who used Hammer's binding matrices to get at the allergy-causing epitopes in ryegrass.

With more and more fields beginning to use the various algorithms, Brusic predicts that "in a few years' time this will be a standard methodology for epitope [identification]." For Sahin and his fellow users, the tools have already become standard. "They're indispensable," he says.

There's still plenty of room for improvement, though. "On average only about 20% of all predicted epitopes are actually recognized by cytotoxic T cells," says Hansjörg Schild, a colleague of Rammensee's at Tübingen. One possible reason is that the unrecognized peptides are not produced by the cell's protein-cleaving machinery. Schild and Rammensee have recently developed another computer program that might help—an algorithm for predicting how a protein will be cleaved. Combining that with epitope prediction should further reduce the number of epitopes that need testing, says Schild.

Immunologists caution that, despite the number of promising preclinical studies, the algorithms still have to prove their worth on the real-life battlefield between pathogen and host, by pointing the way to new vaccines. Says vaccine researcher Anne DeGroot of Brown University in Providence, Rhode Island, who has herself developed a prediction algorithm based on MHC binding motifs, "You can do a lot of neat things on your computer, but ultimately you have to put [the predicted peptides] in vaccines and test whether they protect [from infection]." With clinical trials picking up speed on several fronts, the first answers are likely to come in before too long.

–MICHAEL HAGMANN

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NEWS

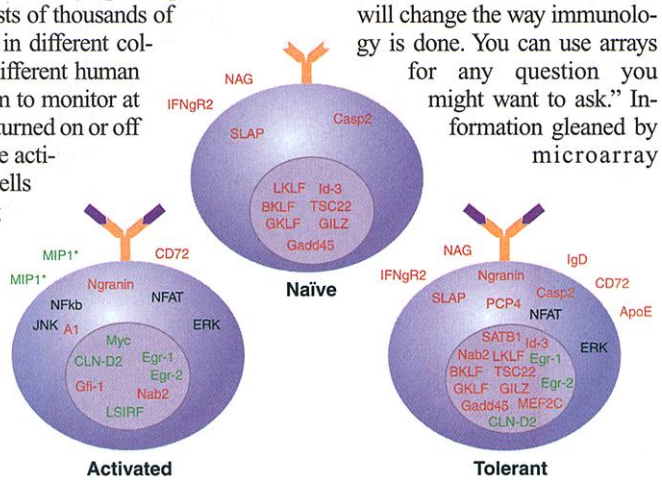
Doing Immunology On a Chip

Using microarrays to measure gene expression patterns may reveal the mysteries of normal immune cells and also of diseases in which immune cells go astray, such as autoimmunity and cancer

For Louis Staudt, an immunologist at the National Cancer Institute (NCI) in Bethesda, Maryland, the key to the enigmas of the human immune system lies buried in what looks like a miniature painting by Piet Mondrian, the Dutch abstractionist who elevated colored rectangles to high art. Dubbed Lymphochip, Staudt's work of art consists of thousands of tiny squares that light up in different colors, each representing a different human gene. This chip allows him to monitor at one time which genes are turned on or off when, say, killer T cells are activated by pathogens, or B cells turn into life-threatening leukemias.

Used by a small but growing number of immunologists, the Lymphochip and similar DNA microarrays mark the entry of immunology into the presumably golden age of genomics, a quantum leap that promises a new level of understanding of the genetic programs underlying immune responses, both normal and abnormal. "This approach allows you to look at the whole system at once instead of looking at one gene [at a time]. It's a 'Let the system tell you what's going on' approach," Staudt says.

Immunologists are already intrigued by what they are seeing. Microarray analysis is helping define the changes in gene activities that cause certain leukemias as well as those that govern the immune system's response to a range of pathogens. Microarrays are also illuminating the developmental paths leading from immature precursor cells to a plethora of immune warriors such as B and T cells. Indeed, says Lars Rogge, an immunologist at Roche Milano Ricerche in Milan, Italy, "this will change the way immunology is done. You can use arrays for any question you might want to ask." Information gleaned by microarray analysis should also help to both diagnose diseases and find new therapies.



Turned on. Microarrays reveal that lymphocyte activation has as much to do with down-regulating inhibitory genes (red) as turning up genes with activating functions (green). The genes' positions indicate the locations of their protein products.

