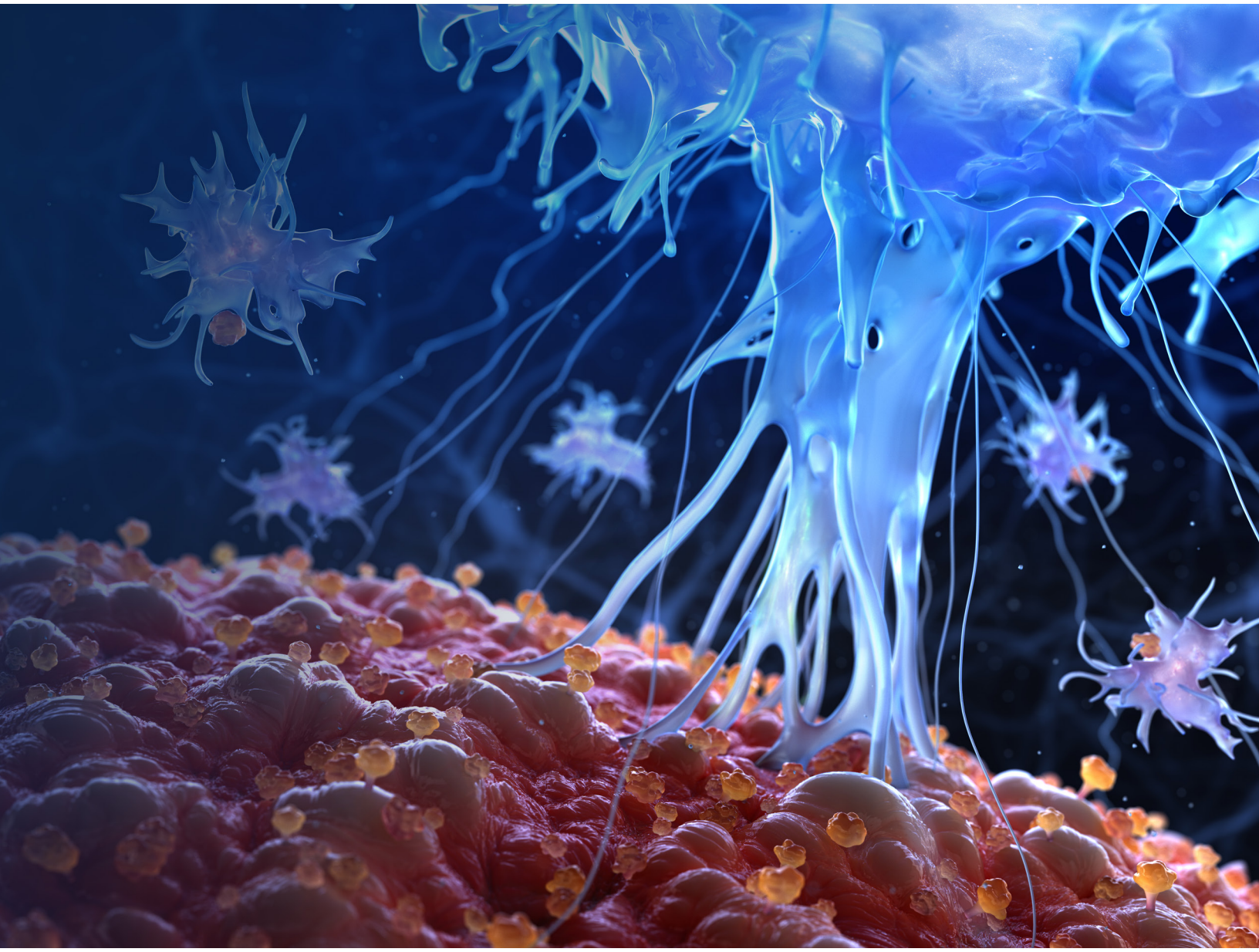
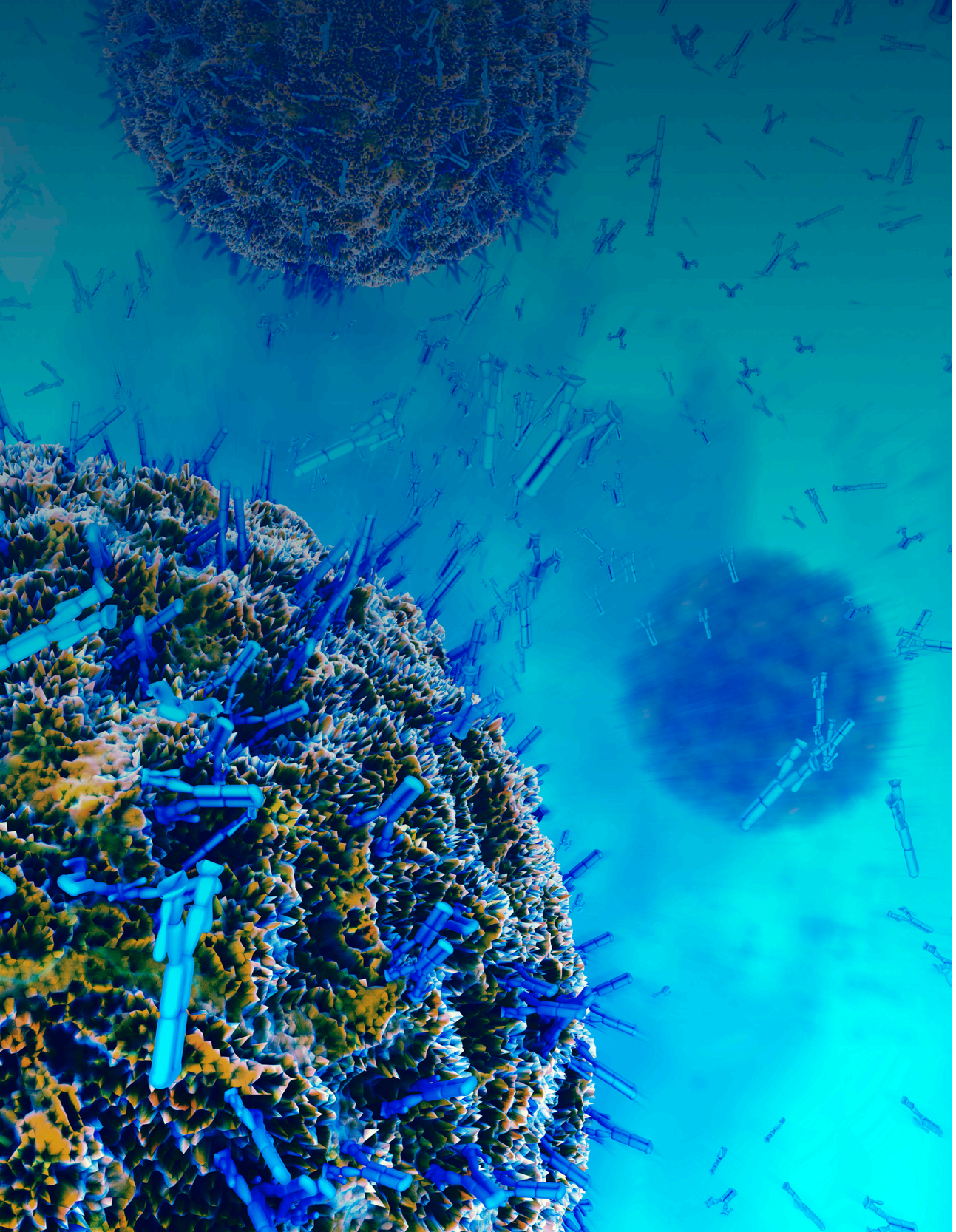
10x GENOMICS

Breaking new ground in immunology

Through the lens of single cell and spatial multiomics





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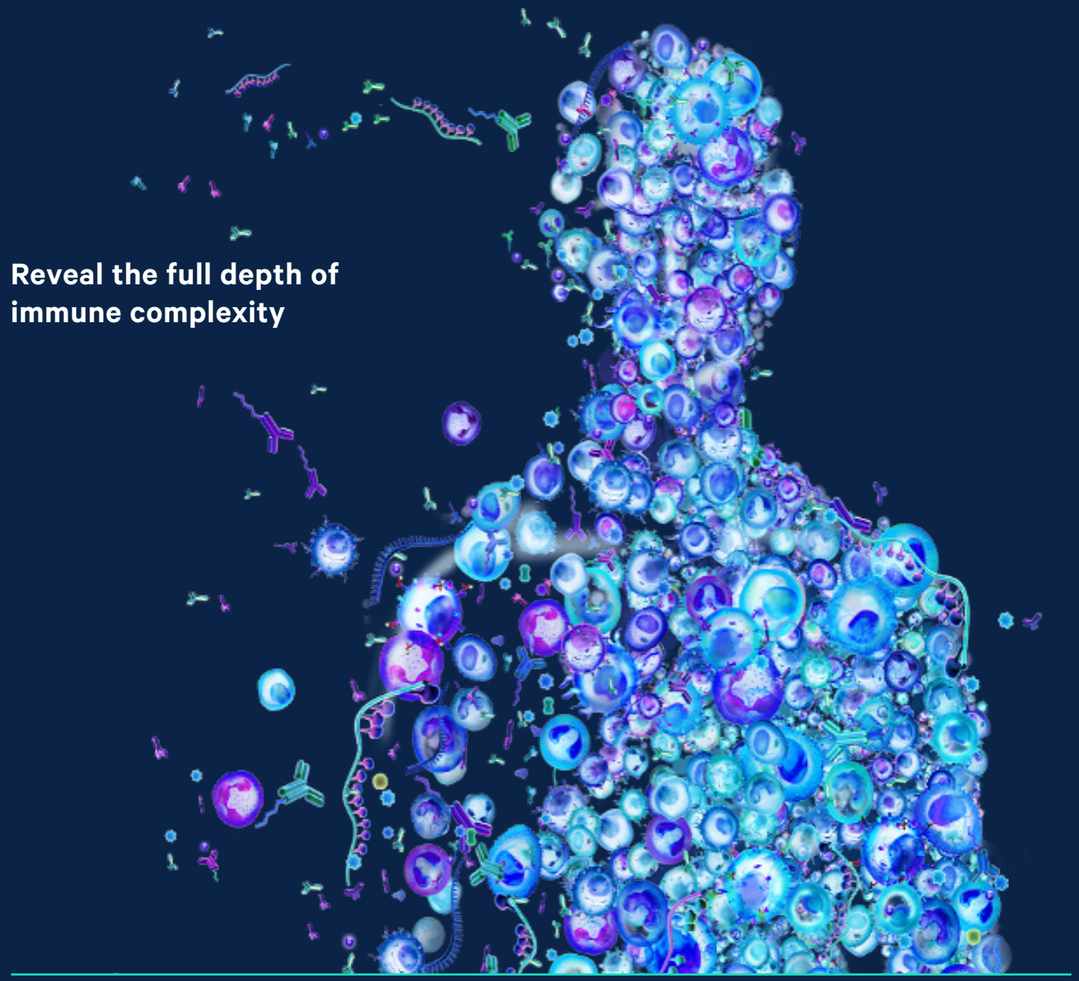


Discover

Immune signatures of inflammation in solid tissue

Cell types and their functional capabilities within any tissue or sample

Innate and adaptive responses without prior selection

Identify

T-cell and immunoglobulin responses useful for novel immunotherapies

Multiomic phenotypic and clonotypic biomarkers of graft rejection

Regulatory networks controlling immune cell responses

Track

Development paths of immune cells in homeostasis and disease

Clonal and phenotypic dynamics of cellular and humoral responses to disease

Characterize

Common immune responses to antigens or disease across individuals

Unique immune response to specific antigens

Specificity of signal transduction pathways

Introduction

Working in harmony: Unmasking the heterogeneity of the immune system

Much like an orchestra, where every instrument must be in perfect harmony, the many players of the immune system need to operate as a seamless unit to maintain our health. A single instrument out of tune or a single musician not in time can throw off the whole piece. Such an imbalance can have disastrous implications in the immune system, leading to over- or under-reaction of the immune system to pathogens or disease (1).

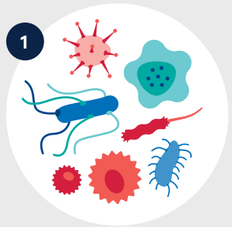
Given our current understanding of immunity, it is easy to forget that the term “immunology” first appeared in scientific literature in 1902 (2). The foundations of immunology began with Emil Behring’s and Paul Ehrlich’s identification of neutralizing antibodies as well as Illya Mechnikov’s identification of phagocytic cells (3). These discoveries became the foundations for acquired and innate immunity, respectively.

Since those early days of immunology, researchers have been working tirelessly to understand cellular and molecular mechanisms driving immune homeostasis and response due to injury, disease, or environmental exposures (Figure 1). Initial characterization of immune cells enabled by microscopy, cytometry, and functional assays have expanded our understanding of the immune system. However, while these tools have provided a great starting point for interrogating immune function, they don’t provide the resolution necessary to unmask the heterogeneity vital to the immune system’s operation (4).

Figure 1. (shown left) Critical applications in immunology. From immune homeostasis to response, much of current immunology research is focused on addressing the intricacies of the immune system to advance our knowledge of the heterogeneity and developmental progression of immune cells.

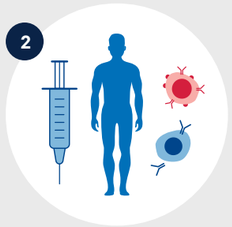
Seven major research areas in immunology

Immunology research is at the forefront of many important scientific and medical breakthroughs, including profiling emerging pathogens, developing vaccines, development of immuno-oncology therapies, understanding autoimmune diseases, tissue matching for safe organ transplantation, and beyond.



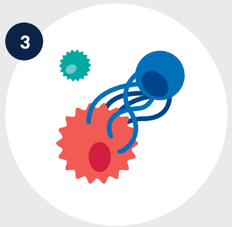
Infectious disease and therapeutic discovery

Uncover how pathogens infect host cells and elicit immune responses. Link the host immune response to recovery or severity. Generate targeted immunotherapies.



