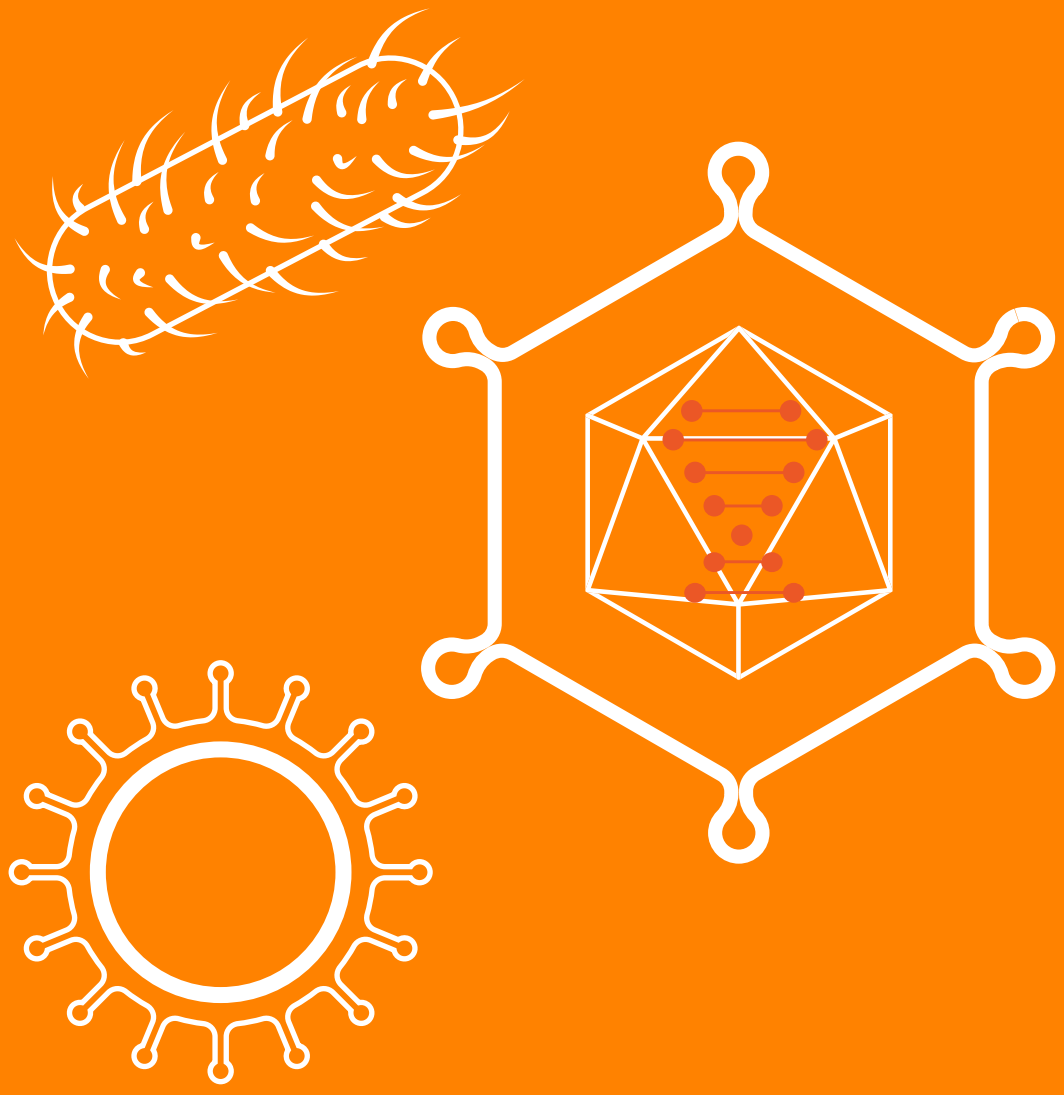

immunoSEQ®

Infectious Disease and the Immune System

An overview of publications featuring the immunoSEQ® Technology

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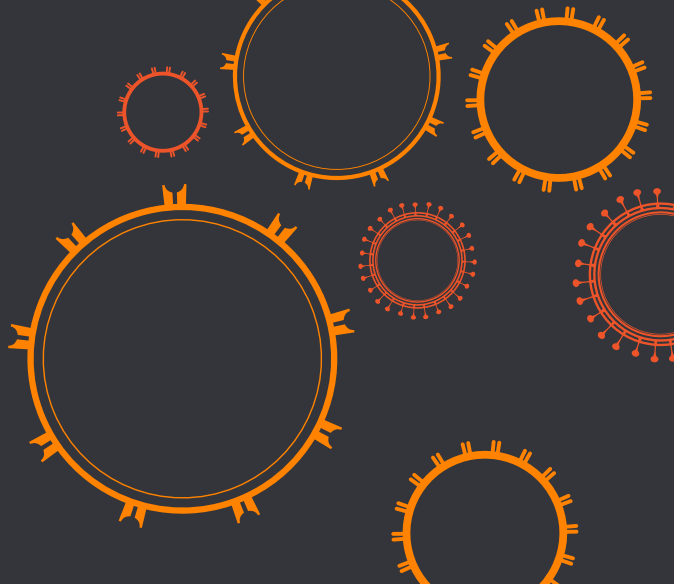
Introduction



Infectious diseases are a leading cause of death worldwide, particularly in low-income countries and in young children. Approximately 7.9 million deaths worldwide in 2019 were caused by infectious disease.[1] Vaccination programs and general improvements in global health have already reduced the global burden of infectious disease,[2] and further introduction of health policies, vaccines, and treatments can improve the global situation even more.

