Perspective

Entropy, Disease, and New Opportunities for Chemical Engineering Research

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Executive Summary

Randomness, diversity, fluctuations, and correlations play a significant role in disease, disease treatment, and the immune response to disease. I suggest that statistical mechanics, the physical theory of randomness that uses a system's physical behavior at the molecular or atomic scale to synthesize a picture of the behavior at a larger level, can address some of these issues. Theory and mathematical modeling can help design or redesign treatment strategies. Theory can also help determine what makes redesign necessary. I describe three lines of research in my group seeking this new mathematics of biology.

Efficacy of the Influenza Vaccine — The Response of the Immune System to a Perturbation

Influenza epidemics are annually responsible for the deaths of 25,0000 to 50,0000 people in the world and cause illness in 5 to 15% of the total population each year (World Health Organization, 2003). The total cost associated with influenza in the U.S. is roughly $10 billion (Lave et al., 1999), and the economic cost of an influenza pandemic has been estimated to be $71–167 billion (Meltzer et al., 1999) in the U.S. alone. The primary method employed to prevent infection by influenza and its associated complications is vaccination. Mutation and antigenic change, combined with the high rate of transmission of influenza strains, means that the vaccine must be redesigned each year. This is currently done with phylogenetic, animal model, and epidemiological analysis.

The effectiveness of the influenza vaccine varies each year due to changes in the molecular structure of the influenza strains that are circulating. Three strains are customarily included in the annual influenza vaccine, with these three strains chosen to be as similar as possible to those estimated to be the most widespread circulating strains in the upcoming flu season. Currently, the vaccine contains a H3N2 and a H1N1 influenza A component and an influenza B component. Due to mutation of the influenza virus, vaccine efficacy is rarely 100%, and is more typically 30 – 60%, against influenza-like illness. The estimated worldwide mortality rises by another 160%–260% if influenza-induced complications in patients with other conditions are included (Neuzil et al., 1999; Sprenger et al., 1993). It is believed that the influenza vaccine, on average, significantly reduces such excess mortality (Hak et al., 2002). Vaccine efficacy can be negative, however, due to original antigenic sin (Davenport et al., 1953; Fazekas de St. Groth and Webster, 1966; Deem and Lee, 2003), which is the tendency for antibodies produced in response to exposure to one influenza vaccine antigen to suppress the creation of new, different antibodies in response to exposure to a new version of the influenza virus. The efficacy of the annual influenza vaccine, and whether original antigenic sin occurs, depends delicately on the similarity between the vaccine and circulating viral strains. The current standard of practice is to measure antigenic distance by ferret antisera hemagglutinin inhibition assays (Smith et al., 2004; Smith et al., 1999; Lee and Chen, 2004), and these distances have been assumed to correlate well with vaccine efficacies in humans. However, to my knowledge no such significant correlation has ever been shown for an experimental, animal model, or theoretical measure of antigenic distance. Besides being useful for the annual flu shot design, a reliable measure of antigenic distance would help to stem the spread of a newly emerged influenza strain by allowing for streamlined decision making if preparation and rush production of a modified vaccine is necessary (Ault, 2003).

So, what is the best order parameter to describe antigenic distance, and which also correlates well with vaccine efficacy? Using the tools of statistical mechanics, we provided a quantitative definition of the difference between the dominant epitope regions in the vaccine and circulating strain, p_{peptide} (Deem and Lee, 2003; Gupta et al., 2005). We showed that this definition of antigenic distance correlates well with human influenza vaccine efficacy over the past 35 years (see Figure 1) (Munoz and Deem, 2005; Gupta et al., 2005).

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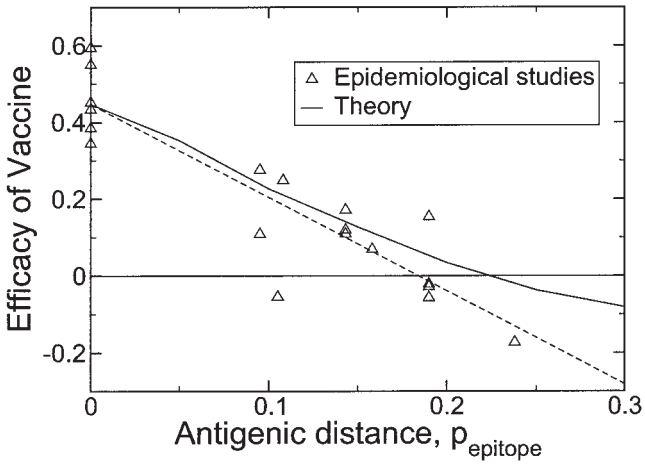

Figure 1. Vaccine efficacy for influenza-like illness as a function of pepitope as observed in epidemiological studies and as predicted by theory.

