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Researchers Determine Fundamental Mechanisms Involved in Immune Response

Scientists from the Howard Hughes Medical Institute and their colleagues have unraveled some of the fundamental mysteries about the genetic mechanisms that endow the immune system with its life-saving ability to generate specialized antibodies.

Without genetic fine-tuning, antibodies would be relatively ineffective in finding a good match on the surface of viruses, parasites, and other potentially dangerous foreign pathogens. The findings also reveal the workings of a gene mutation process that can go awry, leading to the development of certain forms of cancer or allergic reactions.

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— Frederick W. Alt

HHMI investigator Frederick W. Alt at Children's Hospital in Boston and Harvard Medical School directed the studies. His team's findings explain the genetic line dance by which an otherwise generic immunoglobulin, or antibody, molecule acquires the genetic components that encode for the structural characteristics it needs to activate appropriate pathways to eliminate specific types of invaders, or antigens. The appropriate class of immunoglobulin can then mark invading cells for elimination by other cells of the immune system.

The researchers' findings are published in two articles in the online editions of *Nature Immunology* and *Nature* on April 7 and April 9, 2003, respectively. Alt's research team members include three current or former HHMI research associates, Jayanta Chaudhuri, Reiko Shinkura and Ming Tian.

The studies focus on how B cell lymphocytes, one of the major cell classes that is deployed in an immune response, assemble the genes that encode for the specific classes of Immunoglobulin on their surface. Unlike other genes, which retain their integrity through each cell division, multiple segments of

immunoglobulin genes from disparate parts of the chromosome mix together numerous times to provide a diverse repertoire of functional antibodies. Subsequently, a second form gene shuffling creates various specialized classes of antibodies.

One part of the chromosomal gene segments encoding for immunoglobulin can vary enormously and provide specific recognition of foreign entities while the other remains relatively constant but can be changed in a few specific ways to provide specialization of the antibody. Alt's papers address two hotly disputed controversies about the process of immunoglobulin specialization. The first controversy involves how the enzyme, activation-induced cytidine deaminase (AID), which is synthesized by the B cell, acts on a specialized region of immunoglobulin DNA, known as the constant region, to initiate the process of antibody specialization. The second is why the enzyme acts only on those particular DNA sequences and not on any others elsewhere in the genome.

For the *Nature Immunology* paper, Alt and colleagues studied mice to delineate specific gene structures and the alterations necessary for the regional gene swapping to occur. The *Nature* paper showed the results of the Alt team's study of the effects of purified AID on test genetic (DNA) sequences.

In order for the specialized antibody properties to arise in immunoglobulins, the genes that encode for it must undergo a refining process known as class switch recombination (CSR). CSR is a highly specific blending of genes in which one part of the immunoglobulin DNA is swapped out for a more specialized class. Alt's laboratory had previously shown that to activate immunoglobulin differentiation via CSR, a genetic transcription process is necessary within a highly localized region. However, little was known about the actual machinery of CSR or what role AID played in initiating the process.

Working with mice with targeted gene mutations, Alt's group focused on the sequence of the DNA involved in CSR. When a B cell is activated by an antigen, specific double stranded segments of DNA in the immunoglobulin split off and loop out in a very controlled pattern known as an R-loop. One part of the strand is transcribed into RNA while another single strand of DNA is not transcribed. Alt's team showed that transcription leading to the R-loop or other higher order DNA structures containing single-strand regions of DNA is important to generate the primary substrate needed for CSR to take place.

Alt's team then showed, using AID protein, that AID actually modifies DNA and also has a strong preference for acting on the single-stranded segment of DNA. They also showed that AID cannot act on double-stranded segments unless they are transcribed. The AID catalyzed reactions on the single-stranded DNA to mutate it into a specialized form that can then be processed by normal cellular components to complete the CSR process.

The study demonstrates why the gene recombination occurs within such a tightly limited gene segment. It also proves that AID can function to target a specific DNA region to initiate the CSR process resulting in the immunoglobulin molecule's antibody class specialization.

While this explains one of the fundamental mechanisms underlying the immune response, it also generates new avenues for understanding the genetic dislocations that can potentially initiate some forms of cancer. "The AID enzyme can be extremely dangerous," says Alt. "It could potentially mutate or recombine any gene." Were it to do so, it could contribute to genomic instability leading to cancer. Instability in the Immunoglobulin switch region is known to be involved in some forms of lymphoma. Alt's laboratory is studying AID in laboratory mice to see how, when it goes awry, it could initiate a gene shuffling leading to cancer.

Understanding the immune response mechanism could also lead to new insights into allergic reactions. Allergies result from overproduction of immunoglobulin E, which causes an excessive response by the immune system to relatively benign antigens. Regulating CSR could potentially result in novel ways to treat allergies.