Perspective

# Complexity in the Immune System: New Opportunities for Chemical Engineering Research

**Michael W. Deem**
Rice University, Houston, TX 77005

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## Executive Summary

One may imagine that an analysis of the immune system would be immune from chemical engineering analysis. This Perspective shows how methods from statistical mechanics and computer simulation can be used to construct quantitative models of limitations in the immune system. Applications to dengue fever, which strikes 100 million people each year and is one of the most important human viral diseases, and cancer are given. Our challenge is to translate the understanding that these models provide into strategies, treatments, and the large-scale commercial production of vaccines and therapeutics.

## Dengue Fever as a World-Wide Challenge

"You don't die from it, but you wish you could" (Clarke, 2002). Transmitted by mosquitoes, dengue virus causes an estimated 100 million cases annually. Symptoms include raging fever, agonizing limb pains, hair loss, measles-like rash, bleeding gums, and a depression that can last for several weeks. With the presence of certain risk factors, dengue fever can progress to dengue hemorrhagic fever, in which blood fluids leak from capillaries. Rapid clinical deterioration and collapse occurs in 5–15% of dengue hemorrhagic fever cases (Kurane and Takasaki, 2001).

The earliest probable record of dengue fever is found in a Chinese medical encyclopedia published between 265–420 A.D. (Gubler, 1998). Epidemics in the French West Indies in 1635, and in Panama in 1699 may have been dengue. Major epidemics occurred in Asia, Africa, and North America in 1779–1780. Dengue virus is now common in over 100 countries (Kurane and Takasaki, 2001), in which 2.5 billion people live (Gubler, 1998), and is the most important human viral disease transmitted by a living species other than humans (Worobey et al., 1999).

Despite this long history, the immunological response to dengue fever remains, in many ways, a mystery. There are four major strains of the dengue virus. Troublingly, vaccination against one strain tends to make an individual more susceptible to the other three strains (Gubler, 1998). Contracting one strain also tends to make an individual more susceptible to the other strains. Any vaccine for dengue must, therefore, provoke a strong and uniform response to all four strains.

Despite the interest of the World Health Organization, the Bill and Melinda Gates Foundation, the U.S. military, and others, there currently is no effective four-component dengue vaccine. The difficulty is that the response to each of the components of a multistrain vaccine is not uniform: the components seem to interfere with each other (Rothman et al., 2001). Typical vaccine patients develop significant protection to only one or two of the strains, which leaves them at a greater risk for infection with the remaining strains.

## Statistical Mechanics of the Immune Response to Vaccination

The development of quantitative models of the limitations in the immune system response to highly variable viral diseases such as dengue fever is an area ripe for investigation. Of the 14 "Grand Challenges in Global Health" (Varmus et al., 2003), six Grand Challenges are vaccine related, and two Grand Challenges are addressed by such quantitative models. Any such models of immunology must take explicit account of the dynamics of the repertoire of $\approx 10^8$ distinct B cell and T cell sequences that exist within each individual. Moreover, such models must consider the year-to-year evolution and mutation of the virus, and the existence of multiple viral strains. One reason for developing these models is so that design rules for multistrain vaccines can be deduced. The finite size of the immune system repertoire, and the existence of immune system memory makes the immune system response to variable viral diseases nontrivial.

The human immune system is a stunning achievement of modern evolution. Under most circumstances, the dynamics of the immune system is well-matched to the growth dynamics of an infection. Some pathogens, however, have evolved escape mechanisms that subvert in subtle ways the dynamics of the

M. W. Deem's e-mail address is mwdeem@rice.edu.

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immune system. Moreover, negative interactions between the immune system, which has evolved over the last 400,000,000 years, and vaccination, which has been performed for only the last 200 years, are possible.

There are several fundamental issues that arise in the design of vaccines for antibody and T-cell-mediated protection. One such phenomenon is original antigenic sin (Janeway et al., 2001), in which a primary response of the immune system to an initial viral disease selects memory cells that are recruited in a secondary response to a related, but distinct virus. These recruited memory cells, moreover, are nonoptimal in that they lead to a less effective clearance of the virus in the secondary response than does a response forgoing use of memory cells. Roughly speaking, in the secondary response, the immune system responds only to the antigen fragments, or epitopes, that are in common with the original virus. As a result, individuals vaccinated against the original strain may become more susceptible to infection by mutated strains of the virus than would individuals receiving no vaccination. Essentially, original antigenic sin is a malfunction of the memory system: memory sequences out-compete the nonvaccinated immune response upon exposure to a strain that is distinct from, but related to, the vaccine; however, these memory sequences lead to a poorer overall response than would have the random sequences of a nonvaccinated individual. Original antigenic sin was first noticed for the influenza virus (Fazekas de St. Groth and Webster, 1966), and has since been observed in hepatitis B and C (Harcourt et al., 2003; Anderson et al., 2001), malaria (Good et al., 1993), dengue fever (Rothman et al., 2001), Chlamydia (Berry et al., 1999), and HIV (Anderson et al., 2001). Of course, the above description of original antigenic sin is only a rough picture. Some basic unanswered questions include how similar do the vaccine and mutated viral strain need to be in order for original antigenic sin to occur, how different do the vaccine and mutated strain need to be in order for vaccination not to be useful, and what happens if the vaccine contains multiple strains? To answer fundamental questions such as these in a general way, and to develop a strategy for vaccine design, one needs a model of the immune system dynamics.