Take Staudt's work. He and his colleagues have been focusing on identifying the gene changes that distinguish various malignancies of immune cells, or lymphocytes, from one another and from healthy cells. With this in mind, the NCI team, in collaboration with Stanford's Pat Brown, a founder of DNA chip technology, began developing the Lymphochip in 1998. The researchers collected 15,000 genes that are highly active in immune cells at various stages of development as well as in some leukemias. They added another 3500 genes known to be important in lymphocyte or cancer biology and started profiling a variety of B and T cell tumors.

The initial results have been surprising. Staudt and his colleagues reported in the 3 February issue of *Nature* that diffuse large B cell lymphoma is not one disease, but rather "two separate diseases with two distinct profiles hiding within one clinical category," says Staudt. What's more, patients with the disease vary in their responses to therapy, and the gene activity profiles correlate with that finding (*Science*, 8 September, p. 1670). The hope now is that clinicians can use such gene profiling to select the most appropriate treatment options for their patients and perhaps eventually to design better therapies.

And what works for immune cell cancers should work for other immune disorders as well, Staudt predicts. "It's fairly obvious that autoimmune diseases or immune deficiencies are pretty heterogeneous, and one can easily surmise that there are different subtypes. For example, only a subset of multiple sclerosis patients responds to interferon β treatment. I think we should look at their gene expression profiles" to see if they determine the patients' responses.

Infectious disease expert David Relman of Stanford University is pursuing similar goals. "The idea is to recognize specific [hard-to-diagnose] infections based on the gene expression profile in cells from the host," he says. That could be advantageous, because "the clinical sample doesn't even have to contain the bug you're after," says Relman. "With current diagnostics, if you're looking at the wrong time or place, you may end up empty-handed." A glimpse at the host response might also indicate the stage of infection, which could influence therapy.

Relman says his ultimate goal is to understand more precisely how microorganisms cause disease. In as-yet-unpublished work, he

ILLUSTRATION BY C. CAIN, ADAPTED FROM C. GOODNOW

and his colleagues have taken a step in that direction for *Bordetella pertussis*, the cause of whooping cough. The researchers used microarrays to compare the gene expression patterns of cells infected with either of two different *B. pertussis* strains, one with and one without the pertussis toxin. Seeing the genes that are turned on by the toxin, Relman hopes, will tell him how the toxin is doing the pathogen's dirty work of causing disease. He plans to do similar analyses to track the bacterium's counterresponse to the onslaught of the host immune system. "You'll eventually be able to listen to a two-way conversation" between pathogen and host at different times after their first encounter, he says.

Other researchers are using microarrays to help uncover the genetic programs that cause lymphocytes to follow one developmental path instead of another or to become activated when they "see" a pathogen during normal immune responses. Such analyses can also provide clues to what goes wrong either in autoimmunity, when an overachieving immune system attacks the body's organs, or in immune deficiencies. An example comes from Rogge's group in Milan, which has applied the technology to the great coordinators of the immune system—the T helper cells.

These come in two forms: T_H1 cells, which mainly regulate cell-mediated immunity, including the activity of killer cells, and T_H2 cells, which coordinate antibody production. In a study reported in the May issue of *Nature Genetics*, Rogge and his colleagues used microarrays to compare the expression patterns of some 6000 genes in the two types of helper cells and found 215 that are differentially expressed.

One key difference is that genes involved in apoptosis, or cellular suicide, are upregulated in T_H1 cells. "T_H1 cells are more susceptible to apoptosis" than T_H2 cells, Rogge says, and this difference may explain why. Because apoptosis helps keep T_H1 cells in check, it's also possible, he notes, that this failsafe mechanism may be defective in autoimmune diseases such as rheumatoid arthritis. The chips can now be used to check that hypothesis. In contrast, allergies and asthma are due to overzealous T_H2 cells, and the identification of the genes that regulate the two cell types may lead to new treatments for these disorders, Rogge says.

