Identifying the key genes to infection resistance

September 17 2012, by Aaron Dubrow

Examples of two genetic mutations discovered in the Beutler Lab. The mouse on the left exhibits a mutation named piglet. The black and white mouse on the right, known as Dalmatian, has a mutation in the gene known as Sox10. This gene produces a protein which is 98% similar in the mouse and in humans.

Manning the gates of our immune system are toll-like receptors (TLR)—tiny hairs that stick out of the cell membrane, recognize foreign bodies, and rally an organism's defense mechanisms. The molecular building blocks of TLRs are present in bacteria and plants, and are believed to be one of the most ancient, conserved components of the immune system. These tiny receptors and other molecular messengers play a big role in human health as well.

Bruce Beutler and colleagues discovered the first mammalian TLR in the 1990s, showing that it recognizes a bacterial molecule called endotoxin. Beutler received the Nobel Prize in medicine for this discovery in 2011, along with Jules Hoffmann and Ralph Steinman. But this toll-like receptor was not the end of the road.

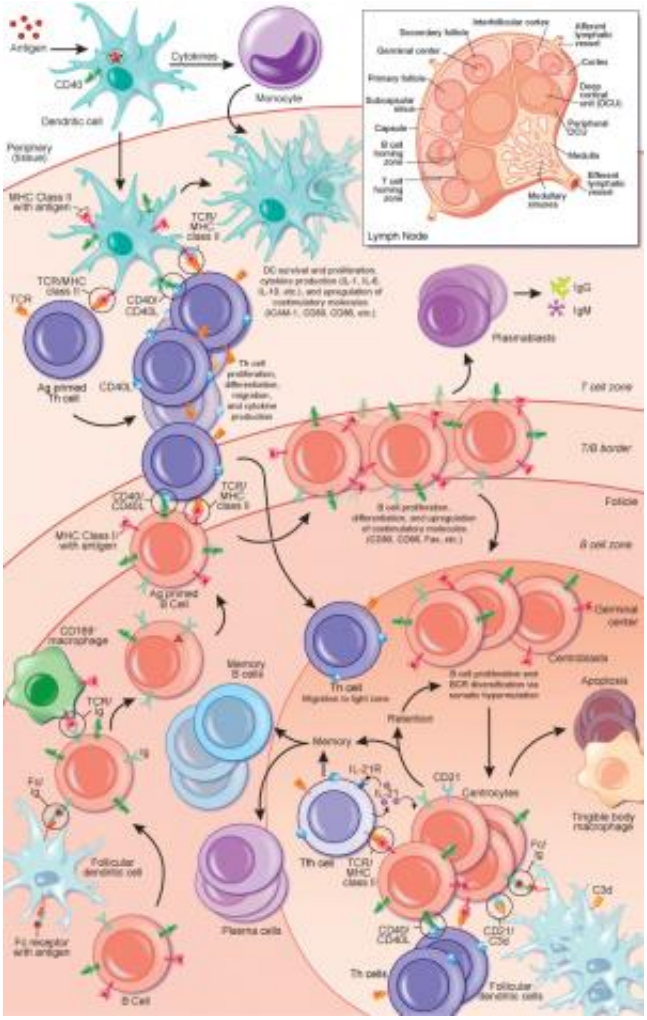
In subsequent years, Beutler has found many new TLRs in humans and mice, as well as dozens of additional genes involved in our innate immune reaction. Many more remain to be discovered.

The long-range goal of Beutler's laboratory at The University of Texas Southwestern Medical Center is to identify the key genes required to resist infection—the so-called mammalian "resistome"—and to determine how these genes interact with one another to defend the body (or in the case of auto-immune diseases, set the body against itself).

To date, the Beutler lab has discovered 375 transmissible mutations that cause discernable changes to mice. 253 of these mutations have been mapped to chromosomes, and in 228 instances, the group has discovered the molecular basis of the mutation. This is just the tip of the genetic iceberg, according to Beutler.

With the assistance of powerful supercomputing resources at the Texas Advanced Computing Center (TACC) at The University of Texas at Austin, Beutler is growing and speeding up his research significantly.

Over the next five years, he hopes to sequence the exomes of 8,000 mice. (The exome represents the coding portions of genes that synthesize proteins and other functional gene products.) This body of genomic data will form the most comprehensive database of functionally meaningful mammalian mutations available to science. It will provide invaluable insights into the hundreds of genes involved in the immune response pathway and other important biological processes.



Overview of the T-dependent humoral immune response. The diagram shows many of the key cells involved in the body's reaction to antigens.