A related limitation of the immune system is immunodominance, in which dominant antigen fragments suppress generation of T cell activity toward other nondominant antigen fragments. Immunodominance becomes especially significant when one virus, or vaccine, shares an antigen fragment with another virus. For example, exposure to influenza may increase susceptibility to hepatitis C through immunodominance (Brehm et al., 2002). As with original antigenic sin, there are some same basic unanswered questions. A sequence level model of the immune response allows an investigation of these effects.

My group has been pursuing some of these questions with a philosophy that is based on random energy models from statistical mechanics. This type of theory allows an analysis of the interaction between the immune system, the variable virus, and vaccination. The random energy model captures the essence of the correlated ruggedness of the interaction energy in the variable space, the variables being the amino acid sequences of the antibody or T cell receptor and the identity of the disease proteins, and the correlations being mainly due to the physical structure of the antibodies or T cell receptors. The random energy model allows the study of the sequence-level dynamics of the immune/antigen system, which would otherwise be an intractable problem at the atomic scale, with $10^4$ atoms per antibody or T cell receptor, $10^8$ distinct antibodies and T cell receptors per individual, $6\times10^9$ individuals on the earth, and many possible viruses, each with several strains. The use of random energy theory to treat correlations in otherwise intractable physical systems started with Bohr's random matrix theory for nuclear cross sections (Bohr, 1936) and has continued in applications to quantum chaos, QCD, and quantum gravity. Applications to condensed matter systems include the study of spin glasses, protein folding, and evolutionary systems.

These random energy models, while coarse-grained in their description of the protein–protein interactions within the immune system, capture much of the thermodynamics of protein folding and ligand binding. The specific antibody or T cell repertoire of an individual is represented in the models by a specific set of amino acid sequences. Moreover, a specific antigen or viral strain is represented in the models by a specific instance of the random parameters. An immune response that finds a T cell receptor or a B cell that produces an antibody with a high affinity constant for the antigen corresponds in the model to finding an amino acid sequence having a low energy for a specific parameter set.

These models have been very successful at reproducing and explaining several of the intriguing limitations of the immune system. For example, original antigenic sin in the flu was shown to stem from the localization of the immune system response in antibody sequence space (Deem and Lee, 2003). This localization is a result of the roughness in sequence space of the evolved antibody affinity constant for antigen, and is observed in general for diseases with high year-to-year mutation rates, such as influenza (see Figure 1). Cross-reactivity in the T cell response to mutated viral antigens was studied with a sequence-level model of the T cell repertoire dynamics (Park and Deem, 2004). Predicted specific lysis curves—the immunological analog of the Langmuir adsorption isotherm—were

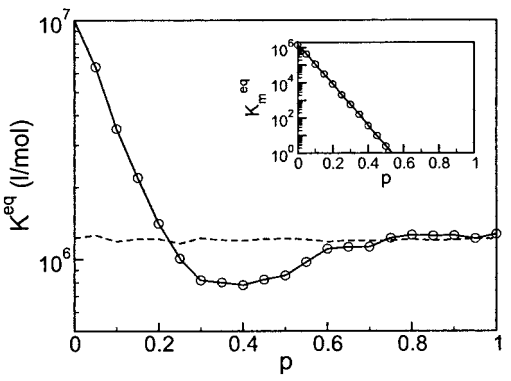


**Figure 1: Evolved affinity constant to a second antigen after exposure to an original antigen that differs by probability p (solid line).**

Affinity constant without previous exposure (dashed line). Original antigenic sin occurs in the region where the secondary response is below the primary response. In inset is shown the affinity of the memory sequences for the mutated antigen, which decreases to nonspecific values at p = 0.36, in agreement with experimental observations that indicate cross-reactivity ceases in the range p= 0.33–0.42 (see Deem and Lee, 2003).