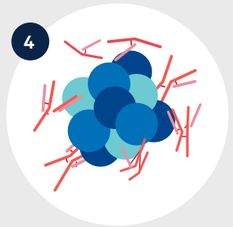
Vaccines

Define the receptor repertoire and antigen specificity of adaptive immune cells. Advance vaccine development and identify prophylactic antibodies.



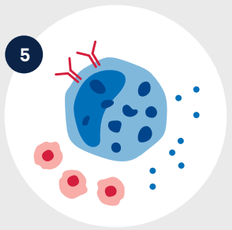
Immuno-oncology

Identify infiltrating immune cells within the tumor microenvironment. Characterize immune cell functions and receptor repertoire.



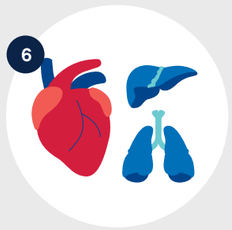
Autoimmunity

Decipher the underlying mechanisms of misdirected immune responses. Explore the pathophysiology of disease in single cells and organ systems.



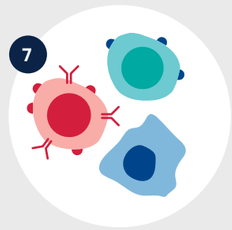
Allergies and inflammation

Investigate the biology of innate and adaptive immune activation. Decode the mechanisms of immune hyperactivation in response to bodily insult or injury.



Transplantation

Advance clinical management of solid organ and hematological stem cell transplants. Understand the immunological basis for transplantation disease conditions.



Cellular and molecular immunology

Explore the fundamental biology of the immune system in health and disease. Characterize immune cell identity, function, and organization in the body.

To obtain deeper insights into the immune system, improved experimental methods are needed when studying cellular phenotypes and functions, cell location and morphology, and signaling pathways. This will allow immunologists to build a more detailed map of the cellular and molecular signatures of immune cells in health and disease (4). While this prospect may seem daunting, advancements in single cell sequencing and spatial profiling have paved the way for the next generation of breakthroughs in immunology research (4, 5). As immunologist and Nobel Laureate César Milstein said, “Although the way ahead [for immunology] is full of pitfalls and difficulties, this is indeed an exhilarating prospect. There is no danger of a shortage of forthcoming excitement in the subject” (6). In this eBook, we examine how immunologists are using single cell and spatial multiomics to pursue this prospect and advance our knowledge in various immunology fields (Figure 2).

References

1. Crimeen-Irwin B, et al. Failure of immune homeostasis — the consequences of under and over reactivity. *Curr Drug Targets Immune Endocr Metabol Disord* 5(4): 413–422, 2005.
2. Brines R. Trends in Immunology — yesterday, today and tomorrow. *Trends Immunol* 25(12): 621–622, 2004.
3. Kaufmann SH. Immunology’s coming of age. *Front Immunol*. 10: 684, 2019.
4. Stubbington M, et al. Single-cell transcriptomics to explore the immune system in health and disease. *Science* 358(6359): 58–63, 2017.
5. Vierra Braga FA, Teichmann SA & Chen X. Genetics and immunity in the era of single-cell genomics. *Hum Mol Genet* 25(R2): R141–R148, 2016.
6. Milstein C. From the structure of antibodies to the diversification of the immune response. *Science* 231(4743): 1261–1268, 1986.

Figure 2. (shown left) Applications of single cell and spatial multiomics in immunology research areas.

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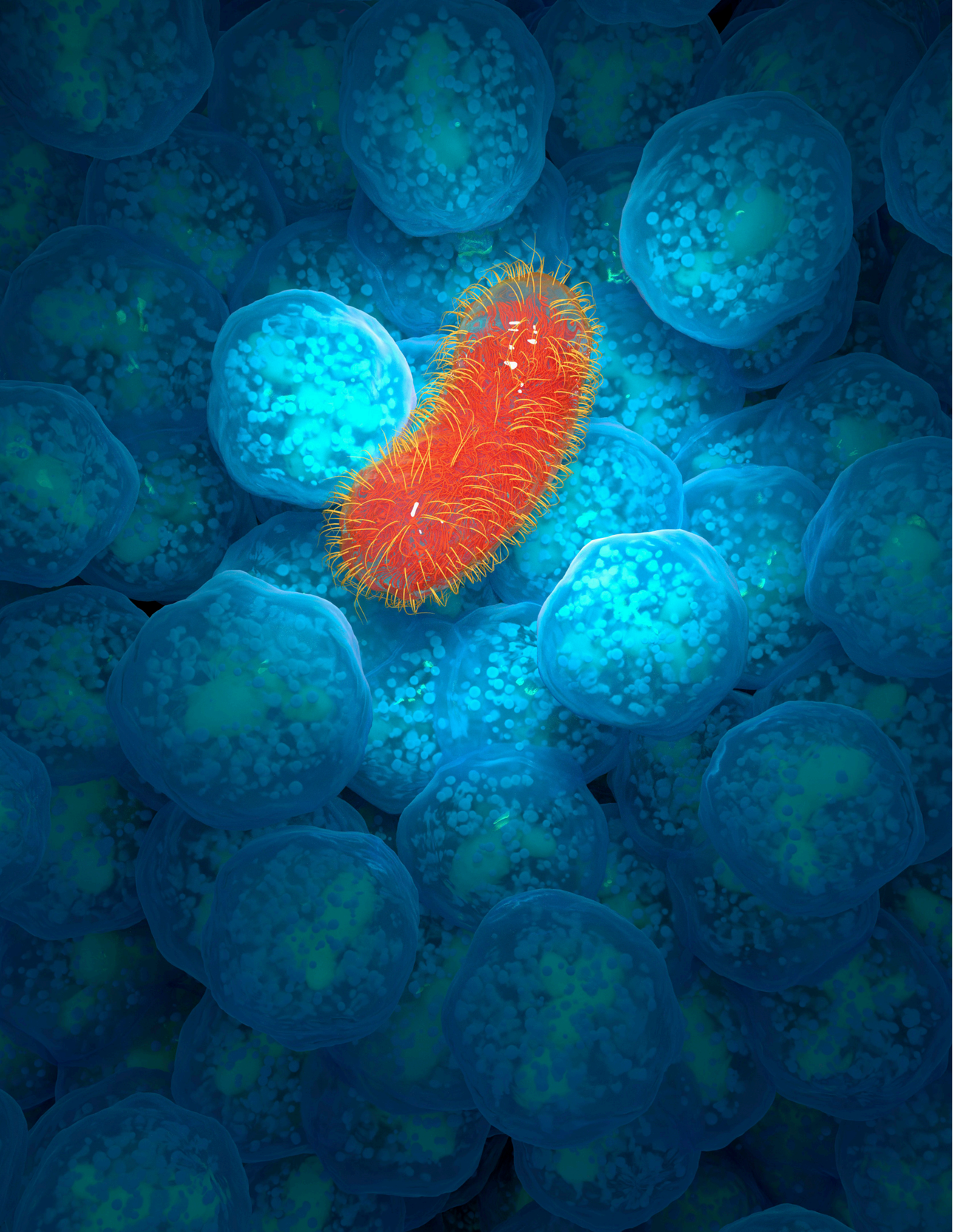

Chapter 1

Infectious disease and therapeutic development

Science vs. pathogens: the fight against infectious disease

Infection by pathogenic microorganisms has been a part of the human experience since the days of our early hominid ancestors and, especially so, since the shift to agricultural societies (1, 2). In his 1935 book (3), biologist Hans Zinsser powerfully describes our ever-present battle with pathogens, “However secure and well-regulated civilized life may become, bacteria, Protozoa, viruses, infected fleas, lice, ticks, mosquitoes and bedbugs will always lurk in the shadows ready to pounce when neglect, poverty, famine, or war lets down the defenses.” Today, the emergence and re-emergence of new and previously identified infectious diseases, respectively, are also aided by microbial adaptation, climate phenomena, increased international travel, and bioterrorism (4).

Characterizing the pathogenic microorganisms involved in disease and the host immune response to infection are primary infectious disease research objectives. However, the heterogeneity and complexity of pathogens and their hosts complicate our interpretation of data derived from bulk studies. Therefore, researchers are turning to single cell analyses to understand the pathogens and host cells that influence the course of infection (5). Single cell transcriptomics is proving to be an incredibly powerful tool to pinpoint heterogeneity in what are otherwise genetically identical cells (5).

Single cell and spatial transcriptomic analysis of infection-induced tissue damage

In this study, the authors were interested in understanding the molecular genetic basis for lung damage caused by infiltrating immune cells responding to viral infection. Their work indicates that inflammatory fibroblasts influence the outcome of infection by integrating signals from epithelial and resident immune cells to modify the local tissue environment.

Read more about this study ⟶

Getting to know thy enemy

Different species of a particular pathogen can be associated with infectious diseases exhibiting similar but unique characteristics. Using single cell RNA-sequencing (scRNA-seq) to profile and compare three different malaria-causing *Plasmodium* species, Howick et al. published the Malaria Cell Atlas—the first high-resolution transcriptional atlas of a unicellular eukaryote (6). Focusing on the blood-stage populations, during which the parasite asexually replicates in blood and can cause disease, the researchers examined the rate of transcriptomic changes between the ring, trophozoite, and schizont stages. They found that the three species follow similar developmental trajectories, although the pace of these changes varied to accommodate the species-specific length of the entire blood-stage cycle (6).

Sà et al. also sought to better understand transcriptional changes in blood-stage *P. vivax*, which have not been as well characterized due to difficulties culturing this species in vitro (7). The scRNA-seq data, derived from infected monkeys' blood, revealed gene expression signatures consistent with trophozoite and schizont blood-stage parasites, supporting the conservation of blood-stage cycle transitions across *Plasmodium* species (6, 7). Furthermore, the study pinpointed that gene expression changes were very tightly restricted to specific developmental stages, raising concern about current diagnostic methods that rely on limited stage-specific genes. Another important finding from this study was a significant number of *P. vivax* genes, particularly in the schizont stage, with no counterparts in other species, highlighting the need to properly characterize each species to develop appropriate diagnostic and therapeutic strategies (7).

Many types of infectious diseases, including malaria, put affected patients at risk for sepsis. However, this dysregulation's cellular and molecular basis has been unclear due to the many factors contributing to sepsis development, especially patient-specific immune responses. Reyes et al. turned to scRNA-seq to identify the specific cell types, and their transcriptional signatures, involved in response to bacterial sepsis (8). The study identified 16 immune cell states, including one unique sepsis-specific monocyte state, which they termed MS1, that likely expands during sepsis-induced myelopoiesis. Differential gene expression analysis allowed the researchers to identify surface markers on these MS1 cells that could be used as a monitoring tool for people at risk of sepsis. As highlighted by the authors, "This study demonstrates the utility of single-cell genomics in discovering disease-associated cytologic signatures and provides insight into the cellular basis of immune dysregulation in bacterial sepsis" (8).

Uncovering the cell biology of SARS-CoV-2 infection with single cell CRISPR screening

In this study, the authors coupled pooled CRISPR perturbations of genes previously observed to be important for SARS-CoV-2 infection with a simultaneous single cell transcriptomic and proteomic readout. The study revealed an essential role for the cholesterol biosynthesis pathway in SARS-CoV-2 infection and identified additional potential therapeutic targets that may also limit viral load in human cells.

Read more about this study ⟶

Systems immunology analysis of COVID-19

Through an integrated analysis of clinical measurements, single cell immune profiling, and analysis of plasma cytokines for 139 COVID-19 patients representing all levels of disease severity, the authors described a major immunological shift between mild and moderate infection.

Read more about this study ⟶

Tackling a global crisis, Part one: Understanding the disease

Amid infectious disease epidemics and pandemics, basic and translational immunology research plays a pivotal role in response efforts. The coronavirus disease 2019 (COVID-19) pandemic that emerged in early 2020 is a prime example of how basic research initially done to characterize infectious diseases can inform appropriate therapeutic responses. In May 2020, just five months after the initial reports of pandemic cases, Sungnak et al. used publicly available scRNA-seq profiles representing various tissue samples from healthy individuals to guide studies that would use precious, limited clinical material from COVID-19 patients (9). Their analysis suggested that the ACE2 receptor, rather than TMPRSS2, is the limiting factor for viral entry at initial infection and that nasal epithelial cells showed the highest expression of this receptor. Based on these findings, the authors hypothesized spatial distribution of receptor accessibility along the respiratory tract might mediate transmissibility of SARS-CoV-2, the virus that causes COVID-19 (9).

A key question that researchers aimed to answer during the pandemic was why many patients experienced mild symptoms while others experienced severe, sometimes fatal infections that required ventilation (10). One research study performed by Liao et al. aimed to characterize the immune response to COVID-19 in patients with moderate and severe infections by performing scRNA-seq on bronchoalveolar lavage fluid (11). The study found several differences, including a highly proinflammatory macrophage microenvironment and a high degree of phenotypic heterogeneity in severe infections. In contrast, samples from patients with mild or moderate infection were characterized by highly clonally expanded tissue-resident T effector cells (11).

Tackling a global crisis, Part two: Searching for therapeutic targets

A second study by Lee et al. examined what drives severe progression of COVID-19 using scRNA-seq to compare the transcriptomic signatures of peripheral blood monocytes to those of patients with influenza (12). Patients with severe COVID-19 had unique hyperinflammatory signatures, with particular upregulation of tumor necrosis factor/interleukin-1β (TNF/IL-1β)–driven inflammation, but shared a type I interferon (IFN) signature seen in patients with severe influenza. Based on these findings, the authors proposed anti-inflammatory strategies targeting TNF, IL-1β, and IFN-I response to be investigated as a specific treatment for severe COVID-19 (12).

High-throughput mapping of B-cell receptor sequences to antigen specificity

In this research, scientists from Vanderbilt University Medical Center reported an antibody discovery technique, linking paired heavy- and light-chain B-cell receptor (BCR) sequences to their target antigens. They validated the technique by identifying the antigen specificity of thousands of single B cells and confirming these specificities with broadly neutralizing antibodies using Chromium Single Cell Immune Profiling.

