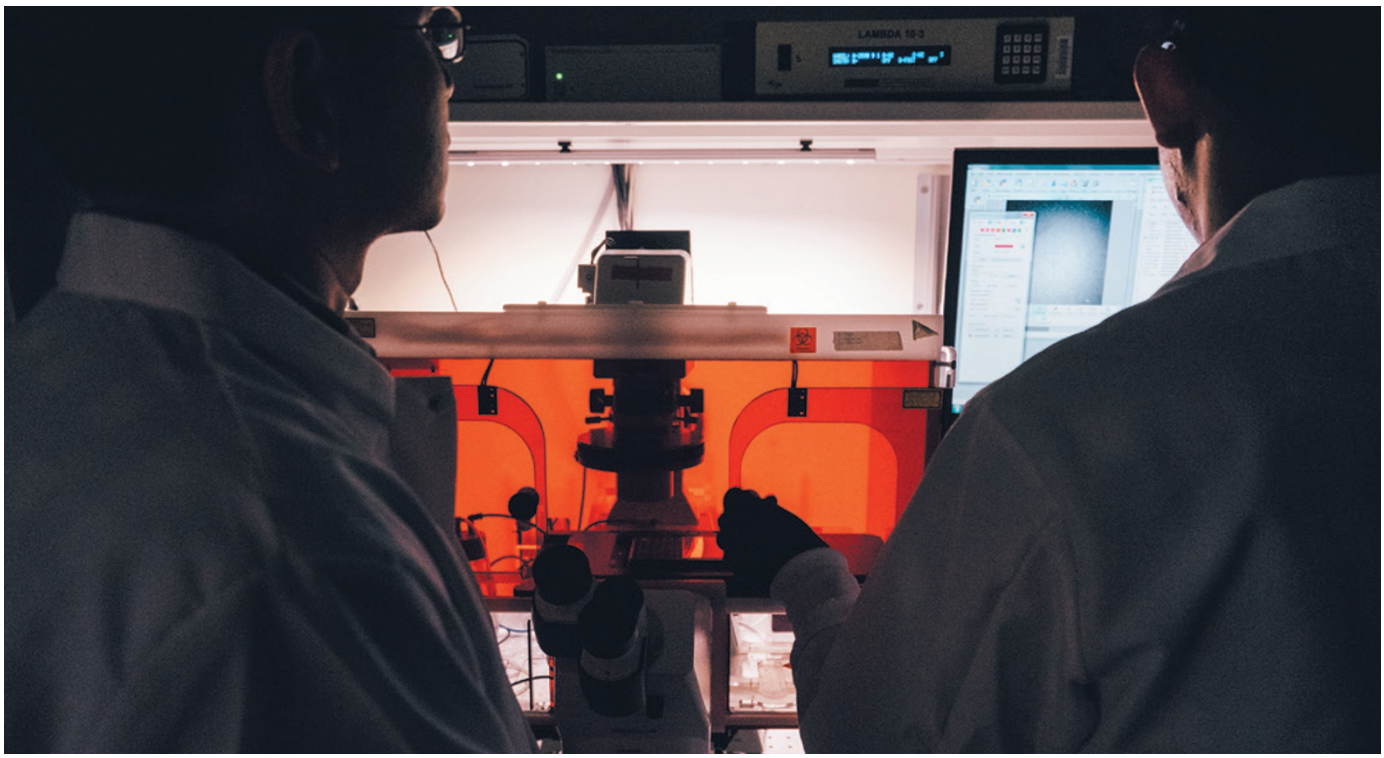
TECHNOLOGY FEATURE

THE CELL MENAGERIE: HUMAN IMMUNE PROFILING

Cutting-edge tools and analyses are digging deeper than ever before to unveil the intricacies of the diverse human immune system.

LILLIE PAQUETTE, MIT SCHOOL OF ENGINEERING



Advanced technologies enable researchers at the Massachusetts Institute of Technology to observe individual immune cells attacking tumour cells.

