reasoned, the transporter must be somehow crippled. But how? The new findings may hold an answer: They show that glutamate-stimulated cells produce substances that sabotage the transporters, leaving the destructive flood of glutamate to linger in the synapse.

Sakire Pogun and Michael Kuhar of the National Institute of Drug Abuse reported that one accomplice is nitric oxide (NO), a volatile and reactive gas that got lots of attention several years ago as the newest (and weirdest) neural signaling molecule. NO is made when glutamate activates a receptor called the NMDA receptor, something that happens during both learning and excitotoxicity. Researchers had already found one way in which NO release might abet excitotoxicity: As a glutamate-stimulated cell makes more of the gas, the NO feeds back on the glutamate-producing cell, boosting glutamate release.

But Pogun thought that NO might inhibit the glutamate transporter as well. She decided to check, by treating a preparation of neuron membranes with nitric oxide, and found that NO reduced glutamate uptake by half. Pogun has not yet discovered how the nitric oxide exerts its effects. It could be acting directly on the transporter-containing cells, but some researchers suggest that NO may be converted into an even more reactive free-radical molecule called peroxynitrate, which actually does the damage. In support of that possibility, Andrea Volterra, of the University of Milan, reported that highly reactive oxygen radicals like peroxynitrate have an inhibiting effect on the transporter.

Whether NO acts directly or not, it doesn't act alone. Volterra also reported a second, apparently independent, avenue of self-destructive behavior in glutamate overstimulated cells. Along with NO, they produce arachidonic acid, a fatty acid that, like NO, diffuses through membranes and can affect nearby cells. Pak Chan, of the University of California, San Francisco, and David Attwell, of University College, London, had already found that arachidonic acid inhibits the glutamate transporter. But Volterra reported that it does so by a mechanism separate from that used by free radicals.

These reports fill in a missing piece in the picture of excitotoxicity, says Johns Hopkins University neurologist Jeff Rothstein, who studies glutamate transporters: "Now we find you have direct transporter toxins that are made as part of the excitotoxic process." That knowledge, he adds, coupled with Volterra's finding of separate mechanisms of action, offers not one but two potential new targets for drugs for stroke and head-trauma patients. By unshackling the glutamate transporter, such drugs might limit excitotoxicity's toll.

–M.B.

IMMUNOLOGY

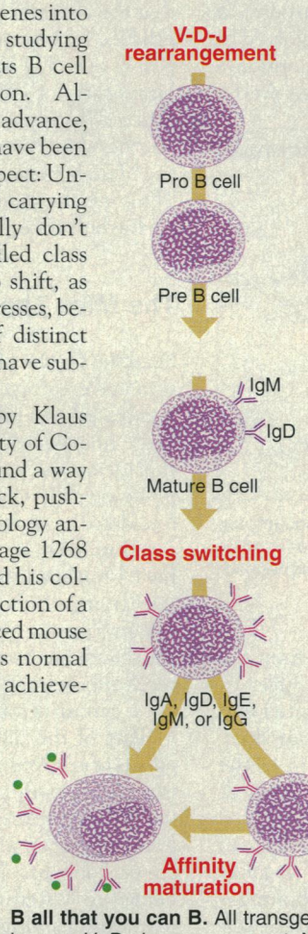
Transgenic Mice Display a Class (Switching) Act

Over the past decade, genetically engineered mice have transformed immunology. Using transgenic animals, researchers have, for example, helped unravel many mysteries of the development of B cells—the immune system cells that produce antibodies—by inserting specific antibody genes into fertilized mouse eggs and studying how their presence affects B cell and antibody production. Although that was a great advance, these transgenic animals have been deficient in one major respect: Unlike normal B cells, those carrying the inserted genes usually don't show a phenomenon called class switching—the ability to shift, as an immune response progresses, between the production of distinct classes of antibodies that have subtly different functions.

Now, a group led by Klaus Rajewsky of the University of Cologne in Germany has found a way to overcome this drawback, pushing the study of B cell biology another step forward. On page 1268 of this issue, Rajewsky and his colleagues describe the production of a mouse carrying an introduced mouse antibody gene that shows normal class switching. Their achievement opens the way to investigate how class switching is controlled. "It allows you to study, in vivo, the biology of the B cell response," says immunologist Dennis Loh of Washington University in St. Louis.

