Secrets of antibody diversity

Originally considered heretical, the idea of genes rearranging during development has been proved to be true. Rearrangement occurs not once, but twice, in the differentiation of antibody-producing cells. And that is only one of several schemes now known to contribute to the variation among antibodies.

Cells that produce antibodies in laboratory culture, analysis of tiny amounts of protein and genetic material and computer methods to handle the resulting data are yielding a rapid proliferation of information on the workings of the immune system. "What is absolutely marvelous now is that we can understand all the major features of B cell [the antibody-making white blood cell] differentiation," says Leroy Hood of the California Institute of Technology. He was speaking recently at the National Institutes of Health in Bethesda, Md., on research results of the past few months.

Antibodies are made up of two pairs of polypeptide chains. Each chain has a variable region that determines what substance the antibody will bind and a constant region that determines the biological action of the antibody. Early in development, rearrangement of genes first brings together the DNA segments that code for a variable region and for a constant region (SN: 12/11/76, p. 372). In the case of the light chains, the genes are connected by one of several joining (J) segments. In the heavy chains two connecting regions are involved to provide even more diversity, Hood says. He estimates that in mice there are 250 genes for variable regions, four J segments and 10 D (for "diversity") segments that act as the second joining element, giving 10,000 possible variations.

The second genetic rearrangement comes in the specifying of the biological activities of the antibody. Some antibodies, such as IgG and IgM, trigger the "classical" complement pathway in which a cascade of reactions destroys invaders and mediates inflammatory processes. Other antibodies use different pathways to fight viruses in body secretions (IgA) or trigger allergic responses (IgE). The class of the antibody is determined by the constant region of the heavy polypeptide chain. In the mouse there are eight neighboring genes for this region. The immature B cell initially expresses the first gene and makes IgM; later the B cell secretes antibody molecules with the same variable region but a different heavy-chain constant region.

Segments of DNA next to the constant-region genes mediate the "class switching," Hood concludes from research in his own and several other laboratories. Unlike other instances in which DNA segments line up and recombine, the switching segments are not homologous in their nucleic acid sequences. Hood postulates, therefore, that a member of a group of special "switching" proteins recognizes and attaches to each switching segment. Then the proteins bind to one another, juxtaposing the segments so the DNA can break and rejoin. Production of these proteins at the appropriate time would program a cell to make a specific class of antibody.

Yet another type of diversity among antibodies arises from mutations in the DNA in B cells. Hood and colleagues have looked at a variety of antibodies that bind a single chemical, phosphorylcholine. In undifferentiated cells, they find four variable-region genes, one of which has two stop signals within it and thus is not functional but is a pseudogene (SN: 12/20 & 27/80, p. 397). Examination of 16 lines of hybrid cells making antibody to phosphorylcholine showed that some of the variable-region genes develop scattered mutations during development. All the somatic mutations were in IgG antibodies, not in IgM. Therefore, somatic mutation only takes place after the class switch; how it is controlled still is not understood.

Not all the strategies for diversity involve gene changes; some involve the mechanisms by which genes are expressed. Hood reports that a difference in trimming of messenger RNA by cellular enzymes determines whether a B cell makes monomeric IgM that attaches to the cell membrane, as it does early in development, or whether it secretes IgM as a 5-subunit protein.

Hood speculates that families of rearrangeable genes coding for segments of proteins encode several groups of proteins characterized by diversity — including receptors for hormones, transplantation antigens and olfactory receptors. He also suggests such genes may encode molecules on cell surfaces that specify patterns of cell migration and interaction during development. All the proposed families may be distantly related; a "split-gene" family could have evolved early and then diverged to take on a variety of functions. Recently, Hood compared the gene for a mouse transplantation antigen with a constant-region heavy-chain gene, and using a powerful computer program he found "striking homology" in a pattern that argues for a common ancestral gene.

New instruments being developed by Hood and associates promise further details of immunology through rapid analysis of smaller and smaller amounts of material. They expect within a few months to be able to determine amino acid sequences of samples as small as 1 picomole and to be able to automatically synthesize DNA at a rate of 12 residues per day. Hood envisions centralized microchemical facilities to generate data that scientists can take home to analyze. "It will be the equivalent of high speed nuclear physics," Hood concludes. □

Heroin addiction and T cell depletion

The heroin high may be in the head, but evidence is accumulating to suggest that opiates do more than affect brain and nerve cells. Studies of street opiate addicts, for instance, have found a variety of abnormalities in their lymphocytes, or white blood cells. And now researchers report direct evidence that it is the opiates the addicts use that bind to and affect white blood cells and can thus affect the human immune system. The research was done by Robert J. McDonough and colleagues and is reported in the December JOURNAL OF IMMUNOLOGY.

Blood samples taken from addicts were compared with samples taken from non-drug-using control subjects. The three types of white blood cells (B, T and null) were measured, and in each addict there was a significant reduction in the number of T cells — cells responsible for one aspect of the immune response. The decrease in T cells was accompanied by an increase in null cells.

Naloxone, a drug known to prevent the binding of opiates, was used to show specifically that opiates were responsible for the T cell decrease. When the blood samples were treated with naloxone, the cell balance changed: After three hours, the percentage of T cells increased in addict blood and the null cell percentage decreased. There was no similar reaction in blood taken from control subjects. The ability of naloxone to produce a time- and concentration-dependent increase in T lymphocyte percentage argues strongly, say the researchers, "for a direct effect of the opiate on the T lymphocyte."

The successful three-hour treatment of the blood samples with naloxone, however, does not mean that the effects of heroin on the immune systems of addicts can be reversed as quickly. After three weeks of drug-free detoxification, all five ex-addicts studied still showed T cell depletion. This, say the researchers, suggests that lymphocytes "have a long-term memory for previous narcotic treatment."

All of this, of course, is bad news for opiate users, including those who use opiates for relief of chronic, severe pain. One researcher, for instance, found that the lymphocytes of opiate addicts have a reduced capacity for repair of certain types of damage to DNA. Another studied 12,000 opiate addicts in Iran and found much higher rates of bladder cancer among opiate users who smoke tobacco than among smokers who do not use opiates. The opiate either potentiates the carcinogenic effect of the tobacco or reduces the immune system activity of the T cells toward the cancer cells. The possible presence of opiate-binding sites on T cells (and other human tissues), say the researchers "has profound health implications." □