Infectious diseases refer to the group of conditions caused by bacteria, viruses, fungi, or parasites and can be transmitted from person to person, via insects or other animals, by consuming contaminated food or water, or via environmental exposure. Frequent handwashing helps protect from many infectious diseases, and several can be prevented by vaccines.[2]

T cells play an important role in the immune response to infectious disease. An infected or antigen-presenting cell contains disease-specific markers on its surface: antigens presented by Human Leucocyte Antigens (HLAs). T-cell receptors (TCRs) specifically recognize HLA-presented antigens and activate an immune response, by directly killing infected cells through production of cytokines such as IL-2 and IFNγ or helping to direct the humoral response.

When an individual's immune system is exposed to an antigen, hundreds or even thousands of T cells, with different TCRs may be activated and expand, of which most are rare in a population and difficult to associate to a specific disease. Some TCRs, however, are observed in multiple individuals in a population. These can be considered "public sequences" and can be helpful in the development of disease classifiers, i.e., tools that allow the assessment of an individual's past or current infection state with a specific disease.

The T-cell repertoire, therefore, gives insight into the immune status of an individual or a population, and better understanding across temporal or population scales can help drive further vaccine and treatment development.

1. Global Burden of Disease: GBD cause and risk summaries. Accessed September 10, 2021. https://www.thelancet.com/gbd/summaries.

2. Vos T, et al. *The Lancet*. 2020;396(10258):1204-1222. doi:10.1016/S0140-6736(20)30925-9.

3. Fu L, et al. *J Infect*. 2020;80(6):656-665. doi:10.1016/j.jinf.2020.03.041.

4. Zuhair M, et al. *Reviews in Medical Virology*. 2019;29(3):e2034. doi:10.1002/rmv.2034.

5. Bristow BN, et al. *PLoS Negl Trop Dis*. 2011;5(4):e1140. doi:10.1371/journal.pntd.0001140.

6. Boeckh M, et al. *J Clin Invest*. 2011;121(5):1673-1680. doi:10.1172/JCI45449.

7. Tokars JI, et al. *Clin Infect Dis*. 2018;66(10):1511-1518. doi:10.1093/cid/cix1060.

8. FastStats. Published August 3, 2021. Accessed September 13, 2021. https://www.cdc.gov/nchs/fastats/flu.htm.

9. Khan G, et al. *BMJ Open*. 2020;10(8):e037505. doi:10.1136/bmjopen-2020-037505.

10. Wutzler P, et al. *Expert Rev Vaccines*. 2017;16(8):833-843. doi:10.1080/14760584.2017.1343669.

11. US EPA O. Climate Change Indicators: Lyme Disease. Published July 1, 2016. Accessed October 15, 2021. https://www.epa.gov/climate-indicators/climate-change-indicators-lyme-disease.

Moreover, understanding the TCR repertoire of an individual can help contextualize results in oncology and transplant studies.

This publication review will focus on viral and bacterial infectious diseases, their interaction with T cells, and related research. SARS-CoV-2, the virus responsible for COVID-19, will not be discussed in this review as it has been discussed in detail in our COVID-19 Publication Review, which you can download here. This document is an overview of infectious disease publications featuring the immunoSEQ Technology. Some of the research studies described in this document were performed by or in collaboration with Adaptive. The diseases, infectious agents, and their key characteristics are summarized in Table 1.

Infectious disease	Name of infectious agent	Type of infection	Prevalence rate per 100,000/ year	Incidence rate per 100,000/ year	Death rate per 100,000/ year	Key symptoms	Route of transmission
COVID-19	SARS-CoV-2	Viral	2,822.3	No data available	57.6	Fever, dry cough[3]	Droplets, aerosol
CMV infection		Viral	83k[4]	No data available	1.7	Typically asymptomatic[5]	Via bodily fluids (saliva, breast milk, blood, semen, vaginal secretions)[6]
HIV infection, can lead to AIDS	HIV	Viral	454.3[1]	25.2[1]	10.7[1]	Can lead to AIDS if untreated, opportunistic infections[1]	Sexual transmission, unsafe needle use
Influenza (Flu)	Influenza Virus	Viral	No data available	8.3k[7]	15.2[8]	Fever, cough	Droplets, aerosol
Epstein-Barr	Epstein-Barr Virus	Viral	No data available	3.5[9]	2.2[9]	Extreme fatigue, fever, sore throat	Via body fluids, especially saliva
Hepatitis B	Hepatitis B Virus (HBV)	Viral	115.7[1]	1002.4[1]	0.4[1]	Liver infection[1]	Sexual transmission, unsafe needle use, from mother to child[1]
Hepatitis C	Hepatitis C Virus (HCV)	Viral	8.5[1]	73.9[1]	0.1[1]	Liver infection[1]	Unsafe needle use and during medical procedures[1]
Hepatitis E	Hepatitis E Virus (HEV)	Viral	20.5[1]	267.4[1]	0.0[1]	Liver disease[1]	Fecal-oral[1]
Zika	Zika Virus	Viral	0.1[1]	3.4[1]	0.0[1]	Mild febrile illness, birth defects associated with infection during pregnancy[1]	Mosquitos[1]
Chickenpox	varicella-zoster virus	Viral	No data available	300-1,291[10]	0.5[10]	Rash, mild fever	Direct contact with vesicular fluid or aerosol inhalation
Tuberculosis	Mycobacterium tuberculosis	Bacterial	23,085.1[1]	106.7[1]	14.6[1]	Cough	Droplets, aerosol
Lyme disease	Borrelia burgdorferi	Bacterial	No data available	7.21[11] (US)	No data available	Fever, fatigue, joint pain, skin rash, and more serious joint and nervous system complications[11]	Ticks

Infectious disease	Name of infectious agent	Type of infection	Prevalence rate per 100,000/ year	Incidence rate per 100,000/ year	Death rate per 100,000/ year	Key symptoms	Route of transmission
Malaria	Plasmodium falciparum, Plasmodium vivax	Parasite	2,466.3[1]	3,247.0[1]	9.0[1]	Fever, flu-like symptoms	Mosquitos[1]
Intestinal nematode infections	Ascaris umbricoides, Trichuris trichiura, Ancylostoma duodenale, Necator americanus	Parasite	12,095.9[1]	No data available	0.01	Abdominal cramping and swelling, fever, vomiting, nausea	Fecal-oral, consumption of uncooked meat or contaminated water

Table 1. Key infectious diseases and their characteristics.