BY MARISSA FESSENDEN

Vaccines save lives — but they don't always work. Take the annual influenza shot: by some estimates, flu vaccines are only 50–70% effective even when well matched to the virus strains in broad circulation. Despite all the research, scientists still cannot predict whether a given vaccine will work for any specific person.

Learning to make vaccines that protect more people means getting a better handle on the immune system — a bewildering militia of cells that communicate to detect and destroy pathogens. So far, attempts to parse the system's complexity have involved work on mice, rats, rabbits, dogs, non-human primates and even lampreys and sea urchins. Yet results do not always translate to the one species that medicine cares most about. "There has been a vast zoo of animal models, but the one animal model we haven't yet exploited is us — *Homo sapiens*," says Bali Pulendran, an immunologist at Emory University in Atlanta, Georgia.

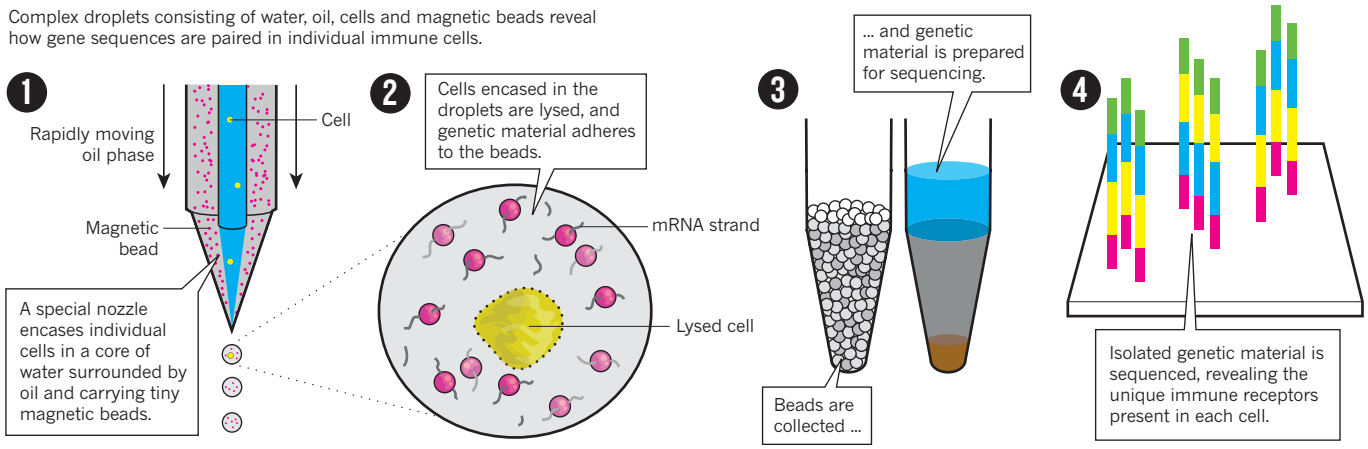
Now, researchers are tackling the most difficult animal to study as never before. Advances in technology are helping scientists to dive deeper into the inner workings of single cells and carry out analysis on greater numbers of cells at once. Efforts in data analysis, sharing and collaboration promise to enable work that is too expensive for individual labs. Ultimately, researchers hope to bring fresh insights to the clinic to protect and treat people using the power of an individual's own immune defences.

The human immune system is incredibly diverse. Each class of immune cell is actually an army of subtypes. The elite forces — the lymphocytes, which recognize specific pathogens or wayward body cells — consist of natural killer (NK) cells, which quickly dispatch infected or cancerous cells, and B and T cells, which bear receptors on their surfaces designed to recognize specific invaders. But B and T cells break down further: there are regulatory T cells, T helper cells, memory B cells, naive B cells and more, each with its own unique role. These lymphocytes coordinate in turn with cells such as macrophages and monocytes, which are further specialized for other functions.