And the ramifications of this work aren't limited to fundamental science, since the technique could have clinical applications. In a second paper on page 1271, Rajewsky's team reports the creation of a mouse in which an antibody gene is replaced by the corresponding gene from human cells. This animal can produce part-mouse, part-human antibodies against any antigen—and may provide a short cut in the production of "humanized" monoclonal antibodies for clinical use.

The key to these achievements is the technique that is usually employed to make knockout mice: gene targeting. Conventional transgenic techniques, which rely on microinjecting foreign DNA into a fertilized egg, introduce genes randomly into the genome. Knockout mice, on the other hand, are made by cloning a particular gene, disrupting it by introducing a short DNA sequence, and inserting it into a mouse stem cell. The disrupted gene replaces the normal gene by a process called homologous recombination. These cells are injected into mouse embryos, yielding mice that are then bred to produce a strain in which all cells carry the knocked-out gene. Instead of disrupting a gene, Rajewsky's group used this method to directly replace an antibody gene with the corresponding DNA from a particular clone of B cells.



B all that you can B. All transgenics bypass *V, D, J,* rearrangement. Normal transgenics usually express only one class of antibodies; Rajewsky's mice show class switching.

ILLUSTRATION: K. SUTLIFF

The introduced gene comes from a B cell line that produces antibodies to the chemical phosphorylcholine. Antibodies consist of four polypeptides: Two heavy chains, each some 440 amino acids long; and two light chains, of about half that length. Both types, in turn, consist of constant regions, which are identical for antibodies of the same class from the same animal; and variable regions, which differ from B cell to B cell. To generate a huge diversity of antibodies, the immune system takes the gene fragments that code for these variable regions—called *V*, *J*, and *D* segments—and rearranges them in different combinations. The Cologne researchers isolated a particular rearrangement of these fragments—coding for the heavy chain variable region of an antibody to phosphorylcholine—and used gene targeting to make a mouse in which all cells carry this sequence in place of the DNA containing the unrearranged gene segments.

In homozygous animals, which carry two copies of the inserted gene, the result is a mouse all of whose B cells make antibodies with antiphosphorylcholine heavy chains. During the course of an immune response, these cells in the transgenic mouse switch class—just as in a normal mouse—to produce antibodies that recognize the same antigen but which carry different heavy chain

constant regions. Early on, they produce two classes of antibody called IgM and IgD, which are responsible for initial antigen recognition and, in the case of IgM, activating the complement system—a series of proteins that attack invading microbes. Later, they shift to make one of the three classes of antibody secreted by mature B cells: IgG, which both stimulate complement and bind to macrophage cells that engulf microbes; IgE, which bind to mast cells, making them release histamines that aid the immune response by dilating blood vessels; and IgA, which are released in secretions such as tears.

The key to this class switching ability is that the introduced variable region gene sits right next to the assortment of sequences that encode the different heavy chain constant regions carried by different classes of antibody. This means that the process of DNA recombination that allows B cells to shift between the production of antibodies with different heavy chain constant regions can take place normally. "The strength of this paper is that they put [the gene] where it belongs," says Washington University's Loh. "The attractive feature of the mouse we've made," concludes Rajewsky, "is that its B cells are physiologically normal."

Indeed, the mouse also seems normal for another key feature of the B cell immune response: affinity maturation, the process by which circulating B cells mutate rapidly and are selected to make antibodies that bind progressively more strongly to a given antigen. Although standard B cell transgenic mice undergo the same process, they may contain many copies of the inserted gene, carried at various positions in the genome, which "makes a mess for trying to analyze affinity maturation," says Stanford University immunologist Chris Goodnow. The new mice, containing a single, precisely placed copy, will give immunologists a model in which they can more confidently assume that they're studying a correct version of the process.

Rajewsky is already embarking on experiments to explore the class switching mechanism. These studies involve crossing the animal with knockouts, made by Frederick Alt of the Boston Children's Hospital, in which the *RAG* genes have been disabled. *RAG* genes are involved in rearrangement of *V*, *J*, and *D* gene segments, so *RAG* knockouts don't produce mature B cells. But it should be possible to make a *RAG*-deficient mouse with working B cells by crossing these animals with the Cologne team's mouse and a third strain containing a standard transgene encoding antibody light chains. If these mice do not show class switching, it would indicate that *RAG* genes help control the process. Immunologists believe that may be the case since both class switching and *V*, *J*, *D* rearrangement involve DNA recombination. "It is possible that some components of the machinery are shared," says Ursula Storb of the University of Chicago.