Cytomegalovirus infection

Human cytomegalovirus (CMV) is one of the most common viruses that infect humans, with a seroprevalence of 50-100%.[1]

CMV spreads from person to person via bodily fluids, including saliva, breast milk, blood, semen, and vaginal secretions.[1] In most cases, CMV infection is asymptomatic and dormant. For immunocompetent individuals, a CMV infection will remain latent throughout their lifetime.[1]

However, infection has severe consequences in immunocompromised populations and is a significant cause of morbidity and mortality in people living with HIV/AIDS and transplant recipients.[2] Moreover, CMV infection has been associated with an increased risk of cancer progression and other diseases,[1] and research has shown an impact on longevity.[3] Congenital CMV infection (transmission from seropositive mother to fetus) is a leading non-genetic cause of hearing loss in children.[1]

While CMV is asymptomatic in most cases, its lack of clearance by the immune system and persistent, latent presence in the body results in the immune system acting on constant high alert through an interplay between the virus that is focused on the survival, and the host immune system that continuously ensures viral suppression.[4] The persistent viral infection drives the expansion of memory T cells, also known as T-cell memory inflation (Figure 1).[5]

1. Boeckh M, et al. *J Clin Invest*. 2011;121(5):1673-1680. doi:10.1172/JCI45449.

2. Emery V. *J Clin Pathol*. 2001;54(2):84-88. doi:10.1136/jcp.54.2.84.

3. Jergovic M, et al. *Med Microbiol Immunol*. 2019;208(3-4):263-269. doi:10.1007/s00430-019-00605-w.

4. Khairallah C, et al. *Front Immunol*. 2017;8:105. doi:10.3389/fimmu.2017.00105.

5. Taylor-Robinson A, et al. *Journal of Immunology and Infectious Diseases*. 2015. doi:10.15744/2394-6512.1.205.

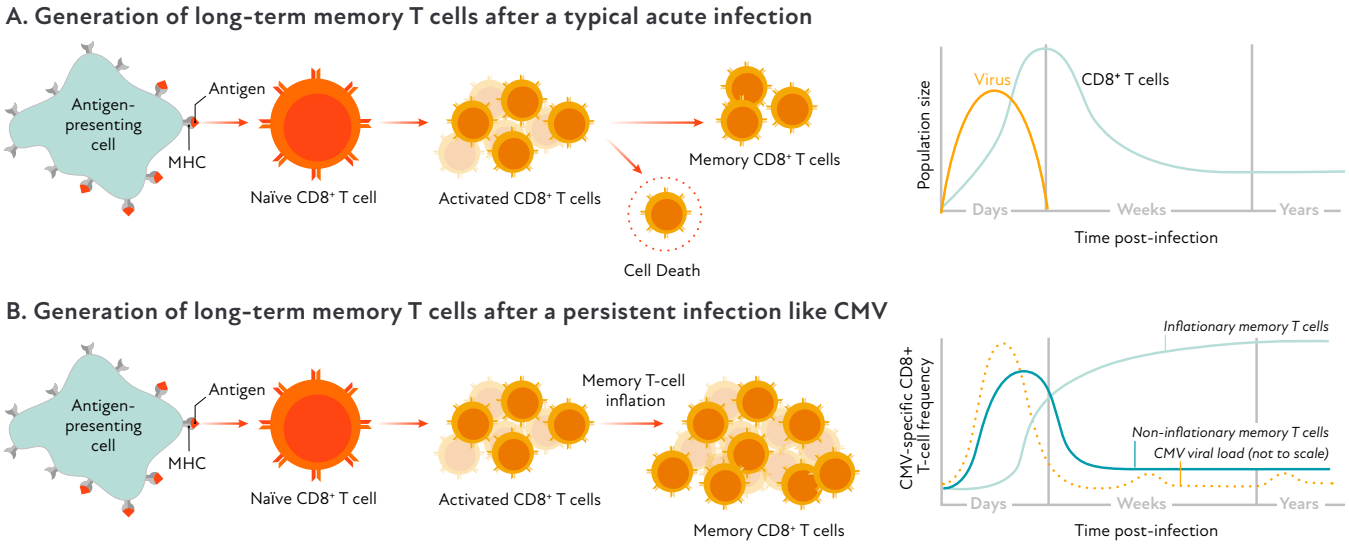


Figure 1. Normally, in acute infection (A), virus-specific CD8+ T cells expand and contract, leaving behind a small population of memory T cells to recognize subsequent viral exposure. In a persistent infection like CMV (B), numbers of virus-specific CD8+ T cells remain high and can constitute a significant portion of the T-cell repertoire. Adapted from Taylor-Robinson and Chapman.[5] APC: antigen-presenting cell; tv1HC: major histocompatibility complex.

It's essential to understand if and how an individual's immune system has been affected by CMV, especially in the context of vaccine or transplant studies, to better understand trial outcomes.[6] To this end, the immunoSEQ® assay has been used to characterize the effects of CMV infection on the T-cell repertoire, including in the context of transplantation and during aging.