Diversity manifests between people, too. Even identical twins vary in terms of the exact molecules and cell profiles that fight off disease. From an evolutionary point of view, variability ensures that some members of a species will survive a deadly disease outbreak — but it confounds researchers.

PROFILE OF AN IMMUNE CELL

Complex droplets consisting of water, oil, cells and magnetic beads reveal how gene sequences are paired in individual immune cells.



SOURCE: REF. 3

Gender, ethnicity, genetic background and disease history all affect a person's immune response in unpredictable ways. They influence whether a vaccine will work, and whether someone has allergies or an autoimmune disease — both resulting from an overactive immune system — or whether a person will develop cancer, which is caused in part by an inattentive system that fails to remove errant cells.

VACCINES UNVEILED

Instead of seeing confusion in such diversity, researchers such as Pulendran see opportunity. With the right combination of sophisticated technologies and data analysis, human variation can offer a natural experiment in what underlies an effective immune response.

This reasoning led Pulendran and his team to some groundbreaking research on why a vaccine for yellow fever works so well. Since immunization against the sometimes-deadly tropical disease began in 1937, only 12 cases have been reported among the hundreds of millions of people immunized.

Scientists have long known that the vaccine spurs the body to produce T cells that can kill cells infected with the yellow-fever virus — but they did not know how. In 2009, Pulendran's team published an analysis[1] of changes in the state, number and types of immune cells in the body before and after vaccination. The group found that quantities of a protein called EIF2AK4 spike in key immune cells (mainly dendritic cells, which help T cells to identify invaders) just days after vaccination. The higher the spike in protein levels, the more anti-yellow-fever T cells are later produced.

The close correlation suggests the existence of components that foster strong immune responses — at least for the yellow-fever vaccine. Pulendran and his colleagues[2] have since discovered other proteins that predict similarly strong responses to vaccines for flu and meningococcal disease. Now, they are linking these types of marker to subpopulations of cells and classifying variation across individuals.

One major reason that immune responses vary is the vast collections of receptors on the surfaces of T and B cells, which correspond to antibodies that are secreted by the latter cells. To produce a near-endless assortment of these Y-shaped molecules, lymphocytes shuffle their genes as they mature. The myriad receptors and antibodies that result enable the immune system to recognize many different pathogens.

Researchers want to sequence genes for these receptors to work out what makes a potent immune response, and so gain clues for developing vaccines and for designing therapies that could spur the immune system to fight cancer.

But because each receptor is made from proteins encoded by at least two types of separately shuffled gene segment, sequences alone are not enough. Researchers must also learn how these proteins are paired in an individual cell — and which combinations show the most promise for fighting disease.

At the University of Texas at Austin, chemical engineer George Georgiou has tackled this challenge by studying B cells one at a time. He and his team[3] first encase individual cells in complex droplets: ones with an aqueous core that preserves the cell's genetic material; an outer oil layer to keep the cells separated; and magnetic beads that allow researchers to manipulate each droplet and so capture and extract the genetic material from individual cells. These data can reveal the antibody repertoire elicited by various stimuli — crucial information for designing vaccines (see 'Profile of an immune cell').

Instead of seeing confusion in such diversity, researchers such as Pulendran see opportunity.

Georgiou's group hopes to publish manual-like methods so that others can use the technique. Investigators who are unfamiliar with it should prepare themselves for a steep learning curve, he says: "Every method, especially new methods from academic labs, has some nuances." But for those who are willing to put in the time and elbow grease, precise answers about individual immune cells await.

Another sequencing approach relies on specially formulated beads to tag individual cells before DNA analysis. The tags fuse with the cells' genetic material and function as barcodes that can be traced back to the original cell, even when cells are analysed in pools. Hedda Wardemann, an immunologist at the German Cancer Research Center in Heidelberg, has used this strategy to analyse genes encoding paired receptor proteins in more than 46,000 B cells at a time[4].

