Immunity
Forum

A Prescription for Human Immunology

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DOI 10.1016/j.immuni.2008.12.003

Inbred mice have been an extremely successful tool for basic immunology, but much less so as models of disease. Thus, to maximize the use of immunologic approaches to improve human health, we need more strategically directed efforts in human immunology. This would also open up new opportunities for basic research.

Immunology as a branch of the biological sciences has advanced tremendously over the last 50 or so years. In this time, clonal selection has advanced from theory to established fact and the basic structures of antibodies and T cell receptors have been determined, together with their remarkable (and thus far, unique) mechanisms of diversification. A whole system of innate immune receptors and responses has been discovered and elucidated very rapidly, and lymphocytes and other hematopoetic cells can now be subdivided into at least 15 different distinct types. Dozens of cytokines and chemokines have been identified as mediators of cellular communication and we are the proud possessors of 350 CD antigens. A field that was once known chiefly for its impenetrable jargon, the byzantine complexity of its experiments, acrimonious disputes, and excessive theorizing is now the very model of a modern, superbly integrated, and rich biological field, one of the most successful in biology (we still have the impenetrable jargon, but, oh well). We can even claim to have saved the most lives through vaccines (albeit indirectly, as most were formulated before immunology could offer much help) and helped bring about a whole new type of pharmacology in the form of specific antibodies as drugs, not to mention the promise of direct immunomodulation made possible by knowledge of specific pathways.

And yet, amid this euphoria, there is a serious problem, which is that virtually none of the advances in basic immunology cited above have been incorporated into standard medical practice; specialized clinics, yes, to an extent, but you can go to the most prestigious medical center in the world and ask "How is my immune system?" and, after a short period of eye rolling and looks of amused incomprehension, you might (if they don't just throw you out) be offered a white blood cell count (which you should probably decline). Ask about blood lipids, though, and you'll be greeted with warm smiles, minor bloodletting, and morality tales about "good" and "bad" cholesterol.

So what's the story here? Is this because the immune system is not important for health? No, at least since AIDS and bubble boys, everyone and their grandmother knows that the immune system is central to health and that a particular deficiency or misregulation can have severe consequences. Which is also not news to the millions of people suffering from the almost ninety different types of autoimmunity or more than 100 inherited immune deficiencies or increased susceptibility to infectious diseases or cancer because of drug treatments or aging.

It is also becoming clear that many diseases that were not previously thought of as immunological, such as atherosclerosis or Alzheimer's disease, have a basis in immunological mechanisms. The list of these will only grow as more research is done. In fact, people are so aware of (and worried) about their immune system that there is a booming business in products labeled "immune boosters" available at pharmacies and health food stores near you. And yet there can be no basis for such a claim unless there are "metrics" of immune function that can show such a boost. A central thesis of this essay will be that immunologists should establish metrics of immunological health in humans (and mice too, for that matter) in order to both better understand the diseases that we study and to make what we know more accessible to the public and the general medical community.

Overreliance on the Mouse Model

How did we arrive at this state of affairs? A good case can be made that the mouse has been so successful at uncovering basic immunologic mechanisms that now many immunologists rely on it to answer every question. Where it was once common to use a variety of species, there is now such an abundance of reagents available in mouse immunology that one has to have an overpowering reason to work in any other species, including humans. It also has raised the bar of evidence required for journals and grant reviews, as pointed out by Steinman and Mellman (2004) and by Hayday and Peakman (2008). This has skewed the field so much that most clinically trained immunologists keep at least a few (and usually a lot more) mice in the "back room" so that they can have a steady flow of papers, grant funding, etc., and some have abandoned human work entirely as a lost cause. But this is just the price of progress, no? Well, except that mice are lousy models for clinical studies. This is readily apparent in autoimmunity (von Herrath and Nepom, 2005) and in cancer immunotherapy (Ostrand-Rosenberg, 2004), where of dozens (if not hundreds) of protocols that work well in mice, very few have been successful in humans. Similarly, in neurological diseases, the mouse models have also been disappointing (Schnabel, 2008).