To reach this goal of 8,000 mice, Beutler's lab will investigate 48 exomes every week—scanning, enriching and sequencing 500 billion base pairs and finding the points of discrepancy from the reference sequence that is itself millions of letters long. The project represents a "Big Data" problem of the highest order.

"We need awesome computing power to sequence 8,000 whole exomes in the mouse," Beutler explained. "We weren't even talking about doing anything like this a couple of years ago. But I think with TACC, it really is conceivable."

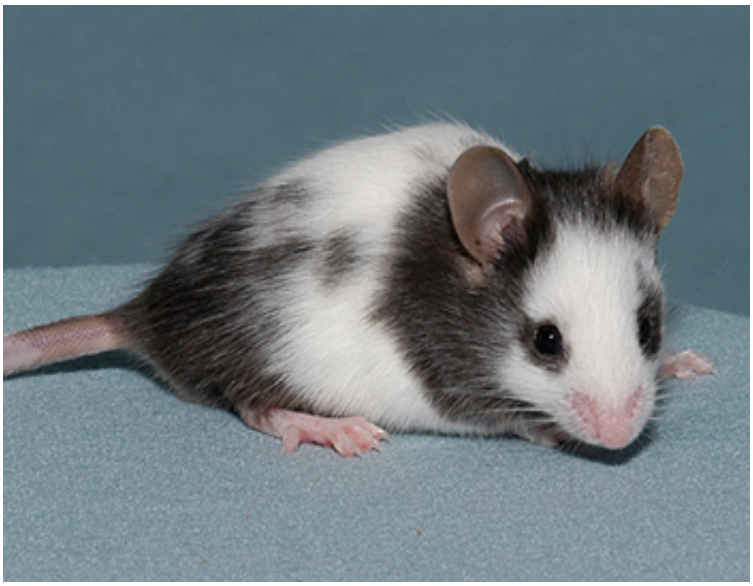
John Fonner, a research associate in the Life Sciences Computing Group at TACC, described Beutler's work as both ambitious and visionary, but achievable thanks to cutting edge sequencing machines and high performance computing.

"Analyzing these data often expends tens of thousands of CPU hours in a day, not to mention the data storage requirements. The research problem is a great match for TACC's resources," he said.

To isolate potentially interesting mutations, Beutler's lab follows a multipart protocol. The first step uses chemical mutagens to induce mutations in mice in order to find the genes involved in innate immunity. It is well known that the mutagen N-ethyl-N-nitrosourea (or ENU) causes approximately 60 base pair changes per mouse genome. But where these changes will be and what effects they will have are unknown.

To find out, the lab breeds mice to the 3rd generation to create individuals who are homozygous (have two sets of the same gene) for mutations. First and second generation mice may not pass the mutation on or show changes to the phenotype, the observable traits exhibited by the mice. However, by the third generation some homozygous mice must

exist. This allows his group to detect a new phenotype even if the trait is recessive.



Heterozygous Dalmatian animals exhibit a classical piebald black and white coat. Dalmatian mice have a mutation in a protein that is associated with some known human genetic diseases. The mutation occurs in the Sox10 gene, which produces the SOX10 protein. In humans, mutations in SOX10 are associated with Waardenburg-Shah syndrome, the symptoms of which are deafness, hypopigmentation of the skin, hair, and eyes, and disorders of the digestive system.

Using TACC supercomputers, Beutler will sequence the exome of every first generation mouse. By the time a third generation mouse is born (6 months after the original mouse was exposed to the mutagen), Beutler will already know all of the mutations that it might contain.

Beutler's laboratory then examines the immune status of every third generation mouse by fluorescence-activated cell sorting, testing how the mice respond to antigens, challenging the animals with viruses that are