Read more about this study ⟶

Chua et al. also investigated factors associated with COVID-19 severity with a focus on the upper respiratory tract, which ideally functions to eliminate pathogens and prevent lower respiratory tract infections (13). The study, which used scRNA-seq, identified a subpopulation of epithelial cells with a strong interferon gamma (IFNγ) response signature. Intriguingly, these cells differentiated directly to ciliated cells from immature secretory cells, representing a previously unidentified differentiation pathway. Additionally, a distinct inflammatory macrophage phenotype was found to be present in patients with critical COVID-19. Detailed characterization of epithelium–immune cell interactions showed that the upregulation of the ACE2 receptor in infected epithelial cells is correlated with IFNγ signals, potentially making patients more susceptible to SARS-CoV-2 infection. Lastly, this study's findings also pinpointed a few chemokine receptors that could serve as targets for new therapeutic interventions (13).

Convalescent plasma was identified as a useful therapeutic modality during the course of the pandemic (10). Unfortunately, the widespread use of this treatment method is not possible given limited access, potentially poor or inconsistent viral neutralizing specificity, limited manufacturing scale, and the need for high purity. To address issues inherent to convalescent plasma, Cao et al. wanted to isolate neutralizing monoclonal antibodies (mAbs) from recovered patients' memory B cells and identify those with the highest potency as mAbs can be produced on a large scale (14). The authors performed combined high-throughput single cell RNA- and V(D)J-sequencing of antigen-binding B cells, a high-throughput antibody screening strategy that proved successful for the discovery of HIV-neutralizing monoclonal antibodies (15). The method takes advantage of oligonucleotide-tagged antigens that bind to cell-surface B-cell receptors (BCRs) and uses these barcodes, once they are detected during single cell sequencing, to map the antigen back to the BCR sequence (15). Out of 14 potent SARS-CoV-2-neutralizing mAb candidates, the one with the highest potency was shown to have high preventative and therapeutic efficacy in mice. "Altogether, we showed that human neutralizing antibodies could be efficiently discovered by high-throughput single B cell sequencing in response to pandemic infectious diseases," noted the authors (14). Promising candidates from this study have been registered for Phase I clinical trials in Australia (16–17) and a multinational Phase II clinical trial (18).

Conclusion

The studies reviewed above emphasize how basic and translational researchers help inform the development of surveillance tools, such as novel biomarkers of response and disease severity, and identify new therapeutic targets during infectious disease outbreaks. Single cell multiomic technologies represent a key tool in the arsenal of infectious disease researchers seeking to understand pathogens and the diseases they cause.

References

1. Han XY & Silva FJ. On the age of leprosy. *PloS Negl Trop Dis* 8(2): e2544, 2014).
2. Mummert A, et al. Stature and robusticity during the agricultural transition: Evidence from the bioarchaeological record. *Econ Hum Biol* 9(3): 284–301, 2011.
3. Zinsser H. *Rats, Lice and History.* Boston: Little, Brown and Company, 1935.
4. Morens DM, Folkers GK & Fauci AS. The challenge of emerging and re-emerging infectious diseases. *Nature* 430: 242–249, 2004.
5. Mills E & Avraham R. Breaking the population barrier by single cell analysis: one host against one pathogen. *Curr Opin Micro* 36: 69–75, 2017.
6. Howick VM, et al. The Malaria Cell Atlas: Single parasite transcriptomes across the complete *Plasmodium* life cycle. *Science* 365: eaaw2619, 2019.
7. Sà JM, et al. Single-cell transcription analysis of *Plasmodium vivax* blood-stage parasites identifies stage- and species-specific profiles of expression. *PLoS Biol* 18(5): e3000711, 2020.
8. Reyes M, et al. An immune-cell signature of bacterial sepsis. *Nat Med* 26: 333–340, 2020.
9. Sungnak W, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat Med* 26: 681–687, 2020.
10. Dhama K, et al. Coronavirus Disease 2019–COVID-19. *Clin Microbiol Rev* 33(4): e00028-20, 2020.
11. Liao M, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat Med* 26: 842–844, 2020.
12. Lee JS, et al. Immunophenotyping of COVID-19 and influenza highlights the role of type I interferons in development of severe COVID-19. *Sci Immunol* 5: eabd1554, 2020.
13. Chua RL, et al. COVID-19 severity correlates with airway epithelium–immune cell interactions identified by single-cell analysis. *Nat Biotechnol* 38: 970–979, 2020.
14. Cao Y, et al. Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells. *Cell* 182: 73–84, 2020.
15. Setliff I, et al. High-throughput mapping of B cell receptor sequences to antigen specificity. *Cell* 179: 1–11, 2019.
16. Yao, Z. (2020, Dec -). A Phase 1, Randomized, Double-Blind, Placebo Controlled Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Immunogenicity of BGB-DXP604 Alone and in Combination With BGB DXP593 in Healthy Subjects. Identifier NCT04669262. https://clinicaltrials.gov/ct2/show/study/NCT04669262
17. Yao, Z. (8, Sep -). A First-in-Human, Randomized, Double-Blind, Placebo Controlled, Single Dose Escalation Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Immunogenicity of SARS-CoV-2 Neutralizing Antibody BGB-DXP593 in Healthy Subjects. Identifier NCT04532294. https://www.clinicaltrials.gov/ct2/show/study/NCT04532294
18. Yao, Z. (30, Oct -). A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of SARS-CoV-2 Neutralizing Antibody BGB-DXP593 in Patients With Mild-to-Moderate COVID-19. Identifier NCT04551898. https://www.clinicaltrials.gov/ct2/show/study/NCT04551898

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Chapter 2

Vaccines

Bridging the gap between immune response and treatment development

Few stories in science have captivated the general public like Louis Pasteur's supposedly accidental discovery of attenuation and his subsequent development of vaccines for chicken cholera, anthrax, and rabies (1). Much has changed since those early days of vaccinology thanks to our ever-growing understanding of the immune system and technological advances in vaccine development. For instance, we now understand that successful vaccination is dependent on the induction of protective antibody and T-cell responses (2, 3). However, the variability in immunity imparted to individuals by the same vaccine underscores that there is still much to learn about the mechanisms governing vaccine response (4).

Unlike other immunological fields, single cell sequencing, particularly multiomic strategies, has not yet been as widely adopted in the context of vaccine research. However, researchers are beginning to recognize that it can "...elucidate complex networks of cell interactions and immune responses and the potential to identify novel or unanticipated response profiles, which have been beyond the scope of bulk RNA and other sequencing technologies" (3). These cellular profiles can then serve as a basis for predicting and monitoring vaccine responsiveness.

Understanding antibody response to the influenza vaccine

What is the relationship between transcriptional response, activation of memory B cells, and clonal expansion? Researchers at Stanford University used Chromium Single Cell Immune Profiling to define the transcriptional profile for clonally expanded B cells activated by influenza vaccine and identify vaccine-responsive clones.

Learn more about profiling vaccination response ⟶

Understanding vaccine response

Protective antibody memory responses are known to be mediated by memory B cells, but the transcriptional programs controlling this process—namely clonal expansion and antigen specificity—are still unclear. Horns et al. sought to address this gap in knowledge by combining single cell transcriptomics and antibody repertoire sequencing of samples collected at several time points before and after influenza vaccination (5). The transcriptomic analysis revealed that vaccination results in an activated memory B-cell state, displaying the hallmarks of an effector B-cell response and characterized by the expression of the transcription factor T-bet and a gene, *CD11c*, associated with humoral activation. From the single cell antibody heavy-chain sequencing, the researchers determined that these cells made predominantly class-switched, somatically mutated antibodies. Surprisingly, 60% of the antibody clones identified were unable to bind the vaccine and likely represent antigen-independent activation. On the other hand, the vaccine-binding antibodies broadly bound several hemagglutinin proteins, a major antigenic determinant of influenza. The authors commented that,

"Our results show that this strategy yields a unified portrait of the molecular and cellular features of the memory B cell response to vaccination, giving insights into mechanisms of immune memory."

Recognizing that the biology underlying protective responses in vaccination can negatively impact patients with autoimmune disease, Kotliarov et al. looked to identify the baseline immune response predictors for these events (6). A more detailed analysis of a B-cell population correlated with influenza vaccine response (7) enabled identification of a 10-gene signature (TGSig) with a good predictive performance for high and low responders in influenza and yellow fever vaccination datasets. Interestingly, the presence of this TGSig in patients with clinically quiescent systemic lupus erythematosus correlated with the magnitude of plasmablast-associated flares in the patients, suggesting a shared mechanism between vaccination response and lupus disease activity. To identify what cell types might be involved, the researchers coupled oligonucleotide-labeled antibody staining of cell-surface protein epitopes with scRNA-seq to simultaneously profile 82 surface proteins and the transcriptomes from high and low influenza vaccine responders. The data obtained from this approach pointed to a cellular activation network where activation of plasmacytoid dendritic cells leads to an increase in type I interferon (IFN), which then activates lymphocytes (6).

Informing vaccine development

While there are many diseases and pathogens for which vaccines have been developed, there are many more for which a vaccine does not yet exist or current vaccination strategies are inefficient. For example, vaccine development for dengue virus (DENV) has proven particularly tricky because an effective vaccine needs to protect against four antigenically distinct serotypes and prevent immune-mediated enhancement of infection severity (8). Waickman et al. examined the immune response elicited by TAK-003, a recombinant DENV vaccine that has demonstrated promise in animal studies and responds to all four serotypes. Using flow cytometry, the researchers found that TAK-003 administration led to activation of T cells expressing CD8, a co-receptor of the T-cell receptor (TCR). Paired single cell sequencing and TCR sequencing was then used to further characterize the clonal dynamics and cell states of this T-cell population committed to the memory repertoire. These cells, determined to be memory precursor cells, contained a large degree of clonal diversity and displayed a unique transcriptional signature dominated by metabolic and proliferation pathways. From these signatures, the researchers were able to identify a panel of metabolic markers that can be used to assess the effector/memory potential of T cells (8). The authors note the data points to potential therapeutic targets that might enhance the efficacy of DENV vaccinations.

HIV is another virus for which vaccine development has proven difficult. Arunachalam et al. set out to determine if a strategy eliciting both neutralizing antibodies (nAbs) and cellular responses provides enhanced protection compared to the induction of broadly nAbs alone (9). Rhesus macaques with simian HIV were immunized with a nAb-inducing HIV envelope trimer alone or alongside a heterologous viral vector regimen to also elicit memory T cell–based immunity. While both strategies protected against several vaginal challenges, the combinatorial strategy reduced the threshold of nABs required for the protective response and was more durable in resisting additional challenges five months later. To understand the role of T-cell subtypes in antiviral response, simultaneous evaluation of gene and protein expression by coupling oligonucleotide-labeled antibody staining of cell-surface protein epitopes with scRNA-seq was performed on antigen-stimulated vaginal tissue to activate T cells in situ. This analysis indicated that the observed antiviral response was mediated by myeloid cells and helper T cells expressing CD4 (9). Thus, this two-pronged vaccination strategy imparts a strong, lasting protection against HIV acquisition.

Conclusion

These studies demonstrate that single cell transcriptomics and multiomics are helping researchers gain a more comprehensive understanding of the cellular and molecular basis of immune responses. As we expand our comprehension of these mechanisms, vaccinologists will be better equipped to tackle vaccine development for more complex diseases and pathogens.

References

1. Berche P. Louis Pasteur, from crystals of life to vaccination. *Clin Microbiol Infect* 18: 1–6, 2012.
2. Plotkin SA & Plotkin SL. The development of vaccines: how the past led to the future. *Nat Rev Microbiol* 9(12): 889–893, 2011.
3. Noé A, et al. The application of single-cell RNA sequencing in vaccinology. *J Immunol Res* 2020: 8624963, 2020.
4. O'Connor D & Pollard AJ. Characterizing vaccine responses using host genomic and transcriptomic analysis. *Clin Infect Dis* 57(6): 860–869, 2013.
5. Horns F, Dekker CL & Quake SR. Memory B cell activation, broad anti-influenza antibodies, and bystander activation revealed by single-cell transcriptomics. *Cell Reports* 30: 905–913, 2020.
6. Kotliarov Y, et al. Broad immune activation underlies shared set point signatures for vaccine responsiveness in healthy individuals and disease activity in patients with lupus. *Nat Med* 26: 618–629, 2020.
7. Tsang JS, et al. Global analyses of human immune variation reveal baseline predictors of postvaccination responses. *Cell* 157: 499–513, 2014.
8. Waickman AT, et al. Dissecting the heterogeneity of DENV vaccine-elicited cellular immunity using single-cell RNA sequencing and metabolic profiling. *Nat Comms* 10: 3666, 2019.
9. Arunachalam PS, et al. T cell-inducing vaccine durably prevents mucosal SHIV infection even with lower neutralizing antibody titers. *Nat Medicine* 26: 932–940, 2020.