Also shown is a linear least squares fit to the data (long dashed, $R^2 = 0:81$). From (Gupta et al., 2005).

A different and highly lethal strain of influenza, the H5N1 avian influenza strain, was first detected in humans in Hong Kong in 1997 (Saw et al., 1998; Claas et al., 1998). Since then, it has spread to at least eight other Asian countries (Normile and Enserink, 2005; Cyranoski, 2005a), and Russia (Allakhverdov and Enserink, 2005), and it is widely expected to enter the rest of Europe through migrating birds. To date there have been roughly 60 reported deaths due to the H5N1 strain. The initial mortality of 70% decreased to roughly 20% (Normile, 2005b), which suggests the bird flu is evolving to become less fatal but concurrently more able to persist and, thus, to create an epidemic. Avian influenza has also been observed in pigs, a classic mixing vessel for influenza (Cyranoski, 2005b). Person-to-person transmission has been suggested (Ungchusak et al., 2005). Avian influenza is, thus, evolving (Normile, 2005a; Hulse-Post et al., 2005). Vaccines, antivirals, animal culling, and public health measures (Longini et al., 2005; Ferguson et al., 2005) are the main weapons against spread of the bird flu. Various countries are stockpiling bird flu vaccines, and a vaccine produced from one test strain of the bird flu has produced an immune response in healthy adults (World Health Organization, 2005). However, since the bird flu is mutating, which strains should be stockpiled? The National Institute of Allergy and Infectious Disease is making sequences of the H5 strains available (Kaiser and Vogel, 2004). The U.S. Centers for Disease Control and Prevention are, controversially, investigating the potential reassortments of the bird flu that might create an epidemic (Khamsi, 2005). However, the key question remains: What is the required or optimal diversity of vaccine stockpile? In particular: How cross-protective will a bird flu vaccine be against other strains that exist or will evolve into existence? The extent of cross-protection is needed to determine the optimal vaccines to stockpile and how to administer them (Patel et al., 2005).

To protect against the strains observed to date, how many vaccine components are needed? We used results from data (Gupta et al., 2005) and theory (Deem and Lee, 2003) of the influenza vaccine effectiveness to estimate how cross-protective the bird flu vaccine will be (Zhou and Deem, 2005). We used the 50 strains of H5N1 observed to date (Macken et al., 2001) to characterize the diversity of a typical pandemic. While vaccines against typical influenza A strains contain only the dominant strain, as subdominant strains typically last only a few seasons (Fitch et al., 1997), a bird flu pandemic may well be over in one season. There may be significant mortality from multiple strains, and so protection against all strains may be important. We considered that the vaccine will contain only those strains that have been observed in the wild, due to the ethical questions arising from developing and vaccinating with mutant strains. We examined all combinations of the wild-type strains, searching for the combination with the least number of components. To cover all these strains with at least 15% vaccine efficacy, we predicted that 3 vaccine strains would be needed.

Evolution of the H5N1 strain may well render existing vaccine stockpiles ineffective, and public health authorities may have to depend almost completely on the production of new vaccine strains, in addition to antivirals, animal culling, and quarantine. The value of pepitope can be used to estimate the expected efficacy of vaccine stockpiles and, thus, to decide whether to deploy them. Our results suggest that reverse genetics or DNA-based vaccines, both able to rapidly deploy a new vaccine, will be useful in the prevention of wide-spread infection during an H5N1 epidemic given the slow and unreliable nature of the traditional vaccine production in hen's eggs. Thus, a chemical engineering approach may contribute not only to the vaccine design, but also to the production (Khosla, 2002).

Evolution in Antibody Sequence Space and Autoimmune Disease

The immune system normally protects the human host against death by infection. The immune system tends to produce antibodies with binding constants of at most 10^6 - 10^7 l/mol. Experimentally, however, it is possible to find binding constants between antibodies and substrates on the order of $\approx 10^{11} - 10^{13}$ l/mol (Schier et al., 1996), and the laboratory techniques to find these antibodies (Maynard and Georgiou, 2000; Swers et al., 2004) mimic mechanisms that exist within the natural hierarchy of evolutionary events (Kidwell and Lisch, 2001; Earl and Deem, 2004). The method that the immune system uses to search sequence space is rather slow — the same mechanisms that can find antibodies with higher affinity can also find them more quickly. Thus, one would think that these more powerful evolutionary mechanisms would give an immune system that responds faster and more effectively against disease. So, why didn't we evolve that kind of adaptive response?

