



Immunodominance complexity: lessons yet to be learned from dominant T cell responses to SARS-CoV-2

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Immunodominance is a complex and highly debated topic of T cell biology. The current SARS-CoV-2 pandemic has provided the opportunity to profile adaptive immune responses and determine molecular factors contributing to emerging responses towards immunodominant viral epitopes. Here, we discuss parameters that alter the dynamics of CD8 viral epitope processing, generation and T-cell responses, and how immunodominance counteracts viral immune escape mechanisms that develop in the context of emerging SARS-CoV-2 variants.

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Introduction

The emergence of SARS-CoV-2 and the resulting global pandemic has presented an opportune moment to review our understanding of T cell immunodominance and the myriad of data required to investigate this complicated idea. Years of lessons from studying other viral infections such as influenza and HIV-1 have paved the way for SARS-CoV-2 research and contributed to the rapid data generation and understanding of this novel virus. Here, we discuss factors that can contribute to the generation of an immunodominant response and described the data already generated for SARS-CoV-2, particularly

highlighting where the gaps in our current understanding of this virus are.

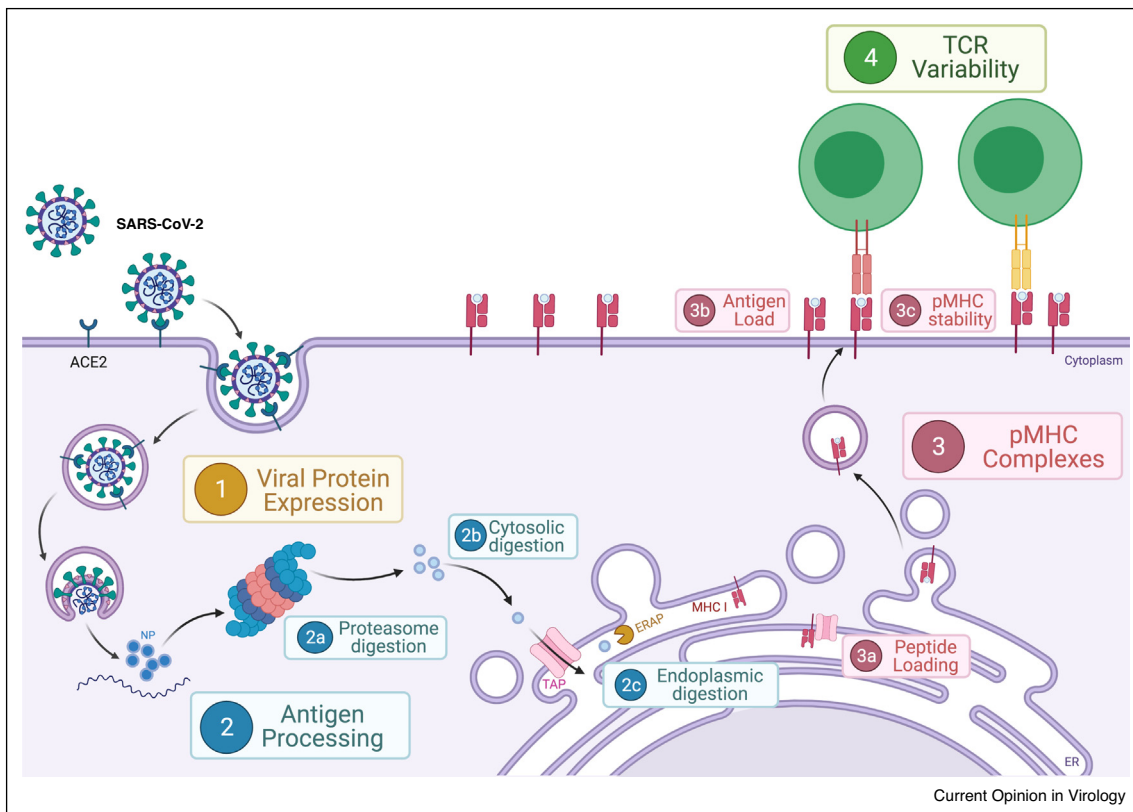
Immunodominance refers to the idea that only a selection of T cell epitopes that are capable of eliciting an immune response will be generated during an acute infection. Thereby creating a specific, robust, T cell response as opposed to a broad response. Here we discuss factors that can influence generation of immunodominant epitopes, which may be shared across many people. As opposed to immunodominant responses in an individual, which will be influenced by an individual's HLA expression. Although it should be noted that an immunodominant epitope across populations will likely be the most dominant response in individuals with that HLA. While immunodominant epitopes also exist for CD4 responses, here we will focus our discussions on CD8 cytotoxic lymphocyte (CTL) responses.

