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# Clinical implications of Epstein-Barr virus strain diversity

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## Article Info

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## ABSTRACT

The Epstein-Barr virus (EBV), a ubiquitous  $\gamma$ -herpesvirus, has been implicated in the etiology of several acute and chronic inflammatory, autoimmune, and malignant diseases. Although considered a genetically stable virus, recent sequence information obtained from a large number of viral isolates from around the world revealed that numerous viral variants exist. Whether these different strains differ in pathogenicity and immunogenicity and thereby contribute to the varying incidence rates of several EBV-associated diseases in different geographical regions is now studied intensively. The recent identification of amino acid sequence polymorphisms in a high percentage of all known virus-specific CD4+ and CD8+ T-cell epitopes, and of holes in the individual T-cell repertoire against epitopes derived from strain variants, may suggest that antiviral immunity is incompletely cross-protective against diverse EBV strains. These findings may have implications for immunological approaches seeking to prevent, monitor, or treat EBV-associated diseases.

## Introduction

EBV is one of the most common viruses in humans and involved in the pathogenesis of several non-malignant and malignant diseases<sup>1,2</sup>. Primary infection with EBV usually occurs within the first three years of life by parent-to-child oral transmission. Following uptake, EBV is assumed to replicate in the oropharynx from where it colonizes the host by latently infecting B cells. The reservoir of latently infected cells can seed foci of virus replication at mucosal sites, and this sporadic reactivation allows the virus to persist for life in the infected host and to spread within the human population<sup>3</sup>. Carried by more than 90% of the worldwide population, EBV infection is usually asymptomatic and well controlled by T cells specific for epitopes derived from various latent and lytic cycle antigens of the virus<sup>4,5</sup>. However, a small percentage of virus carriers eventually develop EBV-associated disorders including malignant diseases that account for 1-2% of all human cancers<sup>1,2</sup>. Incidence rates of some EBV-associated diseases appear geographically clustered, such as nasopharyngeal carcinoma (NPC) and endemic Burkitt lymphoma that are highly prevalent in southern China and sub-Saharan Africa, respectively<sup>6,7</sup>. Together with the histological heterogeneity of tumor subtypes, these observations have led to the assumption that besides regional dietary, genetic, and environmental co-factors, EBV strains with distinct pathogenicity may exist in locally restricted areas and contribute to this geographic predilection<sup>2,8</sup>. Recent sequence analyses of a large number of whole virus genomes have identified numerous viral variants and provided new insights

into virus transmission patterns and disease-associated genotypes<sup>9-12</sup>. To which extent sequence disparities among different EBV isolates impact on antiviral immunity, and whether immunotherapeutic and vaccine approaches that are based on a reference strain will be sufficiently cross-protective against strain variants, is yet to be discovered.

### EBV strain variations

Based on their EBV nuclear antigen (EBNA) 2 and EBNA-3 gene sequences, EBV strains are grouped into type 1 or type 2 (formerly denoted as type A and B)<sup>1,2,6</sup>. Several groups have recently reported on the sequences of more than 150 viral isolates from different healthy virus carriers and tumor specimens. Approximately two thirds of these isolates were derived from tumor specimen (NPC, gastric cancer, Burkitt and Hodgkin lymphoma, etc.) and one third from non-malignant tissue (spontaneous LCL, PBMC, saliva). These studies identified complex variations in EBV strains within the type 1/type 2 classification system, and, when clustered geographically, a clear separation of Asian strains<sup>6,9-12</sup>. Moreover, a recent comparative analysis of whole genome sequences identified a number of polymorphisms that were shared by tumor-derived strains but absent in viral isolates from healthy study participants<sup>10</sup>. Together with the recent observation that the NPC-derived viral strain M81<sup>8,13</sup> shows enhanced epitheliotropism and lytic activity, these findings suggest that some sequence variations may result in altered pathogenicity. Furthermore, polymorphisms have been identified that result in amino acid exchanges in known T-cell epitopes<sup>9,10</sup>. Whether these sequence variations are by-products of random genetic drift, positively selected for reasons related to the function of the protein, or the results of an immune-driven selection process based on different MHC types that are prevailing in different geographical regions, is still unknown<sup>14</sup>.