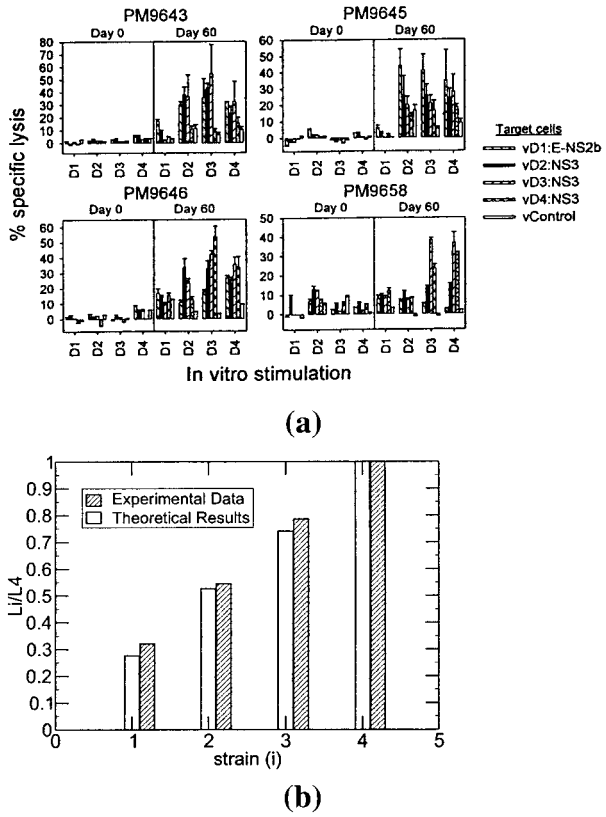


**Figure 2. (a) Specific lysis data from dengue fever vaccine trials, used with permission from Vaccine (Rothman et al., 2001); (b) specific lysis ratios for the least to the most dominant antigen fragment of dengue fever. Predictions from the model (open bars) are very close to data from experimental vaccine trials (hatched bars, from data in (a)).**

in excellent agreement with *ex vivo* and *in vitro* altered peptide ligand experiments.

With these models of the basic immunology in hand, it is possible to look at issues such as the immunodominance in multistrain dengue vaccination. Striking agreement is observed between predictions of immunodominance from the model and observations from multistrain vaccine trials (Rothman et al., 2001) (see Figure 2). There are no adjustable parameters in this comparison, as all model parameters were previously determined when the theory was validated on the altered peptide ligand experimental datasets.

## Relationship to Chemical Engineering

One might ask how the science of vaccination relates to chemical engineering. In addition to the important role of chemical engineering in the large scale production of therapeutics (Khosla, 2002), I suggest that the quantitative training of chemical engineers allows for an unique appreciation of the interplay between the dynamics of the immune system and the viral diversity. Quantitative models that can be developed by chemical engineers are an important tool in the development of design rules for multistrain vaccines toward viruses with high mutation rates or multiple subtypes.

Statistical mechanics, a standard part of many graduate curricula and an optional part of most, provides a framework and some of the tools that we need to understand and manipulate the complexity of the immune system. To recognize invading pathogens, the immune system performs a search of the effectively infinite amino acid sequence space of possible antibodies and T cell receptors. How the immune system is able to find effective structures against a vaccine or virus with only the roughly $10^8$ sequences available in a typical human is one of the great evolutionary success stories. Random energy theory from statistical mechanics allows one to understand this sculpting of the diversity of the antibody and T cell receptor diversity that occurs during the immune response.

Evolution of diversity within the immune system is analogous to evolution of configuration space density that occurs in Monte Carlo computer simulation, in which the true strengths of chemical engineering can be brought to the problem. In the immune system, a search of antibody or T cell amino acid sequence space is performed. In Monte Carlo simulation, a search of configuration space is performed. The important intuition and insight which those familiar with simulation bring to immunology is first, that the diversity within the immune system is a key variable, and second, that the elementary moves of the immune dynamics can themselves be evolved and adjusted. Only by explicit recognition of the importance of diversity can the issues of immunodominance and original antigenic sin be addressed. Moreover, in addressing these issues, the interplay between the dynamics of the immune system and the dynamics of the viral mutation must be modeled. Randomness—diversity— is a natural concept in statistical mechanics, and through statistical mechanics one naturally expects to find the order parameters that characterize the important degrees of freedom of complexity. Finally, as we have seen, random energy models of statistical mechanics can mimic the Hamiltonians of complex immunological systems.

Many, many open questions remain in this field of immunological complexity, and much help is needed from new researchers. With this Perspective, I hope to have persuaded a few of the readers to join me on this quest. Some of the specific issues include:

- Quantification of the impact of immunodominance and original antigenic sin on vaccine design for influenza, dengue fever, hepatitis C, Epstein-Barr, varicella zoster, malaria, and HIV among other diseases.
- Development of the model dynamics to handle both the therapeutically-important *in vivo* and the commonly-measured *in vitro* responses.
- Prediction of how recombination enhances viral survival and transmission, and suggestion of optimal treatment strategies in light of models for recombination.
- Determination of the best way to use models of the immune response in the vaccine design effort, as a function of the mutation rate of the virus, number of strains, and the level of initial exposure to antigen.
- Development of models for immunosenescence—the decline in immune system performance with age.