To answer this question, we first sought to understand the evolutionary rules that govern the way the immune system responds to an infection. With that framework in place, we identified a biologically-plausible strategy that would allow the immune system to react more quickly and with more effective antibodies. Our analysis revealed that such a system would be about 1,000 times more likely to produce antibodies that attack healthy tissues (See Figure 2) (Sun et al., 2005). Such cross reactivity due to increased affinity has recently been observed (Holler et al., 2003).

Antibodies that bind with a molecule other than the antigen they evolved to attack are called cross-reactive, and cross-reactivity can cause autoimmune disease. For example, chronic infection has been found to be correlated with increased probability of autoim-

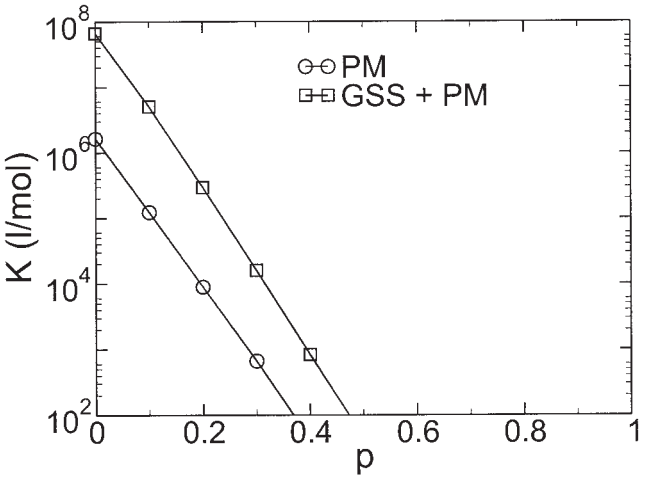

Figure 2. Affinity of memory antibody sequences after a primary immune response for the two different immune system strategies (PM and GSS+PM) to altered antigens.

The binding constant is K, and the antigenic distance of the new altered antigen from the original antigen is p. Cross-reactivity ceases at larger distances in the GSS+PM case (no cross-reactivity for p > 0:472) than in the PM only case (no cross-reactivity for p > 0:368). Theory shows that these results imply the antibodies evolved by the GSS+PM dynamics will recognize on average 10^3 more epitopes than the antibodies evolved by the PM dynamics alone. From (Sun et al., 2005).

mune disease (Leirisalo-Repo, 2005; Kaplan et al., 1997). However, the strength and significance of this correlation is controversial (Carty et al., 2003). Our model suggests a broad distribution for the time of onset of autoimmune disease due to chronic infection. Researchers have been looking for a clear, significant correlation in time, but a long distribution of onset times would lead to weaker statistical correlations, particularly in those cases where the infection persisted the longest. Searching for this distribution could elucidate this immunological puzzle and settle the scientific controversy.

We found that the human immune system evolved to minimize the risk of cross-reactivity. For example, each cell in our bodies contains about 100,000 proteins with an average of 500 amino acids apiece. Consequently, there are about 10^{12} potential docking sites, or epitopes, where antibodies could mistakenly attach themselves to proteins in a healthy cell. The mutation response method employed by our adaptive immune system seems keyed to this number, producing antibodies that are statistically likely to mistakenly bond with healthy proteins slightly less than one in 10^{12} times, meaning that on average, they recognize only invading pathogens.

Randomness of Cancer

The percentage of Americans dying from cancer is the same as what it was in 1970. . . and the same as what it was in 1950 (Leaf, 2004). Although some progress has been made, especially for childhood cancers, cancer remains a largely unsolved purge of modern society. Mouse models remain largely unpredictive, and cancer seems a tremendously complicated disease. Heterogeneity and randomness are two factors that distinguish cancer from other, more treatable diseases. New fundamental concepts are desperately needed in the fight against cancer.

I suggest that the tools of statistical mechanics, the physical theory of randomness, may provide a conceptual framework for cancer drug and vaccine discovery. The design of therapeutic cancer vaccines to effectively besiege a cancer epitope is a pivotal piece of the current war on cancer. Indeed, a molecular-level understanding of cancer is vital to develop much needed diagnostic and therapeutic tools. Thus, the immune response to cancer vaccines must be dissected at the molecular biophysics level.