### EBV immunity and immunotherapy

EBV-specific T cells recognizing viral latent and lytic cycle antigens play a crucial role in antiviral immunity, including the control of EBV-driven lymphoproliferation<sup>4,5</sup>. Consequently, patients with primary or secondary T-cell deficiency and/or dysfunction are at risk of developing life-threatening EBV-associated lymphoproliferative disease. Reconstitution of EBV-specific immunity in these patients by the adoptive transfer of virus-specific T cells has been shown to be effective in preventing and treating such EBV-associated disorders<sup>15-19</sup>. Virus-specific T-cell lines for adoptive transfer were initially generated by repeated stimulation of peripheral blood T cells with autologous EBV-transformed lymphoblastoid B-cell lines (LCL) *in vitro*<sup>20</sup>. More recently, modified LCL were introduced as antigen presenting cells, e.g. LCL pulsed with synthetic peptide pools encompassing viral antigens, or transduced

with vectors expressing viral proteins. Although safe and efficacious, LCL-based approaches take several weeks to yield a product suitable for clinical use. Therefore, new protocols for a faster and more efficient production of virus-specific T-cell lines have been developed. These are based on the selection of EBV-specific T cells with MHC-multimers, the immunomagnetic selection of IFN $\gamma$ -secreting T cells after stimulation of peripheral blood mononuclear cells (PBMC) with viral peptide epitopes, or on using dendritic cells transfected with selected EBV genes to expand virus-specific T cells<sup>19-22</sup>. Although first clinical evidence indicates similar efficacy as traditionally manufactured virus-specific T-cell lines, such rapidly generated EBV-specific T-cell preparations are usually prepared using EBV epitope sequences from the widely used reference strain B95.8<sup>4,23</sup>. Given the significant sequence diversity among EBV strains, these studies still left unanswered whether immunotherapeutic and vaccine approaches that rely on B95.8 epitopes are sufficiently cross-protective against other EBV strains.

### Impact of EBV sequence variation on the T-cell response

Soon after variations in genome sequences were identified and EBV classified into type 1 and 2, differences in T-cell recognition of target cells infected with different viral strains were observed<sup>24</sup>. The notion that sequence diversity may affect immune recognition was substantiated when the first epitope recognized by EBV-specific CD8<sup>+</sup> T cell was molecularly defined and shown to differ between the prototypic type 1 strain B95.8 and the prototypic type 2 strain AG876 in two amino acids<sup>25</sup>. With the identification of a large array of CD8<sup>+</sup> T cell epitopes<sup>23</sup>, a complex picture of the antiviral T-cell response against different viral strains emerged. While several CD8<sup>+</sup> T-cell epitopes were found to be conserved in different viral strains<sup>26</sup>, some were found to differ between virus types<sup>27</sup>, whereas others were reported to vary even within the same virus type<sup>28-33</sup>. Moreover, amino acid exchanges in CD8<sup>+</sup> T-cell epitopes were shown to retain, reduce, or abolish T-cell recognition<sup>28-31,33,34</sup>. Collectively, these studies clearly demonstrated that sequence variations in EBV strains may reside within T-cell epitopes and potentially compromise the antiviral CD8<sup>+</sup> T-cell response.

These studies, however, left unanswered, which proportion of the EBV-specific CD8<sup>+</sup> T-cell epitopes is polymorphic, whether polymorphisms also occur in CD4<sup>+</sup> T-cell epitopes and how they affect T-cell recognition, and whether the individual T-cell response encompasses specificities that recognize epitope variants. These questions were addressed in a recent study comparing 65 and 118 published epitopes recognized by EBV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells, respectively, in the three distantly related viral strains B95.8, AG876, and M81<sup>35</sup>. When the

type 1 strains B95.8 and M81 were compared to the type 2 strain AG876, more than 70% of all CD4+ and more than 40% of all CD8+ T-cell epitopes and/or their flanking regions were found to be polymorphic. Even between the two type 1 strains B95.8 and M81, more than 50% of all CD4+ T-cell epitopes and almost 40% of all CD8+ T-cell epitopes and/or their flanking regions proved to be polymorphic in that study<sup>35</sup>. As reported for CD8+ T cells, polymorphisms in CD4+ T-cell epitopes impaired T-cell recognition to various degrees<sup>35</sup>. Amino acid exchanges in epitopes or flanking regions may impact on T-cell responses in different ways. Examples of changes in MHC or TCR binding affinity<sup>33,34,36</sup> and HLA restriction<sup>36</sup> have been reported, but also different steps in antigen processing and presentation may be affected, e.g. cleavage by cytoplasmic or lysosomal proteases, transport of cytosolic peptides into the ER by TAP, and trimming of the peptides in the ER by ERAP<sup>37-39</sup> (Figure 1).

