A Systematic Review of T Cell Epitopes Defined from the Proteome of Hepatitis B Virus

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Abstract: Hepatitis B virus (HBV) infection remains a worldwide health problem and no eradicative therapy is currently available. Host T cell immune responses have crucial influences on the outcome of HBV infection, however the development of therapeutic vaccines, T cell therapies and the clinical evaluation of HBV-specific T cell responses are hampered markedly by the lack of validated T cell epitopes. This review presented a map of T cell epitopes functionally validated from HBV antigens during the past 33 years; the human leukocyte antigen (HLA) supertypes to present these epitopes, and the methods to screen and identify T cell epitopes. To the best of our knowledge, a total of 205 CD8+ T cell epitopes and 79 CD4+ T cell epitopes have been defined from HBV antigens by cellular functional experiments thus far, but most are restricted to several common HLA supertypes, such as HLA-A0201, A2402, B0702, DR04, and DR12 molecules. Therefore, the currently defined T cell epitope repertoire cannot cover the major populations with HLA diversity in an indicated geographic region. More researches are needed to dissect a more comprehensive map of T cell epitopes, which covers overall HBV proteome and global patients.

Keywords: hepatitis B virus; T cell epitope; HLA restriction

1. Introduction

Hepatitis B virus (HBV) infection still disseminates across the world and causes the most common and fatal liver diseases including acute liver failure, chronic hepatitis, liver cirrhosis (LC), and hepatocellular carcinoma (HCC) [1,2]. Nucleoside analogs and/or interferon are widely utilized antiviral drugs, which can effectively suppress virus replication, decrease serum HBV DNA to undetectable levels, mitigate liver fibrosis, and reduce HCC risk [3–5], however cannot eliminate the virus in patients. Recurrence after therapy discontinuation is emerging to be a common etiology of morbidity and mortality in patients with chronic HBV infection [6].

Numerous researches have demonstrated the important influence of HBV-specific T cell responses on virus clearance [7], disease progression [8–10], antiviral efficacy [11,12], and recurrence [13–15], particularly the CD8+ T cells, which act as vital effector cells to kill virus-infected hepatocytes and secret cytokines. Patients with acute-resolving HBV infection show robust HBV-specific CD8+ T cell responses, while the patients with chronic HBV infection present a phenomenon termed CD8+ T cell functional exhaustion with multifactorial heterogeneity [9], and differs depending on the targeted antigen for HLA-A02 restricted epitopes located in the core antigen versus polymerase [16]. Furthermore, the heterogeneity of HBV-specific T cells also responds differently to therapeutic stimuli [17]. Therefore, T cells specific for HBV not only are the potential markers for monitoring the effects of antiviral therapy and predicting the recurrence [18], but also are the promising modulators in specific immunotherapy. Identifying the T cell epitopes as many as possible from HBV antigens will greatly contribute to the design and development of epitope-based and T cell-based therapies and the detection of host HBV-specific T cell immunity. Although
a systematic review of T cell epitopes in HBV antigens was reported in 2008 [19], an updated map of the T cell epitopes is urgently needed.

Here, this review comprehensively collected the CD8+ T cell epitopes and CD4+ T cell epitopes defined from HBV proteome during the past 33 years. Information resources are the English language journals collected in Pubmed, Scopus, Embase, SinoMed, and Google Scholar databases. The latest online search was conducted on October 8, 2021. “T cell epitopes” and “HBV or hepatitis B virus” were used as specific searching terms. An initial search identified 451 studies from multiple databases and manual searches. All articles were imported to Endnote software X8 (Thompson and Reuters, Philadelphia, PA, USA) and 121 duplicates were removed. In total, 330 studies from 1988 to 2021 were collected. Then, 233 articles were filtered out after abstract and full-text screening, according to the exclusion criteria below: (1) not related to the screening or identification of T cell epitopes; (2) just using in silico prediction or molecular structure bioinformatic analysis rather than satisfactory cell functional experiments, tetramer staining, binding assay, stabilization assay, or immunization; (3) with incomplete information regarding epitopes sequences. Finally, 97 articles were analyzed and referenced in this review.

2. Polymorphism of HLA Alleles and Association with HBV Infection

Human leukocyte antigens (HLA) are coded by human major histocompatibility complex and have multiple important functions. In particular, they present antigenic peptides (T cell epitopes) in the form of peptide/HLA complex to T cell receptors onto specific T cells by which to initiate the adaptive immune response. HLA class I molecules (classically HLA-A, -B, and -C) are constitutively expressed onto almost all nucleated cells with distinct levels and present antigenic peptides to specific CD8+ T cells, while HLA class II molecules (classically HLA-DR, -DQ and -DP) are mainly expressed onto professional antigen-presenting cells (APCs, including monocytes, macrophages, dendritic cells and B cells) and present peptides to specific CD4+ T cells. In virus infection, HLA class I molecules expressed by virus-infected cells present the viral endogenous epitope peptides to specific CD8+ T cells, thus initiating the naive CD8+ T cells to activate, proliferate and differentiate to cytotoxic T lymphocytes (CTLs). The resulting CTLs mediate the cytolyis of virus-infected cells by Fas/FasL, TNF/TNFFL, and perforin/granzyme pathways [20]. HLA class II molecules expressed by APCs present exogenous viral peptides to CD4+ helper T cells, thus eliciting the naive CD4+ T cells to differentiate into effective Th1 or Th2 cells. The former help virus-specific CD8+ T cells activation and the latter help virus-specific B cells differentiate to plasma cells and produce antibodies [21]. However, HLA molecules are highly polymorphic in the general population. As of October 2021, a total of 24,284 alleles have been described at HLA class I and class II loci in the global populations, including 6921 HLA-A, 8181 HLA-B, 6779 HLA-C, 3801 HLA-DRB1, 2033 HLA-DQB1, and 1862 HLA-DPB1 alleles, according to the International Immunogenetics Information Project/HLA database (IMGT; www.ebi.ac.uk/imgt/hla/stats.html, accessed on 11 November 2021). HLA allotypes are distinctive from individual to individual, and each HLA allotype presents distinctive antigenic peptides, thus leading to different immune patterns in different individuals against the same pathogen such as HBV [22,23]. Among the different ethnic populations in different geographic regions, the distribution of prevalent HLA alleles is markedly different. For an instance, 13 kinds of predominant HLA-A allotypes (each allotype has a gene frequency of >1% in Chinese herd) gather a total HLA-A allele frequency of around 95.5% in the Chinese population while 94%, 83%, 80%, 70% and 63% in Northeast Asia, Southeast Asia, Europe, South America, and North America populations, respectively (http://www.allelefrequencies.net, accessed on 11 November 2021).

Consequently, some alleles of HLA molecules have increasingly been linked to the occurrence of the indicated diseases, which are usually associated with abnormal immune function and genetic tendency [24,25]. Although the association of HLA alleles with HBV infection is not well clarified, a few studies have indicated that HLA-DRB1*13 and HLA-DRB1*07 are related to susceptibility to chronic HBV infection, and DRB1*15 is
negatively related to persistence to chronic HBV infection in the populations of Africans [26], Europeans [27], Koreans [28] and Northwestern Chinese [29]. In addition, HLA-A*33 is closely associated with susceptibility to persisting HBV infection, and HLA-DRB1*13 is closely related to protection against persisting HBV infection in an Iranian population [30]. A*0301 and DRB1*1302 are relevant to viral clearance and B*08 is associated with viral persistence in Caucasians [31]. However, although the correlation between HBV infection and HLA alleles has been studied for several decades, in accordance with what we described in the above review, it often has conflicting results. These variations partly result from host HLA polymorphism in different races and regions [32,33]. Further studies should be explored in different regions to reduce the heterogeneity of results.