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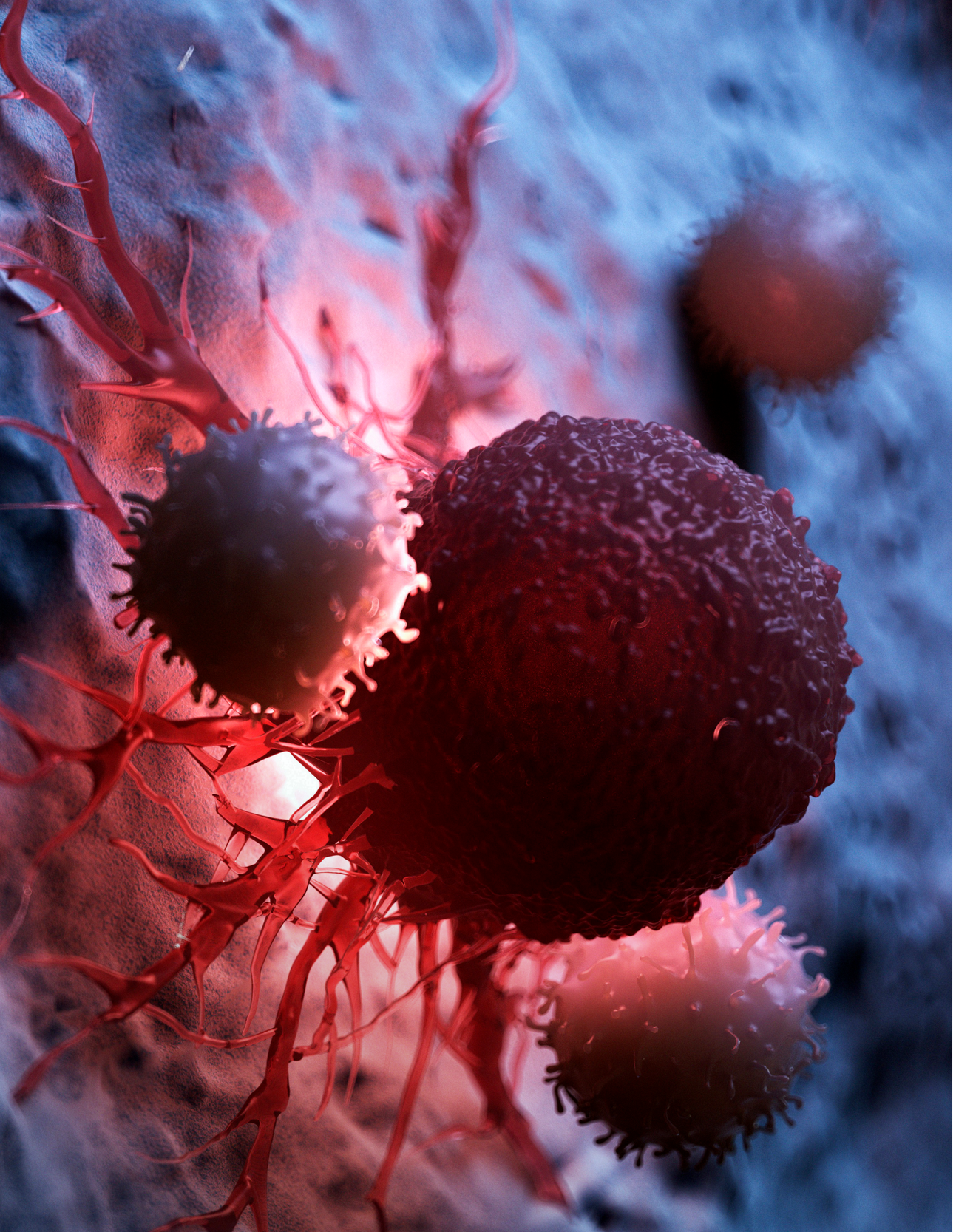


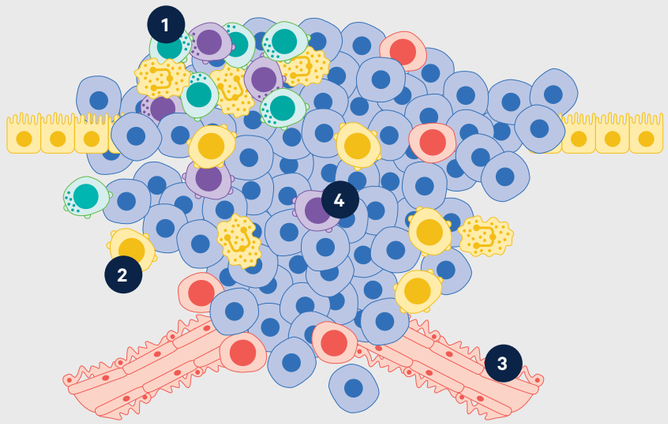
Chapter 3

Immuno-oncology

A complicated relationship: Deciphering tumor–immune interactions

The role of immune cells in tumor microenvironments (TME) is as complex as the immune system itself (1). While some immune cells play a critical role in immunosurveillance, or rejection of cells recognized as malignant tumor cells, chronic inflammation has been linked to the promotion of tumor cell growth, survival, and angiogenesis (2). Our growing understanding of the communication between tumor and immune cells, such as immune checkpoint pathways, underlying these diverse functions has been critical to revolutionizing cancer treatment with immunotherapies.

Key focus areas of immuno-oncology studies include defining the immune cell types in the TME, understanding the fundamental mechanisms of these cells in cancer pathogenesis, searching for novel immunotherapy targets, and/or characterizing therapeutic responses of potential candidates. Regardless of the goal, a common challenge faced by scientists is the frequently low abundance of immune cells within the TME and their heterogeneous nature. Bulk analysis methods, such as RNA-seq and microarray studies, whose readouts may be dominated by the most abundant cell populations, lack resolution for these investigations. Single cell multiomics technologies, however, are enabling the high-resolution molecular phenotyping of immune infiltrating cells and other cells present within a tumor (1). In addition to parsing the transcriptional and/or protein expression profiles of single cells, single cell immune profiling enables full-length, paired T-cell or B-cell receptor sequencing, providing a direct correlation between cell phenotype and clonotype, as well as clonal lineage tracking.



1 How does cellular function and spatial organization affect immune cell accessibility and recruitment?

2 What immune cell types shape the tumor microenvironment during cancer progression and metastasis?

3 What are the growth factors and receptors induced by inflammatory pathways that contribute to tumor angiogenesis?

4 What cells and molecular pathways have the highest therapeutic potential?

Figure 3. Exploring the complex interactions of the immune and tumor microenvironment contexture.

Characterizing response to immune checkpoint blockade

Several immune checkpoint blockade therapies, such as anti-CTLA-4 and anti-PD-1/PD-L1, are currently on the market with many patients experiencing durable clinical response (3). However, the mechanism of T-cell response to checkpoint blockade is still being characterized. Yost et al. investigated the origins of T-cell receptor (TCR) clones and transcriptional phenotypes observed following anti-PD-1 blockade therapy in patients with basal or squamous cell carcinoma (4). From paired 5' scRNA-seq and TCR sequencing data of 79,046 cells, an enrichment of exhausted $CD8^+$ T cells featuring transcriptional signatures consistent with chronic activation, tumor reactivity, and T-cell dysfunction was observed post-treatment (4). Immune repertoire analysis revealed that the TCR clonotypes identified following blockade were largely novel when compared to the pre-treatment repertoire, which suggests that T cells from peripheral blood are responsible for the T-cell response generated by anti-PD-1 blockade.

Insights into T-cell expansion in response to checkpoint therapy

To understand the mechanisms involved in differing patient responses to immunotherapy, researchers at Genentech used Chromium Single Cell Immune Profiling and found clonal expansion of effector-like T cells from patient tumors in both normal adjacent tissue and peripheral blood. A review of clinical data suggests that, in patients who responded to anti-PDL1 therapy, T cells within tumors are replenished with non-exhausted cells from outside the tumor.

Read more about single cell sequencing for clonal expansion studies ⟶

Despite the successful application of immune checkpoint blockade approaches, some tumors have proven resistant while others acquire resistance over time (3). This diversity in response indicates that there are secrets yet to be unearthed when it comes to comprehending the intricate interplay between cancer and immune cells. Some researchers are turning to the epigenome for those hidden clues. Satpathy et al. performed a single cell assay for transposase-accessible chromatin using sequencing (scATAC-seq) to dissect the role of epigenomic landscapes in T-cell responsiveness to PD-1 blockade (5). Focusing on exhausted $CD8^+$ T cells, the authors discovered that PD-1 blockade leads to large-scale remodeling of the chromatin accessibility landscape and identified key regulatory elements with accessibility specific to the post-treatment exhaustion state (5). Importantly, the team discovered that this exhausted T-cell state is not reversed by checkpoint blockade, further corroborating the finding that the anti-tumor response is driven by a replenishment of T cells from outside the tumor.

Profiling responses to engineered cell therapies

Targeting T cells to tumors via engineered chimeric antigen receptors (CARs) and TCRs represents another major cancer immunotherapy strategy (6). Since the success of these therapies is based on the expansion of the engineered T cells in vivo, Sheih et al. turned to paired single cell TCR sequencing and RNA-seq to understand the fate of these cells post-infusion (7). In particular, the group was interested in understanding the phenotypic behaviors driving clonal expansion and shrinkage observed over time in patients. The transcriptional profiles of $CD8^+$ CAR-T cells circulating in the blood increasingly diverged from the initial activated signatures of the infusion product throughout post-infusion timepoints without evidence of an exhaustion state (7). Tracking of the TCR clonotypes revealed a variation in the contribution of the various clonotypes to the circulating CAR-T cell pool (7).

Stadtmauer et al. similarly used scRNA-seq for tracking the transcriptomic evolution of CRISPR-engineered T cells (8). A novel multiplex editing strategy was implemented to target three genes simultaneously. The genes encoding the cells' TCR alpha and beta chains were deleted to prevent mispairing of the transgenic TCR with these endogenous chains. Additionally, the gene encoding PD-1 was also knocked down to improve anti-tumor immunity (8). Transcriptomic analysis allowed the tracking of the engineered T cells in one of the patients for up to four months in vivo. The infused cells showed phenotypic heterogeneity over time with a subset going on to establish a gene expression signature state associated with acquired central memory (8). It is worth noting that a culture of these cells obtained from the patient nine months after infusion still retained anti-tumor cytotoxicity.

Profiling the breast tumor microenvironment

The tumor microenvironment has broad effects on immune cell phenotypes. To understand how these changes occur, researchers at Memorial Sloan Kettering Cancer Center in New York used Chromium Single Cell Immune Profiling for large-scale analysis of the human breast tumor microenvironment to construct a cell atlas and found there was a continuum of activation and differentiation states of T cells and macrophages in tumors.

Discover how to profile tumor microenvironments ⟶

Much like traditional cancer therapies, immune cell therapies are not exempt from acquired resistance. Comprehending the fundamental biology that drives this process is crucial to allow the development of actionable strategies for its prevention or treatment (3). Paulson et al. investigated the mechanism of acquired resistance to a combination immunotherapy strategy, using both autologous $CD8^+$ T cells and immune checkpoint inhibitors, for metastatic Merkel cell carcinoma (9). For one patient's samples, scRNA-seq of tumor digests showed that resistance was driven by a novel immune escape mechanism involving the transcriptional downregulation of the human leukocyte antigen gene, meaning that the antigen targeted by the combo immunotherapy was lost. This result was validated in a second patient (9). Excitingly, the study went on to show that this downregulation was reversible with hypomethylating agents in vitro (9).

Conclusion

To harness the full power of immunotherapies, scientists must more completely understand the foundations of immune cell function in the context of cancer. Single cell multiomics is paving the way for immuno-oncology researchers to decipher the complex communication between immune cells—both endogenous and from adoptive cell therapies—and cells in the tumor microenvironment.

References

1. Valdes-Mora F. Single-cell transcriptomics in cancer immunobiology: The future of precision oncology. *Front Immunol* 9: 2582, 2018.
2. Zamarron BF & Chen WJ. Dual roles of immune cells and their factors in cancer development and progression. *Int J Biol Sci* 7(5): 651–658, 2011.
3. Sharma P, et al. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell* 168(4): 707–723, 2017.
4. Yost KE, et al. Clonal replacement of tumor-specific T cells following PD-1 blockade. *Nat Med* 25(8): 1251–1259, 2019.
5. Satpathy AT, et al. Massively parallel single-cell chromatin landscapes of human immune cell development and intratumoral T cell exhaustion. *Nat Biotech* 37: 925–936, 2019.
6. June CH, Riddell SR & Schumacher TN. Adoptive cellular therapy: A race to the finish line. *Sci Transl Med* 7(280): 280ps7, 2015.
7. Sheih A, et al. Clonal kinetics and single-cell transcriptional profiling of CAR-T cells in patients undergoing CD19 CAR-T immunotherapy. *Nat Commun* 11: 219, 2020.
8. Stadtmauer EA, et al. CRISPR-engineered T cells in patients with refractory cancer. *Science* 367(6481): eaba7365, 2020.
9. Paulson KG, et al. Acquired cancer resistance to combination immunotherapy from transcriptional loss of class I HLA. *Nature Commun* 9: 3868, 2018.

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Chapter 4

Autoimmunity

Fighting with oneself: Uncovering mechanisms of autoimmunity

Distinguishing self from nonself, a central tenet of immunology since the late 1960s, involves a complex interplay between immune tolerance mechanisms and dynamic modulation of the immune response (1). Autoimmune diseases occur when there is a loss of tolerance to self-antigens, leading to increased levels of autoantibodies and/or autoreactive immune cells (2). The diverse responses of patients with similar clinical presentations to the same treatment and, conversely, the ability of some targeted therapies to treat multiple distinct diseases underscores the importance of characterizing these diseases on a more fundamental level to achieve improved outcomes in the clinic (2).

In the early 2000s, genome-wide association studies identified hundreds of single nucleotide polymorphisms (SNPs) associated with several autoimmune diseases, but linking these loci to functional disease has proven tricky (3). Fully characterizing the immune cell types, states, and functions unique to each disease is a critical area of focus in identifying new therapeutic candidates and novel biomarkers for better diagnostics. Given the multifactorial nature of these diseases and evidence of an important role for environmental influences on disease manifestations, single cell multiomic technologies that can profile functional outputs of the genome will be crucial to truly understand disease pathogenesis (4).

Deciphering mechanisms of misdirected immune response

Recently, Borcherding et al. built the first single cell transcriptomic map characterizing 18,231 immune cells derived from healthy mice of a murine model of alopecia areata (AA). Single cell gene expression data identified myeloid dendritic cells in AA mice that showed an enrichment in pro-inflammatory and interferon (IFN) signatures. Intriguingly, paired full-length receptor sequencing revealed that AA mice showed a significant overlap in clonotypes expressed by the clonally expanded T cells expressing the CD4 or CD8 co-receptor. Most promising of all was corroborating sequencing data in human samples, and the ability of these T-cell gene signatures to distinguish healthy from diseased samples (5).