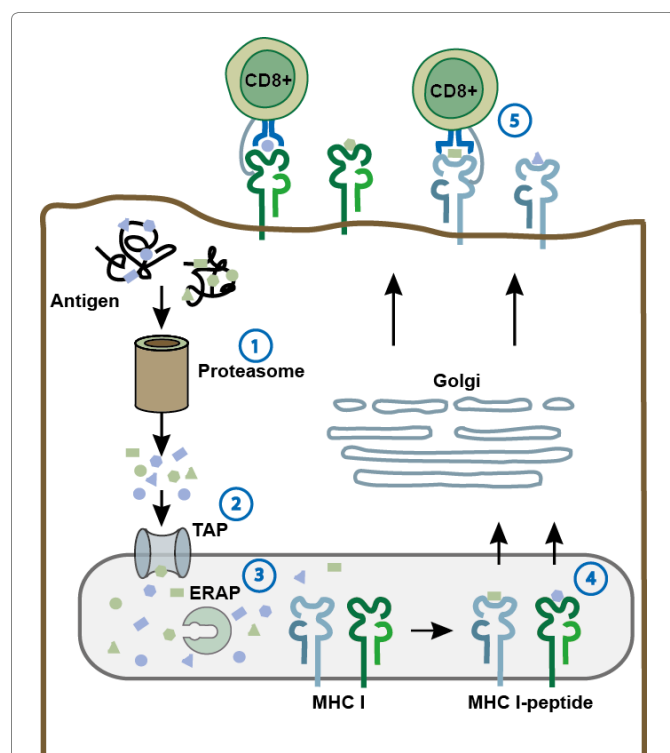
Moreover, the individual CD8+ T-cell response against polymorphic epitopes appears to vary between donors and to be focused on some epitope variants while others

are ignored<sup>35</sup>. To which extent holes in the epitope-specific T-cell repertoire impair antiviral immunity and allow co-infection with strain variants is still unknown. Given the recent technical advances that facilitate full-length EBV genome sequencing from different tissues, first insights may be provided in the near future by comparing sequences of EBV genomes from individuals co-infected with more than one strain<sup>9-11,40,41</sup>. Similar experiments may also shed light on the role of the immune system on the time-dependent decrease in viral strain diversity as noted in IM patients<sup>40</sup>.

In patients with EBV-associated disorders, virus-specific T-cell responses are often monitored using overlapping peptide libraries covering mostly latent cycle proteins e.g. EBNA3<sup>42,43</sup>. These libraries are based on B95.8 sequences and may incompletely measure virus-specific T-cell responses if the patient is infected with a different viral strain. Likewise, current approaches for the design of therapeutic EBV vaccines, and for the generation of virus-specific T-cell preparations for the treatment of EBV-associated disorders, are mostly based on latency proteins from B95.8<sup>21,22,44-50</sup>. Since these proteins are highly polymorphic, clinical efficacy may be impaired should the pathogenic strain be divergent. For example, therapeutic EBV vaccines, which aim at increasing and sustaining the number of virus-specific T cells in persistently infected individuals, may fail to boost relevant T-cell responses in vaccinees infected with strain variants. Likewise, EBV-specific T-cell preparations for the treatment of EBV-associated disorders in stem cell or organ transplant recipients may show diminished clinical efficacy when T-cell donor and recipient are infected with different viral strains. Consequently, peptide-selected T-cell preparations for clinical use should be directed against a broad set of viral antigens to increase the likelihood of targeting shared epitopes. In addition, the incorporation of sequence information from all published virus isolates into epitope databases is expected to identify viral epitopes that are conserved in all strains or at least in subgroups, such as those prevalent in certain geographical regions. Alternatively, diagnostic methods for rapid virus typing in patients may be developed to facilitate the identification of customized sets of antigens for tailored therapy.

## Conclusion

The identification of a growing number of EBV strains continues to add to a global view on EBV genome diversity and to a better understanding of EBV biology and the relationship between viral sequence variation and EBV-associated diseases. These studies have also identified substantial diversity in proteins targeted by the adaptive immune system. Incorporating information on sequence heterogeneity into immune monitoring assays, vaccine design, and immunotherapeutic approaches may ultimately improve clinical outcome of patients with EBV-associated disorders.



**Figure 1:** Amino acid exchanges in epitopes may affect antigen processing, presentation, and recognition by T cells.

As shown for the MHC class I pathway, polymorphisms in epitopes may affect different steps in antigen presentation such as cleavage by the proteasome (1), transport by TAP (2), or trimming by ERAP (3). Also, MHC (4) and T-cell receptor binding affinity (5) may be affected. Similar mechanisms are thought to apply to MHC class II antigen processing and presentation. ERAP, ER aminopeptidase associated with antigen processing; TAP, transporter associated with antigen processing.

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