As a newly emerged virus, many studies have been carried out to identify the CD8 epitopes derived from SARS-CoV-2 proteins. Immunogenic peptides from S, M, N and ORF protein have been reported to be able to activate CD8⁺ T cells [1[•],2[•],3[•],4,5[•]] (reviewed in Ref. [6[•]]). Several HLA-A*02 restricted CD8 epitope variants within S protein were correlated with infection susceptibility and severity [7]. T cell responses against structural and accessory proteins have been identified in ~70% of acute and convalescent patients [2[•]]. Thus far, these studies have been limited to exogenous peptide and further work is required to identify CD8 epitopes from SARS-CoV-2 that are naturally processed and immunodominant [8–11].

One of the most immunodominant CD8 epitopes identified so far is found within the SARS-CoV-2 nucleoprotein, 105-SPRWYFYLL-113 (N_{105–113}) [1[•],2[•],3[•],4,5[•]]. The frequency of this epitope is potentially due to high frequency of naïve precursor T cells that can recognise this epitope [12^{••}]. Several other immunodominant epitopes for SARS-CoV-2 are predicted to exist.

In this review, we discuss some of the factors that contribute to generating an immunodominant response (Figure 1) and how emerging data for SARS-CoV-2 infection is building a picture of immunodominance for this novel viral infection. We also discuss mechanisms of immunoevasion that viruses have employed to decrease the activation of a CTL response.

Figure 1



Factors in the CD8 T cell response to epitopes that can influence immunodominance.

CD8 epitopes are peptides bound to MHC class I molecules that can elicit a cytotoxic lymphocyte (CTL) response. Several factors between viral infection and CTL activation can influence whether these epitopes are dominant or subdominant. (1) The level of viral (or bacterial) protein expression will affect the amount of material available for antigen processing. (2) During antigen processing proteins are broken down into smaller peptide fragments, firstly by proteasomal digestion (2a), followed by digestion within the cytosol (2b) or endoplasmic reticulum (ER) (2c) to generate peptides of 8–10 amino acids in length for MHC Class I binding. (3) The affinity of these peptides for MHC Class I molecules will determine which peptide-MHC (pMHC) complexes leave the ER and are expressed on the cell surface. Within the ER, peptide loading is supported by the peptide loading complex (PLC) (3a) and once high affinity peptide is loaded the pMHC complexes are exported to the cell surface. At the cell surface both the antigen load (3b) and the stability of pMHC complexes (3c) will affect T cell engagement. (4) Once pMHCs are at the cell surface they can engage CD8⁺ T cells, however there are many possible permutations of the T cell receptor (TCR) and the correct TCR is required to engage with pMHC and elicit a T cell response. Created with [BioRender.com](https://www.biorender.com).

Viral protein expression

The first factor in any infection that can affect immunodominance is the relative expression, availability and turnover rate of each of the viral proteins. The general assumption would be that the higher the expression of each protein the more potential peptides will be created. In line with this idea, the kinetics and magnitude of CTL responses have been shown to be dependent on viral gene expression in the context of human cytomegalovirus (HCMV) infection [13]. However, the viral load, in the case of lymphocytic choriomeningitis virus (LCMV) infection, has also been shown to influence which epitopes are dominant with epitopes derived from nucleoprotein dominant in low viral load infections and a switch to a gp-dominant phenotype with high viral load [14]. This suggests that it is not as simple as high expression equals high epitope generation but

understanding the relative abundance of each viral protein in an infection setting will still help in our understanding of immunodominant epitopes.