Most specialized-sequencing approaches are developed by individual labs, such as Georgiou's or Wardemann's, that have engineering know-how. But as the field grows, companies are getting into the game. One of the biggest players in the microfluidics field is Fluidigm in South San Francisco, California.

This September, the company began to ship high-throughput chips, which can be used on the company's C1 microfluidics platform to interrogate genomes of 800 individual cells in a single 6.5-hour run. Although it has a much lower throughput than Georgiou's droplet method (which can process 6 million B cells in a day), Fluidigm's technology requires less expertise. The company plans to increase throughput to nearly 100,000 cells per run in the near future.

PERTURBED POPULATIONS

In addition to profiling individual cells (see 'Nanoarenas for cell attacks'), researchers want to track how cell populations change in response to vaccination or infection. To identify specific cell types, the scientists rely on protein markers studded on the cells' surfaces.

For example, two markers dubbed CD4 and CD8 both show up on certain types of memory T cells — but CD8 is also on NK cells, and CD4 is on monocytes and dendritic cells. So, to measure only memory T cells, researchers may need to screen for three different markers. To isolate an even more-specific subset, the number of markers must increase.

Conventionally, researchers have relied on a cell-profiling technology called flow cytometry, in which coloured, fluorescent proteins are attached to specific cell markers so that combinations can be easily detected

and the cells scored or sorted. But overlaps in colour spectra generally limit analyses to as few as a dozen markers.

The latest iteration of cell-profiling technology — mass cytometry — uses rare-earth metals instead of fluorescence and can detect more than 40 markers. Because mass cytometry can identify so many cell types in a single sample, more types of experiments can be done.

Studies in babies, for example, are key to understanding the immune system's development. But infants generally cannot tolerate blood withdrawals of more than 4–5 millilitres — and even simple flow-cytometry experiments can require more than 10 ml. Mass cytometry, by contrast, can run on less than 4 ml.

Mark Davis, a molecular immunologist at Stanford University in California, used mass cytometry to track hundreds of parameters — including 72 different immune-cell populations — in the blood of 210 twins. His team found[5] that much of the variation between people's immune systems can be attributed to environmental factors, rather than to genetic ones. Without mass cytometry, this work would have been too complex to perform, he says.

DVS Sciences, now a part of Fluidigm, has invented a mass cytometer called the CyTOF for use in cell profiling. The latest version (as well as upgrades for the older system) boosts sensitivity and sample-processing speed, and can run multiple samples at a time.

But these technologies are expensive. The June version of the CyTOF — the 'Helios' system — starts at roughly US$500,000, not counting service contracts. At Stanford, Davis and other researchers rely on shared facilities.

ASSEMBLING THE PIECES

Although scientists are making progress, many tools have been slow to reach the clinic, says Padmanee Sharma, a physician–scientist at the University of Texas MD Anderson Cancer Center in Houston. Every new clinical technology needs standards and quality assurances, which require extensive testing to establish. Clinical trials are only now adopting procedures that might help clinicians to track their patients' immune responses and feed in to treatment decisions.

Communication is another bottleneck. Information is accumulating rapidly and needs to be shared by collaborators as diverse as statisticians, clinicians, basic biologists and technologists. Coordinating research that involves human participants places huge demands on logistics, resources and expertise, and one major effort to facilitate such work is the Human Immunology Project Consortium (HIPC) funded by the US National Institutes of Health (NIH). The HIPC doles out grants to advance methods, and endeavours to extend the fruits of researchers' labour to all.

The consortium offers an online data-analysis and management platform called ImmuneSpace, which helps researchers to place data in a long-term archive called the Immunology Database and Analysis Portal (ImmPORT), also funded by the NIH. The HIPC is spearheading efforts to standardize procedures for commonly performed assays in cytometry as well as alternate methods of immune profiling, such as measuring antibodies in serum samples.