3. HBV Proteome and the Approaches Identifying T Cell Epitopes

HBV is one of the smallest viruses with a genome length of 3.2 Kb [34]. Its genome contains four open reading frames (ORFs) coding four partially overlapping proteins as displayed in Figure 1: (1) preS/S ORF encodes large (L), middle (M), and small (S) surface antigens (HBsAg). HBsAg is being widely investigated in clinical fields and quantified as a diagnostic marker of HBV infection as it can reflect the level of covalently closed circular DNA (cccDNA) and intrahepatic HBV DNA in chronic infection [35,36]. (2) Pre-core/core ORF encodes hepatitis B e antigen (HBeAg), core antigen (HBcAg) or in combination core-related antigen (HBcrAg). HBeAg has long been advocated as a serum marker for guiding the clinical practice of chronic hepatitis B virus [37,38]. HBcrAg has been demonstrated more recently as a potential surrogate marker of cccDNA [39]. (3) X ORF encodes HBx antigen (HBxAg), which plays an important role in virus genome transcription and is correlated with liver cancer. The expression of HBxAg in HBV-associated HCC patients is significantly higher than other viral proteins [40]. (4) P ORF encodes the viral DNA polymerase (HBpol), which is responsible for the replication of the viral genome and is an effective target for the therapeutic intervention of chronic HBV infection [41]. Human HBV strains occur in nine genotypes A-I, and its major HBV surface antigen (HBsAg) has several immune protective conformational B cell epitopes a, d or y, w1–4 or r [42]. The entire amino acid sequences of each protein from different genotypes were obtained from the UniProt database and aligned in Figure 2.
Figure 2. Cont.
Figure 2. Homologous analysis of HBsAg, HBeAg, HBx and HBpol proteins from HBV C, A, B, and D genotypes. The entire amino acid sequences of each protein from different HBV genotypes were obtained from the UniProt database, aligned and used for in silico prediction of HBV antigen T cell epitopes presented by HLA-A allotypes.
The process of T cell epitope identification begins with the selection of candidate epitope peptides. The first strategy is using overlapping peptides (OLPs) spanning the entire proteome or selected antigens of interest (peptide scanning). Chen et al. expanded HBV-specific T cells in vitro by co-culturing the overlapping peptide pools spanning the entire sequence of HBV genotypes B and C and the peripheral blood mononuclear cells (PBMCs) from patients with chronic HBV infection, followed by the detection of T cell response in each co-culture using IFN-\(\gamma\) enzyme-linked immunospot (IFN-\(\gamma\) ELISpot) assay, IFN-\(\gamma\) intracellular staining and flow cytometry [43]. However, peptide scanning is a high-cost and laborious method due to a large amount of OLPs spanning overall HBV proteins. For CD8\(^+\) T cell epitopes, HBsAg, HBeAg, HBx and HBpol contain 131, 68, 49, and 279 OLPs, respectively, when overlapping 6 amino acids in each 9-mer peptide. An alternative strategy is to focus on the in silico predicted T cell epitopes binding to the indicated HLA super-types as calculated by multiple epitope prediction tools and algorithms. Brinck-Jensen et al. predicted 20 HBV-specific epitopes using combined in silico methods and evaluated for the immunogenicity of these epitopes through exposure to patients’ PBMCs by IFN-\(\gamma\) ELISpot [44]. More recently, a similar in silico approach was also employed to assess all previously verified HBx- and HBpol-derived epitopes and to predict novel HLA-binding peptides for 6 HLA supertypes. Then, a part of reported epitopes were chosen for experimental validation. A total of 13 HLA binders derived from HBx and 33 binders from HBpol were described across HLA subtypes by this strategy [45]. Predicted epitopes are based on the indicated HLA restrictions and limit the number of research objects with diverse HLA subtypes to a reasonable range, yet the inaccuracy of theoretical prediction may omit some real-world epitopes.

The methodologies to validate the immunogenicity of candidate epitope peptides have been improved remarkably over the last two decades. Different assays are utilized for the detection of peptide-induced T cell response or peptide-specific T cells with individual advantages and disadvantages in terms of practicability, cost, sensitivity, function evaluation. The following approaches are currently widely used, such as cytotoxicity assay, proliferation assay, intracellular cytokine staining (ICS), ELISpot/FluoroSpot, and peptide-MHC multimers staining (tetramers, pentamers, or dextramers). The cytotoxicity assay was initially performed to validate CD8\(^+\) T cell epitopes by co-culturing patients’ PBMCs with target cells labeled with Chromium-51, after the PBMCs were stimulated by the indicated candidate epitope peptides [46]. Additionally, lymphocyte proliferation assay is mostly applied to CD4\(^+\) T cell epitopes validation. The PBMCs from HBV-infected or HBV-vaccinated individuals were co-cultured with HBV-derived peptides for several days and \(^3\)H-thymidine pulses were administered eventually followed by quantifying the incorporated radioactivity [47]. One more common approach currently utilized is ICS or ELISpot/FluoroSpot. Patient’s PBMCs are in vitro or ex vivo stimulated with the candidate epitope peptides and simultaneously cytokine release is blocked followed by ICS and flow cytometry to define whether CD4\(^+\) T cells or CD8\(^+\) T cells activation [48]. The ELISpot or FluoroSpot technology enables the detection of single activated cells among one million PBMCs. The accuracy, sensitivity, reproducibility and durability have led to its widespread applications in researches and the broad prospects in the clinical detection of antigen-specific T cells [49,50]. An issue encountered with ELISpot, FluoroSpot, ICS, and related assays is that they may ignore T cells that produce different cytokines or trace cytokines during the window of time of the assay (e.g., Follicular helper CD4\(^+\) T cells generally produce very low amounts of cytokines). Peptide-MHC tetramer staining has been the gold standard to quantify antigen-specific T cells with high sensitivity and precision, thus is often used to identify T cell epitopes in many researches. However, the preparation of peptide-MHC tetramers or multimers is high-cost, complicated, and time-consumption [51,52]. A pioneering study focused on all possible peptides of the entire HBV genome and 484 unique HLA-A1101-restricted epitopes predicted by NetMHC algorithms were validated using mass cytometry and multiplex peptide-tetramers staining [53]. Many researchers also have established a transgenic mouse model to map HLA-restricted epi-
topes. Ru et al. developed and immunized HLA-A2/DP4 mice with epitopes derived from HBsAg to identify four new HLA-DP4-restricted epitopes [54]. Besides cellular functional experiments, peptide-HLA molecule binding and stabilization assays were commonly used to identify epitopes. Pan et al. defined 16 HBV epitopes by analyzing the different binding affinities of candidate epitope peptides with HLA-A3303 using RMA-S cells binding and stability assay. More recently, Ferretti et al. used a high-throughput genome-wide screening technology to identify the target cells expressing candidate epitopes productively recognized by T cells (T-Scan) and determined 29 epitopes in SARS-CoV-2 for the six most prevalent HLA types [55]. Chikata et al. employed immunocapture and liquid chromatography mass spectrometry (LC-MS) subsequent to pre-treatment of the target protein to disrupt its three-dimensional structure to characterize HIV-1 epitope peptides on a large scale presented by HLA-C1202 [56]. A variety of epitope assay strategies have been utilized with their own features and potential.

4. Defined T Cell Epitopes in HBV Proteins during the Past 33 Years

Table 1 collected the CD8\(^+\) T cell epitopes and CD4\(^+\) T cell epitopes defined from HBV proteome during the past 33 years and displayed their HLA restrictions and the methods used to validate their immunogenicity. Notably, we performed manual management in this review, only the epitopes of 8–14 or 12–25 amino acids in length presented by HLA class I molecules or class II molecules are displayed since they reflect the standard size of the peptide-binding groove of HLA molecules. According to the previous report, if the epitope peptides are too short or long, the experiment tends to represent false positives instead of the result caused by the binding of peptide and HLA molecule [57].

Overall, 82 and 19 studies reported the epitopes presented by HLA class I molecules and class II molecules, respectively, and totally contained 284 unique epitopes including 205 CD8\(^+\) T cell epitopes and 79 CD4\(^+\) T cell epitopes (Table 1). Of these, 121 (59.0\%) CD8\(^+\) T cell epitopes are restricted by HLA-A0201, A2402 or B0702 (Figure 3A), which are common supertypes in Caucasians and less predominant in Asia and Africa [58,59]. The remainder are restricted mainly by 12 HLA-A, 5 HLA-B and 1 HLA-C supertypes. For the CD4\(^+\) T cell epitopes, the majority of currently described restrictions apply to 8 DRB1 supertypes (Figure 3B). The cumulative frequency of the HLA-A supertypes described in Figure 3A was highest in Europe (66.6\%), followed by Asia (53.1\%), Africa (50.7\%), and North America (52.3\%) while the HLA-B supertypes showed an accumulative frequency of 32.7\% in Europe, 20.1\% in Asia, 19.2\% in Africa and 18.8\% in North America. The DRB1 supertypes in Figure 3B displayed little difference in the cumulative gene frequency in Europe (30.5\%), Asia (32.2\%), Africa (31.1\%) and North America (34.1\%). (Data from http://www.allelefrequencies.net/, assessed on 11 November 2021). Obviously, the 284 validated T cell epitopes of HBV cannot cover the major populations in an indicated geographic region. More T cell epitopes restricted by more HLA supertypes are urgently needed. Further efforts are required to identify more T cell epitopes restricted to the regional prevalent HLA supertypes, especially for the HLA alleles prevalent in Asian populations with a high HBV incidence [59,60].

In addition, although the validated T cell epitopes have derived from all HBV proteins, the CD8\(^+\) T cell epitopes mainly distribute in HBpol and HBsAg (72\%) (Figure 3C), while the majority of CD4\(^+\) T cell epitopes concentrate in HBeAg and HBsAg (78\%) (Figure 3D). The biased distribution of epitopes in proteome may be caused partially by the different lengths of proteins (HBpol 843aa, HBsAg 400 aa, HBeAg 212 aa, HBx 154 aa) and the pitfalls of screening methods.