The immunoSEQ CMV Classifier has been developed to determine if an individual has a CMV infection. The Classifier allows researchers to gain insight into how a latent infection may affect other aspects of the immune system (Table 2).[7]

6. Schober K, et al. *Immunol Rev.* 2018;283(1):113-128. doi:10.1111/imr.12654.

7. Emerson RO, et al. *Nat Genet.* 2017;49(5):659-665. doi:10.1038/ng.3822.

Clinical trial research	• Track drug/vaccine response longitudinally across samples in the context of CMV status • Understand drug-related adverse effects and how they are affected by CMV infection • Investigate the effect of CMV infection on repertoire structure and diversity
Infectious Diseases	• Does CMV infection predispose subjects to other viral infections? • Capture differences or similarities in T-cell immune responses to various diseases in the context of CMV status • Explore the impact of CMV status on vaccine response
Autoimmunity & Transplantation	• Understand the relationship between CMV infection and autoimmune response or development of autoimmune diseases • Study effects of CMV status on immune reconstitution during transplantation • Evaluate the role of CMV infection on transplant outcomes
Oncology	• Assess the relationship between immunotherapy outcomes and CMV status • Add CMV status to hematopoietic cell transplant (HCT) studies to better understand immune reconstitution dynamics

Table 2. CMV classifier research questions and use cases

Key Takeaways

- CMV infection is extremely widespread with a seroprevalence of 50-100%, and while asymptomatic in most, it may cause complications for immunocompromised individuals.
- A chronic and latent CMV infection causes constant strain on the immune system and may affect its response to other infections, transplants, and cancer.
- The immunoSEQ Assay combined with machine learning techniques has been used to identify public T-cell receptor sequences to develop the immunoSEQ CMV Classifier.
- immunoSEQ CMV Classifier allows researchers to assess an individual's CMV status by looking at their T-cell repertoire, with important implications in infectious disease, transplant, and oncology research.

immunoSEQ Publications

Single-cell analysis shows that adipose tissue of persons with both HIV and diabetes is enriched for clonal, cytotoxic, and CMV-specific CD4⁺ T cells
Wanjalla CN, et al. Cell Reports Medicine, 2021.

In this study, researchers showed a correlation between the co-incidence of HIV and diabetes with CMV-specific T cells in adipose tissue. Large-scale sequencing was utilized to sequence TCR repertoires to identify clonality in diabetic patients with or without diabetes.

Impact of HLA type, age and chronic viral infection on peripheral T-cell receptor sharing between unrelated individuals
Johnson SA, et al. PLoS One, 2021.

TCR repertoire sequencing revealed that in a study of 426 healthy participants, CMV negative individuals shared more TCRβ clones than CMV positive individuals. These results suggest that TCRβ become more private after CMV antigen exposure, likely through specific expansion of clones unique to the individual. This study exemplifies how T-cell repertoire sequencing can be linked to HLA status and compared across individuals.

CD8⁺ γδ T cells are more frequent in CMV seropositive bone marrow grafts and display phenotype of an adaptive immune response
Gaballa A, et al. Stem Cells International, 2019.

TCR γ-chain sequencing of bone marrow donors showed a higher frequency of CD8⁺ γδ T cells in CMV seropositive donors compared to CMV seronegative donors. The immunoSEQ Assay was used for initial handling of sequencing data and identifying diversity, clonal space homeostasis, V-J segment usage, CDR3 spectratyping, and repertoire overlap between the samples.

Graft γδ TCR sequencing identifies public clonotypes associated with hematopoietic stem cell transplantation efficacy in acute myeloid leukemia patients and unravels cytomegalovirus impact on repertoire distribution
Arruda LCM, et al. Journal of Immunology, 2019.

This study aimed to analyze the TCR γ-chain repertoire of γδ T cells within donor stem cell grafts and investigate its potential impact on the clinical response in the corresponding transplant recipients. Grafts from CMV seropositive donors in acute myeloid leukemia recipients with disease relapse and acute graft-versus-host disease showed a more private repertoire, lower diversity, and displayed overrepresented clones. These results may help illuminate the role of γδ T cells in transplant-related morbidity and mortality following allogeneic human stem cell transplants due to CMV reactivation, primary disease relapse, and graft-versus-host disease.

Cytomegalovirus exposure in the elderly does not reduce CD8 T cell repertoire diversity
Lindau P, et al. Infectious Disease, 2018.

By sequencing TCR β-chains, this research explores the clonal expansion due to CMV infection and its burden on the aging adaptive immune system. Results show that CMV-associated clones expand in the elderly population sample without altering the diversity of the rest of the T-cell repertoire, suggesting that reduced immunity in seropositive elderly individuals is not directly due to a reduction in $CD8^+$ T-cell repertoire diversity but rather due to alterations in cell function.

Memory $CD4^+$ T cell receptor repertoire data mining as a tool for identifying cytomegalovirus serostatus
De Neuter N, et al. Genes & Immunity, 2018.

The authors show a novel approach to determine cytomegalovirus serostatus using T-cell repertoire data mining of $CD4^+$ memory T cells.

Immunosequencing identifies signatures of cytomegalovirus exposure history and HLA-mediated effects on the T cell repertoire
Emerson RO, et al. Nature Genetics, 2017.

In this study, the T-cell repertoire of 666 subjects with known CMV serostatus was immunosequenced to determine whether pathogen exposure signatures could be identified. This research was crucial for developing the classification framework to diagnose CMV status based on a catalog of known TCRβ sequences with high specificity and sensitivity and accurately predicting HLA-A and HLA-B alleles of the original research cohort.

