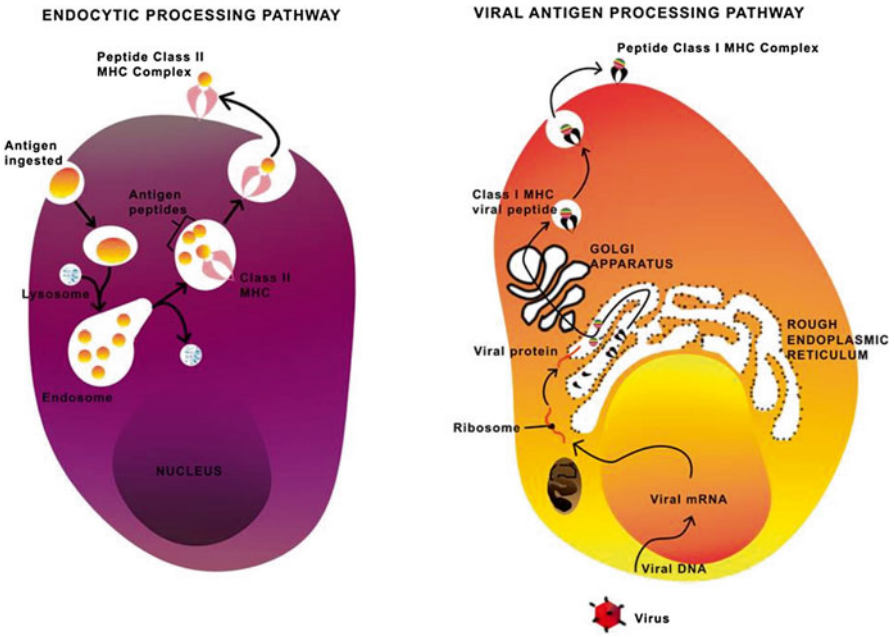


Fig. 8.2 Two different types of antigen processing in APC

Cytokine Signaling

Cytokines play an important role in adaptive immunity. They act using autocrine, paracrine and endocrine mechanism and they are numerous (Fig. 8.2).

Emphasizing Bioengineering Aspect to Immunological Control and Communication: Engineering Vaccines and Rational Vaccine Design (RVD)

A vaccine is any preparation used as a preventive inoculation to confer immunity against a specific disease, usually employing an innocuous form of the disease agent, as killed or weakened bacteria or viruses, to stimulate antibody production. The word vaccine is derived from *vacca*, (Latin for cow) [7]. The science of vaccination began with **Edward Jenner** in 1796 and his observation that milk maids who contracted cowpox due to their exposure to farm cows became immune to small pox from the pus in the blisters formed by cowpox [8]. Jenner subsequently tested his hypothesis on an 8-year old boy and was successful [8]. He inoculated the boy with cowpox blisters initially from which he developed only a mild illness.

Later the same boy was inoculated with small pox or variola particles. The child showed immunity developing no sign of disease [8]. Thus, a new field of preventive medicine was born and vaccine development has been a major biomedical concern ever since.

Despite the overall success of vaccination efforts in this modern era, there is still a great need for new and improved vaccines which cannot be met easily [9]. Even though vaccination is probably the most beneficial therapy that a physician can provide a patient, there are still significant roadblocks to the development and licensing of new vaccines [9]. The greatest roadblock is the lack of a complete understanding of how the human immune system "works". Early vaccines were developed using technology from the 19th and 20th centuries: inactivation by heat, chemicals, and irradiation to produce a killed vaccine, vaccination with a serologically related virus a' la Jenner, and attenuation by tissue culture passage to produce live vaccines with substantially reduced virulence [9]. These methodologies have failed to usher in vaccines against new and emerging diseases. Unmet targets for vaccine development include some of the more difficult infectious agents, such as *human immunodeficiency virus* (HIV), *Ebola*, cytomegalovirus, *Dengue* virus, *Human Parvovirus B19* and *severe acute respiratory syndrome coronavirus*; bacteria, such as *Pseudomonas aeruginosa*, *Neisseria gonorrhea*, *or Mycobacterium tuberculosis*; and parasitic diseases, such as malaria or hookworm disease [9, 10]. In the upcoming years vaccines towards diseases of this caliber will be developed by improvements on the basic techniques mentioned above and through the use of new technologies based on the expanding understanding of the immune response [9, 10].