Rajewsky is also planning to use a variant of his mouse to study autoimmunity. Several groups have made conventional transgenic mice whose B cells produce antibodies, mostly IgD molecules, that recognize antigens carried by their own tissues. Usually, however, these B cells become inactivated, or "anergic." But by using gene targeting to create a similar mouse that's capable of class switching, says Rajewsky, it may be possible to study factors that cause a shift from this self-tolerant state to the production of self-reactive IgG antibodies. "The question is whether cells can escape from the anergic state by class switching," he says.

Although clinical advances from this particular line of study are a distant prospect, the second paper from Rajewsky's lab, describing a mouse that produces humanized antibodies, shows one possible therapeutic application of immune system gene targeting. Monoclonal rodent antibodies have long been touted as weapons in the fight against viral diseases and cancer. But mouse antibodies, when injected into humans, provoke an immune response that over time negates their therapeutic effects.

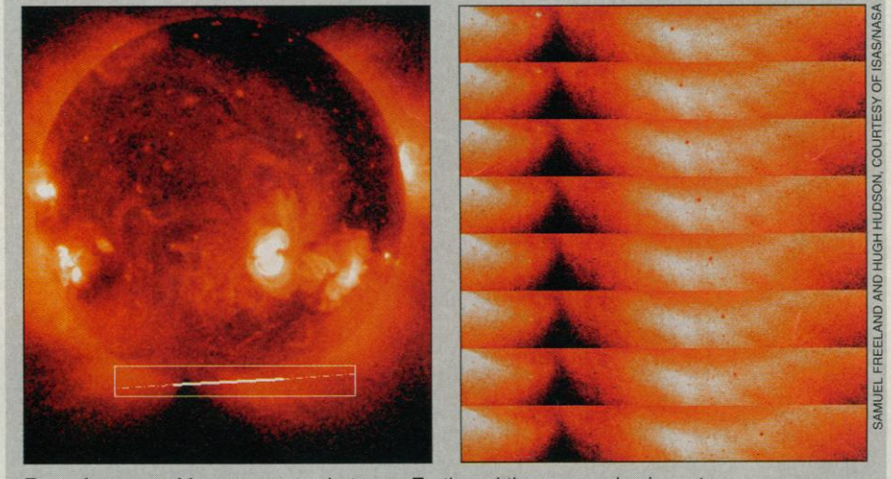
To get around this obstacle, several groups have used recombinant DNA technology to make partially human antibodies in vitro. The advantage of having a mouse that creates such chimerics, however, is that there's no need to engineer a humanized version for each new antibody: Just immunize the mouse against the relevant antigen and it will do the job.

In the current paper, Rajewsky's group describes a mouse in which only the light chain constant region genes have been replaced by human sequences. But the team has already done the same for the heavy chain genes and is now crossing the two animals to produce a strain that makes complete chimeric antibodies. Competing researchers such as Michael Neuberger at the Laboratory of Molecular Biology in Cambridge, England, agree that it's a neat piece of work, but they point out that several labs are now producing antibodies significantly more humanized than those produced by the Cologne group's mouse. While there's no firm proof that these perform better, regulatory bodies may play it safe and favor more humanized versions.

Nevertheless, Rajewsky says his group's achievement in replacing a mouse gene with the corresponding sequence from human cells could have broader implications. "What it shows," he says, "is that you can use gene targeting to test a human gene in the in vivo context of a mouse model." Of course, human genes can be put into mice using standard transgenic techniques. But by directly replacing a mouse gene with its human counterpart, it should be possible to produce more physiologically relevant models for studying human disease genes or testing drugs targeted at human cell surface receptors. Clearly, there's more to gene targeting than knockout mice.

–Peter Aldhous

Mercury Meets the X-Ray Sun



SAMUEL FREELAND AND HUGH HUDSON, COURTESY OF ISAS/NASA

Every few years Mercury passes between Earth and the sun, and solar astronomers can watch its tiny black disk move across the sun's bright face. On 6 November, in images from the Japan/U.S./U.K. satellite Yohkoh, they got their first look at a transit of Mercury against the x-ray glow of the sun's surging, million-degree atmosphere, or corona. Yohkoh investigators say these images, taken at intervals of 4 or 5 minutes, showcase the high resolution of the satellite's soft x-ray telescope, developed by Japan's Institute for Space and Astronautical Science and NASA. By studying how individual bright pixels fade as Mercury moves across them, the researchers also hope to decipher fine details of the corona's structure.