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Researchers Uncover Secrets of Immune Systems Munitions Factory

Howard Hughes Medical Institute researchers have discovered a new component of the machinery immune cells use to generate a remarkably diverse array of antibodies from a relatively small number of genes.

The discovery reveals important links in the molecular pathway by which complex genetic alterations arm the immune system to target myriad potential bacterial and viral invaders with swiftness and precision. The discovery may also provide welcome new information about lymphoma, a form of leukemia in which certain cells of the immune system proliferate uncontrollably.

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— **Frederick W. Alt**

Howard Hughes Medical Institute investigator Frederick W. Alt led the research team that published its findings August 26, 2004, in the journal *Nature*. Alt and lead author Jayanta Chaudhuri are at Children's Hospital, Boston, and Harvard Medical School.

The studies focus on B cells, specialized immune cells responsible for producing antibodies, and how an enzyme in those cells known as activation-induced cytidine deaminase (AID) triggers mutations of antibody gene segments to produce an assortment of antibody proteins. This process enables the immune system to produce antibodies that will recognize billions of different antigens - the fragments of foreign invaders that are used to call the immune system to arms.

The presence of an antigen on the surface of a B cell stimulates it to produce antibodies. An important step in this process is the activation of AID, which causes largely random mutations in the genes for the antibody segments that recognize antigens. These mutations occur about a million times more frequently than spontaneous mutations in other genes. In this process, known as somatic hypermutation, AID selectively "damages" the DNA strand,

prompting the DNA repair system to create the mutations.

AID also triggers class switch recombination, a highly specific process that involves recombining gene segments that encode the part of the antibody molecule that direct it where to take its antigen cargo and how to dispose of it.

A central mystery in the field of immunology, said Alt, has been how AID acts on antibody genes. In previous studies, Alt and his colleagues showed that the enzyme acts on single-stranded DNA and that, for class switch recombination, such single-stranded DNA can be unraveled from native double-stranded DNA during the process of copying its information to RNA, the cell's template for production of antibody proteins. However, this mechanism could not explain how AID works during somatic hypermutation.

In the current work, Chaudhuri developed techniques to isolate purified AID from B cells and test its activity on the target for somatic hypermutation. In this way, he found that the enzyme requires an unknown co-factor that was critical for AID function and that this co-factor specifically interacts with AID.

Subsequent analysis revealed that Chaudhuri's protein was replication protein A (RPA), long known to be part of the DNA replication and repair machinery that attaches to single-stranded DNA.

"RPA was never even suspected to be a candidate for such a role, since it was never suspected that it could get into DNA during RNA transcription," said Alt. "Until now, it was only known to be involved in certain DNA replication and repair pathways." Chaudhuri also found that after AID "damages" the DNA, it leaves the complex, but RPA remains. "We believe that RPA is sitting there on the DNA, and it's recruiting DNA repair factors, which is a great link to the repair machinery needed for the next step in the hypermutation pathway," said Alt. Thus, in retrospect, RPA's involvement makes biological sense.

The other major discovery, said Alt, was that for AID to interact with RPA, AID must undergo some modification in B cells - a clue to the nature of the machinery that initiates somatic hypermutation, and a topic for future studies in Alt's lab.

Identifying RPA's involvement could have implications for understanding lymphomas. "Lymphomas and some mature B cell tumors are known to show aberrant somatic hypermutation and class switching," said Alt. "Now that we know more about how the AID-RPA complex works, we can begin to address questions of how the aberrant processes might occur.

"While this is currently pure speculation, it might be that deregulation of AID activity could lead to mutations that could be involved in the evolution of lymphoma," Alt said. "Also, there might be a mutation in AID or its co-factors that would deregulate it and cause it to target other genes," he said.

Additional studies will seek to connect the AID-RPA complex with the cell's repair mechanism to further elucidate the somatic hypermutation and class switch recombination processes, said Alt. "We'd like to figure out how those downstream steps in these two different situations lead in one case to mutation and in the other case to recombination," he said. "Now that we know more about how AID links to the next steps through RPA, we can begin to address this problem, which is a major question in the field."