As displayed in the sixth column of Table 1, most studies used the in silico prediction strategy to screen the candidate CD8\(^+\) T cell epitopes (92\% of studies) and CD4\(^+\) T cells epitopes (63\% of studies). Relatively, overlapping peptides were more often used in selecting candidate CD4\(^+\) T cell epitopes (7 of 19 studies; 37\%) than CD8\(^+\) T cell epitopes (7 of 82 studies; 8\%), partially due to the lower accuracy and efficacy of predicting HLA class II molecule-binding epitopes as compared with class I molecule-binding epitopes.
Figure 3. HLA restriction and protein distribution of validated CD4+ T cell epitopes and CD8+ T cell epitopes in HBV proteome. (A, B) displayed the number of CD8+ T cell epitopes and CD4+ T cell epitopes restricted by each HLA supertype, respectively. (C, D) showed the fraction of CD8+ T cell epitopes and CD4+ T cell epitopes in each HBV protein, respectively.

Table 1. List of CD4+ T cell epitopes and CD8+ T cell epitopes validated from HBV proteins.

| Sequence       | Protein | Position | Reference | HLA Restriction | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes |
|----------------|---------|----------|-----------|-----------------|-------------------------------------|------------------------------------------|
| MQLFHLCLI      | Core    | 1–8      | [61]      | A*0201          | Predicted                           | Binding assay; ELISpot; Cytotoxicity assay; CTL assay |
| KEFGASVEL(L)   | Core    | 7–15/16  | [62]      | A*0206, B*4001  | Predicted                           | ELISpot; ICS; Binding assay               |
| EFGASVELL      | Core    | 8–16     | [63]      | A*0201, A*0207  | overlapping                         | ICS; ELISpot                            |
| FLPSDFFFPS     | Core    | 18–26    | [64]      | A*0201          | Predicted                           | Tetramer staining                        |
| FLPSDFFFPSV    | Core    | 18–27    | [45, 65–79] | A*0201, A*0202, A*0203, A*0206, A*0207 | overlapping                         | Immunization of mice; Cytotoxicity assay; CTL assay; Binding assay; Tetramer staining; ELISA |
| LPSSDFPSV      | Core    | 19–27    | [74, 80–83] | B*3501, B*51, B*5301, B*5401, B*07, B*51, A*0201 | overlapping                         | Binding assay; CTL assay; Cytotoxicity assay; Tetramer staining |
| FFSIRDL       | Core    | 23–31    | [84]      | A*24            | Predicted                           | Tetramer staining                        |
| DLLDTASALY    | Core    | 39–48    | [81]      | A*0101, A*2902, A*3002 | Predicted                           | Binding assay; Immunization of mice; ELISpot |
| DFFPSIRDL      | Core    | 51–59    | [85]      | A*2402          | Predicted                           | ELISpot                                  |
| Sequence       | Protein | Position | Reference | HLA Restriction | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes |
|----------------|---------|----------|-----------|----------------|-------------------------------------|------------------------------------------|
| LCWGEMLNL     | Core    | 60–68    | [86]      | A*0201         | Predicted                           | Stabilization assay; ELISpot assay       |
| ELMNLATWV     | Core    | 64–72    | [87]      | A*02           | Predicted                           | Binding assay; ELISpot; Cytotoxicity assay |
| SYVNMNMGL     | Core    | 87–95    | [88]      | A*2402         | Predicted                           | Binding assay; CTL assay                 |
| SYVNTNMGL     | Core    | 87–95    | [89]      | A*02           | Predicted                           | Tetramer staining                        |
| YVNVMNLK      | Core    | 88–96    | [63]      | A*1101 overlapping | ICS; ELISpot                     |
| MGLKFRLQ      | Core    | 93–100   | [90]      | A*0201         | Predicted                           | Immunization of mice; FACS               |
| LLWFHISCL     | Core    | 101–108  | [43]      | A*0201         | Predicted                           | Proliferation assay; ICS; Cytotoxicity assay |
| LWFHISCLTF    | Core    | 101–110  | [85]      | A*2402, A*2301 | Predicted                           | ELISpot                                  |
| HISCLTFGR     | Core    | 104–112  | [91,92]   | A*33           | Predicted                           | Cytotoxicity assay; ICS; Tetramer staining |
| CLTFGRETV     | Core    | 107–115  | [93]      | A*02           | Predicted                           | Tetramer staining                        |
| EYLVSFGVVW    | Core    | 117–125  | [81,84,88] | A*2402, A*2407, A*2301 | Predicted                           | Stabilization assay; CTL assay; Cytotoxicity assay; Tetramer staining; Binding assay; Immunization of mice; ELISpot |
| YLVSGVW      | Core    | 118–126  | [43]      | A*0201         | Predicted                           | Proliferation assay; ICS; Cytotoxicity assay |
| LVSFGVWIR     | Core    | 119–127  | [91]      | A*33           | Predicted                           | Stabilization assay; ELISpot; Cytotoxicity assay; Immunization of mice             |
| GLKILQLL     | Core    | 123–130  | [82]      | B*08           | overlapping                         | ICS; Tetramer staining                   |
| AYRPNNAPI     | Core    | 131–139  | [94]      | A*0201         | Predicted                           | ELISpot; Cytotoxicity assay              |
| LTFGRETVLEN   | Core    | 137–147  | [95]      | A*0101, A*02, A*2902, A*3002 | Predicted                           | ELISpot                                  |
| ILSTLPETTV    | Core    | 139–148  | [75]      | A*02           | Predicted                           | CTL assay                               |
| STLPTTVVVR    | Core    | 141–150  | [74,96]   | A*11, A*6801, A*02 | Predicting                         | Binding assay; CTL assay; Cytotoxicity assay; ELISpot |
| STLPTTVDVRR   | Core    | 141–151  | [17,76,81, 92,97] | A*31, A*68, A*02, A*0201, A*6801, A*03, A*11, A*3101, A*0201 | Predicted                           | Cytotoxicity assay; Immunization of mice; CTL assay; Binding assay; ELISpot |
| TLPETTVVRR    | Core    | 142–151  | [63]      | A*1101         | overlapping                         | ICS; ELISpot                            |
| GVWIRTPPA     | Core    | 152–160  | [98]      | A*0201         | Predicted                           | ELISpot                                  |
Table 1. Cont.

| Sequence            | Protein | Position | Reference | HLA Restriction | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes                  |
|---------------------|---------|----------|-----------|----------------|-----------------------------------|------------------------------------------------------------|
| STLPETAVVRR         | Core    | 170–180  | [9]       | A*1101         | Predicted                         | Proliferation assay; Tetramer staining                      |
| RTQSPRRR           | Core    | 196–203  | [9]       | A*1101         | Predicted                         | Proliferation assay; Tetramer staining                      |
| RTQSPRRRRR         | Core    | 196–204  | [9]       | A*1101         | Predicted                         | Proliferation assay; Tetramer staining                      |
| RSQSPRRRSSK        | Core    | 196–206  | [9]       | A*1101         | Predicted                         | Proliferation assay; Tetramer staining                      |
| RLCCQLDPA          | HBx     | 4–12     | [99]      | A*0201         | Predicted                         | Binding assay; ELISpot; Cytotoxicity assay                  |
| AYFKDCVFKDHW       | HBx     | 6–16     | [45]      | A*2402         | Predicted                         | ELISA                                                      |
| QLDPARDVL          | HBx     | 8–16     | [45,65,73,99–101] | A*0201 | Predicted                         | ELISpot                                                   |
| VLCLRPVGA          | HBx     | 15–23    | [45,99,102]| A*0201         | Predicted                         | ELISpot                                                   |
| RGRPVSGPF          | HBx     | 26–34    | [85]      | A*2402         | Predicted                         | ELISpot                                                   |
| PVSGPFGL           | HBx     | 29–37    | [100]     | A*0201         | Predicted                         | ELISpot                                                   |
| AVPADHGAHL         | HBx     | 44–53    | [100]     | A*0201         | Predicted                         | ELISpot                                                   |
| HLSLRGLPV          | HBx     | 52–60    | [65,99–103]| A*0201, A*02   | Predicted                         | Immunization of mice; CTL assay; ELISpot; Cytotoxicity assay |
| LPVCAFSSA          | HBx     | 58–66    | [45]      | B*0702         | Predicted                         | ELISA                                                      |
| AFFSSAPCALRF       | HBx     | 62–73    | [45]      | A*2402         | Predicted                         | ELISA                                                      |
| ALRTSARR           | HBx     | 70–78    | [45]      | A*0301         | Predicted                         | ELISpot                                                   |
| ALRTSARRM          | HBx     | 70–79    | [100]     | A*0201         | Predicted                         | ELISpot                                                   |
| NAHQILPKV          | HBx     | 84–92    | [99]      | A*0201         | Predicted                         | Binding assay; ELISpot; Cytotoxicity assay                  |
| (K)VLHKRTLGL       | HBx     | 91/92–100| [65,100,102]| A*0201         | Predicted                         | Binding assay; ELISpot; Tetramer staining                   |
| VHLKRTLGL         | HBx     | 92–100   | [99,101,104]| A*0201, A*02   | Predicted                         | Binding assay; ELISpot; Cytotoxicity assay; Proliferation assay; ELISpot; ICS |
| TLGLAAMST          | HBx     | 97–105   | [100]     | A*0201         | Predicted                         | Binding assay; ELISpot; Cytotoxicity assay                  |
| GLSAMSTTDL         | HBx     | 99–108   | [99,100,104]| A*0201, A*02   | Predicted                         | Binding assay; ELISpot; Cytotoxicity assay                  |
Table 1. Cont.