Unraveling immune tolerance mechanisms with single cell multiomics

Using Chromium Single Cell Immune Profiling and Single Cell ATAC solutions, scientists at the Geisel School of Medicine at Dartmouth performed transcriptome, T-cell repertoire, and open chromatin analysis in a mouse model. They discovered the role that V-type immunoglobulin domain-containing suppressor of T-cell activation (VISTA) plays in immune tolerance as an early checkpoint regulator.

Learn more about multiomic analysis ⟶

Multiple sclerosis (MS) is a devastating autoimmune disease that is systemic within the central nervous system and impacts immune cell expression in regionally distinct ways. Schafflick et al. set out to better understand immune cell behavior in the blood and cerebrospinal fluid (CSF) compartments of healthy controls and patients with MS. They uncovered that the CSF compartment in MS patients experienced cell composition changes not seen in the control group, including an expansion of cytotoxic T helper cells and late-stage B lineage cells. In contrast, blood cells exhibited transcriptional changes characterized by enrichment in immune activation, memory formation, and interferon responses (6). Importantly, this study shows the feasibility of profiling samples with limited numbers of cells, such as CSF, with single cell transcriptomics. As the authors note,

"Clinically, CSF facilitates the diagnosis of inflammatory and degenerative diseases and limited volumes can be safely sampled in every patient. Technical approaches must therefore be compatible with low input."

Despite being one of the most common autoimmune diseases, the mechanisms underlying rheumatoid arthritis (RA) are still unclear. While macrophages have been shown to promote joint inflammation, whether the inflammatory macrophages originate from tissue-resident populations or blood monocytes has not yet been determined. Culemann et al., therefore, sought to investigate the origins and cellular functions of macrophage subsets to RA (7). Combining single cell sequencing with fluorescence microscopy, they discovered tissue-resident macrophages formed a renewable membrane-like structure that acts as an immunological barrier in the absence of inflammation. However, the onset of arthritis is associated with an influx of pro-inflammatory monocyte-derived macrophages, distinct from the tissue-resident population, to the synovial space (7). A separate study by Alvivernini et al. sought to further dissect synovial tissue macrophage (STMs) subtypes during drug-free remission of RA. Nine phenotypically distinct STM profiles whose frequencies varied in each clinical state examined—early/active RA, treatment-refractory/active RA, and RA in sustained remission—were identified with single cell sequencing (8). In agreement with the Culemann et al. study, these researchers found evidence for one population of STMs that likely play a joint-protective function in healthy patients and those experiencing sustained remission (7, 8).

Exploring the single cell transcriptomic immune landscape of lupus nephritis

How do immune cells interact to drive lupus nephritis? Researchers at the Broad Institute of MIT and Harvard, Harvard Medical School, and the University of Michigan used Chromium Single Cell Gene Expression analysis in the kidneys of patients with lupus nephritis to uncover 21 subsets of active leukocytes and high expression of two chemokine receptors that may be involved in cell trafficking.

Read more about characterizing heterogeneous, diseased populations ⟶

Conclusion

Detailed cellular characterizations like those described in the studies above are providing a critical first step to inform new therapeutic and diagnostic development avenues for autoimmune diseases. Moving forward, integrating these cellular profiles with epigenomic, proteomic, and spatial analyses will help provide even richer insights.

References

1. Leng Q & Bentwich Z. Beyond self and nonself: Fuzzy recognition of the immune system. *Scand J Immunol* 56(3): 224–232, 2002.
2. Banchereau R, et al. Understanding human autoimmunity and autoinflammation through transcriptomics. *Annu Rev Immunol* 35: 337–370, 2017.
3. Hu X & Daly M. What have we learned from six years of GWAS in autoimmune diseases, and what is next? *Curr Opin Immunol* 24: 571–575, 2012.
4. Donlin LT, et al. Insights into rheumatic diseases from next-generation sequencing. *Nat Rev Rheumatol* 15(6): 327–339, 2019.
5. Borcherding N, et al. A transcriptomic map of murine and human alopecia areata. *JCI Insight* 5(13): e137424, 2020.
6. Schafflick D, et al. Integrated single cell analysis of blood and cerebrospinal fluid leukocytes in multiple sclerosis. *Nat Commun* 11: 247, 2020.
7. Culemann S, et al., Locally renewing resident synovial macrophages provide a protective barrier for the joint. *Nature* 572: 670–675, 2019.
8. Alivernini S, et al. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. *Nat Med* 26: 1295–1306, 2020.

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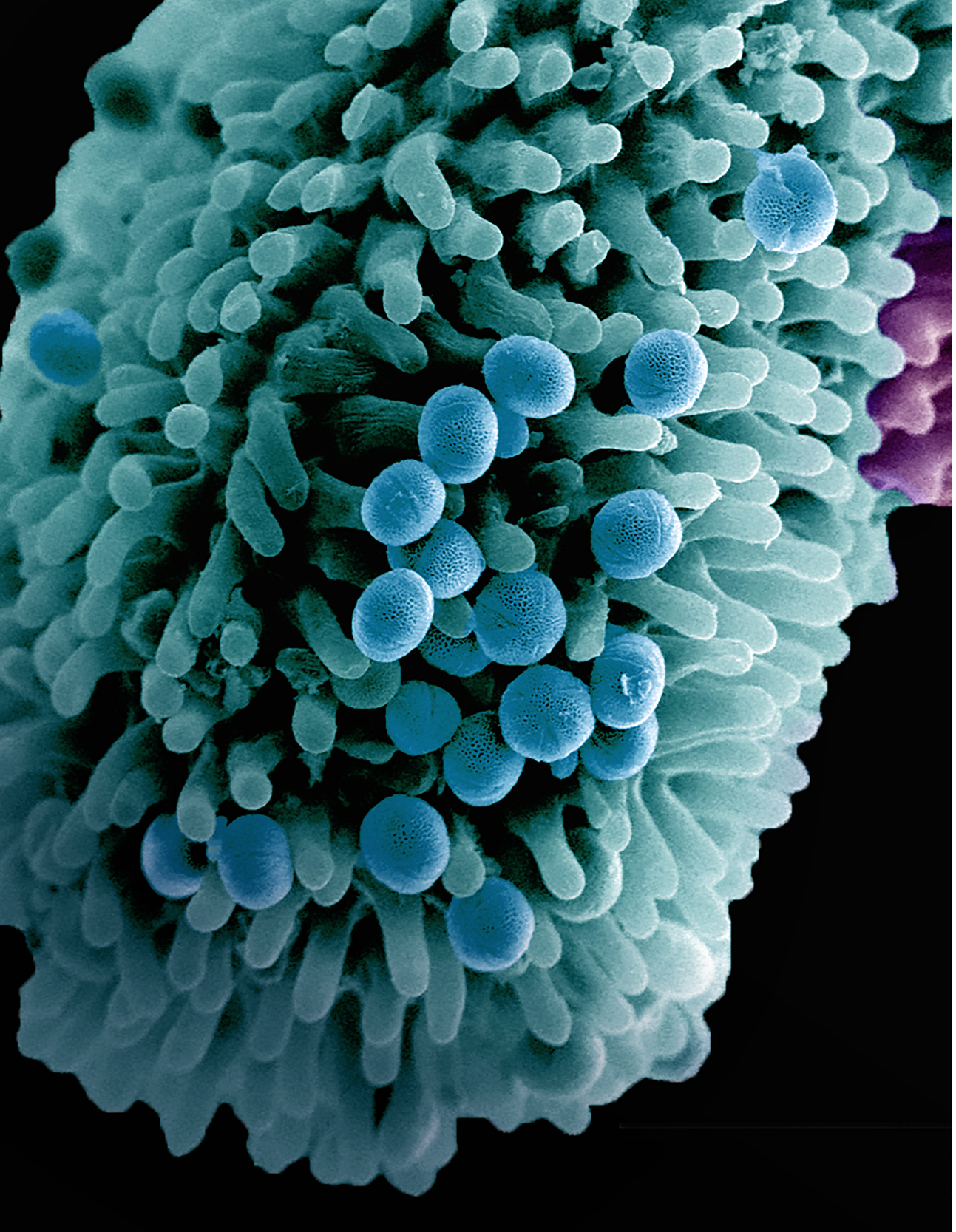

Chapter 5

Allergies and inflammation

Investigating the biological framework of allergic and inflammatory immune response

Response to injury, allergen or toxin exposure, and infections leverages an essential protective immune response—inflammation (1). When this tightly controlled process gets derailed and does not resolve, inflammation becomes chronic and can eventually lead to the development of diseases such as asthma, allergies, cancer, and autoimmunity (1, 2). Control of inflammation is a crucial focus for the treatment regimes of some disorders, but more effective induction of the inflammatory process may also help treat certain conditions (1). A more detailed framework of the cellular and molecular mechanisms of inflammation must be built to effectively implement either strategy. This will involve pinpointing the cell types and subtypes involved in the pathogenesis of inflammation.

Uncovering the cellular landscape of inflammation

In the context of chronic obstructive pulmonary disease (COPD), a key question has been determining whether the dysregulated immune response results from the development of novel pathogenic cell subsets or the expansion of pre-existing subsets (3). To investigate the origins of COPD immune responses, Rao et al. used single cell cloning and sequencing techniques to perform a comparative analysis of clonogenic cells derived from patients with COPD and healthy individuals (4). Using single cell RNA-sequencing (scRNA-seq), the researchers were able to confirm that the distal airways of COPD lungs contained four major clusters: normal epithelia, goblet cell metaplasia (GCM), squamous cell metaplasia (SCM), and inflammatory squamous cell metaplasia (iSCM). Combined with functional analyses, distinct roles could be tied to each of the clusters, with GCMs linked to excess mucin secretion, SCMs playing a role in fibrosis, and iSCMs promoting neutrophilic and fibrotic responses. Interestingly, while predominated by normal epithelia, the healthy and fetal lungs contained a small proportion of the metaplastic variants (4). These findings expand the understanding of the cellular drivers of COPD pathogenesis and present new cell types that can be further investigated for novel biomarkers and therapeutic candidates.

Identifying therapeutic targets for drug-induced hypersensitivity syndrome

By combining Chromium Single Cell Gene Expression and Immune Profiling analyses, researchers discovered a possible therapeutic target for the treatment of drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms. Patients were successfully treated with the therapeutic options identified based on data from the single cell results.

Discover how single cell sequencing can help guide treatment decisions ⟶

Carlberg et al. also took advantage of unbiased transcriptomic analysis to characterize inflammation sites of rheumatoid arthritis (RA) and spondyloarthritis (SpA; 5). Uniquely, this group employed Spatial Transcriptomics, an emerging technology that combines histology and mRNA analysis for whole transcriptome mapping in intact tissue sections. The differential gene expression analysis revealed that adaptive immune response signatures dominated in RA, while tissue repair signatures were prevalent in SpA. Meanwhile, layering the transcriptomic data on top of histological images of the joint tissue biopsies showed an overrepresentation of central memory T cells in patients with RA, and effector memory T cells in patients with SpA (5). Thus, Spatial Transcriptomics provided a unique glimpse of the inflammatory pathways, in a morphological context, that distinguish these similar but distinct conditions.

Understanding cellular mechanisms in allergy and asthma

Seumois et al. aimed to characterize allergen-specific T cells in asthma and allergy using unbiased single cell transcriptomics (6). In all, this study examined roughly 50,000 house dust mite (HDM) allergen-reactive $CD4^+$ T helper (T_H) cells and regulatory T (T_{reg}) cells from asthmatics with and without HDM allergy as well as non-asthmatics with and without the allergy. Notably, the researchers had previously performed bulk RNA-seq but noted, "The heterogeneity observed within the HDM-reactive T_H population…is likely to have limited the resolution of bulk transcriptome data to distinguish asthma-specific features" (6). In contrast, the single cell RNA-seq data was able to identify an increase of IL-9–expressing HDM-reactive T_H cells with enhanced pathogenic properties as a hallmark of asthmatics with HDM allergy compared to HDM-allergic non-asthmatics. When looking at asthmatics with and without HDM allergy, T_H and T_{reg} cells expressing interferon response signatures and *TNFSF10*, whose protein product may dampen these cells' activation, distinguished asthmatics without the allergy (6). This study highlights the power of single cell transcriptomics to resolve biological heterogeneity masked by bulk RNA-seq and uncover novel, distinct cell subsets.

Turning bedside insights into benchside discoveries

While translating transcriptomic data into actionable insights in the clinic is an important goal for inflammation research, Gruber et al. demonstrate that sometimes the benefits can flow from the bed to the benchside with clinical insights driving mechanistic discoveries (7). In this study, the cause of early-onset immunodysregulatory syndrome in the patient profiled was determined to be the result of a mosaic gain-of-function (S703I) mutation in *JAK1*, a kinase which functions in cytokine signaling. Single cell 3' RNA-seq was performed alongside a custom S703I-targeting scRNA-seq assay to examine the impact of this mutation on immune cells. This strategy allowed simultaneous transcriptome profiling and *JAK1* genotyping, revealing an expansion of $CD56^{hi}$ natural killer (NK) cells and a preferential distribution of the mutant mRNA in these cells (7). Intriguingly, it was the unique asymmetric clinical manifestations of this disease that guided the genetic analysis.

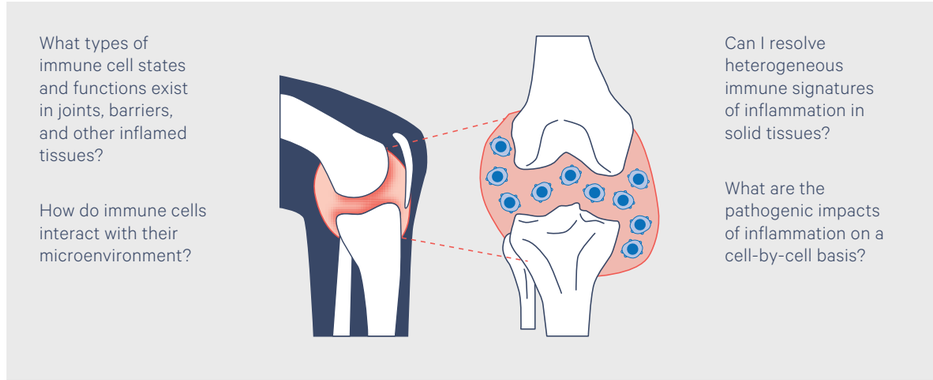

Figure 4. Unraveling mechanisms of inflammatory immune responses.