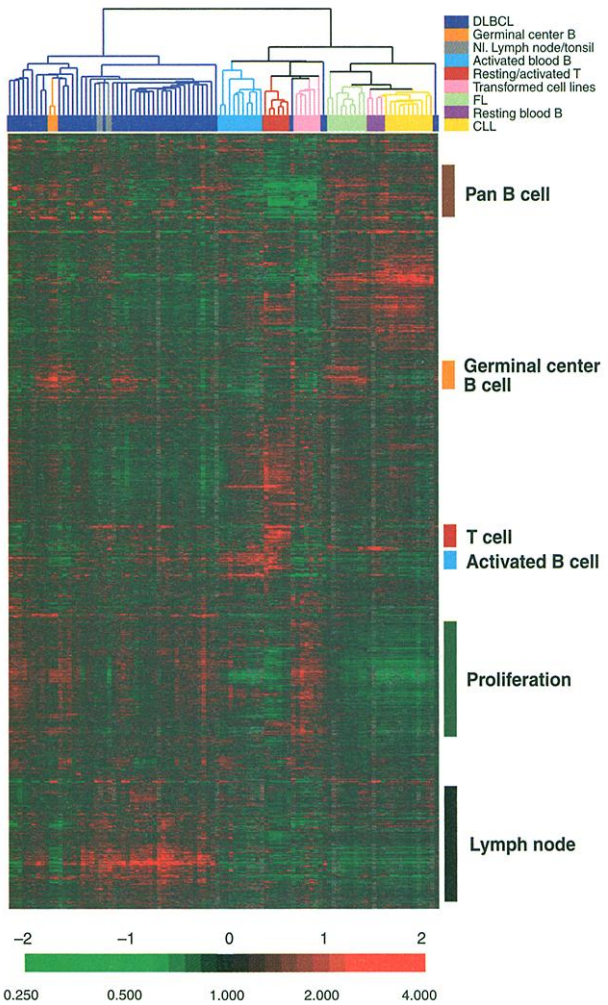
Autoimmune diseases are also high on the agenda of Christopher Goodnow, an immunologist at Australian National University (ANU) in Canberra. His team is trying to understand the genetic pathways that instruct immune cells to become tolerant of the body's own tissues and that are somehow perturbed in autoimmunity. In a study in the 10 February issue of *Nature*, Goodnow and his colleagues compared how an antigen affected gene expression patterns in normal and so-called anergic B cells, which, in the course of development, have somehow become refractory to self-antigens. In normal cells, the team found, the antigen switched off a whole series of genes that inhibit cell proliferation, enabling the cells to multiply in response. But that didn't happen in the anergic cells. "B cell activation may be more a question of down-regulating inhibitory genes than turning on [cell proliferation] genes, which is quite a surprise," says Goodnow.

What's more, the researchers found that a common immunosuppressive drug, FK506, turned off the inhibitory genes as well as blocking proliferation genes in B cells. "This may be the reason why immunosuppressive drugs don't do a good job at inducing tolerance," speculates Goodnow. The gene profile of an anergic cell could now be used as a standard "to screen other drug candidates to find one that more exactly mimics the tolerance-inducing process." Such a drug might only have to be given until the immune system has learned to tolerate, say, a liver transplant—instead of for life, as is necessary with today's immunosuppressives.

Goodnow is also using microarrays to help him in another venture. Convinced that making real headway in understanding the immune system requires good animal models for all sorts of immune defects, in 1998 he established a brand-new facility at ANU aimed at churning out thousands of random mouse mutants over the next 5 years (*Science*, 2 June, p. 1572). "There are only five or six natural mouse mutants with defects in the immune system," Goodnow explains. Analysis of the first 200 mutant mouse pedigrees has already yielded a rich harvest: 26 mutants with disrupted immune systems, including one where T cell development is blocked and another with hyperactive T helper cells. Goodnow is now using microarray analysis to relate clinical symptoms in the mutants to the underlying changes in gene activity.

But while chips can reveal gene expression patterns, they are "only the first step," warns Michael Cooke, an immunologist at the Novartis Institute for Functional Genomics in La Jolla, California, and himself a chip enthusiast. "They just give you a list of genes that are up or down [under certain conditions]. The real challenge is to come up with functional assays" that tell what the genes really do.

Like other chip users, immunologists also face a looming data overload, as every single chip harbors thousands of genes. Distilling the few that actually matter for the biological process under investigation "requires a good team of computer scientists who will present and visualize those data in a way the human mind can comprehend," says NCI's Staudt. Still, he is optimistic that the problems can be solved: "Very soon DNA arrays will change from being an emerging technology to becoming just another lab technique" for immunologists.



Sorting them out. Normal and malignant lymphocytes (key at upper right) can be grouped according to their gene expression patterns, with green indicating genes turned down in the various cell types and red indicating genes that were turned up. The analysis also shows that diffuse large B cell lymphomas (DLBCLs) split into two subgroups.

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