Why has the mouse been so unsuccessful as a clinical model? A number of possibilities have been put forward. One is that the use of inbred strains creates a wealth of homozygous recessive defects that skew the regulation of the

Takeda Vaccines EX2021
IPR2025-00776
U.S. Patent No. 11,730,802

immune response (von Herrath and Nepom, 2005). Another potential culprit is the artificiality of many disease-inducing protocols (Quintana-Murci et al., 2007), and third is the sheer evolutionary distance (65 million years) between mice and humans and the likelihood that the immune system of a short-lived, ground-dwelling mammal that can replicate quickly may be substantially different than that of a long-lived, somewhat higher off-the-ground mammal that replicates very slowly (and thus has more of an evolutionary investment in individual survival). In this regard, Mestas and Hughes (2004) have carefully delineated the many differences between mice and humans with respect to various immune markers, as have recent reports contrasting human versus mouse phenotypes (von Bernuth et al., 2008; Cohen et al., 2006) in specific gene deficiencies.

Although it is not clear which of these possible explanations is most important, it seems that mouse-human differences are not even being studied systematically. We seem to be in a state of denial where there is so much invested in the mouse model that it seems almost unthinkable to look elsewhere, and yet, if we had objective criteria for particular human responses, we might be able to use mice or other animal models more appropriately. One possible answer could lie in the renewed interest in "humanized" mice, which in most cases means immune-deficient mice into which are introduced either human hematopoetic stem cells or white blood cells. Although promising, it should not be assumed that such mice are equivalent to a human immune system in any respect unless it is demonstrated to be so by a variety of objective measures. Many of these can come only from developing metrics of human immune function. Thus one very important reason to develop such metrics is that they would allow us to use any particular animal model with much more confidence.

The Human "Model"

This argument leads inevitably to the conclusion that if we are to make more rapid progress in clinically useful immunology and metrics for immunological health, we need to encourage more efforts in human immunology and somehow compensate for all the disadvantages that have discouraged so many people in our field. Here, a good illustration of what can happen is the field of human genetics. Long ago, in the 1970s, human genetics was one of the least "happening" fields around. There was really not much you could do in most cases besides describing a mutation and constructing a family tree. Among geneticists, human genetics was considered a backwater compared to what could be done in bugs, flies, and worms. Then, gene cloning came along and especially the Human Genome Project (I'm compressing things a bit here) and suddenly, genes could be identified and mechanistic work could be done, and so forth. This has transformed the field and hardly a day goes by now without some new discovery in human genetics. This is largely because the Genome Project put in place a massive chunk of infrastructure that made looking for genes and polymorphisms almost trivial. And so, people could focus on more interesting things. The other useful lesson of the Genome Project is that it showed that the typical academic lab is not the be-all, end-all of how science should be done, but that more industrial models can, in some cases, be more appropriate. It is worth remembering that this was a pretty hot debate at the time, as many thought that the "big science" model being proposed for the Genome Project, with its emphasis on economies of scale, would fatally pollute biological science as we knew it then. Luckily, the "big science" proponents won that argument (Collins et al., 2003; with significant help from Craig Venter and Celera), or we would still be working toward finishing the human genome today. The point is that although the relatively small academic labs as we know and love them are great for innovation and out-of-the-box thinking, some problems in biology (and other sciences for that matter), particularly those that involve a great deal of repetitive assays and data collection, are much better suited to a larger-scale organization and execution. The data are both more uniform and considerably cheaper.

What to Do?

Hopefully, the preceding discussion has convinced the reader that mouse models are not the answer to everything in immunology and that we need to make greater efforts in human immunology if we are to realize the potential health benefits. But how to do this is an important question. Naturally, one could just increase funding for what's being done now and that would certainly help, but I could argue that like the recent history of human genetics, we could be much bolder. In addition, even with massive new funding, it's pretty clear that human immunology will never "catch up" to mouse immunology if they pursue parallel paths, with each lab doing "its own thing" largely independent of everyone else. This is because there are just too many reagents and tools available to mouse immunology and nowhere near the restrictions and limitations that are involved in human work.