During an infection, depending on the virus, there is a difference in the time that some genes are transcribed and translated. Positive-stranded RNA viruses, such as SARS-CoV-2, do not require any transcription steps before translation, but are required to replicate their RNA to increase protein production. For coronaviruses, the non-structural proteins encoded by ORF1 are translated first generating the replicase-transcriptase complex for RNA replication. Following RNA replication, the structural RNA is translated to spike, membrane, nucleoprotein, envelope and accessory proteins for new virion generation [15].

In the context of SARS-CoV-2 infection, it has been shown that the nucleoprotein is the highest expressed protein between days 2–7 post infection of Vero E6 cells [16]. This was also shown to be the most abundant RNA transcript and produced the highest ribosomal density suggesting highest translation rate following infection [17^{••}]. The majority of identified CD8 epitopes for SARS-CoV-2 are derived from the nucleoprotein [1[•]].

However, additional factors such as cellular localisation may also play a role. In influenza virus infection, there are many more identified CD8 epitopes from internal proteins such as nucleoprotein than there are from the structural envelope proteins haemagglutinin and neuraminidase [18]. This is likely due to the cellular localisation, abundance and the turnover rate of these proteins during an infection. Structural membrane proteins generally require insertion in membranes for stable protein expression. This membrane-bound state reduces their availability for proteasomal digestion and limits degradation of these proteins in this manner to situations where the protein is misfolded or overexpressed and enters the ER-associated degradation pathway (ERAD) [19]. A similar scenario is likely occurring in the case of SARS-CoV-2 infection, supported by the low number of spike CD8 epitopes identified so far, particularly in comparison to nucleoprotein CD8 epitopes [1[•]].

The turnover rate of each protein is also a key factor that can determine the number of antigenic epitopes generated. Well folded, stable proteins will have a slow turnover rate and therefore may generate less epitopes than others. In contrast, short-lived or defective ribosomal products (DRiPs) are rapidly degraded and therefore can generate more antigenic peptides [20].

Finally, the size of each protein and gene can also affect the number of epitopes it can generate. The ORF1 gene of SARS-CoV-2 makes up 70% of the viral genome encoding sixteen non-structural proteins. Saini *et al.* identified the highest number of epitopes from SARS-CoV-2 from within the ORF1 gene, with their data generally showing that the number of epitopes was proportional to gene size [21[•]]. Indeed, this has been previously shown in the context of HIV, Hepatitis C virus and influenza although the correlation between size and epitope number is not exact and some additional factors such as cellular localisation can also play a role, as discussed above [18].

Antigen processing

MHC class I antigen processing and presentation reflects a critical constituent that controls immunodominance of antigenic peptides. Antigen processing is coupled to intracellular protein degradation, occurring in all nucleated cells and eliciting a CD8⁺ T cell response, which is critical for virus clearance and long-term protection in the

host. Peptides presented by MHC class I are mainly derived from cytosolic and nuclear proteins. Intracellular proteins are ubiquitinated and subsequently broken down to peptide fragments by the 26S proteasome, generally measuring 3–24 amino acids in length [22].

The majority of these peptides then undergo further processing by peptidases in the cytosol or, following translocation of peptides into the ER, by transporter associated with antigen presentation (TAP), and further trimming is carried out by ER aminopeptidases. Peptides of 8–11 amino acids will then replace the ER chaperone proteins on MHC class I molecule to stabilize MHC class I heterodimer for export to the cell surface (explained in more detail in the next section) [23].