| Sequence       | Protein | Position | Reference | HLA Restriction | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes |
|----------------|---------|----------|-----------|----------------|-------------------------------------|--------------------------------------------|
| AMSTTDLEA      | HBx     | 102–110  | [99]      | A*0201         | Predicted                           | Binding assay; ELISpot; Cytotoxicity assay |
| STTDLEAYFK     | HBx     | 104–113  | [45]      | A*1101         | Predicted                           | ELISA                                      |
| DLEAYFKDCL     | HBx     | 107–116  | [100]     | A*0201         | Predicted                           | Immunization of mice; CTL assay; Cytotoxicity assay |
| CLFKDWEEL      | HBx     | 115–123  | [99,100,102] | A*0201        | Predicted                           | Immunization of mice; CTL assay; Cytotoxicity assay; Binding assay; ELISpot |
| ELGEEIRLKV     | HBx     | 122–131  | [100]     | A*0201         | Predicted                           | Immunization of mice; CTL assay; Cytotoxicity assay |
| EIRLKVFVL      | HBx     | 126–134  | [100]     | A*0201         | Predicted                           | Immunization of mice; CTL assay; Cytotoxicity assay |
| VLGCCRHKL      | HBx     | 133–141  | [99,101]  | A*0201, A*02   | Predicted                           | Binding assay; ELISpot; Cytotoxicity assay; ELISpot |
| VLGCCRHKL(V)   | HBx     | 133–141/142 | [98]    | A*0201         | Predicted                           | Immunization of mice; CTL assay; Cytotoxicity assay |
| LLDDEAGPL      | Pol     | 13–21    | [105,106] | A*0201         | Predicted                           | Binding assay; Immunization of mice; CTL assay; Cytotoxicity assay |
| PLEEELPRRL     | Pol     | 20–28    | [105,106] | A*0201         | Predicted                           | Binding assay; Immunization of mice; CTL assay; Cytotoxicity assay |
| DLNLGNLN       | Pol     | 40–48    | [106]     | A*0201         | Predicted                           | Binding assay; Immunization of mice; CTL assay; Cytotoxicity assay |
| NLGNLNVI       | Pol     | 42–50    | [106]     | A*0201         | Predicted                           | Stabilization assay; ELISpot; Cytotoxicity assay; Immunization of mice; Proliferation assay; Tetramer staining; Binding assay |
| NVSIPWTHK      | Pol     | 47–55    | [9,74,81] | A*03, A*11, A*6801, A*0301, A*1101 | Predicted                           | Binding assay; CTL assay; Cytotoxicity assay; ELISA |
| KVGNFTGLY      | Pol     | 55–63    | [45,74]   | A*0301, A*03, A*11 | Predicted                           | Binding assay; CTL assay; Cytotoxicity assay; ELISA |
| GLYSSTVPV      | Pol     | 61–69    | [73,105,106] | A*0201         | Predicted                           | Binding assay; Immunization of mice; CTL assay; Cytotoxicity assay; Tetramer staining |
| Sequence       | Protein | Position | Reference | HLA Restriction | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes |
|---------------|---------|----------|-----------|-----------------|-------------------------------------|------------------------------------------|
| LYSSTVPVF     | Pol     | 62–70    | [79]      | A*24            | Predicted                           | ELISpot                                  |
| STVPCFNPK     | Pol     | 65–73    | [9]       | A*1101          | Predicted                           | Proliferation assay; Tetramer staining    |
| TVPCFNPK      | Pol     | 66–73    | [9]       | A*1101          | Predicted                           | Proliferation assay; Tetramer staining    |
| PSFPHIHLK     | Pol     | 77–85    | [9]       | A*1101          | Predicted                           | Proliferation assay; Tetramer staining    |
| QYVGPLTVN     | Pol     | 94–102   | [85]      | A*2402          | Predicted                           | ELISpot                                  |
| YLHTLWKAGI    | Pol     | 147–156  | [65]      | A*02            | Predicted                           | ELISpot assay; Tetramer staining          |
| (H)TLWKAGILYK | Pol     | 149/150–159 | [81] | A*03            | Predicted                           | Binding assay; Immunization of mice; ELISpot |
| HTLWKAGILYK   | Pol     | 149–159  | [74,76,98]| A*03, A*11, A*3101, A*3301, A*6801, A*02, A*11 | Predicted                           | Immunization of mice; Cytotoxicity assay; Binding assay; CTL assay |
| TLWKAGILY(K)  | Pol     | 150–158/159 | [74] | A*03, A*11 | Predicted                           | Binding assay; CTL assay; Cytotoxicity assay |
| RSASFCGSPY    | Pol     | 164–173  | [45]      | A*1101          | Predicted                           | ELISA                                    |
| ASCFGSPYSW    | Pol     | 166–175  | [45,62,63]| A*2402, B*5801  | overlapping                        | ELISA; ELISpot; ICS                       |
| SFCCGSPYSW    | Pol     | 167–175  | [45]      | A*2402          | Predicted                           | ELISA                                    |
| ASCFGSPY      | Pol     | 166–173  | [81,95,107]| A*0101, A*2902, A*3002 | overlapping                        | Binding assay; Immunization of mice; ELISpot; Tetramer staining |
| SPYWSEWQEL    | Pol     | 171–179  | [17]      | A*0201, B*3501  | Predicted                           | Tetramer staining                         |
| QSSGILSR      | Pol     | 200–207  | [9]       | A*1101          | Predicted                           | Proliferation assay; Tetramer staining    |
| GILPRSSVGPR   | Pol     | 205–215  | [9]       | A*1101          | Predicted                           | Proliferation assay; Tetramer staining    |
| CLHQSAVRK     | Pol     | 274–282  | [45]      | A*0301, A*1101  | Predicted                           | ELISA                                    |
| KTAYSHLTSK    | Pol     | 283–293  | [9]       | A*1101          | Predicted                           | Proliferation assay; Tetramer staining    |
| SSARSQSER     | Pol     | 310–318  | [9]       | A*1101          | Predicted                           | Proliferation assay; Tetramer staining    |
| CLSLIVNLL     | Pol     | 338–346  | [65]      | A*02            | Predicted                           | ELISpot assay; Tetramer staining          |
| TPARVTGGVF    | Pol     | 354–362  | [45]      | B*0702          | Predicted                           | ELISA                                    |
| TPARVTGGVF    | Pol     | 354–363  | [45]      | B*0702          | Predicted                           | ELISA                                    |
| RVTTGGVFLV    | Pol     | 357–365  | [45]      | A*0201          | Predicted                           | ELISA                                    |
| VTTGGVFLVDK   | Pol     | 358–367  | [45]      | A*1101, A*03    | Predicted                           | ELISA                                    |
| RIPRTPSRV     | Pol     | 361–369  | [65]      | A*02            | Predicted                           | ELISpot assay; Tetramer staining          |
| TPARVTGGVF    | Pol     | 365–374  | [74,76,108]| B*0702, B*3501, A*03, B*07, A*02, B*51 | Predicted                           | Immunization of mice; Cytotoxicity assay; Binding assay; CTL assay |
| Sequence      | Protein | Position | Reference | HLA Restriction | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes         |
|---------------|---------|----------|-----------|-----------------|-------------------------------------|--------------------------------------------------|
| RVTGGVFLVDK   | Pol     | 368–378  | [74]      | A*11            | Predicted                           | Binding assay; CTL assay; Cytotoxicity assay     |
| VTGGVFLVDK    | Pol     | 369–378  | [74]      | A*03, A*11      | Predicted                           | Binding assay; CTL assay; Cytotoxicity assay     |
| FLVDKNPHNT    | Pol     | 374–383  | [62]      | A*0203          | Predicted                           | ELISpot; ICS; Binding assay                      |
| LVVDFLHQFSR   | Pol     | 377–386  | [9]       | A*1101, A*3301, A*6801 | Predicted                           | Proliferation assay; Tetramer staining; Binding assay; Immunization of mice; ELISpot; CTL assay; Cytotoxicity assay |
| SRLVVDPSQF    | Pol     | 386–395  | [63]      | B*1301          | overlapping                        | ICS; ELISpot                                      |
| VVDFSQFSR     | Pol     | 389–397  | [74, 91]  | A*11, A*6801, A*33 | Predicted                           | Stabilization assay; ELISpot; Cytotoxicity assay; Binding assay; Immunization of mice; CTL assay |
| SWPKFAVPNL    | Pol     | 392–401  | [45]      | A*2402          | Predicted                           | ELISA                                            |
| WPKFAVPNL     | Pol     | 393–401  | [45]      | B*0702          | Predicted                           | ELISA                                            |
| FAVPNLQSL     | Pol     | 396–404  | [45]      | A*0201          | Predicted                           | ELISA                                            |
| NLQSLTNLL     | Pol     | 411–419  | [105, 106]| A*0201         | Predicted                           | Cytotoxicity assay; Immunization of mice; Binding assay; CTL assay |
| LLSSNLSWL     | Pol     | 418–426  | [65, 105, 106]| A*0201      | Predicted                           | Cytotoxicity assay; Immunization of mice; Binding assay; CTL assay; ELISpot; Tetramer staining |
| NLSWLSLDV     | Pol     | 422–430  | [101, 105, 106]| A*0201, A*02 | Predicted                           | Cytotoxicity assay; Immunization of mice; Binding assay; CTL assay; ELISpot |
| LSDVSAAFY     | Pol     | 426–435  | [81]      | A*0101, A*2902, A*3002 | Predicted                           | Binding assay; Immunization of mice; ELISpot |
| HPAAMPHLL     | Pol     | 440–448  | [74]      | B*0702          | Predicted                           | Binding assay; CTL assay; Cytotoxicity assay     |
| HLLVGSSGL     | Pol     | 446–454  | [105, 106]| A*0201         | Predicted                           | Cytotoxicity assay; Immunization of mice; Binding assay; CTL assay |
| GLPRYVARL     | Pol     | 453–461  | [65, 71, 73, 74, 81, 92, 93, 100, 101, 106, 109–111]| A*0201, A*0202, A*0203, A*02, A*0207 | Predicted                                           | Cytotoxicity assay; Immunization of mice; Binding assay; CTL assay; ELISpot; Tetramer staining |
| RIINNQHR      | Pol     | 466–473  | [9]       | A*1101          | Predicted                           | Proliferation assay; Tetramer staining           |
| Sequence          | Protein | Position | Reference | HLA Restriction | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes                                                                 |
|-------------------|---------|----------|-----------|----------------|-------------------------------------|----------------------------------------------------------------------------------------------------------|
| RNLYVSLLL         | Pol     | 484–492  | [85]      | A*2402         | Predicted                           | Cytotoxicity assay; Immunization of mice; Binding assay; CTL assay; ELISpot; Tetramer staining           |
| NLYVSLLLL         | Pol     | 485–493  | [65,106]  | A*0201, A*02   | Predicted                           |                                                                                                          |
| KLHYSHPI          | Pol     | 500–508  | [45,62,93, 101,106] | A*0201, A*02, A*0203, B*0801     | Predicted                           | Cytotoxicity assay; Immunization of mice; Binding assay; CTL assay; ELISpot; Tetramer staining; ELISA    |
| HLYSHPIIL         | Pol     | 502–510  | [65,105, 112–114] | A*0201, A*02, A*0203 overlapping | Predicted                           |                                                                                                          |
| IPMGVGGLSP        | Pol     | 504–512  | [45]      | B*0702         | Predicted                           | ELISA                                                                                                   |
| ILGFRKIPM         | Pol     | 509–517  | [45]      | B*0801         | Predicted                           | ELISA                                                                                                   |
| FLLAQFTSAI        | Pol     | 524–533  | [65,101]  | A*0201, A*02   | Predicted                           | ELISpot; Tetramer staining                                                                                         |
| LLAQFTSAI         | Pol     | 525–533  | [65,101,106] | A*0201, A*02   | Predicted                           | Cytotoxicity assay; Immunization of mice; Binding assay; CTL assay; ELISpot; Tetramer staining           |
| SAICSVVRR         | Pol     | 531–539  | [74]      | A*11, A*3301, A*6801 | Predicted                           | Binding assay; CTL assay; Cytotoxicity assay                                                                 |
| SVVRRAFPF         | Pol     | 535–542  | [9]       | A*1101         | Predicted                           | Proliferation assay; Tetramer staining                                                                 |
| FFPHCLAFSYM       | Pol     | 539–550  | [81]      | B*07           | Predicted                           | Binding assay; Immunization of mice; ELISpot                                                                 |
| FPHCLAFSYM        | Pol     | 540–550  | [74]      | B*0702, B*3501, B*51, B*5301, B*5401 | Predicted                           | Binding assay; CTL assay; Cytotoxicity assay                                                                 |
| YMDDVVVLG         | Pol     | 549–556  | [81]      | A*0201, A*0202, A*0203, A*0206, A*6802 | Predicted                           | Binding assay; Immunization of mice; ELISpot                                                                 |
| YMDDVVVLGA        | Pol     | 549–557  | [45,71,72, 99,101,114–116] | A*0201, A*02, A*0101 overlapping | Predicted                           | Cytotoxicity assay; Immunization of mice; Binding assay; ELISpot; CTL assay; ELISA                         |
| YMDDVVVLGAK       | Pol     | 549–558  | [74]      | A*03           | Predicted                           | Binding assay; CTL assay; Cytotoxicity assay                                                                 |
Table 1. Cont.