Conclusion

As the cells involved in inflammation pathogenesis associated with diseases are identified, new mechanistic insights begin to emerge. These new insights also raise new questions, including: How do these disease-associated cells communicate with and impact their microenvironment? What regulatory networks control the inflammatory functions of these cells? How do these processes vary at different inflammation sites? Single cell and spatial multiomics will continue to play a crucial role in uncovering these answers.

References

1. Nathan C. Points of control in inflammation. *Nature* 420: 846–852, 2002.
2. Medzhitov R. Inflammation 2010: New adventures of an old flame. *Cell* 140(6): 771–776, 2010.
3. Seumois G & Vijayanand P. Single-cell analysis to understand the diversity of immune cell types that drive disease pathogenesis. *J Allergy Clin Immunol* 144(5): 1150–1153, 2019.
4. Rao W, et al. Regenerative metaplastic clones in COPD lung drive inflammation and fibrosis. *Cell* 181: 1–17, 2020.
5. Carlberg K, et al. Exploring inflammatory signatures in arthritic joint biopsies with Spatial Transcriptomics. *Sci Rep* 9: 18975, 2019.
6. Seumois G, et al. Single-cell transcriptomic analysis of allergen-specific T cells in allergy and asthma. *Sci Immunol* eaba6087, 2020.
7. Gruber CN, et al. Complex autoinflammatory syndrome unveils fundamental principles of *JAK1* kinase transcriptional and biochemical function. *Immunity* 53: 672–684, 2020.

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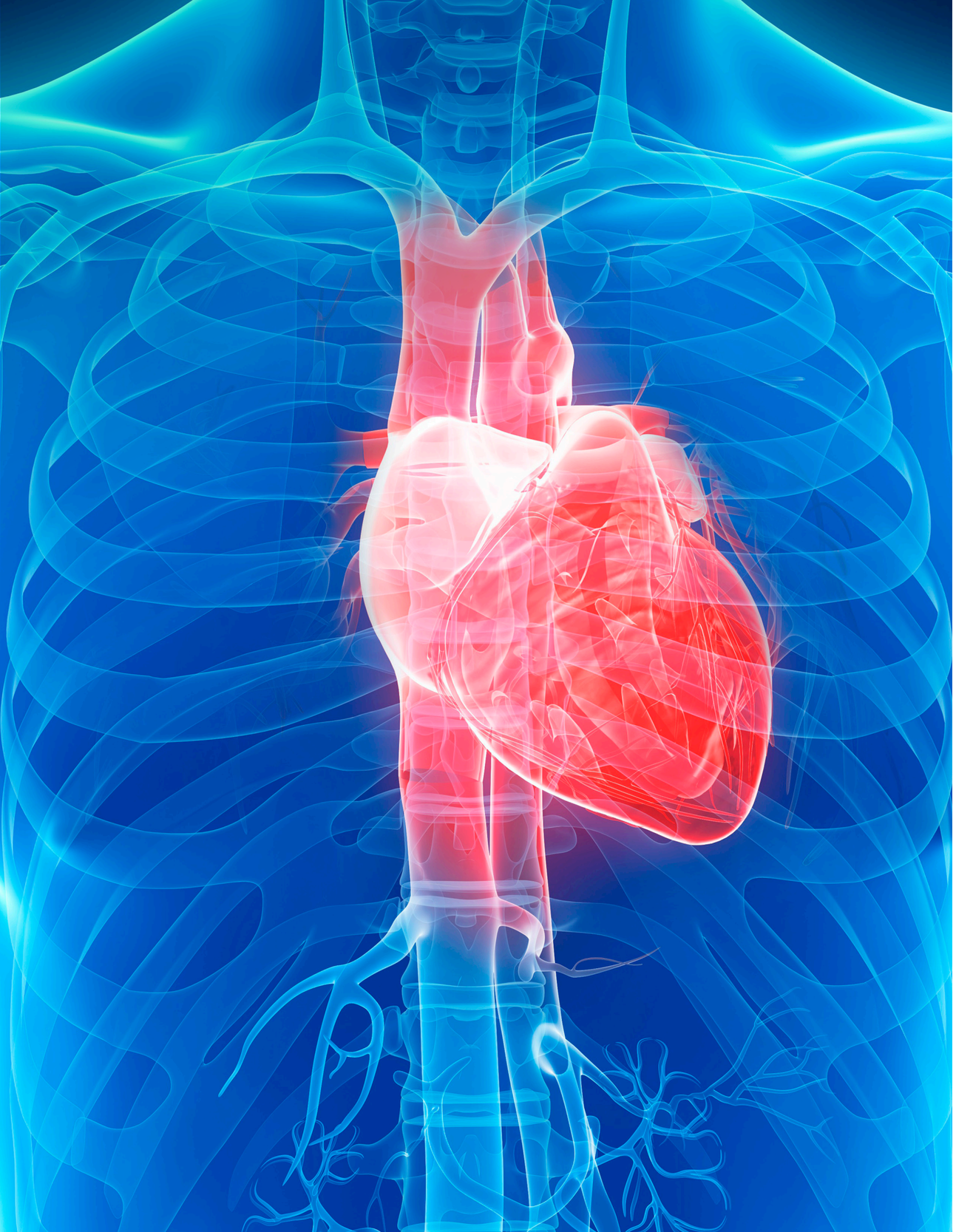

Chapter 6

Transplantation immunology

New tools for survival: Advances in transplant immunology

When disease or injury has damaged an organ beyond the body's ability to heal, transplantation may become one of the only remaining options. In half a century since the first successful kidney transplant (1), many of the immune system features—human leukocyte antigens, antibodies, helper and cytotoxic T-cell subsets, and more—mediating graft tolerance, rejection, and graft-versus-host disease (GvHD) have been identified and studied (2). With these discoveries and developments in immunosuppressive treatments, the field has seen extraordinary advancements in one-year patient and graft survival rates (1, 3). Unfortunately, long-term graft rejection remains an unavoidable reality, and researchers are digging deeper into the immunologic factors at play in this process.

Distinguishing donor from recipient

Transplant immunology research is complicated by the diversity of immune cell types and the rarity of some of these populations. In particular, one major challenge in studying recipient samples is the need to distinguish the origins of infiltrating immune cells (4). As demonstrated by Zheng et al., single cell RNA-sequencing (scRNA-seq) data provides the resolution to determine host and donor cell chimerism after transplantation (5). Since the donor and recipient genotypes were unknown, the authors developed a method that uses the transcriptome data to predict single nucleotide variants and then classify the cells. Therefore, it was possible to monitor subpopulation changes and the interplay between the donor and host cells in response to hematopoietic stem cell transplantation. In a separate study, Byrne et al. revealed that mature recipient-derived airway macrophages (AMs) dominated bronchoalveolar lavage cell samples derived from lung transplant recipients using scRNA-seq (6). Furthermore, the authors were able to determine that these AMs originated from circulating precursors.

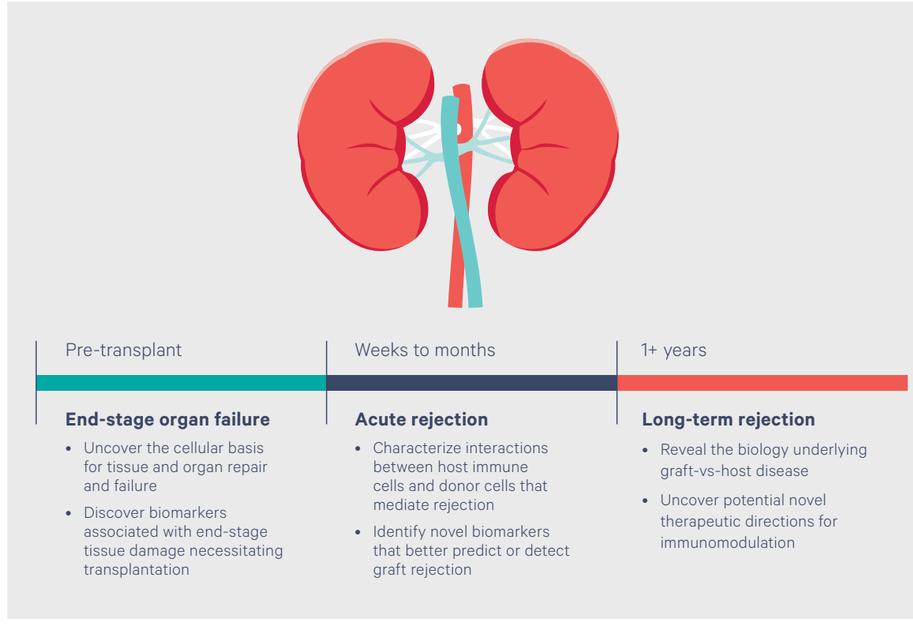

Figure 5. Applications of single cell and spatial multiomics in transplant immunology.

Unraveling mechanisms of rejection

In terms of allograft loss, a primary culprit of this failure is the development of transplant arteriosclerosis, an alloimmune-mediated arterial dysfunction (7). However, this process's pathogenesis is not well understood, thus limiting the development of effective therapeutic approaches. Cai et al. used scRNA-seq to develop the first transcriptional landscape of aortic allografts in mice experiencing early- and late-stage transplant arteriosclerosis (8). The analysis revealed that macrophage-mediated immune responses play an essential role in early disease, while B cell–mediated immune responses become important later. Additionally, T cell–mediated responses dominated throughout both stages. Interestingly, further characterization indicated that CD8-expressing T cells, and not CD4-expressing helper T cells, are the likely interferon-gamma producers, key contributors to transplant arteriosclerosis. The study also uncovered cell–cell communication networks coordinated by the CXCR3-CC21 receptor-ligand pair that have therapeutic potential.

Graft-versus-host disease (GvHD), characterized by the attack of healthy recipient tissues by alloreactive donor T cells, is another common contributor to graft loss and patient mortality (9). Kim et al. observed that somatic mutations are acquired by activated T cells due to constant activation and proliferation caused by chronic GvHD (10). The authors identified that one specific T-cell receptor (TCR) subtype, Vβ20, underwent a large clonal expansion in their index patient, who developed chronic GvHD after a bone marrow transplant. The T cells expressing this Vβ20 TCR harbored a gain-of-function *mTor* mutation, which increased proliferation and decreased apoptosis. Paired single cell RNA-seq and TCR sequencing revealed a large percentage of cytotoxic CD4-expressing T cells in the patient sample, with the *mTor*-containing Vβ20 clonotype being the most expanded fraction. These findings were used to guide a drug sensitivity screening of the cytotoxic T cells, which showed the patient was more sensitive to HSP90 inhibitors and less sensitive to *mTOR* inhibitors.

Chronic kidney transplant rejection (CKTR) is a poorly understood immune response in renal transplant recipients. To better understand the pathogenesis of CKTR, Liu et al. turned to single cell RNA-seq to classify the cell types and states that drive this process (11). Several cell subclasses showed enrichment in immune activation activities within the CKTR-derived biopsy samples, including cytotoxic T cell lymphocytes, CD8-expressing T cells, memory B cells, as well as classical and non-classical monocytes. Interestingly, the authors also identified a myofibroblast population with high expression levels of collagen and extracellular matrix molecules that were CKTR-specific, indicating an important role of stromal cells. The authors highlight that,

"By identifying cell subpopulations and distinct signaling signatures and by analyzing the expression levels of key molecular functions in cell subtypes, our data will help advance the diagnosis and treatment of CKTR."

Identifying predictive biomarkers for organ failure

Although improving graft survival rates is an important goal, the number of patients needing transplants far exceeds actual organ availability (1). Genomic analyses can help identify better biomarkers that indicate when transplantation will be the most likely outcome and to guide the development of possible therapeutic interventions that may delay the need for transplantation in the first place. Wilson et al. performed single cell nucleus sequencing of renal samples to understand cell-specific gene expression changes in diabetic nephropathy, a leading cause of end-stage renal disease that often requires transplantation (ESRD; 12). The researchers identified increased potassium secretion and angiogenic signaling as hallmarks of early diabetic nephropathy. Additionally, several key immunologic markers from infiltrating immune cells and monocytes were identified, including *TNFRSF1B* which may be a useful urinary marker for diabetic nephropathy progression. In another study, Luo et al. used single cell RNA-seq of peripheral blood mononuclear cells to identify several ESRD-specific transcriptional changes and the transcription factors likely driving those changes (13). Patients with ESRD exhibited decreased cellular proliferation but increased inflammation and cellular metabolism. The authors noted that,

"...the distinctive metabolic features of ESRD-derived immune cells highlighted here...could provide exciting therapeutic opportunities for treating immune aberrations in ESRD."

COVID-19 poses a unique challenge to the field of lung transplantation. Patients with severe SARS-CoV-2 infection may rapidly progress to respiratory failure, Acute Respiratory Distress Syndrome (ARDS), or end-stage organ failure (14). While lung transplantation is a life-saving option, there are many immunologic concerns, including SARS-CoV-2 recurrence and allograft infection by pathogens associated with ventilator-associated pneumonia (15). Bharat et al. performed successful lung transplants of three patients with non-resolving SARS-CoV-2 infection–associated pneumonia. Explanted lung tissue from these patients and postmortem lung biopsies from patients who succumbed to COVID-19 were extensively characterized by various techniques. Although viral transcripts were not detected in the explanted lung tissue, histology and extracellular matrix imaging uncovered the extensive fibrosis left behind by the infection. These findings led the researchers to perform single cell RNA-seq and compare the transcriptional profiles of cells in COVID-19-associated fibrosis with already characterized pulmonary fibrosis populations. Indeed, several cell populations were shared by these two patient populations—basaloid-like epithelial cells, profibrotic macrophages, and myofibroblasts. The authors made special note of biomarkers from a subset of basaloid cells and profibrotic alveolar macrophages that could be useful biomarkers for irreversible lung fibrosis. Altogether, this study demonstrates the potential of scRNA-seq as a valuable tool in the evaluation of transplant candidates.