In today's world, vaccine design is not limited towards elimination and prevention of infectious diseases. For example, Bioterrorism has brought renewed interest to new and large-scale vaccine development [9]. Furthermore in the developed world chronic illnesses are of greater concern than infectious diseases. Thus, there are the trials for the vaccines that will also be developed as therapies against disease for autoimmune diseases (lupus—SLE), cancer, hypertension, Alzheimer's dementia, contraception, and to promote the cessation of bad habits, such as smoking [9].

What all the aforementioned diseases have in common, particularly those of an infectious nature, is the involvement of the immune system. Understanding the immune system requires the work of not only natural scientists—biologists, chemists, physicists etc.—but also the involvement of applied scientist's as well; namely engineering and computational experts. Such an interdisciplinary approach is promising as research endeavors are now a part of the post genomic era, a common acceptance that all diseases have a genetic component. In microbiology the pathogen's genome is equally important to the host's genome in the establishment of a disease state. Experts in human and microbial genomic exploitation and information extrapolation, both of which rely on the domain expertise of the applied scientist must form a bridge with the biologist in an effort to develop better and more effective vaccines. While it is no secret that disciplines such as bioengineering, bioinformatics, and artificial intelligence cannot replace traditional wet-lab biology, it cannot be disputed that these disciplines and their associated tools have

accelerated biomedical research at astounding rates. Undeniably, the development of a successful vaccine towards one ailment may very well serve as a gateway to the elucidation of novel immune system mechanisms as well as a vaccine model towards other disease targets.

Rational Vaccine Design

The idea behind ration or cellular vaccine design as it pertains to viruses is that viral properties (proteins of their "body") can be exploited for sensible vaccine design. This is similar to the design of subunit vaccines which are made from microorganism fragments such as viral surface proteins. Vaccines towards the Human Papilloma Virus (HPV) and Hepatitis B Virus (HBV) are both subunit vaccines. The difference here is the design of "super vaccines" which rely on epitopes-antigenic determinants, usually made of protein, that are recognized by the immune system. Supervaccines are therefore compact forms of "pseudo-virus" that cover the diversity of the virus being studied.

Rational vaccine design seeks to manipulate the immune system to "work harder". This might be possible if the number of responding immune cells targeted by a vaccine is increased upon vaccination and later on during an immune challenge. Thus it is practical to explore improved vaccine design that is based on the cellular arm of the immune system while focusing on a specific pathogen—Parvovirus B19, Dengue virus, Ebola virus, etc. In the post genomic era all potential antigens, which are coming into consideration for inclusion into a vaccine formulation, are well known [9, 10]. This knowledge has been exploited in the context of reverse vaccinology–driven approaches, which in combination with comparative genomics enable us to select the most highly conserved and promising antigens for vaccine design [9, 10]. Therefore the issue isn't identification of the best epitopes. Rather the roadblocks to rational vaccine design (RVD) are as follows.

Roadblocks Toward RVD [11]

1. Knowledge on the effector mechanisms responsible for the clearance of these pathogens is by and large fragmentary.
2. The availability of tools enabling the stimulation of predictable immune responses of the adequate quality following vaccination. In fact, highly purified antigens are often less immunogenic than more complex preparations, rendering essential their co-administration with potent adjuvants (chemical agents that stimulate the immune system).
3. The need to bridge the translational gap, as well as current stringent regulations for vaccine testing

There are 3 roadblocks listed but 1 holds more importance than the remaining 2, the first. There are still unanswered questions as to how exactly the immune system

responds to pathogens. This is why both computer simulation studies and clinical studies of infected individuals are necessary. Yet the latter can be enhanced by the former. Mathematics can help us understand some of the complex cellular and molecular processes that make up the immune system [7]. Thus modeling the activity of a vaccine's impact on the immune system can provide insight which can ultimately lead to the development—roadblock #2—and clinical testing—roadblock #3—of novel vaccines. Hence overcoming the first roadblock must happen first, and modeling and simulation studies may help that to occur sooner while enhancing our knowledge base of how exactly the immune system processes pathogens. This greater understanding can be achieved both by analyzing models that formalize the biological ideas and by using mathematical methods to extract information from experiments that may not be accessible to a more intuitive biological approach [12].