HIV

Human immunodeficiency virus, or HIV,[1] includes two species of the Lentivirus retrovirus that infect humans, and overtime cause acquired immunodeficiency syndrome (AIDS), a progressive failure of the immune system that allows other infections or cancer to thrive.[2]

According to the WHO, almost 36.3 million lives have been claimed by HIV since it first appeared, and in 2020 an estimated 37.7 million individuals were living with HIV worldwide. Thanks to education and preventative measures, the number of new infections per year has been reduced by 31% since 2010, but there are still an average of 1.5 million newly infected people annually. (WHO)

The most common method of HIV transmission is through sex and the use of contaminated needles.[1] Sexual transmission can be prevented by condom usage and availability of pre-exposure prophylaxis (PrEP),[3] especially among high-risk populations. Mother-to-child transmission initially contributed significantly to transmission, but rates are now below 5% of total cases, thanks to preventative measures.[3]

Due to rapid mutations of HIV, the virus is able to evade the immune system.[4] One mechanism involves the HIV accessory protein Nef, which causes downregulation of HLA class I molecules in infected cells, resulting in the suboptimal presentation of HIV peptides to cytotoxic T cells.[5]

HIV preferentially targets $CD4^+$ T cells, leading to cell death through direct and indirect mechanisms.[1] This reduction in $CD4^+$ cell count, coupled with an increase in $CD8^+$ cell count, leads to an inversion of the $CD4^+$:$CD8^+$ ratio, an indicator typically used to predict non-AIDS morbidity.[6] Research has shown that antiretroviral therapy only partially remedies this ratio inversion and may need to be taken into account when predicting the risk of non-AIDS morbidity.[7]

1. Deeks SG, et al. *Nat Rev Dis Primers*. 2015;1(1):15035. doi:10.1038/nrdp.2015.35.

2. Douek DC, et al. *Annu Rev Med*. 2009;60:471-484. doi:10.1146/annurev.med.60.041807.123549.

3. Ghosn J, et al. *The Lancet*. 2018;392(10148):685-697. doi:10.1016/S0140-6736(18)31311-4.

4. Koup RA, et al. *J Virol*. 1994;68(7):4650-4655. doi:10.1128/JVI.68.7.4650-4655.1994.

5. Schwartz O, et al. *Nat Med*. 1996;2(3):338-342. doi:10.1038/nm0396-338.

6. Leung V, et al. *PLOS ONE*. 2013;8(10):e77665. doi:10.1371/journal.pone.0077665.

7. McGettrick PMC, et al. *Germs*. 2018;8(2):54-57. doi:10.18683/germs.2018.1131.

Markers of T-cell activation and exhaustion have also been seen in people living with HIV (Figure 2), especially in the context of HIV/CMV coinfection, which exceeds 90%.[1,8] Understanding the T-cell status, $CD4^+$:$CD8^+$ ratio, and markers for typically latent or acute opportunistic coinfections can help predict HIV treatment outcomes, aid vaccine development, and gain insight into population effects of HIV infections. Immunosequencing may provide part of the solution to contextualize the T-cell repertoire.

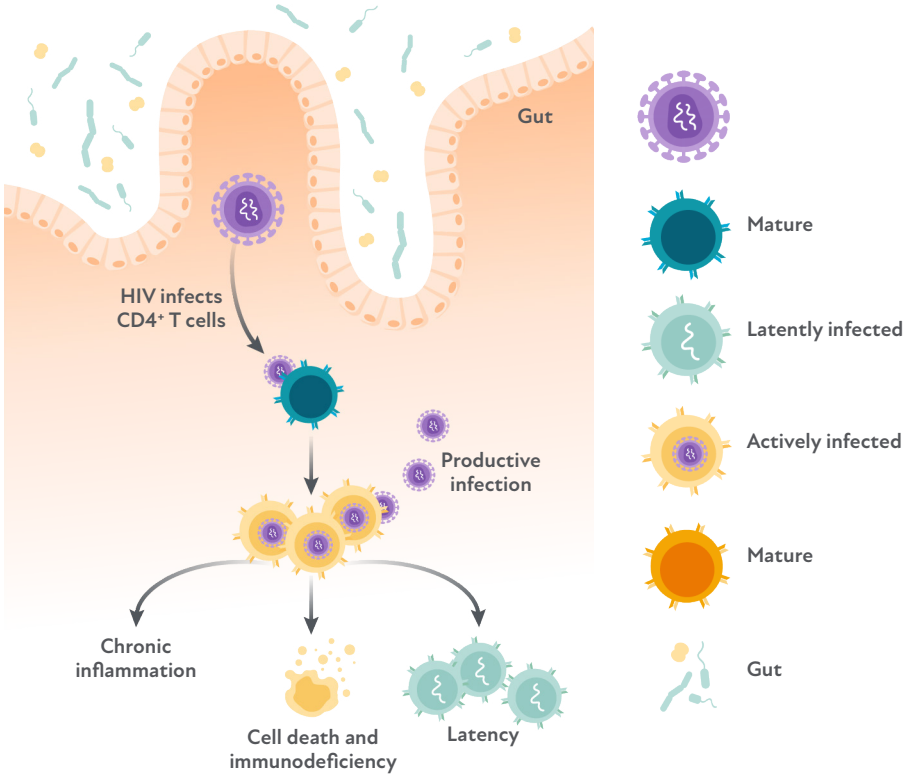


Figure 2. HIV infection affects the T-cell repertoire by preferentially infecting $CD4^+$ cells, leading to cell death and an inverted $CD4^+$:$CD8^+$ ratio. This T-cell depletion causes immunodeficiency and makes way for opportunistic infections and cancers to thrive. Adapted from Deeks et al.[1]

Key Takeaways

- HIV infection preferentially targets $CD4^+$ cells and leads to an inversion of $CD4^+$:$CD8^+$ ratio, as well as T-cell exhaustion.
- Co-infection with CMV is common among people living with HIV and affects the immune repertoire.
- Immunosequencing provides a solution to contextualize the T-cell repertoire in studies aiming to develop HIV vaccines and treatments

8. Booiman T, et al. *PLOS ONE*. 2017;12(8):e0183357. doi:10.1371/journal.pone.0183357.

immunoSEQ Publications

In chronic infection, HIV gag-specific CD4⁺ T cell receptor diversity is higher than CD8⁺ T cell receptor diversity and is associated with less HIV quasispecies diversity
Pilkinton MA, et al. Journal of Virology, 2021.