Another major source of proteins for antigen processing are misfolded proteins which are exported out of the ER and back into the cytosol in a process called ERAD [24]. This process has been hijacked by viruses in a bid to avoid detection. For instance, the HCMV proteins US2 and US11 can co-opt this pathway to increase degradation of the MHC class I heavy chain [25,26].

Proteasome-dependent protein turnover is a major source of CD8 peptide antigens

The proteasome is a multi-subunit cytosolic machinery that can degrade intracellular proteins following ubiquitination, which tags and marks them for destruction. The proteasome is composed of a catalytic core particle (20S) and one or two terminal 19S regulatory particles. The 20S catalytic core is capable of cleaving at the C-terminal side of acidic, basic and hydrophobic amino acid residues [27]. In an infection setting, in the presence of IFN γ or TNF α , a highly related structure termed the immunoproteasome can be generated four times faster than constitutive proteasomes. The immunoproteasome produces more antigenic peptides at a faster rate, making them better at epitope generation [28].

Proteasomal digestion relies on the availability of proteins in the cytosol, returning us to the point of cellular localisation of viral proteins and therefore their availability for epitope generation. As part of this proteolytic process, a small fraction of peptide fragments will contain correctly cleaved C-termini of CD8 epitopes, whereas the N-terminus will generally require further trimming by aminopeptidases [29]. The sequence either side of an epitope can affect proteasomal digestion, potentially decreasing, or even increasing, epitope generation. The presence of two proline residues at position 159 and 178 of HIV-1 gag-p26 were demonstrated to enhance antigen processing and presentation efficiency [30].

The proteasome is the first target in the antigen presentation and processing pathway that viruses use to escape CTL activation. HIV-1 is known to inhibit

immunoproteasome function to prevent epitope generation. Human adenovirus E1A protein interacts with a subunit of the immunoproteasome and prevents upregulation of immunoproteasomes through STAT1 signalling. HCMV can also prevent immunoproteasome transcriptional upregulation following IFN γ treatment [31]. For SARS-CoV-2, an effect on proteasomal function has yet to be demonstrated but it will be important to monitor the flanking regions of known CD8 epitopes for mutations that may alter epitope formation through changes in proteasomal cleavage, thereby directly avoiding dominant T-cell responses.

Cytosolic digestion

Following proteasomal protein degradation, peptides are released into the cytosol where they can undergo further processing by cytosolic peptidases before translocation into the ER by TAP. Below we discuss some of these cytosolic peptidases and their role in epitope generation.

Tripeptidyl peptidase II (TPPII) has both endopeptidase and exopeptidase activity and is heavily implicated in MHC class I antigen processing. Most proteasomal degradation products beyond 15 amino acids will be trimmed by TPPII before binding to other peptidases or MHC class I molecules [32]. A study on the processing and presentation of an immunodominant HIV epitope HIV-Nef (amino acids 73–82) in human dendritic cells showed that TPPII can also function independent of the proteasome [33].

Leucine aminopeptidase (LAP) preferentially catalyses the hydrolysis of N-terminal leucine residues. It is reported that LAP aids in the processing of ovalbumin-derived 11-mer QLESIINFEKL to antigenic 8-mer SIINFEKL following IFN γ stimulation [34].

Thimet oligopeptidase (TOP) is a metallopeptidase that hydrolyses oligopeptides of 6–17 amino acids [35]. TOP is required in the generation of immunodominant CD8 epitopes from Epstein-Barr virus protein EBNA3C and melanoma protein MART-1 [36]. However, too much TOP has been demonstrated to destroy antigenic peptides in the cytoplasm, therefore limiting MHC class I antigen presentation [37].

Puromycin-sensitive aminopeptidase (PSA), a zinc metallopeptidase, and bleomycin hydrolase (BH), a cysteine protease, are two other cytosolic aminopeptidases that were identified to trim a vesicular stomatitis virus nucleoprotein precursor peptide into the correct epitope 52–59 (RGYVYQGL) [38,39].