| Sequence      | Protein | Position | Reference | HLA Restriction       | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes |
|---------------|---------|----------|-----------|-----------------------|-------------------------------------|------------------------------------------|
| FLLSLGIHL    | Pol     | 573–581  | [71,73,74, 81,93,106, 108,110, 116–120] | A*02, A*0201, A*0206, A*0202 | Predicted                          | Cytotoxicity assay; Immunization of mice; Binding assay; ELISpot; CTL assay; Tetramer staining |
| SLNFMGYVI    | Pol     | 592–600  | [106]    | A*0201               | Predicted                          | Binding assay; Immunization of mice; CTL assay; Cytotoxicity assay |
| PVNRPIDWK    | Pol     | 612–620  | [9]      | A*1101               | Predicted                          | Proliferation assay; Tetramer staining |
| PVNRPIDWK    | Pol     | 623–631  | [74]    | A*03, A*11           | Predicted                          | Binding assay; CTL assay; Cytotoxicity assay |
| CGYPALMPLY   | Pol     | 638–647  | [45]    | A*2402               | Predicted                          | ELISA                                    |
| GYPALMPLY    | Pol     | 639–647  | [45]    | A*2402               | Predicted                          | ELISA                                    |
| YPALMPYLA    | Pol     | 651–659  | [74]    | B*0702, B*2501, B*51, B*5401 | Predicted                          | Binding assay; CTL assay; Cytotoxicity assay |
| YPALMPYSA    | Pol     | 651–659  | [62]    | B*5401               | Predicted                          | ELISpot; ICS; Binding assay             |
| ALMPYACI     | Pol     | 653–661  | [71,74,93, 106] | A*0201, A*0202, A*0203, A*0204, A*0206, A*02 | Predicted                          | Cytotoxicity assay; Immunization of mice; Binding assay; ELISpot; CTL assay; Tetramer staining |
| QAFTSPTYK    | Pol     | 665–674  | [74,113] | A*03, A*11, A*6801   | Predicted                          | Cytotoxicity assay; Binding assay; CTL assay |
| VFADATPTGW   | Pol     | 686–695  | [45]    | A*2402               | Predicted                          | ELISA                                    |
| GLCQVFADA    | Pol     | 692–700  | [45]    | A*0201               | Predicted                          | ELISA                                    |
| LPIHTAELL    | Pol     | 712–720  | [45]    | B*0702               | Predicted                          | ELISA                                    |
| PLPIHTAEL    | Pol     | 722–730  | [106]   | A*0201               | Predicted                          | Binding assay; Immunization of mice; CTL assay; Cytotoxicity assay |
| IIGTDNSVV    | Pol     | 744–752  | [65]    | A*0201               | Predicted                          | ELISpot assay; Tetramer staining         |
| RKYTSFPWLL   | Pol     | 744–753  | [45]    | A*2402               | Predicted                          | ELISA                                    |
| KYSFPWLLG    | Pol     | 745–754  | [45]    | A*2402               | Predicted                          | ELISA                                    |
| GTDNSVVLSR   | Pol     | 746–755  | [74]    | A*11                 | Predicted                          | Binding assay; CTL assay; Cytotoxicity assay |
| KYSFPWLL     | Pol     | 756–764  | [63,81,84, 88,93] | A*24, A*2301, A*2402 | overlapping                        | Cytotoxicity assay; Immunization of mice; Binding assay; ELISpot; CTL assay; Tetramer staining; ICS; ELISA |
| LLGCAANWI    | Pol     | 763–771  | [65,106] | A*0201               | Predicted                          | Cytotoxicity assay; Immunization of mice; Binding assay; ELISpot; CTL assay; Tetramer staining |