Another emerging need is for techniques for easy cross-analysis of many data types, says Steve Kleinstein, a computational immunologist at Yale University in New Haven, Connecticut. "There's a lot of subtlety in the data, and it's very easy to pick up a piece of code or tool that somebody put out there on the web, run it with your data and get a plot that looks interesting — but that's a very dangerous thing to do," he says.

To help solve this problem, Kleinstein and his group have developed software called the Repertoire Sequencing Toolkit (pRESTO)[6], which offers a way to process, annotate and correct raw sequencing data from high-throughput platforms such as Illumina. It also allows researchers to run their data in different computing environments and then return to the pRESTO environment.

A separate tool, a web portal known as the VDJServer, is in beta-testing after launching in April. It offers the ability to analyse B- and T-cell-receptor data, with the goal of providing an intuitive interface for users who have not done any programming, says project leader Lindsay Cowell, a bioinformatician and immunologist at the University of Texas Southwestern Medical Center in Dallas. The server will incorporate more analysis tools into the portal as they become available (Kleinstein's pRESTO is already embedded). Moreover, the portal lets researchers share data and even tap into the computing power of the Texas Advanced Computing Center at the University of Texas at Austin.

There is still an acute need for human immunology-specific data repositories, notably for T- and B-cell-receptor sequencing data, says Jamie Scott, a molecular immunologist at Simon Fraser University in Burnaby, Canada, who is co-leading an effort to share such data.

But perhaps the biggest block is a basic one: a dearth of training. Most analysis requires some programming skills, says John Tsang, head of computational systems biology for the Trans-NIH Center for Human Immunology in Bethesda, Maryland. For now, most tools are limited to the specialist, he says; collaboration with those who can understand the programming is still the best way forward.

Creating more collaborations should, in turn, help to ensure that the tools truly further basic knowledge and translate into practical applications. "It is very attractive to apply the latest gee-whiz 'omics' technology to measure things," says Pulendran. "But I think we need to go beyond measuring and accumulation of data — to knowledge and to understanding." ■

Marissa Fessenden *is a science journalist and illustrator based in Bozeman, Montana.*

1. Querec, T. D. *et al. Nature Immunol.* **10,** 116–125 (2009).
2. Li, S. *et al. Nature Immunol.* **15,** 195–204 (2014).
3. DeKosky, B. J. *et al. Nature Med.* **21,** 86–91 (2015).
4. Busse, C. E., Czogiel, I., Braun, P., Arndt, P. F. & Wardemann, H. *Eur. J. Immunol.* **44,** 597–603 (2014).
5. Brodin, P. *et al. Cell* **160,** 37–47 (2015).
6. Vander Heiden, J. A. *et al. Bioinformatics* **30,** 1930–1932 (2014).
7. Yamanaka, Y. J. *et al. Integr. Biol.* **4,** 1175–1184 (2012).

Nanoarenas for cell attacks

YVONNE YAMANAKA

In addition to tracking populations of immune cells, researchers want to know how they interact.

Christopher Love, an immuno-engineer at the Massachusetts Institute of Technology in Cambridge, is using microfluidics to probe how individual immune cells cooperate with each other. His lab engineers devices that he describes as "essentially ice-cube trays": each well in the tray holds sub-nanolitre volumes, as opposed to the tens of microlitres held by wells in more-conventional plates.

Using these tiny arenas to watch natural killer (NK) cells home in on leukaemia cells, the team has discovered[7] — unexpectedly — that even a single NK cell will attack a cell that does not belong. In the past, researchers suspected that NK cells coordinated their actions through secreted chemical signals, but now it seems that such cooperation may be necessary only among larger cell groups.

Love and his team hope to map their understanding of interactions at this single-cell level to the immune system as a whole, and potentially compare healthy individuals with those who have cancer. "With these technologies, first you ask: can we define normal?" he says. "Then you can think about heterogeneity in disease." M.F.