The authors measured the diversity of T cells responding to HIV Gag proteins, showing that the CD8+ T-cell response consisted of a few dominant TCRs, while the CD4⁺ T-cell subset was highly diverse. This correlated with lover HIV Gag genetic diversity and therefore may act to limit HIV diversity and thus viral evolution.

Single-cell analysis shows that adipose tissue of persons with both HIV and diabetes is enriched for clonal, cytotoxic, and CMV-specific CD4⁺ T cells
Wanjalla CN, et al. Cell Reports Medicine, 2021.

In this study, researchers showed a correlation between the co-incidence of HIV and diabetes with CMV-specific T cells in adipose tissue. Large-scale sequencing was utilized to sequence TCR repertoires to identify clonality in diabetic patients with or without diabetes.

A T cell receptor sequencing-based assay identifies cross-reactive recall CD8⁺ T cell clonotypes against autologous HIV-1 epitope variants
Hok Yee Chan, et al. Frontiers in Immunology. 2020.

This study focused on detecting and quantifying the HIV-1 specific cytotoxic lymphocyte (CTL) response hypothesized to be required for HIV-1 positive elite controllers and suppressors to control viral replication without antiretroviral therapy. Further, the authors wanted to provide a proof-of-principle to demonstrate the utility of a modified version of MANAFEST, ViraFEST, which uses TCRβ sequencing of memory CD8⁺ T-cell responses against autologous HIV-1 Gag and Nef epitope variants in an elite suppressor. Results showed that the ViraFEST Assay (which relied on the immunoSEQ Assay) could detect cross-reactive CD8⁺ T-cell responses in an HIV-1+ elite suppressor. The authors propose that routine use of the ViraFEST Assay to detect and monitor T-cell responses to HIV-1 antigens could identify novel antigens that could be exploited in future T-cell-based immunotherapy or vaccine.

Antigen-driven clonal selection shapes the persistence of HIV-1–infected CD4⁺ T cells in vivo
Simonetti FR, et al. The Journal of Clinical Investigation, 2020.

This study aimed to combine provirus, integration site, and TCRβ analysis to understand antigen-driven proliferation relative to other drivers of clonal expansion. Through isolation of CMV- and Gag-responding CD4⁺ T cells, the authors showed that proviral populations were dominated by large clones, driven by converging adaptive responses. Their findings suggest that antigen-driven selection is a major factor in HIV-1 persistence, which will create challenges in treatment efforts.

Unbiased Profiling Reveals Compartmentalization of Unconventional T-Cells Within the Intestinal Mucosa Irrespective of HIV Infection
Magnoumba M, et al. Frontiers in Immunology, 2020.

The authors set out to study the overall impact of HIV on unconventional T cells across the gut mucosa through mass cytometry, flow cytometry, and T-cell receptor profiling. They found distinct compartmentalization of T cells between blood, duodenum, and colon. However, no significant impact of HIV infection on any of the unconventional T-cell subsets was observed in frequency or TCR repertoire.

Cross-reactive microbial peptides can modulate HIV-specific CD8+ T cell responses
Pohlmeyer CW, et al. PLOST ONE, 2018.

In this study, the authors studied the process of heterologous immunity as a method to modulate the HIV-1-specific CD8⁺ T-cell response. They found that CD8⁺ T cells stimulated with either HIV-1 or cross-reactive peptides effectively suppressed HIV-1 replication in autologous CD4⁺ T cells, suggesting that exposure to cross-reactive microbial antigens can modulate HIV-1-specific immunity.

Other viral infections and vaccine development

immunoSEQ can help reveal the T-cell response to a variety of other viral infections, help vaccine development, and help researchers understand the effect of co-infections on the TCR repertoire. This section highlights key publications that utilized the immunoSEQ Assay in the context of viral diseases not yet covered.

Novel vaccine development and proofing can be accelerated with the immune response tracking that immunoSEQ helps enable. In supporting efficacy studies, it is paramount to quantitatively track longitudinal expansion or contraction of specific T-cell clones and monitor the T-cell response over time. immunoSEQ allows monitoring immunologic response tracking during vaccine trials. For more details on the use of immunoSEQ during vaccine development specifically, you can download our publication review on "Vaccine & Drug Development" **here**.

immunoSEQ Publications

Epstein-Barr Virus Epitope–Major Histocompatibility Complex Interaction Combined with Convergent Recombination Drives Selection of Diverse T Cell Receptor a and b Repertoires
Gil A, et al. Host-Microbe Biology, 2020.

Using immunosequencing, the authors show a high degree of diversity of EBV-specific clonotypes in acute infectious mononucleosis (AIM), shedding light on the factors driving the selection of TCR repertoires from primary through persistent human virus infections. immunoSEQ was used to analyze both TCRα and TCRβ repertoires.

Deciphering and predicting CD4⁺ T cell immunodominance of influenza virus hemagglutinin
Cassotta A, et al. Journal of Experimental Medicine, 2020.

The authors combined ex vivo stimulation of memory T cells, screening of naive and memory T-cell libraries, T-cell cloning, and TCR sequencing to study the human naive and memory CD4⁺ T-cell repertoire against the influenza pandemic H1 hemagglutinin (H1-HA). Naïve CD4⁺ T cells were found to have a broad repertoire capable of recognizing peptides spanning the whole H1-HA sequence.

Defining Virus-specific CD8⁺ TCR Repertoires for Therapeutic Regeneration of T Cells against Chronic Hepatitis E
Soon CF, et al. Journal of Hepatology, 2019.