The Adaptive Immune System

The human immune system has two primary components: **the innate system** and the **adaptive system**. *The innate*, *nonspecific system* is present at birth and treats all infectious agents equally, meaning it does not distinguish between different species or types of viruses etc. [13]. Consequently vaccination efforts are not targeted towards stimulation of the innate line of defense. On the contrary, the *adaptive*, *specific*, *immune system* refers to defenses that involve specific recognition of a microbe once it has breached the innate immunity defense [13]. It is this system that is the target of vaccine development as it possesses the ability to confer memory or "immunity" to the individual or host.

The adaptive immune system is very complex and is based on the activity of white blood cells (WBCs) called lymphocytes. There are two major types of lymphocytes: B cells and T cells, each of which is involved in a specific branch of adaptive immunity. B cells contribute to the fluid or humoral response (by secreting antibodies into the blood), while T cells regulate and integrate the cell mediated response [13]. Both responses collectively represent the adaptive immune system.

The high degree of microbe specificity seen in adaptive immunity is a testimony to the complex molecular interactions that take place between the cells involved. These lymphocytes have to be activated by other white blood cells (WBCs). It is this comingling of cells that allows the human host to develop memory against pathogens it has seen before, and in terms of vaccination to create effective memory against the pathogen.

The Humoral Arm of Immunity

The humoral arm of immunity involves two major players: B cells and antibodies. B cells are a type of lymphocyte capable of secreting antibodies, and antibodies are proteins capable of binding antigens [13, 14]. An antigen is simply any chemical or

particle that triggers an immune response. Antigens are typically recognized as being foreign to the host by the immune system. Humoral refers to the fact that antibodies are generally found in body fluids or humor. Thus the humoral response is most effective against pathogens such as viruses and bacteria that are circulating freely where the antibodies can contact them [13, 14].

Antibodies

Antibodies are a type of globular proteins that are very soluble [13]. They are often referred to as immunoglobulins due to their structure and function. Antibodies are generally produced in response to an antigen on a pathogen that has invaded the human host, and are capable of recognizing this antigen [13]. A single pathogen typically possesses several antigens which trigger the activation of several different antibodies at the same time [13].

The role of antibodies in immunity is of great significance. Antibodies bind the epitopes of a pathogen with both specificity and affinity [13]. The closer the fit is between the antibody and its epitope—a fragment of an antigen—the higher the affinity or binding energy between the pair [8]. Regarding specificity, antibodies are capable of discerning between structural isomers as well as minor differences in the amino acid sequence of a protein [13]. Though the antibody does nothing to the epitope, it marks the pathogen for elimination from the system by specific immune mechanisms carried out by other immune components. Ultimately this can lead to clearance of the pathogen.

B Lymphocytes

B Lymphocytes or B cells are primarily involved in antibody production. They are produced in the bone marrow where they undergo maturation before entering circulation [13]. Each B cell bears fixed immunoglobulins or antibodies on its surface which serve as a B cell receptors (BCR) capable of recognizing the same antigen or epitope [13, 15]. This accounts for the pronounced specificity of B cells towards specific pathogens. B cells must be activated by a specific epitope in order to trigger an immune response.

Prior to activation B cells are considered naïve. When a B cell's immunoglobulins bind to the epitope for which they become specific, the B cell is activated [13]. Once activated the B cell undergoes a process referred to as proliferation or clonal expansion [13, 15]. During clonal expansion the activated B cell proliferates into two classes of cells: plasma or effector cells and memory cells [8]. Effector B cells or plasma cells secrete antibodies while memory cells are long-lived and responsible for the enhanced secondary response to an antigen [13]. B cell proliferation serves two purposes. The first is to produce additional cells that can search the body

for the pathogen bearing the epitope and the second is to confer immunity to the human host. This is achieved by the effector cells and memory cells respectively. Without B cells there are no antibodies, the two immune system components that mediate the humoral response. Thus vaccination towards any pathogen must seek to activate B cells.

The Cell Mediated Arm of Immunity

It can be argued that the cell mediated arm of immunity or cell mediated response picks up where the humoral arm leaves off. Intracellular antigens, such as a virus within an infected cell are not exposed to circulating antibodies and are therefore inaccessible to the humoral response [8]. T cells, the major players in the cell mediated response, probably evolved in response to this aspect of pathogenicity—the need to combat intracellular parasites [13].