Table 1. Cont.

| Sequence       | Protein | Position  | Reference         | HLA Restriction       | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes |
|----------------|---------|-----------|-------------------|-----------------------|------------------------------------|------------------------------------------|
| WILRGTSFV      | Pol     | 770–778   | [65,105]          | A*0201, A*02          | Predicted                          | Immunization of mice; Binding assay; ELISpot; Tetramer staining |
| ILRGTSFVVV     | Pol     | 771–780   | [65,71]           | A*0201, A*02          | Predicted                          | Cytotoxicity assay; ELISpot; Tetramer staining |
| DPSRGRLGL      | Pol     | 789–797   | [74]              | B*0702                | Predicted                          | Binding assay; CTL assay; Cytotoxicity assay |
| RLGLSRPLLL     | Pol     | 794–802   | [106]             | A*0201                | Predicted                          | Binding assay; Immunization of mice; CTL assay; Cytotoxicity assay |
| GLSRPLLRLL     | Pol     | 796–804   | [65]              | A*02                  | Predicted                          | ELISpot assay; Tetramer staining |
| LVYRFTTGR      | Pol     | 804–812   | [9]               | A*1101                | Predicted                          | Proliferation assay; Tetramer staining |
| SLYADSPSV      | Pol     | 814–822   | [65,71,73, 90,93,106, 114,116] | A*0201, A*02          | Predicted                          | Cytotoxicity assay; Immunization of mice; Binding assay; ELISpot; CTL assay; Tetramer staining; FACS |
| FLLTRILTI      | S       | 20–28     | [66–68,77, 100,121] | A*0201                | Predicted                          | ICS; Tetramer staining; Cytotoxicity assay; Degranulation assay |
| PLGFPPPVDH     | S       | 21–28     | [122]             | A*11                  | Predicted                          | ELISpot |
| NLLGWSFQA      | S       | 73–81     | [63]              | A*0201, A*0207        | overlapping                        | ICS; ELISpot |
| LTTVPAASLLA    | S       | 85–95     | [95]              | A*02                  | Predicted                          | ELISpot |
| TTSTGPCK       | S       | 115–122   | [9]               | A*1101                | Predicted                          | Proliferation assay; Tetramer staining |
| LLDPVRGL       | S       | 131–139   | [75]              | A*02                  | Predicted                          | CTL assay |
| AILSKTGDVP     | S       | 160–169   | [116]             | A*02                  | Predicted                          | Tetramer staining |
| FGLPLVLQLA     | S       | 182–190   | [62,63,107]       | C*0801                | overlapping                        | Cytotoxicity assay; Binding assay; ELISpot; Tetramer staining |
| VLQAGFFL       | S       | 188–195   | [62]              | C*0801                | Predicted                          | ELISpot; ICS; Binding assay |
| VLQAGFFLL      | S       | 188–196   | [65,71,101, 116,123] | A*0201, A*02          | Predicted                          | Cytotoxicity assay; Immunization of mice; Binding assay; ELISpot; CTL assay; Tetramer staining |
| SWWTSLNFL      | S       | 192–200   | [85]              | A*2402                | Predicted                          | ELISpot |
| FLLTRILTI      | S       | 194–202   | [54,74,76, 81,90,93,94, 101,108,111, 114,116,119, 120,123–128] | A*0201, A*0202, A*0203, A*0206, A*02 | Predicted                          | Cytotoxicity assay; Immunization of mice; Binding assay; ELISpot; CTL assay; Tetramer staining; ICS; FACS |
| Sequence          | Protein | Position | Reference | HLA Restriction | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes                           |
|-------------------|---------|----------|-----------|-----------------|-------------------------------------|---------------------------------------------------------------------|
| IPQSLDSWWTSL      | S       | 202–213  | [129,130] | A*0201, A*02    | Predicted                           | Cytotoxicity assay; Immunization of mice; Binding assay; ELISpot    |
| SILSPFPLL         | S       | 207–216  | [131]     | A*0201          | Predicted                           | Binding assay; ELISpot                                             |
| NILSPFMPLL        | S       | 208–216  | [131]     | A*0201          | Predicted                           | Binding assay; ELISpot                                             |
| TLSPFPLL          | S       | 208–216  | [131]     | A*0201          | Predicted                           | Binding assay; ELISpot                                             |
| SWWTSLNFLL       | S       | 208–216  | [84]      | A*24            | Predicted                           | Tetramer staining                                                  |
| FLGGTPVCL         | S       | 215–223  | [95,116,123,125] | A*0201, A*02, A*24 | Predicted                           | Cytotoxicity assay; Immunization of mice; Binding assay; ELISpot; CTL assay; Tetramer staining |
| SWLSLLVPF         | S       | 226–234  | [85]      | A*2402          | Predicted                           | ELISpot                                                           |
| RWMCRRFII         | S       | 236–245  | [85]      | A*2402          | Predicted                           | ELISpot                                                           |
| CGYRWCMCL         | S       | 243–251  | [108]     | B*07            | Predicted                           | Cytotoxicity assay                                                 |
| GYRWMCRRR         | S       | 245–253  | [91]      | A*33            | Predicted                           | Stabilization assay; ELISpot; Cytotoxicity assay; Immunization of mice |
| RWMCRRFII         | S       | 247–256  | [81]      | A*2301, A*2402  | Predicted                           | Binding assay; Immunization of mice; ELISpot                       |
| ILLLCLIFL         | S       | 260–268  | [73,125]  | A*0201          | Predicted                           | Cytotoxicity assay; Immunization of mice                           |
| LLLLCLIFLL        | S       | 261–268  | [72]      | A*02            | Predicted                           | Cytotoxicity assay                                                 |
| LLLLCLIFLLV       | S       | 262–269  | [65,115,123] | A*0201, A*02    | Predicted                           | Stabilization assay; ELISpot; Cytotoxicity assay; Tetramer staining; Immunization of mice |
| LCLIFLLVL         | S       | 263–271  | [85]      | A*2402          | Predicted                           | ELISpot                                                           |
| (L)VLLDYQGML      | S       | 269/70–278 | [75]     | A*0201          | Predicted                           | CTL assay                                                         |
| LLDYQGMLP         | S       | 271–279  | [123]     | A*0201          | Predicted                           | Immunization of transgenic mice; Cytotoxicity assay; ELISpot; Binding assay |
| LLDYQGMLPV        | S       | 271–280  | [72,101,116,125] | A*02          | Predicted                           | ELISpot; Cytotoxicity assay; Binding assay; Tetramer staining     |
| TSMFPSCCCTK       | S       | 305–315  | [9]       | A*1101          | Predicted                           | Proliferation assay; Tetramer staining                            |
| Sequence     | Protein | Position | Reference       | HLA Restriction | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes |
|--------------|---------|----------|-----------------|----------------|-------------------------------------|-------------------------------------------|
| IPIPSSWAF    | S       | 324–332  | [74,76,81,108]  | B*0702, B*3501, B*51, B*5301, A*03, B*07, A*02, B*5101 | Predicted                             | ELISpot; Cytotoxicity assay; Immunization of mice; Binding assay; CTL assay |
| YLWEWASVR    | S       | 335–343  | [91]            | A*33           | Predicted                           | Stabilization assay; ELISpot; Cytotoxicity assay; Immunization of mice |
| RFSWLSLLVPF  | S       | 343–353  | [81]            | A*2301, A*2402 | Predicted                           | Binding assay; Immunization of mice; ELISpot |
| SWLSLLVPF    | S       | 345–353  | [84]            | A*24           | Predicted                           | Tetramer staining                       |
| WLSLLVPFV    | S       | 346–354  | [71–76,99, 105,108,117, 118,120,123, 132,133] | A*02, A*0201, A*0202, A*0203, A*0206, A*0207, A*04, A*6602 | Predicted                             | ELISpot; Cytotoxicity assay; Immunization of mice; Binding assay; Tetramer staining |
| LLVPFVQWFV   | S       | 349–358  | [93,101,111]    | A*02           | Predicted                           | ICS; Degranulation assay; ELISpot; Tetramer staining |
| VGLSPTVWL    | S       | 358–366  | [85]            | A*2402         | Predicted                           | ELISpot                                 |
| GLSPTVWLS    | S       | 359–367  | [123]           | A*0201         | Predicted                           | Immunization of transgenic mice; Cytotoxicity assay; ELISpot; Binding assay |
| GLSPTVWLSV   | S       | 359–368  | [72,73,90, 93,105,111, 114,116,124, 125,128,130, 134] | A*02, A*0201, A*0202, A*0203, A*0207 | overlapping                           | Immunization of mice; FACS; CTL assay; ELISpot; Tetramer staining; Degranulation assay |
| VWLSVIWM     | S       | 364–371  | [90]            | A*0201         | Predicted                           | Immunization of mice; FACS              |
| (L)SVIWMWYW  | S       | 366/367–375 | [62]          | B*5801         | Predicted                           | ELISpot; ICS; Binding assay            |
| SVIWMWYW     | S       | 367–375  | [63,107]        | B*5801         | overlapping                         | Tetramer staining; ICS; ELISpot         |
| SIVSPFIPLL   | S       | 370–379  | [131]           | A*0201         | Predicted                           | Binding assay; ELISpot                  |
| ILSPFPLL     | S       | 371–379  | [131]           | A*0201         | Predicted                           | Binding assay; ELISpot                  |
| MMWYYGPSLY   | S       | 371–380  | [74]            | A*03           | Predicted                           | Binding assay; CTL assay; Cytotoxicity assay |
| NILSPFPLL    | S       | 381–390  | [131]           | A*0201         | Predicted                           | Binding assay; ELISpot                  |
| SILSPFPLL    | S       | 381–390  | [77]            | A*0201         | Predicted                           | ICS; Tetramer staining                  |
| SIVSPFIPLL   | S       | 381–390  | [72,73,116, 123] | A*02, A*0201  | Predicted                           | Immunization of mice; FACS; CTL assay; ELISpot; Tetramer staining |
| Sequence      | Protein | Position | Reference | HLA Restriction | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes |
|--------------|---------|----------|-----------|----------------|-------------------------------------|--------------------------------------------|
| ILSPFLPLL    | S       | 382–390  | [75,90]   | A*0201         | Predicted                           | Immunization of mice; FACS; CTL assay      |
| IVSPFIPLL    | S       | 382–390  | [134]     | A*0201         | Predicted                           | ELISA; Cytotoxicity assay                  |
| ILRSFIPLL    | S       | 382–390  | [95]      | A*02, A*24     | Predicted                           | ELISpot                                    |
| LLPFFCLWV    | S       | 389–398  | [101]     | A*02          | Predicted                           | ELISpot                                    |
| DIDPYKEFGATVELL | Core     | 2–16     | [135]     | DRB1*0401     | overlapping                         | Proliferation assay; ICS                   |
| IDPYKEFGATVELLS | Core     | 3–17     | [135]     | DRB1*0401     | overlapping                         | Proliferation assay; ICS                   |
| DPYKEFGATVELLSF | Core     | 4–18     | [135]     | DRB1*0401     | overlapping                         | Proliferation assay; ICS                   |
| PYKEFGATVELLSFL | Core     | 5–19     | [135]     | DRB1*0401     | overlapping                         | Proliferation assay; ICS                   |
| YKEFGATVELLSFLP | Core     | 6–20     | [135,136]| DRB1*0401,   | overlapping                         | ICS; Proliferation assay                   |
| KEFGATVELLSFLPS | Core     | 7–21     | [135]     | DRB1*0401     | overlapping                         | Proliferation assay; ICS                   |
| EFGATVELLSFLPSD | Core     | 8–22     | [135]     | DRB1*0401     | overlapping                         | Proliferation assay; ICS                   |
| FGATVELLSFLPSDF | Core     | 9–23     | [135]     | DRB1*0401     | overlapping                         | Proliferation assay; ICS                   |
| GATVELLSFLPSDF | Core     | 10–24    | [135]     | DRB1*0401     | overlapping                         | Proliferation assay; ICS                   |
| TVELLPSLPSDFPS | Core     | 12–26    | [135]     | DRB1*0401     | overlapping                         | Proliferation assay; ICS                   |
| VELLSLPSLPSDFPSV | Core     | 13–27    | [135]     | DRB1*0401     | overlapping                         | Proliferation assay; ICS                   |
| LSFLPSLPSDFPSVRD | Core     | 15–29    | [135]     | DRB1*0401     | overlapping                         | Proliferation assay; ICS                   |
| LSFLPSLPSDFPSVRDL | Core     | 16–30    | [135]     | DRB1*0401     | overlapping                         | Proliferation assay; ICS                   |
| FLPSLPSDFPSVRD | Core     | 18–29    | [137]     | DPw4, DRB1*07 | Predicted                           | Cytotoxicity assay                         |
| RDLLDTSALYREALSEPEH | Core   | 28–47    | [138]     | DRB1*07,     | overlapping                         | Proliferation assay                       |
| ALYREALSEPEHCS | Core     | 36–50    | [136]     | DRB1*1202     | overlapping                         | ICS                                        |
| ALESPEHCSPHHTALRQAIL | Core     | 41–60    | [139]     | DRB1*13      | overlapping                         | Proliferation assay                       |
| EHCSPHHTALRQAIL | Core     | 46–60    | [136]     | DRB1*0803     | overlapping                         | ICS                                        |
| PHHTALRQAILCWGELMTLA | Core     | 50–69    | [81]      | DRB1*07,     | Predicted                           | Binding assay; Immunization of mice; ELISpot |
| HHTALRQAILCWGEL | Core     | 51–65    | [136]     | DRB1*1202     | overlapping                         | ICS                                        |
| RQAILCWGELMNLAT | Core     | 56–70    | [136]     | DRB1*0803,   | overlapping                         | ICS                                        |
| LCWGELMTLATWVGVN | Core     | 60–76    | [140]     | DRB1*0101     | Predicted                           | Proliferation assay; ICS; Tetramer staining |
| MNLATWVGNSNLEDPA | Core     | 66–80    | [136]     | DRB1*0803     | overlapping                         | ICS                                        |
| LEDPASRELVSVYVN | Core     | 76–90    | [136]     | DRB1*1202     | overlapping                         | ICS                                        |
Table 1. Cont.

