PNAS COMMENTARY

Connecting the dots: Presentation of EBV antigens on HLA class II risk alleles connects the two main risk factors of multiple sclerosis

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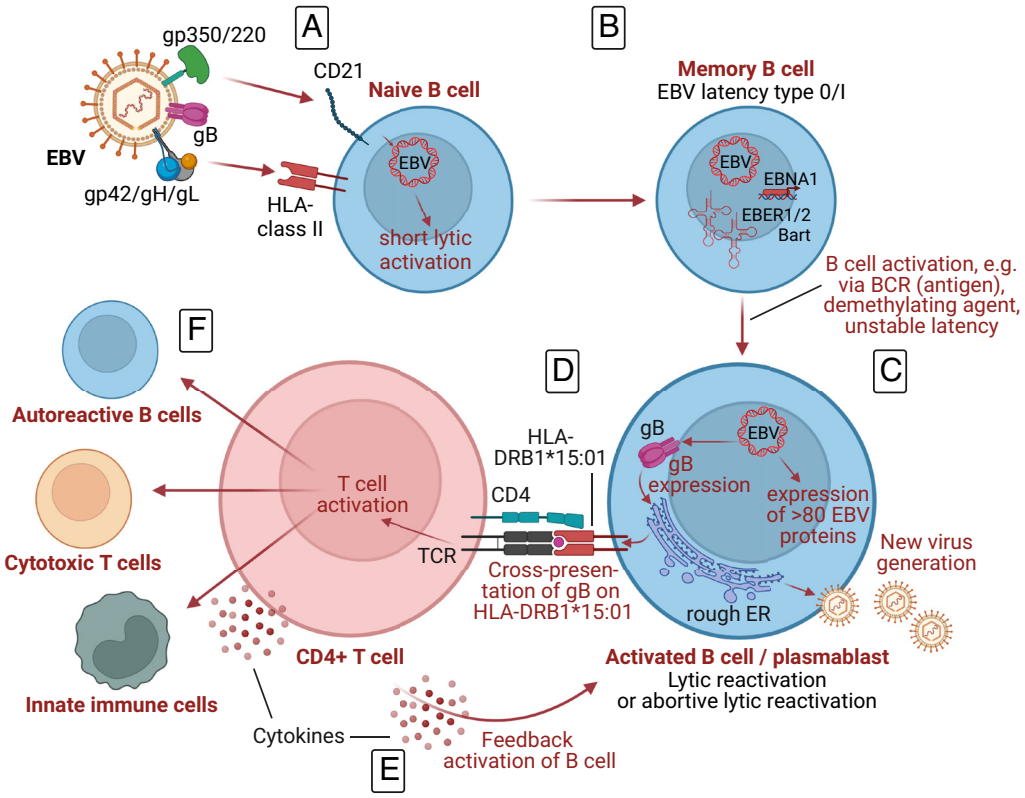

Fig. 1. EBV infecting B cells, cross-presentation of gB, and CD4+ T cell activation. (*A*) EBV infects naïve B cells by binding to CD21 (via gp350) and HLA class II (via gp42). gB facilitates membrane fusion. EBV undergoes a short lytic activation cycle, before (*B*) establishing latency in B cells. EBV remains mainly in latency type 0 and 1, in which gene expression is reduced to only short RNAs (EBER1, EBER2, BARTs) and EBNA1. (*C*) EBV can reactivate, often triggered by B cell activation. Latency in MS patients' B cells is unstable, leading to more frequent lytic reactivation. During the lytic cycle, >80 EBV genes can be expressed, including gB and gH, and new virions are formed. EBV can also enter an abortive lytic cycle with a limited set of lytic genes and no viral replication. (*D*) gB gets channeled to the rough endoplasmic reticulum (rough ER), which enables cross-presentation on HLA class II (HLA-DRB1*15:01). Presentation of gB activates $CD4^+$ T cells, which *(E)* provide stimulatory feedback to the EBV-transformed B cell, independent of BCR specificity, and the B cell can be licensed to secrete antibodies. (*F*) The activated CD4+ T cell further stimulates other B cells, CD8+ T cells, and innate immune cells to promote immune responses against EBV and potentially against self. (graphics: Biorender).

Multiple sclerosis (MS) is the most common autoimmune demyelinating disease of the central nervous system (CNS). The two major risk factors for developing MS are inheritance of the human leukocyte antigen DR15 (HLA-DR15) haplotype (1) and infection with Epstein–Barr virus (EBV) (2). Our understanding of the underlying mechanisms that link these factors to MS is limited. In PNAS, Drosu et al. (3) identified two EBV antigens that are preferentially presented on HLA-DRB1*15:01 and promote strong T cell responses in MS patients carrying this allele.