Like B lymphocytes, T lymphocytes are produced in the bone marrow; however they migrate to the thymus, and organ in the upper chest, to undergo maturation [13, 15]. Also like B cells T cells undergo clonal expansion to form effector and memory cells [15]. The primary difference between the two lymphocytes is that unlike B cells, T cells never interact with native antigen or epitopes. On the contrary T cells are stimulated for activity *via* a process known as antigen presentation.

Antigen Presenting Cells

T cells do not interact directly with native antigens. Rather processed antigens are "presented" to T cells on their surfaces by a special groups of cells commonly referred to as antigen presenting cells (APCs) [13]. APCs take up antigen, process them *via* chemical reactions, and then place an antigenic fragment/epitope into a specialized receptor on their surface [13]. The processed, surface bound epitope is now fit for interaction with T cells. APCs then migrate to specialized regions of the immune system where T cells are abundant [13]. One primary location is the lymph node through which lymphatic fluid flows [13].

There are three major types of APCs all of which are WBCs: dendritic cells, macrophage, and B cells [13]. Macrophages and dendritic cells are found predominantly in lymphatic tissue and fluids and very important in their role as APCs [13]. Both ingest pathogens *via* phagocytosis—cell eating—and present pathogen specific antigens on their surfaces [13]. B cells are a component of the humoral response and their presentation of antigens is slightly different. B cells are not phagocytes; rather they take in antigens *via* endocytosis—cell invagination—after the antigen binds their antibody receptor [13]. All three types of APCs enhance the immune response by their interaction with T cells following antigen ingestion.

The Major Histocompatibility Complex

APCs have the ability to interact with T cells. This is accomplished only after these cells have loaded processed antigens into specific receptors on their cells. These specific receptors are referred to as the Major Histocompatibility Complex (MHC) or Human Leukocyte Antigen (HLA) system [13].

MHC proteins are protein receptors on cells in the human body. They function as cell recognition agents in the immune system. All non-immune system cells contain MHC type 1 receptors (MHC-1) and cells of the immune system contain type 2 MHC receptors (MHC-2). The type 3 MHC receptors are less understood but they are found on a variety of cells in the body [8]. MHC receptors vary among members of the human population. The name is derived from the necessity of a match or similarity between these receptors in organ donors and recipients; hence the histocompatibility—histo meaning tissue [13].

The concept is clear. When a cell, for example an epithelial cell, is infected by a virus, it digests the virus and loads a small epitope into its type 1 MHC receptor. Cells of the immune system that are involved in the cell-mediated response, such as T cells, can then interact with the infected cell to elicit an immune response. The T cell interacts with the MHC receptor *via* its own receptor—T cell receptor (TCR). The communication between the two cells is enough to trigger an immune response.

The existence of different subgroups of MHC molecules is not due to mere coincidence. There are different types of T cells that can interact with infected cells, different APCs, and different MHC receptors [8]. Therefore, this produces an opportunity to develop a vaccine that can target multiple immune systems cells that are involved in cellular immunity. Of primary importance are the cytotoxic T cells and the helper T cells.

Cytotoxic T Cells

Different classes of T cells are recognized by their surface markers or cluster differentiation [CD] protein receptors. Cytotoxic T cells are a group of lymphocytes that are recognizable by their CD8 markers [13]. CD8—cluster differentiation 8—is a transmembrane glycoprotein and also a co-receptor [14]. It has a preference for interacting with the MHC-1 receptors and antigens [13]. Upon activation, these CD8+ positive T cells undergo clonal expansion into memory cells and effector cells and it is the effectors cells that are capable of cytolytic activity [13]. Their ability to kill infected body cells renders these T lymphocytes most effective at eliminating viruses and other intracellular parasites from the human host. Dendritic cells are the APCs that are most effective at activating CD8+ T cells [14, 15].

Helper T Cells

While cytotoxic T cells interact heavily with dendritic cells, another type of T cell favors interaction with macrophages and B cells. This class is referred to as the CD4+ positive group of T cells. CD4 is a surface glycoprotein found on several cell types such as T-helper cells, macrophage, dendritic cells, and monocytes [13]. The latter of the three are phagocytes, which are capable of engulfing pathogens that have entered the body, and later presenting them to the helper T cells [13, 14]. The CD4 functions as a co-receptor and helps to activate the helper T cell. It also interacts with the MHC-2 antigens on the surface of the phagocytic cell [13].