Conclusion

Successful organ transplantation is one of the most pivotal medical advancements of our time, so much so that several Nobel prizes have been awarded to pioneers in this field (1). There are still many more discoveries and advancements to be made, particularly where transplant immunology is concerned. Single cell RNA-seq and its integration with spatial and multiomic analysis will be a vital tool to characterize this complex biology, identify predictive and diagnostic biomarkers, and discover potential therapeutic targets (4).

References

1. Sayegh MH & Carpenter CB. Transplantation 50 years later—progress, challenges, and promises. *N Engl J Med* 351(26): 2761–6, 2004.
2. Chinen J & Buckley RH. Transplantation immunology: Solid organ and bone marrow. *J Allergy Clin Immunol* 125(2): S324–S335, 2010.
3. Morris PJ. Transplantation—a medical miracle of the 20th century. *N Engl J Med* 351(26): 2678–80, 2004.
4. Malone AF & Humphreys BD. Single cell transcriptomics and solid organ *Transplantation* 103(9): 1776–1782, 2019.
5. Zheng G, et al. Massively parallel digital transcriptional profiling of single cells. *Nat Commun* 8: 14049, 2017.
6. Byrne AJ, et al. Dynamics of human monocytes and airway macrophages during healthy aging and after transplant. *J Exp Med* 217(3): e20191236, 2020.
7. Rossum A, Laher I & Choy JC. Immune-mediated vascular injury and dysfunction in transplant arteriosclerosis. *Front Immunol* 5: 0001–0014, 2015.
8. Cai J, et al. Impact of local allo-immunity and recipient cells in transplant arteriosclerosis. *Circ Res* 127(8): 974–993, 2020.
9. Yu J, et al. Mortality, length of stay and costs associated with acute graft-versus-host disease during hospitalization for allogeneic hematopoietic stem cell transplantation. *Curr Med Res Opin* 35(6): 983–988, 2019.
10. Kim D, et al. Somatic mTOR mutation in clonally expanded T lymphocytes associated with chronic graft versus host disease. *Nat Commun* 11(1): 2246, 2020.
11. Liu Y, et al. Single-cell analysis reveals immune landscape in kidneys of patients with chronic transplant rejection. *Theranostics* 10(19): 8851-8862, 2020.
12. Wilson PC, et al. The single-cell transcriptomic landscape of early human diabetic nephropathy. *Proc Natl Acad Sci U S A* 116(39): 19619–19625, 2019.
13. Luo T, et al. A single-cell map for the transcriptomic signatures of peripheral blood mononuclear cells in end-stage renal disease. *Nephrol Dial Transplant*, 2019.
14. Wu C, et al. Incidence of ARDS and outcomes in hospitalized patients with COVID-19: a global literature survey. *JAMA Intern Med* 180(7): 934–994, 2020.
15. Bharat A, et al. Lung transplantation for patients with severe COVID-19. *Sci Transl Med*, 2020.

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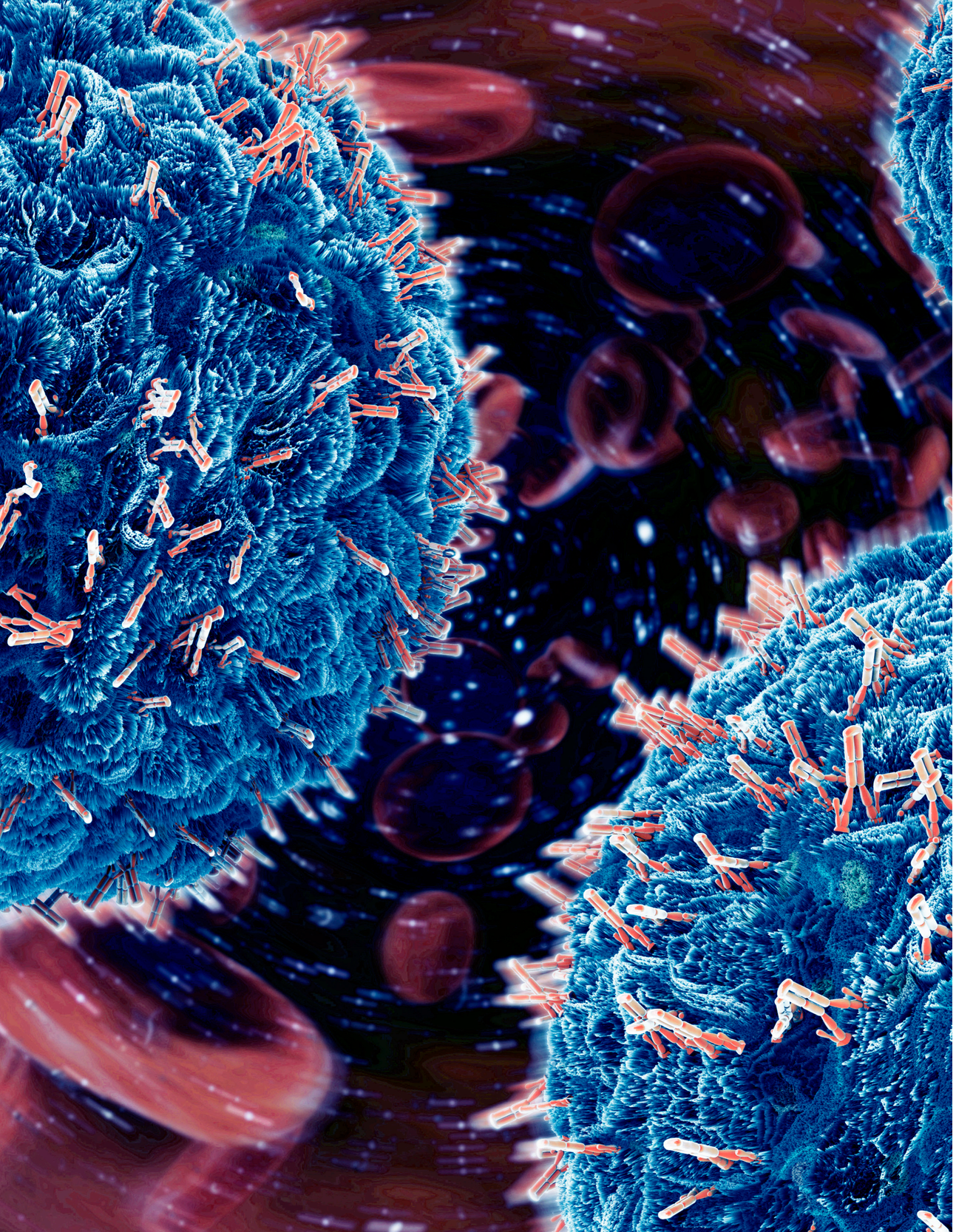

Chapter 7

Cellular and molecular immunology

A roadmap to immunity: Building an immune cell atlas

To truly understand the immune system, scientists must build a detailed immune cell atlas defining the cellular and molecular signatures of immune cells across many different physiological and pathological contexts (1). Even when in steady-state, properly characterizing the immune system is a daunting endeavor due to its sheer vastness and distribution throughout the body. For example, an immune cell type present in different tissues throughout the body may have different subtypes and/or states that allow it to function in the cellular environment where it resides (2). Additionally, the differentiation or response state of any given immune cell will be heavily influenced by its cellular networks and environmental cues. Thus, similar cell types may have widely different transcriptional signatures (2). This complexity and heterogeneity highlight the importance of profiling the immune system at the single cell level and independently within specific tissue contexts (2, 3).

Unlocking the secrets of immunological success in supercentenarians

Performing Chromium Single Cell Gene Expression and Immune Profiling analyses on blood samples from seven supercentenarians (≥110 years), researchers at Keio University School of Medicine in Tokyo discovered a large clonal expansion of rare $CD4^+$ cytotoxic T cells. This suggests that $CD4^+$ T cells expressing a cytotoxic, rather than helper, phenotype may be driving long lives.

Learn more about identifying rare cell populations ⟶

Examining the evolution of innate immune responses

Researchers at the Wellcome Sanger Institute in the UK used Chromium Single Cell Gene Expression analysis to understand the evolutionary divergence of the mammalian innate immune system in humans, macaques, mice, rats, rabbits, and pigs. The expression levels of genes involved in regulating infection response were conserved across species, while genes that diverged rapidly exhibited high levels of expression variability from cell to cell.

Read more about gene expression profiling in the innate immune system ⟶

Tracking immune system development

The adaptive immune system's establishment involves T lymphopoiesis and thymus organogenesis in developing embryos and fetuses (4). Zeng et al. sought to generate a single cell transcriptional atlas of human early T lymphopoiesis throughout embryonic and fetal development using scRNA-seq. Of particular interest was a unique subset of embryonic thymic progenitors (ETPs) transcriptomically similar to fetal liver thymus-seeding progenitors (TSPs). Termed TSP-like ETPs, they hypothesized these cells could be a bridge between TSPs and proliferating ETPs. Trajectory analysis revealed that fetal-liver lymphoid progenitors either retained B-lymphoid lineage potential or deviated towards the T-lymphoid lineage. Additionally, a distinct pre-thymic lymphoid progenitor subtype was identified within the aorta-gonad-mesonephros region, where the first hematopoietic stem cells emerge. Overall, the authors highlight that their "unbiased approach provides informative insight into the biology of hematopoietic cells and [thymic epithelial cells] TEC" and will help to elucidate T cell–related diseases, while informing the creation of several T cell–based therapeutic strategies.

Despite being the hub of T-cell development and education, the thymus gland naturally atrophies as we age, leading to an increased incidence of infection and disease (5). To discern the unique features of human thymic development and function, Park et al. created a detailed cell census of the human thymus across distinct developmental stages (embryonic, fetal, pediatric, and adult) using scRNA-seq and TCR repertoire sequencing (6). This comprehensive census identified more than 50 different human thymus cell states and described how these states change in abundance and transcriptome signatures over time. Several previously undescribed cell types were identified, including an unconventional T-cell subtype expressing CD8aa and GNG4. By combining the insights from single cell sequencing and TCR repertoire analysis, the authors also uncovered that the human thymus shows a strong bias in V(D)J recombination and selection.

Defining immune homeostasis and dsyregulation

Given the importance of the kidneys in maintaining immune homeostasis (7), Stewart et al. sought to define the cellular landscape of the fetal and mature human kidney using scRNA-seq (8). The researchers used specialized bioinformatics analyses to infer the spatial and temporal distribution of both immune and nonimmune cells identified. One interesting finding was that mature kidneys have spatially zonated antimicrobial immunity within the epithelial compartment, but prenatal kidneys do not. Furthermore, proinflammatory and infection-defense transcriptional signatures were found to be acquired by tissue-resident myeloid and lymphoid cells postnatally.

A dysregulated immune system can drive the progression of many diseases, such as atherosclerosis. Fernandez et al. used multiomic single cell analysis, combining transcriptomics with cell surface epitope analysis, to dissect differences in dysregulated immune cells from patients with symptomatic

Profiling macrophage activation states in atherosclerosis

Scientists at NYU School of Medicine wanted to study the full spectrum of macrophage activation states during progression and regression of atherosclerotic plaques. Chromium Single Cell Gene Expression analysis of blood-derived monocytes in mice revealed that, as the cells differentiated, there were more than the two activation states described by the current macrophage polarization model.

Discover how single cell resolution can improve functional characterization of cell states ⟶

and asymptomatic atherosclerosis (e.g., that had or had not recently experienced a stroke or transient ischemic attack; 9). This transcriptional analysis revealed that T cells in plaques from symptomatic patients were enriched for activation, differentiation, and exhaustion markers. Additionally, the analysis found that plaque macrophages from symptomatic patients expressed gene signatures that were suggestive of pro-inflammatory and reparative functions. Interestingly, interleukin-1β signaling was found to occur only in the plaque T cells and macrophages from asymptomatic patients.

Conclusion

As demonstrated by the studies above, single cell sequencing is already contributing to the development of detailed cellular and molecular immune cell profiles. The combination of datasets with insights from spatial, immune repertoire, and other -omic readouts will help advance both basic and translational immunology.

References

1. Chen H, Ye F & Guo G. Revolutionizing immunology with single-cell RNA sequencing. *Cell Mol Immunol* 16(3): 242–249, 2019.
2. Stubbington M, et al. Single-cell transcriptomics to explore the immune system in health and disease. *Science* 358(6359): 58–63, 2017.
3. Gomes T, Teichmann SA & Talavera-López C. Immunology driven by large-scale single-cell sequencing. *Trends Immunol* 40(11): 1011–1021, 2019.
4. Zeng Y, et al. Single-cell RNA sequencing resolves spatiotemporal development of pre-thymic lymphoid progenitors and thymus organogenesis in human embryos. *Immunity* 51: 1–19, 2019.
5. Lynch HE, et al. Thymic involution and immune reconstitution. *Trends Immunol* 30(7): 366–373, 2009.
6. Park JE, et al. A cell atlas of human thymic development defines T cell repertoire formation. *Science* 367(6480): eaay3224, 2020.
7. Tecklenborg J, et al. The role of the immune system in kidney disease. *Clin Exp Immunol* 192(2): 142–150, 2018.
8. Stewart BJ, et al. Spatiotemporal immune zonation of the human kidney. *Science* 365: 1461–1466, 2019.
9. Fernandez DM, et al. Single-cell immune landscape of human atherosclerotic plaques. *Nat Med* 25: 1576–1588, 2019.

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Technology focus

Tools for a new era of immunological discovery

The path to tackling important questions in immunology research is filled with significant challenges due to the complex, dynamic, and heterogeneous nature of the immune system and the limitations of prevailing research tools. Exploration and characterization of immune cells have been historically fueled largely by flow cytometry and mass cytometry. These methods allow the characterization of cells into distinct types and states based on predetermined cell surface and intracellular protein markers (1). However, their readouts are limited in the number of classification parameters that can be measured at a time, masking the immune system's true complexity (2). Lastly, these methods are not able to distinguish the genetic diversity of clonal antigen receptor sequences (1).