Instead, I think a good case can be made for taking some very different approaches in human immunology that take advantage of its strengths and work around its weaknesses. So, what are the strengths? These are, simply, that (1) billions of people screen themselves for illnesses every day and that those who are most ill visit doctors and hospitals and while there contribute millions of blood specimens. (2) Many thousands of healthy volunteers can be recruited for studies of "normal" people that can be assayed in parallel. (3) Hundreds of millions of people are vaccinated every year and this represents a valuable resource for the study of normal immune systems "perturbed" in a safe way. (4) Specific immunological illnesses have been studied intensively—almost 90 different autoimmune syndromes have been described as well as more than 120 inherited immune deficiencies. Thousands of infectious diseases affect humans pathologically, with new variants or whole new organisms arising regularly. We also harbor thousands of commensal bacteria in our bodies and at least some of these influence immunological functions for good or ill. Lastly, as more and more diseases are found to have an immunological component, it is even more imperative that the workings of the human immune system (or its failings) be more fully understood and incorporated into basic medical practice.

This leads to an argument for a broad-scale "systems" approach to immunology in humans that can use high-throughput immune-monitoring assays to perform uniform analyses across many different clinical samples (blood usually), and those

from healthy people as well to establish the basic parameters of immunological health. These will benefit from the many assays that we have available that can assess dozens of soluble cytokines, distinguish between 350 cell-surface proteins (CD antigens), separate the 15+ distinct types of whole blood cells, isolate many signaling pathways, and survey the expression of all 25,000+ genes and regulatory RNAs. Comparing gene expression patterns in normal individuals versus patients with autoimmune disorders has already proved fruitful in identifying aberrant cytokine patterns linked to these diseases and suggests therapeutic options (Allantaz et al., 2007). Further genetic analysis with single-nucleotide polymorphisms (SNPs) will also be valuable, at least in the long term, although it should be noted that patient care is a "real time" activity and that the subtleties of multigenic disorders may be too problematic to be of much use in the clinic. Specifically, studies of susceptibility loci other than HLA in human autoimmunity have shown very modest risk factors, rendering this kind of information not immediately useful clinically, although it certainly can point to commonalities and drug-treatment strategies eventually.

Why focus on defining immunological health versus mechanisms of disease? Because I think that's "what's missing from this picture" in all this, and given the variations in immune parameters in people, we need to get a grip on this before we can properly understand the perturbations of many diseases. Although some of this is "built into" all analyses as the control population, it hasn't really been tackled as an end in itself. And yet, it has to be defined if we are ever to give physicians the "metrics" needed to answer the question posed at the beginning of this essay.

So, how can we define immunological health? First by searching through all the biomarkers mentioned and finding ones that delineate healthy individuals from those with any of the various diseases mentioned (much as has been started already via gene expression data [Allantaz et al., 2007]). Or more simply, health is the absence of disease and the more disease phenotypes that can be integrated into the same data set, the more you should be able to identify the warning signs of a system that is malfunctioning.

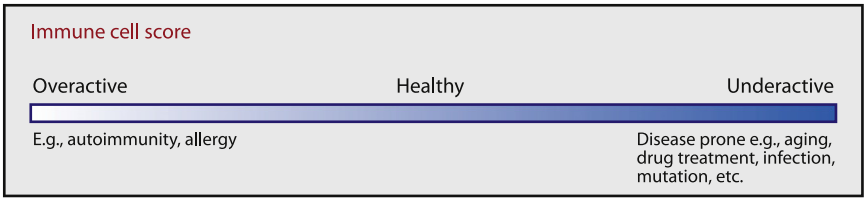

Figure 1. Representative Assay for a Particular White Blood Cell
Here a given white blood cell is assayed for either a function or an array of biomarkers in both normal and diseased/aged individuals such that a range from overactive to underactive properties is registered. For the major cell types, it is likely that a deficiency in any one of them will disable the entire system in some way.