Helper T cells are involved in recruiting or activating other cells of the immune system, namely B cells. B cells are critical as they are mainly involved in the humoral response system which produces antibodies. When activated by helper T cells, B cells also undergo clonal expansion to form memory cells and antibody producing plasma cells [13].

Example from Author's Collaborative Work: RVD for Ebola Virus

The Ebola virus (EBOV) is extremely lethal with mortality rates ranging from 23 to 90 %. Rational vaccine design toward the Ebola vaccine seeks to treat the immune system as a decoder as it is responsible for the processing of incoming "information". Enhancing the immune system's output by controlling its various components could ultimately lead to the discovery of novel vaccine development strategies and deeper understanding of the humoral and cell mediated immune responses of immunity.

No licensed Ebola vaccine exists and classical protocols for vaccine design do not comply. One solution, rational vaccine design (RVD) is based on two parameters:

1. Identification of epitopes, antigenic peptides that mediate the cellular immune system and
2. Exploitation of the immune system's ability to recognize and remember vaccines.

The Ebola virus not only poses a safety threat for bioterrorism, but serves as an excellent model to study for all viruses that cause human disease. In the post genomic era vaccine design will take on new techniques as well as reinvent some of the older means of producing vaccines. This type of progress will require the involvement of not only the microbiologists, but engineers, and informaticians as well to name a few. Current vaccines against the Ebola have yet to be tested at a time of crisis and there is much doubt surrounding their expected rate of efficacy.

The human immune system though very complex can be studied using the Ebola model with the hope of not only saving lives, but setting an example for rational

vaccine design—exploitation of the virus' antigens and the immune systems natural ability to recognize, remove, and repair. Thus a super-vaccine geared towards Ebola should at least contain viral epitopes towards both MHC-2:CD4+ and MHC-1:CD8+ cell interactions. Activation of the former will result in B cell activation and expansion and subsequently antibody production towards the Ebola virus. Activation of the latter will supply the immune system with a fleet of cytotoxic effector cells capable of eliminating virus on contact. Additional epitopes could be used to involve other components of the immune system such as Natural Killer (NK) cells, macrophage, complement and other non-specific responses.

To assess RVD feasibility, EBOV proteins were computationally analyzed for epitope identification. To evaluate vaccine efficacy, mathematical models for virus dynamics were simulated using MATLAB. Models relied on data from EBOV cultivation in cell-cultures, and were extended with novel equations to consider memory B- and T-cell production.

First, RVD towards the EBOV is feasible. Computer-based protein analysis identified novel EBOV peptides for vaccine design. A key epitope–**EAIVNAQPKCNPN**…**MHNQDG**– was extracted from a three-dimensional structure of an EBOV protein bound to human antibody **KZ52**. Secondly, **vaccine efficacy can be assessed using mathematical models**. Multiple simulations of the *models revealed generally unknown parameters such as the virus' birth and cellular infection rates*. The models also quantified the cellular immune response necessary for vaccine efficacy in an individual; the specifications of what the vaccine must accomplish.

These results show that computer-aided (CADE) RVD is feasible and that mathematical models can establish RVD guidelines for the development of an EBOV vaccine, and not only that one (Fig. 8.3).

