the bodyguard

TAPPING THE IMMUNE SYSTEM'S SECRETS

By Bruce Goldman • ILLUSTRATION BY BRIAN CRONIN

So, how's your immune system doing? It's not a question you've likely heard before. Give it about five years, though, and that will all change, if a forward-looking pack of Stanford immunologists have their way.

These scientists are out to generate a simple battery of tests, performed on blood obtained from a single needle-stick in a doctor's office, to let you know what shape your immune system is in. Not just whether it's acting up, or idling too slow, but specifics you and your doctor could use to guide your next medical move. You've got the sniffles: Is it an allergy, or an infection? You're getting older: Do you need a bigger dose of the annual flu shot, or is the standard one going to work just fine? You feel great: Are you cruising asymptomatically toward an autoimmune disease that will flare up five years hence, and if so, how can you prevent it? • For now, from the standpoint of the practicing clinician the immune system remains a black box, says Garry Fathman, MD, a professor of immunology and rheumatology and associate director of the Institute for Immunology, Transplantation and Infection. • "If a patient were to ask me, 'How's my immune system doing today?' I would have no idea how to answer that, and I'm an immunologist. *None* of us can answer that. Right now we're still doing the same tests I did when I was a medical student in the late 1960s," he says. • "What we need is a scorecard: a routine, standardized, easily interpreted blood test you take *before* you get sick — analogous to the ones you get for cholesterol or glucose levels," says Mark Davis, PhD, the director of the institute. "This would let you and your doctor know how well your immune system is functioning in general — and, if it's malfunctioning, how, and with what consequences." • Sounds reasonable. We've got blood tests for cholesterol, blood tests for liver function, blood tests for pregnancy. So, why not blood tests for the state of our blood — or more precisely, the state of that all-important blood- and lymph-borne network of circulating sentinels, soldiers and signals that compose our immune system? • Alas, that's easier said than done. In the last few decades a huge amount has been learned about the basic mechanisms of immune response — a super-smart system of sensors, cells and secretions that has evolved to guard us from invasion by pathogens or betrayal by our


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own tumor-prone tissues. It's staggeringly complex, comprising at least 15 different interacting cell types that spew dozens of different molecules into the blood to communicate with one another and to do battle. Within each of those cells sit tens of thousands of genes whose activity can be altered by age, exercise, infection, vaccination status, diet, stress, you name it.

"That's an awful lot of moving parts. And we don't really know what the vast majority of them do, or should be doing," says Davis, the Bert and Marion Avery Family Professor in the Department of Microbiology and Immunology. "We can't even be sure how to tell when the immune system's not working right, let alone *why* not, because we don't have good metrics of what a healthy human immune system looks like." Despite billions spent on immune stimulants in supermarkets and drugstores last year, we don't know what — if anything — those really do, or what "immune stimulant" even means.

So Davis, Fathman and a cohort of their fellow Stanford scientists have launched a far-reaching effort to create the first-ever program capable of characterizing the human immune system under normal conditions — and thus identifying the multitude of minute changes it undergoes when we get sick, or successfully vaccinated, or old. To fund the endeavor, Davis has received over $40 million in public and private funding, and Fathman, $3 million.

Pulling off such an ambitious undertaking requires a shift in the way immunological research is conducted. In classical laboratory science, a researcher asks a question, then selects a simplified "model system" (such as a lab mouse) to help track down the answer. The researcher keeps everything as close to the same as possible, messing with only a single variable to see what happens when it's tweaked. What happens isn't always so good for the mouse. Maybe not such a great idea to try these tweaks on people. Besides, huge environmental and species differences render the mouse results less than perfectly applicable to us.

But Davis, Fathman and their colleagues think there's a way around that. The marriage between new or improved analytic instrumentation (much of it pioneered at Stanford) and the latest computing technology, they believe, will let them find needles in a haystack.

"Suppose you've got a very complicated system, with a lot of moving parts," Davis says. "You don't know how those parts talk to one another. You don't even know where to start. So instead, you keep your eye on the whole thing, and you watch what happens to the parts when you hit it with a hammer. Some of the parts move together. Some move one after another. Then, you hit it with something else — a bucket of ice water, maybe — and see what moves this time, and when, and how much.

"We can perturb the immune system all kinds of different ways, measure the levels of hundreds or thousands of different things in response to that, and figure out which ones go up or down with different states of health or non-health," Davis says. "Anything that might affect the system — a vaccine, a disease, a drug — can tell you something."

To get answers, Stanford has created the Human Immune Monitoring Center, consisting of a couple of clusters of world-class instruments and expertise. The HIMC operates according to a principle Davis only half-jokingly refers to as "ignorance-driven research." The more formal name is systems biology, an information-technology-rich approach to unraveling complex systems of intensely interacting components.

With systems biology, you don't have to know what you're looking for until you find it — some extremely high or low level of something (a cell count, a secreted immune protein, expression of a gene) that turns out to correlate with a disease or a vulnerability to it. You call that a "biomarker." In a human blood sample, there's an embarrassment of potential biomarkers to pick from, and the HIMC is bringing new sophistication to the task.