The authors sought to investigate the role of CD4⁺ T cells in the protection against neuroinvasive ZIKV disease using a mouse model. Their results show a role for CD4⁺ T cells in protecting the nervous system. The ZIKV antigen-specific TCRβ repertoire showed a high degree of diversity in response to a single epitope and among different mice responding to a CD4⁺ T-cell epitope. Together, these results highlight the need for robust CD4⁺ T-cell responses in vaccine development against ZIKV.

HBV induces inhibitory FcRL receptor on B cells and dysregulates B cell-T follicular helper cell axis
Poonia B, et al. Scientific Reports, 2018.

The authors show expansion of atypical memory subsets of B cells in chronic HBV infection (CD19⁺ CD10⁻ CD27⁻ CD21⁻) that express high levels of FcRL5. This provides evidence that HBV infection results in upregulation of inhibitory pathways in B cells, allowing atypical B cells that lack anti-HBs function to accumulate.

Identifying and Tracking Low-Frequency Virus-Specific TCR Clonotypes Using High-Throughput Sequencing
Wolf K, et al. Cell Reports, 2018.

Using high-throughput TCR sequencing, the authors surveyed circulating TCR repertoires of mice before and after Orthopoxvirus infection to develop a diagnostic assay for Orthopoxvirus infection, which was shown to be 97% accurate up to 9 months post-exposure.

Viral Genetics Modulate Orolabial Herpes Simplex Virus Type 1 Shedding in Humans
Ramchandani MS, et al. Journal of Infectious Diseases, 2018.

This study investigated the role of viral genotype and host immunity in oral HSV-1 shedding rates in monozygotic and dizygotic twins. Mapping the CD4⁺ T-cell response revealed greater agreement in response between monozygotic twins compared to unrelated persons. Thus, viral strain characteristics are likely to contribute the highest to oral HSV-1 shedding rates.

Broad TCR repertoire and diverse structural solutions for recognition of an immunodominant CD8⁺ T cell epitope
Song IY, et al. Nature Structural & Molecular Biology, 2017.

TCR repertoire sequencing revealed that CD8⁺ T cells have distinct TCRs to recognize the influenza HLA-A2-M1 epitope. Broad repertoires lead to plasticity in antigen recognition and protection against T-cell clonal loss and viral escape.

Multidisciplinary study of the secondary immune response in grandparents re-exposed to chickenpox
Ogunjimi B. Scientific Reports, 2017.

A longitudinal study of 36 adults over the course of one year after re-exposure to chickenpox evaluated whether re-exposure boosts varicella-zoster virus (VZV) immunity. Exposed individuals showed a higher percentage of VZV-specific CD4⁺ IL-2 producing T cells and VZV-specific antibodies nine months after exposure, especially in CMV IgG-positive participants. Because only 17-25% of exposed participants showed a boosting effect, and for less than one year, the authors conclude that the protective effect of re-exposure to chickenpox in the elderly is likely limited.

Selective expansion of high functional avidity memory CD8 T cell clonotypes during hepatitis C virus reinfection and clearance
Abdel-Hakeem MS, et al. PLOS Pathogens, 2017.

The authors examined the dynamics and functionality of the CD8 TCR repertoire before, during, and after hepatitis C virus (HCV) reinfection. Results suggest that protection from viral persistence upon HCV reinfection was associated with the virus-specific memory CD8 T-cell repertoire, with established cell lines showing high functional avidity. These results have implications for vaccination strategies aiming to develop a protective human T-cell repertoire.

Tuberculosis and other bacterial infections

Mycobacterium tuberculosis causes the bacterial disease Tuberculosis (TB), which is recognized as the most deadly infectious disease worldwide, annually resulting in up to 1.5 million deaths.[1] It most commonly occurs as a latent infection (latent TB infection or LTBI), which shows no clinical symptoms. In 2016, a WHO-endorsed estimate updated the global prevalence of LTBI to 23%, corresponding to 1.7 billion people infected worldwide.[2]

For approximately 5-15% of individuals with LTBI,[2] the infection progresses to an active disease that primarily affects the lungs, with coughing as the main symptom.[3] The bacterium spreads through airborne droplets and aerosols from an infected person.

Active TB is typically treated with antimicrobials. However, drug-resistant strains of TB pose a challenge to treatment.[3] The only licensed vaccine currently is the partially effective Mycobacterium bovis bacilli Calmette-Guérin (BCG), which was developed in 1921.[4]

Aside from providing potential context of previous or latent TB infections in clinical trial data and possible co-infections, understanding the T-cell response to TB may help support vaccine development.[5] TB proteins pose a particular challenge with regards to vaccination due to conserved T-cell epitopes. For other infections, such as HIV and influenza, antigen variation is the main difficulty for vaccination development. The TB proteins that are targeted by human T cells, however, do not exhibit significant sequence variation.[4,6]

An effective TB vaccine will likely have a number of attributes that activate multiple mechanisms and elicit a comprehensive immune response involving humoral and cell-mediated immunity at different points of time (Table 3).[4]

1. World Health Organization. Global Tuberculosis Report 2020. Organisation mondiale de la Santé. Bureau régional de l'Afrique; 2020. Accessed September 10, 2021. https://apps.who.int/iris/handle/10665/339606.