Endoplasmic digestion

Within the ER the major aminopeptidase responsible for N-terminal trimming of peptide precursors is ERAP1, although ERAP2 also plays a role with opposing

substrate preferences to cover the majority of amino acids. Both proteins sequentially trim amino acids from the N-terminal end of peptides, but ERAP1 is more important for the generation of MHC Class I peptides as it loses interest in peptides smaller than 8 or 9 amino acids, while ERAP2 can continue trimming smaller peptides [37,40]. ERAP1 is essential for the generation of many major epitopes in infection settings and loss of ERAP1 leads to a reduction in cell surfaced pMHC complexes [41].

ERAP1 is a highly polymorphic protein and ten haplotypes have been identified that account for 99% of the natural ERAP1 variation in the population [42]. These haplotypes vary in trimming capacity, meaning that some are better than others in generating CD8 epitopes. They can affect the speed with which ERAP1 trims amino acids, its preferences for certain amino acids and also the overall expression level of ERAP1 in cells, all of which can result in differences in epitope generation.

In the context of SARS-CoV-2, ERAPs have been proposed to play a role [43] and indeed they were shown to be important for generation of 8–11 amino acid peptides from 15 amino acid precursors [44]. Additionally, mutations in ERAP2 were shown to be associated with increased risk of death in SARS-CoV-2 infection. However, the sample size containing this mutation was low [45]. Further investigation as to the contribution of ERAP to the generation of SARS-CoV-2 CD8 epitopes is required in order to clearly determine the link between ERAP genetics and infection severity.

Peptide-MHC class I complex formation and surface expression

Once peptides have been processed and transported into the ER by TAP, they are loaded onto MHC class I molecules with help of the peptide-loading complex (PLC). The PLC is comprised of TAP, protein disulphide isomerase (PDI), ERp57, tapasin and calreticulin. The loading of high affinity peptides onto MHC Class I is a highly dynamic process, and several peptides will enter and exit the MHC Class I peptide binding groove until a peptide of sufficient affinity is bound. Epitopes that have higher affinity are more dominant than others [46].

Once high affinity peptide has bound, peptide-MHC (pMHC) complexes are exported to the cell surface. The stability of these complexes at the cell surface can impact the subsequent T cell response as complexes with low stability (i.e. short half-life) at the cell surface may not be present long enough for establishment of a CD8 T cell response. For an immunodominant response, the goal is to have pMHC complexes with high affinity peptide bound in a very stable way that will stay at the cell surface for a long time.

Across all individuals, a huge number of antigenic pMHC complexes are generated and identified during a viral infection, a necessary step because each individual will only express up to six different MHC molecules out of the ~800 possible HLA class I molecules. As these genes are inherited through generations there is a geographical skew for HLA molecules with several associations between ethnicity and specific HLA molecules. Investigations into which epitopes are dominant can be skewed by HLA that are more common than others, or the ethnicity of the populations being investigated. Currently, most CD8 studies identifying epitopes from SARS-CoV-2 have focussed on Caucasian populations and have identified a strong epitope that binds to HLA-B*07 which is a common allele in this population [4,6*]. Future studies aiming to identify SARS-CoV-2 epitopes should take ethnicity into account.

Viral immunoevasion been demonstrated for this stage of the antigen processing and presentation pathway. ICP47 and US6 gene products from herpes simplex virus (HSV) and HCMV, respectively, have both been shown to inhibit peptide transfer from the cytosol to the ER through TAP [47–52]. Several viral proteins, such as adenovirus E3/19K and HCMV US3, can prevent transport of MHC Class I from the ER to the plasma membrane either transiently or conclusively [53–56].

Peptide loading

While the PLC as a whole facilitates peptide binding, tapasin in particular has been shown to impact the immunogenicity of pMHC complexes by editing the peptide repertoire and selecting for peptides with slow disassociation kinetics [57]. In addition, a correlation can be seen between slow disassociation of pMHC complexes following tapasin editing and T cell responses [58]. In this way, tapasin acts similarly to HLA-DM in the MHC Class II pathway [59].