The idea is to make that huge haystack, human blood, smaller by fishing out a few, or a few dozen, biomarkers that by themselves may not be so great but that, taken together, correlate with various states of health, disease, vulnerability and resistance, or are highly predictive of immune response to particular challenges. With standardized assays, improved methods and increasing efficiency, these markers could someday be measured simultaneously via a simple blood test a patient can get in a clinical lab or doctor's office.

In the past decade, scientists have steadily advanced the technologies capable of pinpointing such biomarkers of immune status. These technologies can capture the tens of thousands of changes that might be induced by a vaccine, a disease or aging. The changes might be in immune cells' activity or numbers, in the amounts and types of molecules they secrete, or in which of their genes are idling or running in overdrive. Among the new techniques the HIMC employs:

Tetramer profiling: This technique, pioneered by Davis, detects attractions between members of a class of immune cells and the foreign or altered biochemical entities they target. For instance, it can measure the presence, or changes in the number, of specialized immune cells targeting a particular entity such as a viral or bacterial component — changes that could signal a state of immune readiness or sluggishness.

Mass-spectrometery of single cells: This was developed in large part in the lab of Garry Nolan, PhD, professor of microbiology and immunology. It involves an instrument — one of five in the world — that busts a single cell's contents into tiny pieces and, effectively, flings them at a wall; different metal tags attached to as many as 30 or more chosen proteins enable researchers to track levels of those proteins in

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individual blood cells. Knowing in such sensitive detail how individual cells' protein contents are altered by challenges with drugs or disease may allow the detection of important events, such as the onset of illness or response to therapy, well before they become obvious.

Luminex panel: This method uses beads carrying fluorescent barcodes to quickly determine, for almost 100 different blood samples at a crack, which and how much of 51 different important immune-signaling molecules called cytokines reside in each sample. Viral infection, bacterial infection, cancer, immune deficiency, age and traumatic injury, to name a few "perturbations" life visits on us, all result in different "cytokine signatures": characteristic patterns of these molecules' presence and activity in our blood. These signatures could be used to quickly map a person's immune health.

IF YOU'RE COLLECTING ABOUT 40,000 DATA POINTS PER BLOOD SAMPLE, FROM HUNDREDS OF PATIENTS PER YEAR, you're going to pile up a staggering amount of raw data. So, taking the systems-biology tack, you hand the entire database to the computer guys and let them sift through it, asking questions like: What's different between samples from, say, older versus younger people or people whose flu shots worked versus those who got sick anyway? Which differences appear to be medically important? Which are the most reproducible? Which could be used in a diagnostic test?

Having started life in 2005 as a bootstrap operation, the HIMC now employs 12 people and works on dozens of projects. Director Holden Maecker, PhD, regularly meets with investigators to help them plan studies, determine their needs (what samples to take, how to bank those samples and which assays to use) and interpret their data. "For them, it's like going to the best restaurant," Maecker says. "Everything's on the menu."

The center's resources are good for much more than simply characterizing the immune system under normal conditions. Researchers and clinicians from more than a dozen departments and divisions within the medical center have teamed up with the HIMC to study everything from anesthesia's impact on wound healing, to opioids' effects on immune function, to potential biomarkers of depression. The search is on for immune biomarkers of aging, Alzheimer's, autoimmune disease, cancer, chronic pain, rejection in organ transplantation and viral infection — both acute (influenza) and chronic (HIV).

Investigators from around the world send samples to be assayed for their own experiments. In fact, the center is one of the medical school's biggest money-makers, with a good half of its $1.2 million annual budget last year generated internally as a service center for both internal and external laboratories.

University of Washington immunology professor Jerry Nepom, MD, PhD, is providing the HIMC with samples in the hope of identifying biomarkers of immune status. "We don't have any standard, clinically validated test to do that," says Nepon, past president of the Federation of Clinical Immunology Societies, a 40,000-member organization of clinical immunologists Fathman founded almost a decade ago. "What they're doing at Stanford is unique — developing the tools that will get us there."

Regardless of its origin or fate, every blood sample — and the data that comes from testing it — becomes part of the center's database. That growing pool of data, combined with clinical comparisons of patient's health status (diseased versus healthy, old versus young, male versus female), promises to reveal solutions to medical riddles that for obvious reasons can't be solved by subjecting humans to the kind of experimentation that immunologists have been using on mice.

The mice themselves may have already told us much of what they can about the human immune system, Davis says. While he's quick to acknowledge the value of mouse studies in puzzling out details of the immune system's interactions, he thinks we're bumping up against an evolutionary limit. Having diverged from a common ancestor 60 million years ago, mice and people are — how to say this gently? — *different*. They've got four legs, we've got two. Their hearts beat 500 times a minute, ours 60. And their immune systems are different, too.

"We've cured cancer and autoimmune disease in mice many times over," muses Davis. He says a colleague of his often starts his talks with the salutation: "For the mice in the audience, I have *wonderful news!*"

Your immune system is there to save your life. Will Stanford's new systems-biology approach speed the day when this 24/7 lifeguard's signals are accurately interpreted in real time, on a patient-by-patient basis, in a clinician's office? That would be wonderful news for the rest of us. **SM**

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