| Sequence                | Protein | Position | Reference | HLA Restriction | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes |
|-------------------------|---------|----------|-----------|-----------------|-------------------------------------|---------------------------------------------|
| SRELVVSYYVNVNMGL        | Core    | 81–95    | [136]     | DRB1*0803       | overlapping                         | ICS                                         |
| LEYLVSFGVWIRTPP         | Core    | 116–130  | [136]     | DRB1*1202       | overlapping                         | ICS                                         |
| EYLVSFGVWIRTPPA         | Core    | 117–131  | [138]     | DRB*05, DRB*06  | overlapping                         | Proliferation assay                         |
| VSFGVWIRTPPA1RPPNAPI    | Core    | 120–139  | [81,138]  | DRB1*01, DRB1*07, DRB1*11, DRB1*12, DRB1*13 | overlapping | Binding assay; Immunization of mice; ELISpot; Proliferation assay |
| NAPILSTLPETTVVR         | Core    | 136–150  | [136]     | DRB1*0803       | overlapping                         | ICS                                         |
| STLPEETVVRRRGRRSSRT     | Core    | 141–160  | [141]     | DRB1*13         | Predicted                           | Proliferation assay; Cytotoxicity assay; ICS |
| PRRTSPRRTRSSQRS         | Core    | 156–170  | [136]     | DRB1*0803       | overlapping                         | ICS                                         |
| PPA1RPPNAP1STL          | Core    | 158–172  | [135]     | DRB1*0101       | overlapping                         | Proliferation assay; ICS                    |
| PAPPRPNAP1L             | Core    | 159–169  | [142]     | DR52, Drw3      | overlapping                         | Proliferation assay; Cytotoxicity assay     |
| PSRRRSSQPSRRRSS         | Core    | 161–175  | [136]     | DRB1*0803       | overlapping                         | ICS                                         |
| RRSSQPRSSQSSQRRE        | Core    | 166–180  | [136]     | DRB1*1202       | overlapping                         | ICS                                         |
| YFKDCLFKDWEELGE         | HBx     | 111–125  | [143]     | DRB1*1301       | overlapping                         | ELISpot; Binding assay; ICS                 |
| EIRLKVFLGCGCRHK         | HBx     | 126–140  | [143]     | DRB1*0101, DRB1*0401, DRB1*1301, DRB5*0101 | overlapping | ELISpot; Binding assay; ICS |
| VFVLGGCRHKLVCAP         | HBx     | 131–145  | [143]     | DRB1*1301       | overlapping                         | ELISpot; Binding assay; ICS                 |
| VGPLTVNEKRRKL1I         | Pol     | 96–111   | [113]     | DRB1*0301       | Predicted                           | ELISpot; Cytotoxicity assay                 |
| RHYLHTLWKAGILYK         | Pol     | 145–160  | [113]     | DRB1*0301, DRB1*07, DRB1*08, DRB1*09, DRB1*11, DRB1*12, DRB1*15 | Predicted | ELISpot; Cytotoxicity assay |
| ESRLVVFDSQFRSGN         | Pol     | 385–400  | [113]     | DRB1*03, DRB1*04 | Predicted                           | ELISpot; Cytotoxicity assay                 |
| LQSLTNLLSSNLSSLWL       | Pol     | 412–427  | [113]     | DRB1*01, DRB1*04, DRB1*07, DRB1*11, DRB1*12, DRB1*13, DRB1*15 | Predicted | ELISpot; Cytotoxicity assay |
| SSNLSWLSLDSAAAF         | Pol     | 420–435  | [113]     | DRB1*01, DRB1*03, DRB1*04, DRB1*13 | Predicted | ELISpot; Cytotoxicity assay |
| LHYSHPIILGFKI          | Pol     | 501–516  | [113]     | DRB1*01, DRB1*04, DRB1*11 | Predicted | ELISpot; Cytotoxicity assay |
| Sequence                  | Protein | Position | Reference | HLA Restriction | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes |
|---------------------------|---------|----------|-----------|-----------------|------------------------------------|--------------------------------------------|
| PFLLAQFTSAICSVV           | Pol     | 525–538  | [81]      | DRB1*01, DRB1*04, DRB1*07, DRB1*08, DRB1*09, DRB1*11, DRB1*15, DRB5*01 | Predicted                                | Binding assay; Immunization of mice; ELISpot |
| KQCFRKLPVNRPIDW           | Pol     | 618–633  | [81,113]  | DRB1*01, DRB1*04, DRB1*07, DRB1*13 | Predicted                                | Binding assay; Immunization of mice; ELISpot; Cytotoxicity assay |
| LCQVFADATPTGWGL           | Pol     | 649–664  | [81]      | DRB1*03, DRB1*04, DRB1*07 | Predicted                                | Binding assay; Immunization of mice; ELISpot |
| KQAFTSPTYKAFLC            | Pol     | 664–679  | [113]     | DRB1*01, DRB1*04, DRB1*07, DRB1*08, DRB1*09, DRB1*11, DRB1*13, DRB1*15 | Predicted | ELISpot; Cytotoxicity assay |
| AANWILRGTSFVVVP           | Pol     | 676–691  | [81]      | DRB1*07, DRB1*08, DRB1*09, DRB1*12, DRB1*13, DRB1*15 | Predicted                                | Binding assay; Immunization of mice; ELISpot |
| LCQVFADATPTGWGL           | Pol     | 694–709  | [113]     | DRB1*03, DRB1*04 | Predicted                                | ELISpot; Cytotoxicity assay |
| AANWILRGTSFVVVP           | Pol     | 767–782  | [113]     | DRB1*01, DRB1*04, DRB1*07, DRB1*08, DRB1*09, DRB1*11, DRB1*13, DRB1*15 | Predicted | ELISpot; Cytotoxicity assay |
| GTSFVVVPASLNPAD           | Pol     | 774–789  | [81]      | DRB1*01, DRB1*04, DRB1*07, DRB1*08, DRB1*09, DRB1*11, DRB1*15, DRB5*01 | Predicted                                | Binding assay; Immunization of mice; ELISpot |
| AGFFLLTRILTPQS            | S       | 17–31    | [144]     | DRB1*07, DRB1*08, DRB1*11, DRB1*13 | Predicted                                | ELISpot; Proliferation assay |
| GFFPDHQLPDAF              | S       | 23–33    | [145]     | DRB1*0405 | Predicted                                | Binding assay; FASC |
| TSLNFLGOSPVCGLQ           | S       | 37–51    | [144]     | DRB1*01 | Predicted                                | ELISpot; Proliferation assay |
| GAFPGPGFTPPhG             | S       | 61–72    | [145]     | DRB1*0405 | Predicted                                | Binding assay; FASC |
| PICPGYRWMCRRFI            | S       | 67–81    | [144]     | DRB1*08, DRB1*11, DRB1*13 | Predicted                                | ELISpot; Proliferation assay |
| Sequence | Protein | Position | Reference | HLA Restriction | Method to Screen Candidate Epitopes | Method to Validate the Candidate Epitopes |
|----------|---------|----------|-----------|----------------|-------------------------------------|-------------------------------------------|
| GWSPQAQGVLTT | S       | 76–87    | [145]     | DRB1*0405      | Predicted                           | ELISpot; Proliferation assay               |
| MQWNSTTFHQTLDPRVRGL | S       | 109–134  | [47]      | DRB1*01       | Predicted                           | Immunization of mice; Proliferation assay; ELISpot |
| TTFHQTLDPRVRGL | S       | 114–128  | [47]      | DRB1*01       | Predicted                           | Immunization of mice; Proliferation assay; ELISpot |
| MQWNSTAHQTLQDP | S       | 109–123  | [146]     | DRB1*02       | Predicted                           | Proliferation assay; Cytotoxicity assay     |
| SLPETTVVRRRGRSPRRRT | S       | 141–160  | [139]     | DRB1*13       | overlapping                         | Proliferation assay                        |
| WASVRFWSLL | S       | 165–176  | [147]     | DRB1*11, DRB1*14 | Predicted                          | CTL assay; Proliferation assay             |
| VPFWQVFGLSPTVW | S       | 177–191  | [144]     | DRB1*11       | Predicted                           | ELISpot; Proliferation assay               |
| QAGFFLLTRILIQPS | S       | 179–194  | [47]      | DRB1*01       | Predicted                           | Immunization of mice; Proliferation assay; ELISpot |
| WLSVIWMMMYWGPSL | S       | 191–205  | [136]     | DRB1*1202     | overlapping                         | ICS                                        |
| TSLNFLGGTTVCLGQ | S       | 200–214  | [47]      | DRB1*01       | Predicted                           | Immunization of mice; Proliferation assay; ELISpot |
| GSPSYSVSPFILL | S       | 202–216  | [144]     | DRB1*07       | Predicted                           | ELISpot; Proliferation assay               |
| LLPFFCLWWYI | S       | 215–226  | [147]     | DRB1*07, DRB1*08, DRB1*14 | Predicted                          | CTL assay; Proliferation assay             |
| PICPCYRWMCLRRFIIFL | S       | 241–258  | [148]     | DRB1*0201     | overlapping                         | Tetramer staining                          |
| FLIVLLDQGMLP | S       | 256–268  | [54]      | DP4           | Predicted                           | Immunization of mice; Proliferation assay; ELISpot |
| WEWASARFSWL | S       | 326–338  | [54]      | DP4           | Predicted                           | Immunization of mice; Proliferation assay; ELISpot |
| WLSLIVPFWQWFSVGL | S       | 335–349  | [149]     | DRB1*0101     | Predicted                           | Immunization of mice; Pentamer staining; ELISpot; Cytotoxicity assay |
| SLIVFPFWQVGLSPTWLSV | S       | 337–357  | [47]      | DRB1*01       | Predicted                           | Immunization of mice; Proliferation assay; ELISpot |
| SVRFWSLIVPFWQWFSV | S       | 343–357  | [148]     | DRB1*0201     | overlapping                         | Tetramer staining                          |
| VGLSPTWLSVI | S       | 347–358  | [54]      | DP4           | Predicted                           | Immunization of mice; Proliferation assay; ELISpot |
| GLSPTWLSVIW | S       | 348–359  | [149]     | DRB1*0101     | Predicted                           | Immunization of mice; Pentamer staining; ELISpot; ICS; Cytotoxicity assay |
| TVWLSVIWMMYW | S       | 352–364  | [54]      | DP4           | Predicted                           | Immunization of mice; Proliferation assay; ELISpot |
5. Conclusions