For the CD8 epitopes of SARS-CoV-2 currently identified some appear to bind to tapasin-dependent alleles of MHC class I [60], suggesting that tapasin may have been involved in their selection although this has yet to be investigated. The implication being that if an epitope is bound to a tapasin-dependent MHC Class I the complex will be more stable and is therefore more likely to elicit a strong response and be immunodominant.

Antigen load at the cell surface

The number of pMHC complexes at the cell surface may also have an effect on T cell activation as the more complexes that are present the higher the chances of an interaction with a passing CD8 T cell. Antigen load at the cell surface is the result of many factors including protein expression of each antigen, the number of high affinity peptides, the amount of processing required and peptide stability. Immunopeptidome studies can identify

the pMHC complexes present on the cell surface but quantification of this can be difficult and so far studies are limited [61].

As a viral immunoevasion strategy, viruses can cause downregulation of pMHC complexes at the cell surface through multiple strategies [62]. K3 and K5 of Kaposi's Sarcoma-associated herpesvirus (KSHV) can promote endocytosis of pMHC complexes from the cell surface resulting in down regulation [63,64]. The Nef protein of HIV-1 can also downregulate pMHC but sequesters complexes in the trans-Golgi network preventing their export to the cell surface [65]. This downregulation affects HLA-B alleles > HLA-A > HLA-C, suggesting that there are allele specific mechanisms at play [66]. In SARS-CoV-2 infection, it has been suggested that the protein encoded by ORF8 can downregulate MHC Class I expression, potentially through targeting of pMHC complexes for lysosomal degradation via autophagy [67*]. Clearly, viruses have found ways to alter antigen load at the cell surface, however, any pMHC downregulation can also be the result of cytokines and the micro-environment of the interaction making this a complex area of research.

Peptide stability

While efficient loading and affinity of peptide is required for stable pMHC complexes to leave the ER, the stability of those complexes and their half-life at the cell surface is actually a better determinant of immunogenicity [68]. Harndahl *et al.* showed that two peptides with equal affinity for MHC have very different stabilities with large differences in half-life which actually correlated to immunogenicity of the peptide. This stability at the cell surface will affect antigen load, engagement of T cells and therefore CD8 responses to pMHC. Successful antigen processing should result in pMHC complexes of high affinity being exported from the ER and remaining stable at the cell surface, at high enough load, for as long as possible to maximise the chances of eliciting a cytotoxic T cell response. For the SARS-CoV-2 epitope N_{105–113}, binding to HLA-B*07 was shown to be highly thermostable with a $T_m > 60^\circ\text{C}$, contributing to this epitope's suggested immunodominance [69]. This information for other SARS-CoV-2 epitopes is not yet available.