For this next stage of discovery, immunologists need tools that allow them to fully appreciate all the possible immune cell types and subtypes, differentiation stages, and response states elicited by a particular pathogen, disease, or therapeutic. Single cell -omics methods provide the means to comprehensively capture the heterogeneity of the immune system through a variety of modular parameters—transcriptomic, proteomic, epigenomic, or a combination of these modalities (3). Furthermore, because immune cell states and functions are influenced by their microenvironment, spatial profiling methods will play a critical role in interpreting how cellular context influences immune response. Here, we provide an overview of single cell sequencing and spatial profiling methods available for the immunologist's toolbox.

Single cell RNA-sequencing

At the core of cell diversity and heterogeneity is differential gene expression. Knowing a cell's transcriptome can provide insight into how it developed, what it's doing, and how it may react to external stimuli. Bulk RNA-sequencing has played an important role in understanding the immune system; however, the readout is an ensemble average that masks any rare cell types present in a sample and also masks the nuances between the unique functional roles that each individual cell type has to play. Single cell RNA-sequencing (scRNA-seq) provides an unbiased readout of gene expression at the single cell level based on the sequencing of cDNA derived from mRNA transcripts. Thus, immunologists are able to identify new cell subtypes and rare cell populations based on transcriptome signatures.

By default, scRNA-seq gene expression libraries provide whole transcriptome profiling, which means they include every transcript expressed and captured within a cell. However, if you are only interested in a subset of genes that are relevant to your particular research question it is possible to enrich your gene expression library for transcripts of interest, focusing your results and reducing your sequencing needs. For discovery applications, or when you want a full characterization of every cell, you can use whole transcriptome analysis.

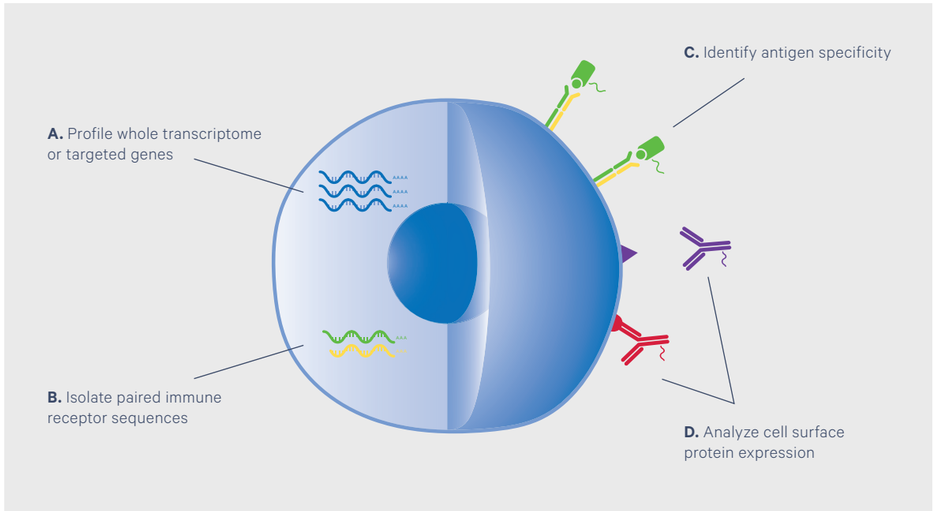

Figure 6. Multiomic integration of immune cell diversity. Immune cells carry a wealth of information that can be profiled to characterize individual cell type and state, including whole transcriptome or targeted gene expression **(A)**; paired, full-length receptor sequences of T or B cells **(B)**; antigen specificity **(C)**; and cell surface proteins **(D)**.

Single cell immune profiling

A rigorous understanding of lymphocyte functionality requires the study of clonotype as well as transcriptome. Single cell immune profiling provides gene expression profiles plus TCR and/or Ig immune repertoires. With full-length, paired T-cell and/or B-cell receptor sequence, you can match clonotype expansion to cell type and state.

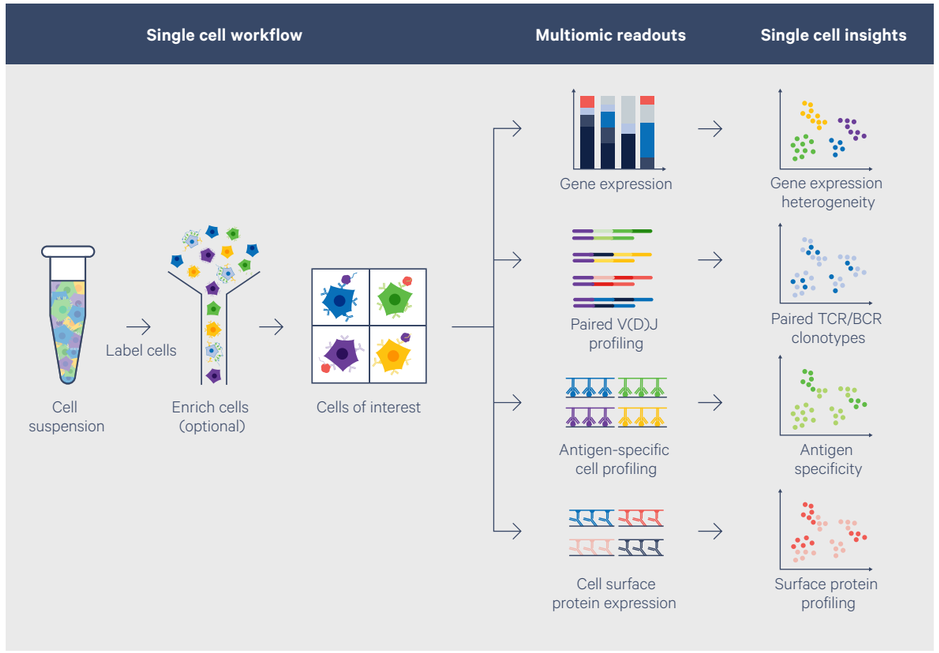

Figure 7. Multiomic immunology lets you access multiple types of information at once for thousands to millions of single cells.

Multiomic cytometry

This method allows you to measure the existence and prevalence of specific biomolecular analytes by turning a next-generation sequencer into an ultra-high parameter cytometric detector. Multiomic cytometry provides a cell-by-cell readout similar to flow cytometry or mass cytometry but overcomes the limitations of traditional methods by tagging antibodies with DNA barcodes rather than fluorophores or heavy metals, creating almost unlimited potential for feature identification. Profile hundreds of cell surface markers at once and, if desired, accompanying transcriptome, TCR/BCR sequence, or antigen specificity in a single workflow.

Immune receptor mapping

Immune receptor mapping provides another level of information beyond single cell immune profiling. In addition to gene expression, immunophenotyping with cell surface markers, and TCR/BCR sequences, you can characterize antigen specificity and immunophenotype with cell surface markers. Similar to multiomic cytometry, cell surface marker antibodies and antigen-specific multimers are barcoded with DNA oligonucleotides, providing almost infinite co-detection possibilities. Immune receptor mapping provides cell type, cell state, clonotype, and antigen-binding information from the same single cells, helping you track vaccine response and accelerate antibody discovery.

Single cell epigenomics

The transcriptome is ultimately the byproduct of a highly coordinated program of gene expression regulated by the epigenome. The assay for transposase-accessible chromatin (ATAC) is a sequencing method that surveys the physical structure of the genome by identifying regions of open chromatin. By providing information about chromatin accessibility, performing ATAC at the single cell level can help reveal areas of active gene transcription, as well as regulatory regions, binding site motifs, and whether lowly expressed genes like transcription factors are likely turned on in individual cells. Even deeper insights into cell identity can be gained through the integration of transcriptomic and epigenomic profiles from the same single cell.

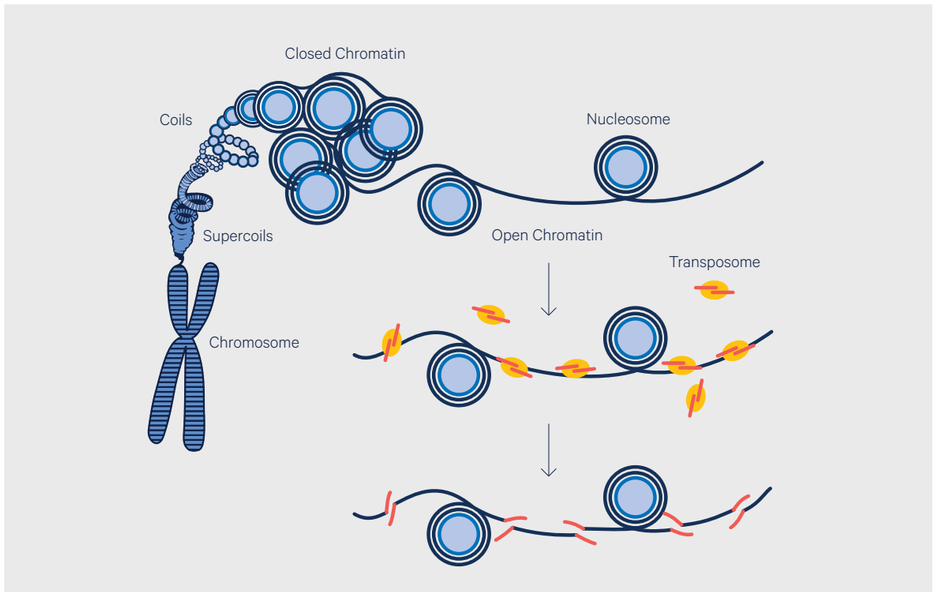

Figure 8. Regions of open chromatin correlate with areas of active gene transcription. The assay for transposase-accessible chromatin (ATAC) works by generating short fragments of DNA specifically within open chromatin regions. Mapping these cut sites back to the genome provides a window into transcription factor motif binding, promoter and enhancer regions, and areas of euchromatin versus heterochromatin.

Spatial transcriptomics and proteomics

How cells are organized within a tissue and the regional context of cell heterogeneity is integral to biological function. Slide-based spatial transcriptomics and proteomics technologies allow for the determination of gene and protein expression patterns in the context of tissue sections.

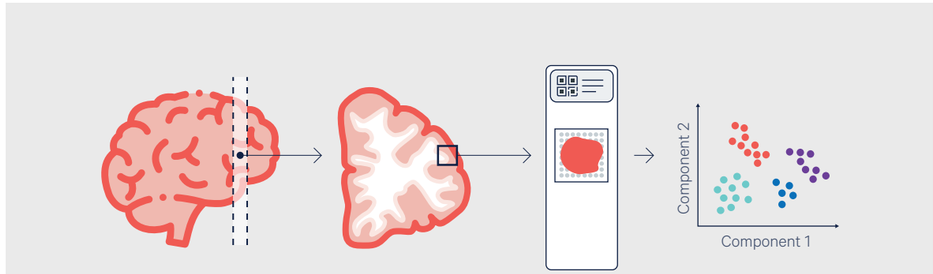

Figure 9. Slide-based spatial capture technologies allow researchers to layer transcriptome data onto histology and immunofluorescence images to understand tissue microenvironments.

Conclusion

Immunology's "Age of Discovery" is far from over (1). In fact, immunologists are entering a critical stage in their understanding of the immune system that will revolutionize how they approach and treat immune-related diseases and allow for the development of actionable insights. Single cell multiomics and spatial profiling will play a crucial role in building a more complete immune cell atlas that will lead to actionable insights.

References

1. Stubbington M, et al. Single-cell transcriptomics to explore the immune system in health and disease. *Science* 358(6359): 58–63, 2017.
2. Chen H, Ye F & Guo G. Revolutionizing immunology with single-cell RNA sequencing. *Cell Mol Immunol* 16(3): 242–249, 2019.
3. Gomes T, Teichmann SA & Talavera-López C. Immunology driven by large-scale single-cell sequencing. *Trends Immunol* 40(11): 1011–1021, 2019.

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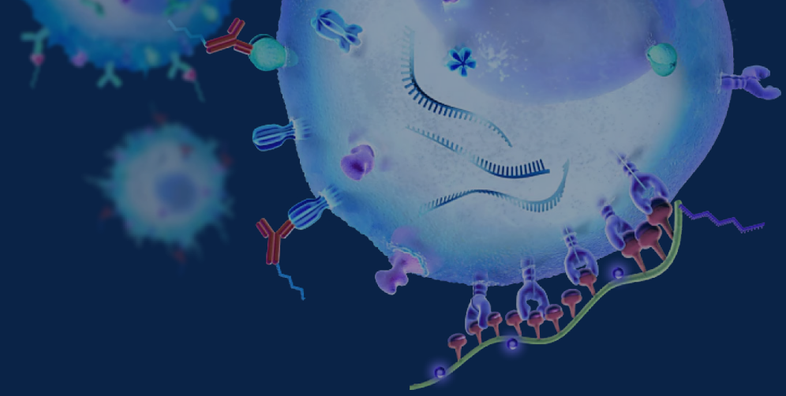

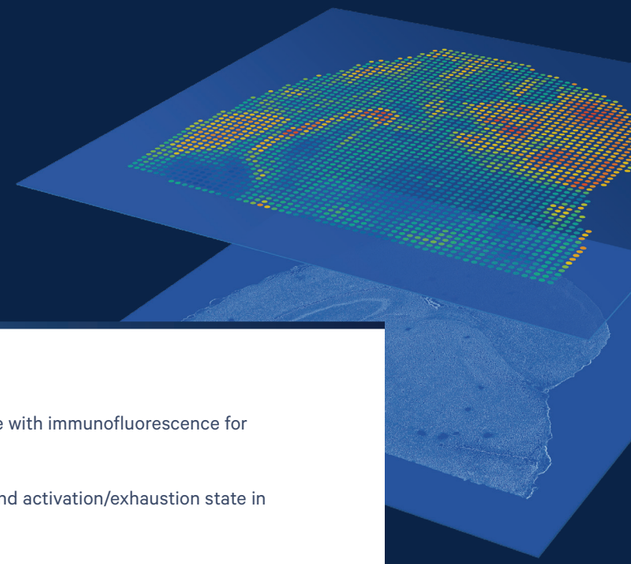

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