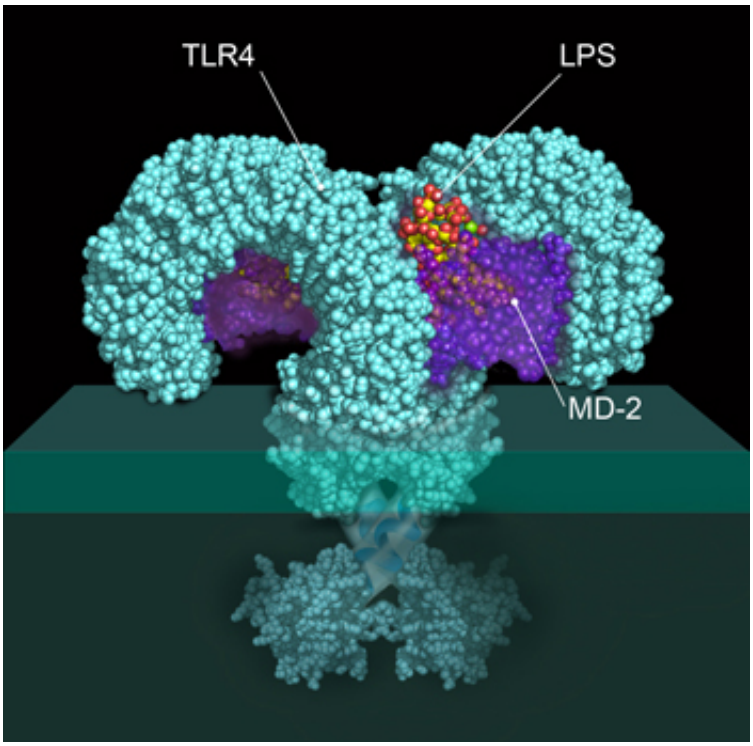
normally harmless, and using several other procedures as well, each designed to measure the performance of the immune system. When they find a mouse with an immune abnormality, the researchers are able to refer to the database of G1 mutations they have already acquired by exome sequencing to see the list of candidate mutations.

Sometimes the cause of the abnormality is obvious; sometimes it is not. In cases of the latter type, a method known as meiotic mapping is used to track down the causative defect. This often involves a mutation in a gene about which nothing was previously known. Bit by bit, the list of all genes with essential function in the immune system is thus deciphered.

The project advances Beutler's own research, but it also allows his lab to act as a seed bank for genetic mutations.

"The archive that we're putting together will be of broad scientific interest," Beutler said. "Whenever someone wants to have a particular mutation and wants it fast, they can simply contact us and we can send them a straw with frozen sperm in it so they can regenerate the mouse immediately. This will have a dramatic stimulatory effect on biology research."

Currently, it is necessary to wait as long as a year to obtain a mouse with a particular germline mutation.



The molecular structure of Toll-like receptor 4 complex. Toll-like receptor 4 (TLR4) forms a complex with myeloid differentiation factor 2 (MD-2) and lippopolysaccharide (LPS). The complex forms when TLR4 encounters LPS molecules on the surface of Gram negative bacteria. Recognition of the LPS molecule triggers responses in the cell to produce factors to fight infection. The molecular model is based on Protein Databank number 3FXI, and was created with the PyMOL.

Beutler estimates that by sequencing 8,000 mice, his lab will have created functionally null alleles in 80 percent of all genes. Put another way, Beutler will have knocked out 4/5ths of the genes in the mouse genome and determined which of those cause significant changes to immunity.

Since approximately 80 percent of all mouse genes have a human ortholog (meaning they come from a common ancestor), the catalog will be valuable for our health as well.

"Time and again, we find a gene that has no known function, or that is not known to function in immunity, and we see that a mutation in that gene creates an obvious immune defect," Beutler said. "If you do this enough times, soon you have hundreds of genes that contribute to the immune response and you begin to be able to build a picture of the molecular machinery that protects us from infections."

Beutler estimates that 50 percent of the genes involved in immunity have been uncovered so far. New discoveries occur regularly in his laboratory.

Recently, his group identified transcription factors called Zbtb1, Zeb1, and Nfkbid that were not known to participate in immunity and found that other known molecules work in different ways than expected.

As an example, Beutler cited a phospholipid flippase, an enzyme that rearranges the lipids in the plasma membrane, that they found is necessary for B-cells to develop in the bone marrow. "Nobody had guessed that such an enzyme would be required for this process. If the mice don't have this flippase, the B-cells can't develop there. And the big question is: Why do you need to have an enzyme that moves lipids from the outer leaflet to the inner leaflet of the membrane in order for b-cells to develop? Hard to imagine." The deployment of B-cells is one of the primary ways the body defends itself against infection.

The research was published in the November 2011 edition of *Nature Immunology*. Beutler's group continues to study the consequences of the finding.

Each gene involved in immunity that his team uncovers fills out the picture of the molecular machinery a little more. A complete understanding would have important implications for medicine.

A large fraction of medicine concerns inflammation. Even diseases like

atherosclerosis, which can lead to heart attacks and stroke, have an inflammatory component to them.

"In our work, we try to understand how inflammation comes about: what starts it, how is it propagated, and ultimately how can it be blocked? These are the kinds of questions we can address if we have a comprehensive understanding of the immune system and all the genes that are necessary for it."

Provided by University of Texas at Austin