TCR precursor frequency and plasticity

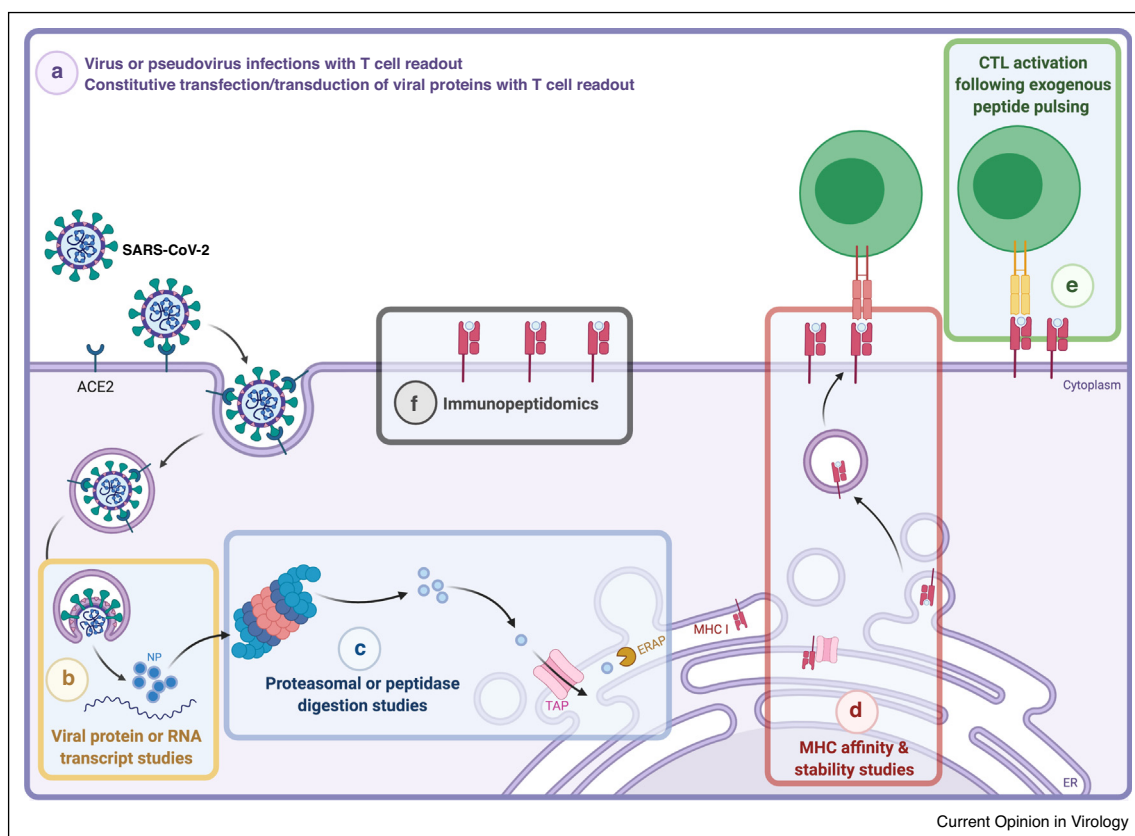
Once pMHC have reached the cell surface they await recognition and binding by T cell receptors (TCR). The actual diversity of TCR sequences is currently unclear but most recent estimates are in the order of magnitude of $>10^8$ [70]. This high diversity is achieved through recombination of the variable (V), diversity (D) and junction (J) regions of the TCR-alpha and TCR-beta genes. During recombination, V and J (TCR alpha) or V, D, and J (TCR beta) gene segments are randomly selected [71]. They

undergo random addition and deletion of nucleotides to form the final mature TCR gene [72]. Recombination can potentially result in 10^{15} unique TCRs [73,74].

Despite the large diversity, each TCR can recognise several pMHC complexes to account for the huge diversity in pMHC due to infectious agents [75,76]. This means that it is possible for a single T cell to respond to multiple pMHC complexes, but the strength of this interaction will vary for different pMHC, thereby potentially generating immunodominant and subdominant responses for different HLA molecules.

Dominance of particular TCRs has been demonstrated previously for several acute infections, while for chronic viral infection there is generally more diversity in TCR usage [77]. However, despite a dominant response against the N_{105–113} epitope, no common TCR sequences could be identified across either SARS-CoV-2 patients or pre-pandemic donors that recognised this epitope [12^{••},69]. This suggests that this epitope, bound to HLA-B*07:02, can be recognised strongly by several TCRs. In contrast, several shared TCR genes were identified for the subdominant S₂₆₉ epitope that binds to HLA-A*02:01, potentially showing a lack of TCR plasticity [12^{••}]. This

Figure 2



Research techniques for analysing generation of immunodominant CD8 T cell epitopes.