Several features of cancer and the immune system limit the effectiveness of traditional vaccination techniques (Dunn et al., 2002; Schreiber et al., 2002; Whelan et al., 2003). In light of the need to deliver multiple, related vaccines to eradicate a cancer (Stuge et al., 2004; Whelan et al., 2003; Markiewicz and Kast, 2004), a multisite vaccination strategy appears promising (Schreiber et al., 2002). In my group, we developed a quantitative theory that explains the success of the new multisite approach (see Figure 3), and we are using this theory use to guide the arduous process of vaccine design (Yang et al., 2005). By inducing a T cell response to each cancer-associated epitope in a distinct lymph node, vaccine efficacy is increased, and immunodominance is reduced. The approach captures the recognition characteristics between the T-cell receptors (TCRs), and tumor, the primary dynamics due to TCR resource competition (Kedl et al., 2003), and the secondary dynamics due to competition between escape of tumor cells by epitope mutation and allele loss, and elimination of tumor cells by TCRs. This approach may be applied to both solid tumors and post-surgical micrometastases.

Sculpting the diversity of the TCR repertoire is a means to reduce immune evasion of tumor cells due to epitope mutation or MHC I allele loss. The TCR diversity provides a recognition reserve to target the unmutated, subdominant epitopes (Nikolich-Zugich et al., 2004). The importance of diversity, the biological analog of entropy in this problem, is naturally appreciated within the context of statistical mechanics.

The solid nature of tumors presents some challenges to immune control, but also some opportunities for engineering contributions. It may be difficult, for example, for T cells to enter the solid tumor. Conversely, high intensity focused ultrasound can disrupt solid tumors and can enhance systemic antitumor cellular immunity (Wu et al., 2004). Although the exact mechanism of this enhancement is unknown, one possibility is that the fragments of tumor after destruction can travel to different lymph nodes and, thus, induce a diverse TCR repertoire, in similar fashion to polytopic vaccination. More prosaically, the physical disruption of the tumor can allow easier entry of the T cells. Another feature of solid tumors is the enhanced probability of uptake of large proteins, due to the highly porous capillaries in tumors (Raucher and Chilkoti, 2001). By using this property, stimulants of T cell activity may be localized to the tumors. The concentration of such stimulants could be increased further by conjugation with elastin-like polypeptides that undergo a thermally triggered phase transition in heated tumors, causing selective aggregation in the tumor (Raucher and Chilkoti, 2001).

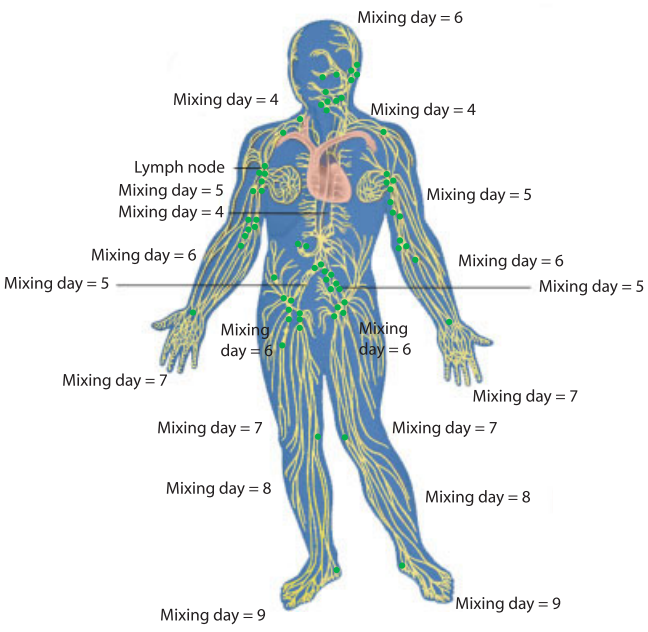

Figure 3. Value of the parameter mixing round for vaccination to different lymph nodes at different distances from the heart.

Humans have several hundred lymph nodes. For effective polytopic vaccination, well-separated sites on different limbs are used.

Summary

By a discussion of three examples, I hope to have convinced the reader that significant unsolved theoretical problems exist in medicine and that statistical mechanics has a pivotal role to play in their solution. The importance of randomness to the proper functioning of the immune system seems an especially ripe topic for statistical mechanical analysis. Many, if not most, problems in immunological diversity remain open, and identification of the models and theories to tackle these problems is just starting. Interaction with immunologists and pathologists has proved helpful to my group as we explore these issues. We are also fortunate as a field and as a profession that graduate students are keen to contribute to the new mathematics of biology.

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