2. Houben RMGJ, et al. *PLoS Med*. 2016;13(10):e1002152. doi:10.1371/journal.pmed.1002152.

3. Glaziou P, er al. *Semin Respir Crit Care Med*. 2018;39(3):271-285. doi:10.1055/s-0038-1651492.

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Cell type/molecule	Role in vaccine pathway
Antibodies	Attachment and entry blocking, high-affinity, broadly neutralizing, FC effector function equipped complement-fixing, opsonophagocytic, antibody-dependent cell phagocytosis and antibody-dependent cell cytotoxicity-capable and Mtb killing
Airway T cells	Increase in "innate-like" sensing and early effector functions
Alveolar macrophages	Increase in microbicidal capacity and trained-immunity
Alveolar epithelial cells (AEC)	Increase in surfactant and antimicrobial peptide production
Innate lymphoid cells (ILC)	Increase in "innate-like" sensing and early anti-Mtb effector functions
Natural killer (NK) cells	"Memory-like" quick recall, increase in trained-immunity and killer potential
Unconventional T cells	Rapid recall and increase in early anti-Mtb effector functions
Tissue-resident memory T cells (TRM)	Increase in sensory and early anti-Mtb effector functions
Plasma cells	Rapid antibody secretion and specificity against critical antigens
Neutrophils	Modulate frequency and increase in microbicidal capacity
Dendritic cells (DC)	Modulate subset frequency; increase in activation and rapid migratory capacity to draining lymph node. Increased antigen presentation by lymph node DCs
Interstitial effector memory T cells (TEM)	Appropriately located, modulate pro- and anti-inflammatory subsets, sustained proliferation and balance multifunctional capacity without exhaustion
Circulating TEM/TEFF	Lung and granuloma-homing, modulate pro- and anti-inflammatory subsets, sustained proliferation and balance multifunctional capacity without terminal differentiation
Circulating central memory T cells (TCM)	Draining lymph node-homing, long-lived, rapid recall and generation of secondary effectors of crucial specificity that recognize Mtb-infected cells with low to intermediate TCR avidity
Circulating memory B cells	Draining lymph node-homing, long-lived, rapid recall and generation of secondary effectors of crucial specificity
Monocytes	Modulate frequency and increase "trained-immunity"

Table 3. Aspects of the immune system to be activated in an "ideal" TB vaccine. Adapted from Sable, et al. (2020)[4]

High-throughput sequencing of the TCR repertoire also has the potential to help create clinical tests and classifiers, screening specifically for disease-related TCRs to determine if an individual has a past or current viral or bacterial infection, as was demonstrated recently by Greissl et al. for Lyme disease.[7]

Key Takeaways

- The most common type of TB infection is latent and asymptomatic, with an estimated 25% of the world living with LTBI.
- There is no fully effective vaccine for TB on the market, and antibacterial resistance poses an extra challenge for treatment.
- The effect of TB on the T-cell repertoire is not entirely understood, and immunosequencing can help elucidate outstanding questions and advance further research and vaccine development.
- T-cell classifiers can be developed based on "public" TCR signatures,

such as the one demonstrated for Lyme disease.

immunoSEQ Publications

Inherited PD-1 deficiency underlies tuberculosis and autoimmunity in a child
Ogishi M, et al. Nature Medicine, 2021 .

A case study of a child with TB and inherited PD-1 deficiency who ultimately died of pulmonary complications from autoimmunity. This highlights the role of PD-1 in immunity against mycobacterial agents and self-tolerance.

TRAV1-2$^+$ CD8$^+$ T-cells including oligoconal expansions of MAIT cells are enriched in the airways in human tuberculosis
Wong EB, et al. Communications Biology, 2019.

This research shows enrichment of Mucosal-associated invariant T (MAIT) cells in humans with TB. High-throughput TCR analysis revealed oligoclonal expansion of TRAV1-2$^+$ MAIT-consistent TCRα within that population of pro-inflammatory TRAV1-2$^+$ CD8$^+$ T cells, demonstrating a role for these cells in the human pulmonary immune response to *Mycobacterium tuberculosis*. These results support targeting these cells for vaccine or immunotherapy development.

Effector TH17 Cells Give Rise to Long-Lived TRM Cells that Are Essential for an Immediate Response against Bacterial Infection
Amezcua Vesely MC, et al. Infectious Disease, 2019.

In an IL-17 tracking-fate mouse-model study to investigate the cellular origin of CD4 tissue-resident memory T (T_{RM}) cells, the authors identify IL-17A-producing effector cells as the source of a significant fraction of CD4 T_{RM} cells, which are maintained in the lungs by IL-7 produced by lymphatic endothelial cells and play an important role in bacterial clearance. This origin and function of airway CD4 T_{RM} cells may offer novel strategies for vaccine development.

Differential skewing of donor-unrestricted and γδ T cell repertoires in tuberculosis-infected human lungs
Ogongo P, et al. Infectious Disease, 2019.

Unconventional T cells, such as mucosa-associated invariant T cells (MAITs), CD1- restricted T cells, and γδ T cells, are of great interest as potential vaccine targets against TB. Through TCR sequencing, the authors investigated the distribution, frequencies, and characteristics of these TCRs in lung tissue. The authors concluded that these T cells of interest are well preserved in the lungs, regardless of disease status or HIV coinfection, and despite depletion of the same T-cell population in blood samples.

CD1b Tetramers Identify T Cells that Recognize Natural and Synthetic Diacylated Sulfoglycolipids from Mycobacterium tuberculosis
James CA, et al. Cell Chemical Biology, 2018.

In this study, the authors describe a new hybrid synthesis method for the development of sulfoglycolipid (SGL)-specific tetramers that could be recognized by TCRs, showing the synthetic SGLs are bioequivalent to their natural counterparts produced by *Mycobacterium tuberculosis*. Thus, the synthetic SGLs have the potential to be investigated for their potential as vaccine agents or for their diagnostic potential to detect SGL-specific T cells.

Immunosequencing of the T-cell receptor repertoire reveals signatures specific for diagnosis and characterization of early Lyme disease
Greissl J, et al. medRXiv, 2021.

Through immunosequencing TCR repertoires in blood samples of 3 independent cohorts with Lyme disease, the authors identified 251 public, Lyme-associated TCRs that were used to train a classifier that can be used to detect early Lyme disease with 99% specificity. In addition, validation demonstrated a 1.9-fold increase in sensitivity compared to the current standard (2-teared

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