Here, we have taken an effort to present a reliable and updated T cell epitope repertoire of HBV. We summarized the statistics of 205 unique CD8\(^+\) T cell epitopes and 79 unique CD4\(^+\) T cell epitopes that have been experimentally validated and reported during the past 33 years, corresponding restricting HLA-molecule, and the methods to screen candidate epitopes and validate candidate epitopes. We hope that this review will be used as a tool for the design and development of therapeutic vaccines and T cell detection kits for HBV-infected patients.

**Author Contributions:** Conceptualization, C.S.; Data curation, Y.W. and Y.D.; Funding acquisition, C.S.; Writing—original draft, Y.W.; Writing—review and editing, C.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by Jiangsu Provincial Science and Technology Fund of China (BE2017714). The sponsors had no role in study design, data collection and analysis, preparation of the manuscript, or decision to submit the article for publication.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

**Abbreviations**

HBV: Hepatitis B virus; HLA: human leukocyte antigen; LC: liver cirrhosis; HCC: hepatocellular carcinoma; CTL: cytotoxic T lymphocyte; ORF: open reading frame; cccDNA: covalently closed circular DNA; OLPs: overlapping peptides; PBMCs: peripheral blood mononuclear cells; IFN-\(\gamma\) ELISpot: IFN-\(\gamma\) enzyme-linked immunospot; ICS: intracellular cytokine staining.

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