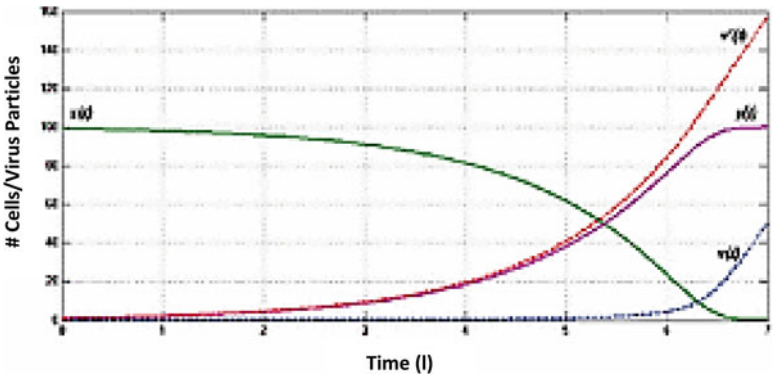


Fig 8.3 Ebola dynamics in unvaccinated system. Sophia Banton, Zvi Roth and Mirjana Pavlovic. Mathematical Modeling of Ebola virus Dynamics as a Step towards Rational Vaccine design. In: KEHerold, We Bentley, and J. Vossoughi (Eds): SBEC 2010 IFMBE Proceedings 32.:196-200 *This chapter and details of theoretical and practical approach are initiated and performed mostly by Sophie Banton, graduate student at FAU at that time, and Dr. Zvi Roth to whom the author is giving the full credit for. My participation as an immunologist was also useful

References

1. Pavlovic, M., Balint, B.: Stem Cells and Tissue Engineering. Springer Briefs in Electrical and Computer Engineering. Springer, New York (2013). pp. i–xii, 1–153. ISBN 978-1-4614-5504-2
2. Pavlovic, M., Cavallo, M., Kats, A., Kotlarchyk, A., Zhuang, H., Shoenfeld, Y.: From Pauling's abzyme concept to the new era of hydrolytic anti-DNA autoantibodies: a link to rational vaccine design? – A review. Int. J. Bioinform. Res. Appl. **7**(3), 220–238 (2011)
3. Pavlovic, M., Kats, A., Cavallo, M., Shoenfeld, Y.: Clinical and molecular evidence for association of SLE with parvovirus B19. Lupus **19**(7), 783–792 (2010)
4. Pavlovic, M., Kats, A., Cavallo, M., Chen, R., Hartmann, J.X., Shoenfeld, Y.: Pathogenic and epiphenomenal anti-DNA antibodies in SLE. Autoimmune Dis. 18 pages (2010). Article ID 462841
5. Cavallo, M.F., Kats, A.M., Chen, R., Hartmann, J.X., Pavlovic, M.: a novel method for real-time, continuous, fluorescence-based analysis of anti-DNA abzyme activity in systemic lupus. Autoimmune Dis. (2012). 814048
6. Banton, S., Roth, Z., Pavlovic, M.: A bioengineering approach for rational vaccine design towards the Ebola Virus. BMC Bioinformatics. **11**(S-10), O12 (2010)
7. Banton, S., Roth, Z., Pavlovic, M.: Mathematical modeling of Ebola virus dynamics as a step towards rational vaccine design. In: Herold, K.E., Bentley, W.E., Vossoughi, J. (eds.): SBEC 2010 IFMBE Proceedings, 32, 196–200
8. Tan, S.Y.: Edward Jenner (1749–1823): conqueror of smallpox. Singapore Med. J. **45**(11), 507–508 (2004). PMID: 15510320
9. Rosenthal, K.S., Zimmerman, D.H.: Vaccines: all things considered. Clin. Vaccine Immunol. **13**(8), 821–829 (2006)
10. Jones, S.M., Feldmann, H., Ströher, U., et al.: Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. Nat. Med. **11**(7), 786–790 (2005). doi:10.1038/nm1258. PMID 15937495
11. Martin, J.E., Sullivan, N.J., Enama, M.E., Gordon, I.J., Roederer, M., Koup, R.A., Bailer, R.T., Chakrabarti, B.K., Bailey, M.A., Gomez, P.L., Andrews, C.A., Moodie, Z., Gu, L., Stein, J.A., Nabel, G.J., Graham, B.S.: A DNA vaccine for Ebola virus is safe and immunogenic in a phase I clinical trial. Clin. Vaccine Immunol. **13**(11), 1267–1277 (2006)
12. Callard, R., Hodgkin, P.: Modeling T- and B-cell growth and differentiation. Immunol. Rev. **216**(1), 119–129 (2007)
13. Guzman, C.A.: Optimizing rational vaccine design. Microb. Biotechnol **2**(2), 136–138 (2009)
14. Tortura, G.J., Funke, B.R., Case, C.L.: Microbiology: An Introduction, 9th edn, pp. 514–516. Pearson Education, San Francisco (2007)
15. Hamilton, S.E., Harty, J.T.: Quantitation of CD8+ T cell expansion, memory, and protective immunity after immunization with peptide-coated dendritic cells. J. Immunol. **169**, 4936–4944 (2002)