Vaccine design is undergoing a resurgence not only because of its importance to public health and biodefense, but also because of the many fascinating associated scientific issues.

Statistical mechanics allows the investigation and determination of the fundamental qualitative and quantitative features that govern the interaction between an effective multistrain vaccine and the variability of the virus. Theories of statistical mechanics complement and provide some guidance to the long and difficult process of experimental multistrain vaccine development. The development of vaccine design rules can not only help traditional vaccine design efforts, but also guide directed evolution experimental efforts at vaccine development (Cohen, 2001), the tunable parameters of which are present in our sequence-level model.

## Therapeutic Cancer Vaccination

For my second example, I apply these ideas to cancer.

Our immune system protects us against a broad spectrum of possible cancers (Whelan et al., 2003), most likely on a daily basis (Dunn et al., 2002). The refractory nature of cancer to many standard therapies lends urgency to efforts to achieve immune control of this disease through strategies such as vaccination (Finn, 2003). Cancerous cells of various origin, however, are exceptionally adept at subverting the immune response, and most likely, multiple immune-stimulation strategies will be necessary to avoid escape from the immune system by cancer (Whelan et al., 2003).

A given cancer typically has several antigen fragments that are recognized by the immune system. It is usual that one of these antigen fragments generates the strongest immune response, i.e., is dominant. This immunodominance dramatically reduces the diversity of the immune response to such a tumor, and the essence of immunodominance is competition of T cell receptors for antigen. Immunodominance, which as we have seen is captured by random energy models, is important to understand in this context because it can render a multistrain vaccine effectively single-strain.

One suggestion for breaking the immunodominance hierarchy of cancer immunology is to inject the different strains of the vaccine in different physical regions of the patient (Schreiber et al., 2002). A sequence-level model of the primary and secondary responses for multisite, T-cell-mediated cancer immunity can gauge the effectiveness of this strategy, considering the diversity of the cancer antigens that arises from the natural antigen expression and from the non-negligible cancer mutation rate. A validated model can also be used to generate design rules for the development of multisite vaccines for cancers with high mutation rates or multiple antigens, with a focus on reducing the deleterious effects of immunodominance. Figure 3 shows how the injection of each strain of a multistrain vaccine in a different anatomical location leads to better control of the cancer proliferation. As far as I know, this is the first theoretical study of spatially localized vaccination, and this model provides a way to think about and engineer this new paradigm for cancer vaccination. For example, I can imagine vaccinating with the different strains not only in different physical locations, but also at different times, with different concentrations, or with time-varying concentrations.

## Summary

In closing, the increasingly large biological datasets, increasingly powerful cluster computers, and increasing interest in systems biology within chemical engineering provide the raw data, muscle, and fuel to address within our community these and other general questions of viral and cancer immunity.

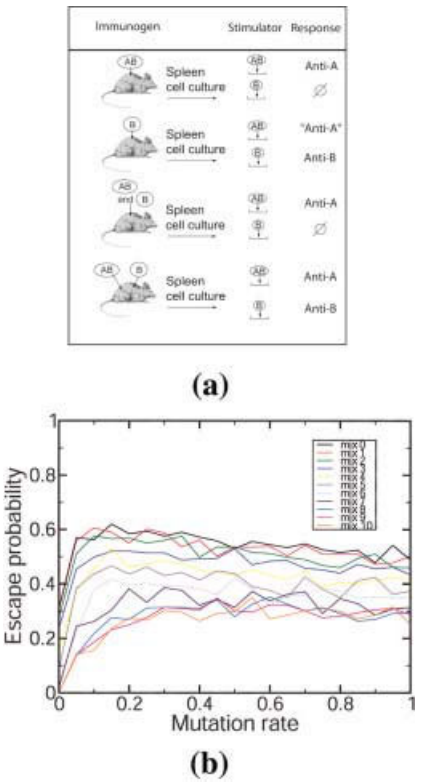


**Figure 3: (a) Experimental observation of superior immune recognition of a cancer vaccine when each strain is injected in a different physical location.**

In this experiment, strain A is immunodominant to strain B. After (Schreiber et al., 2002). (b) Results of the model when the different strains are injected in different physical locations, and provoke an immune response that evolves independently for mix days. Larger values of mix allow for more independent evolution and lead to superior recognition of the cancer strains and less cancerous proliferation. In the model, the cancer cells were allowed to mutate for 10 days, and a cell population that had not been reduced in size by the immune response within 10 days was considered to have proliferated, or escaped.

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