Several experimental techniques are used to elucidate the story for each factor that can affect immunodominance. **(a)** In order to get a full picture of immunodominance, virus infections with a T cell readout can give a full picture, although this system is limited by the HLA expression of target cell lines. Pseudovirus particles expressing one or two viral proteins or protein transfection/transduction systems can also tell you about the epitopes generated from individual proteins, but this data is limited by the exclusion of other viral proteins. **(b)** Viral protein or RNA levels in infected cells can be measured by transcriptomic or proteomic approaches, as well as conventional western blot techniques to give a picture of how protein expression alters across the course of an infection. **(c)** Digestion of exogenous proteins or peptides by the proteasome or different peptidases can offer insight into the peptides that will naturally be available for MHC Class I binding. Inhibitors of proteasomal function and specific inhibitors for particular aminopeptidases are also available to help tease out which proteins are required for epitope formation. **(d)** Studies into peptide affinity for MHC and stability of pMHC studies using surface plasmon resonance, FRET or pulse chase experiments can give insight into antigen load at the cell surface — a key factor in generating immunodominant responses. **(e)** Many studies utilise exogenous peptide pulsing to determine what is an epitope. However, these studies do not take into account antigen processing and CTL activation may be seen in response to peptides that are not naturally processed and presented at the cell surface. **(f)** Immunopeptidomic studies analysing pMHC complexes eluted from the cell surface can give a snapshot of pMHC at a certain time during an infection but fail to tell you much about the stability of those complexes or T cell responses to them. Created with [BioRender.com](https://www.biorender.com).

suggests that in the case of SARS-CoV-2 infection, TCR diversity may increase immunodominant responses.

Concluding remarks

Here, we have highlighted the major factors that we feel can contribute to generation of an immunodominant SARS-CoV-2 specific CD8 T cell response, although there are other minor contributors such as CD8 co-receptor engagement. The majority of approaches can only interrogate one, possibly two, factors within this schematic (Figure 2), with only live virus infection assays with a T cell readout combining all aspects to show which responses are robust and physiologically relevant. This means that it is integral that the virology, antigen processing and T cell communities work together to combine data and build a full picture for immunodominant epitopes. Identification of these immunodominant responses is important for understanding the responses of individuals to viral infection as well for rational vaccine design targeting CTL responses.

In the 18 months since SARS-CoV-2 infections became a worldwide concern, the vast amount of data produced has led to fast adaptations of treatment, prevention and vaccination protocols. We have learnt a lot in a short time due to the lessons and experience immunologists and virologist have established over the years of investigation into other viral infections, but there are still vast gaps in our knowledge.

Thus far, we have data showing the expression of viral proteins, the sequence of the epitopes at the cell surface capable of eliciting a CTL response and some information about TCR usage. We have yet to determine much about how SARS-CoV-2 is processed as most studies have used exogenous peptides, and the host proteins required for this, further work in this area is required. Identification of immunodominant T cell responses to naturally processed and presented epitopes and their association with immune protection and vaccine efficacy is a key area of research focus in the future. It will be important to gain an in depth understanding of the functional correlation of immunodominant T cell responses to disease outcomes, and the impact of the tissue microenvironment on these responses.

We have seen that SARS-CoV-2 is highly polymorphic, with variants of concern and interest dominating the news. However, thus far most data have focussed on how these mutations can alter affinity for ACE2 or antibody responses, as that is the target of current vaccination strategies. Future work into the effect of natural variants on antigen processing and presentation and therefore immunodominance will be interesting to investigate. Additionally, whether the patterns of immunodominance will be altered over time or in response to natural

infection as opposed to vaccination will be important to elucidate.

We have discussed the various methods of viral immunoevasion that other viruses have adopted. Thus far, one study has suggested that the ORF8 protein is capable of downregulating HLA expression at the cell surface [67]. However, further investigation is required to confirm this. Studies into the function and consequences of each viral protein will be essential to understand whether this virus is smart enough to evade host immune responses.

Finally, we commend the efforts of many that have got us to this stage in our understanding of immunodominant CTL responses to SARS-CoV-2. Now we call for unity to work together to establish the role that the remaining factors affecting immunodominance may be involved in this viral infection.

Conflict of interest statement

Nothing declared.

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