A well-known example of this is the loss of $CD4^+$ T cells in advanced HIV infection-AIDS, which leaves the victim open to all kinds of opportunistic infections. This example also illustrates a likely truism here, which is that the immune system is made up of many interacting cell types and the failure of any one of these is likely to have deleterious consequences for the health of that individual. This suggests that we will need to develop a battery of functional tests for each cell type or at least enumerate them by flow cytometry. A functional assay is ideal because you not only count the cells of that type but you test them for a particular attribute or attributes that essentially integrate over many potential defects, inherited or acquired. Ultimately, these cellular assays may be replaced by a simple biomarker (a particular cytokine or gene, etc.) or just the number of cells of a particular phenotype; but perhaps not. So this would seem to be a useful adjunct to the other data sets. Thus, at the end of this, one could imagine a normal range of functional activity, together with correlative biomarkers that define the useful range of a given cell type, on either side of which are examples of where the cell doesn't work as well, as illustrated by one or more disease states. This could be reduced to a "score" for each cell type that could then turn into a useful series of clinical tests, as shown in Figure 1. But many scientific benefits could come from such data as well, such as how the system as a whole (or parts of it) responds to the many possible perturbations. This would tell us about how the different components interact with each other. This may or may not reproduce what we have deduced about cellular interactions from mouse studies, but also would have the ability to reveal interactions that are entirely novel. It would be particularly interesting to monitor how the various new immune modulatory drugs change an individual's overall system and could also show why some people respond to a particular drug but others do not. Here, the diversity of human genetics and immune responses could provide a rich source of new insights into how the immune system functions in the face of constant challenges from the environment.

Another important component of this is to develop the informatics infrastructure that is able to handle this amount of information and perhaps most critically integrate the different types of data in the search for markers and patterns of markers that correlate with particular disease states. This points to another benefit, which is to find commonalities between different diseases that depend on similar mechanisms; this may not correlate with a particular type of disease as they are categorized now (largely by inspection), but may get to a deeper understanding of the underlying causes and lead to potential drug treatments more rapidly (Chaussabel et al., 2008; Tenenbaum et al., 2008).

Another promising area of informatics is that of "text mining," where various data can be culled from the literature to create a kind of general "meta-analysis" of the literature (Muller et al., 2004).

Specifics

How to implement this approach? Obviously, more NIH funding would be needed and there are already some very forward-looking efforts to encourage human immunology ongoing at NIH, particularly the Cooperative Centers for Translational Research and Biodefense created 6 years ago (disclaimer: the author receives funding from this program). But one basic step

is to have fully equipped immune monitoring facilities at all major research medical schools because that's where the blood samples are being taken and there can be more direct links with the research projects at that site. Although there are a number of such facilities, there is not yet enough of a national or international program to link them together and to standardize (and validate) assays, although a very good beginning has been made by the Cancer Immunotherapy Monitoring Program (http://www.c-imt.org/content/view/20/26/). Bioinformatics standards are also needed as well as the deposition of relevant data after a reasonable "blackout" period and/or publication. At Stanford University, we have set up what could be a prototype facility with these features (the Human Immune Monitoring Core), but the need for a broad and uniform approach is ever more apparent if we are to realize all the benefits of this approach quickly. This type of coordination and standardization is also important with respect to clinical trials, and here the Immune Tolerance Network (http://www.immunetolerance.org/) has done this very well. It also would be useful to have a website dedicated to reports of human-mouse differences or similarities in immune function. With a national or international effort, we could, at much less cost than the Human Genome Project, provide something like the richness of that data infrastructure to create a great and continuing resource for human immunology. Efforts along these lines could easily fit into the concept of "personalized medicine" together with the analysis of signaling pathways, soluble biomarkers, and genetics (Hood et al., 2004).

Concluding Remarks

Immunologists have long emphasized the potential benefits for human health of basic research in our field. Although the mouse model has been spectacularly successful in advancing our understanding of basic immunological mechanisms, its record in formulating clinically useful protocols is much less impressive. Thus, to fully realize the potential benefits of immunology for human health, we need to place more emphasis on human studies and make greater efforts to allow it to flourish. This could also create a rich resource for future studies of new immunological principles, especially as humans live "in natura" (Quintana-Murci et al., 2007) more or less, outbred and exposed to many more diseases than laboratory mice.

ACKNOWLEDGMENTS

I thank the many colleagues who have shared their insights on this topic, although they shouldn't necessarily be blamed for my conclusions, including C.G. Fathman, J.-L. Casanova, D.B. Lewis, M.G.v. Herrath, A. Butte, H. Quill, W. Robinson, and M. Brenner. I also thank the reviewers for their helpful comments and suggestions and to the Howard Hughes Medical Institute, the Ellison Medical Foundation, and especially NIH U19 AI053589 for grant support.

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