The HLA class II haplotype HLA-DR15 conveys a fourfold increased risk for developing MS (1). HLA class II molecules are expressed by antigen-presenting cells (APCs) and present extracellular antigens to CD4+ T helper cells. CD4+ T cells, in turn, promote and direct B cell, CD8+ cytotoxic T cell, and innate immune cell responses. Each HLA class II allele has distinct binding affinities to certain amino acids at characteristic positions

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in a peptide sequence, promoting CD4+ T cell responses to some peptide antigens over others. The vast variability of the HLA locus developed during evolution to provide our species with an effective adaptive immune defense against any conceivable pathogen. The evolutionary pressure from infectious diseases likely allowed for a low level of autoimmunity. The HLA-DR15 locus includes the two alleles HLA-DRB1*15:01 and HLA-DRB5*01:01, which are in almost complete linkage disequilibrium. HLA-DRB1*15:01 has been characterized in detail and is believed to favor the presentation of certain encephalitogenic antigens. Several lines of evidence suggest increased CD4+ T cell responses to myelin antigens, viral antigens, and other pathogens' antigens in MS patients carrying the HLA-DRB1*15:01 allele (4, 5).

The most significant environmental risk factor for MS is EBV. A recent retrospective cohort study showed that almost all patients contract EBV before the onset of MS, making EBV a prerequisite for developing MS (2). Infectious mononucleosis and highly elevated antibody titers against EBV nuclear antigen 1 (EBNA1) are additional independent risk factors (6). However, the molecular mechanisms behind this association are incompletely understood. We and others described B cell molecular mimicry between EBV and CNS antigens, including GlialCAM, Anoctamin-2, Alpha-B crystallin, and myelin basic protein (7, 8). B cells are EBV's primary target cells, and since they are central to MS pathogenesis, EBV-mediated changes of B cell phenotypes and activation levels could contribute to the disease (9). EBV latency in transformed B cells from MS patients is unstable and cells enter the lytic cycle more frequently, leading to altered cellular programs and expression of over 80 viral proteins with immunogenic capacity (10).

In PNAS, Drosu et al. identified two EBV antigens that are preferentially presented on HLA-DRB1*15:01 and promote strong T cell responses in MS patients carrying this allele.

The data on anti-EBV T cell responses in MS is somewhat inconsistent: Pender et al. described an exhausted T cell response to EBV in MS (11), resulting in insufficient control of EBV in B cells and enabling uninhibited lytic transformation, increased overall numbers of EBV+ B cells, and elevated levels of EBV antigens stimulating immune responses and facilitating molecular mimicry. In contrast, other studies found increased EBV-specific T cell responses and T cell molecular mimicry between EBNA1 and myelin antigens (12). TCR-β chain sequencing and comparison with publicly available datasets of anti-EBV TCR sequences revealed increased numbers of anti-EBV CD8+ T cells in MS patients, and enrichment of anti-EBV T cells in spinal fluids of MS patients (13, 14).

While EBV infection is as a prerequisite for developing MS, most infected individuals never develop autoimmunity. Additional genetic and environmental factors clearly contribute to the disease, and a connection between HLA-DR15 and EBV seems likely. HLA class II is a coreceptor of EBV cell entry (Fig. 1*A*) and HLA-DRB15*15:01 facilitates EBV infection more strongly than other HLA class II alleles (15). However, many HLA class II alleles enable EBV entry, rendering this explanation less plausible (16). In an EBV infection model (5), humanized mice reconstituted with HLA-DRB1*15:01 HSCs had elevated EBV copy numbers despite increased numbers of CD8+ T cells, suggesting diminished control of EBV despite increased CD8+ T cell activation, potentially due to insufficient help from CD4+ T cells. Interestingly, EBV infection generated CD4+ T cells reactive against several major myelin proteins (5).

In PNAS, Drosu et al. (3) found that antigens from the EBV glycoproteins gB and gH are heavily favored to be presented on HLA-DRB1*15:01 and thus induce strong CD4+ T cell responses. Using the machine learning algorithm MixMHC2pred, the authors predicted binding of all 12 to 18mer peptide sequences in the EBV proteome to HLA-DRB1*15:01 and HLA-DRB5*01:01. Results were compared with HLA-DRB1*15:02 and HLA-DRB5*01:02, respectively, the closest related alleles not associated with MS risk. Two peptides from the EBV protein gB (BALF4) were predicted to bind exceptionally well to HLA-DRB1*15:01, and this interaction was predicted to be stronger than to any other HLA-DRB allele. Both gB peptides potently activated CD4+ T cells from 4 out of 5 HLA-DRB1*15:01 positive MS patients, similar to a previously described immunodominant peptide from the EBV protein BORF1 (17), which stimulated CD4+ T cells from all five patients. Similar results were obtained when adding the full gB protein instead of peptides. In this experiment, gB was presented on the homozygous HLA-DRB1*15:01 B cell line SUDHL4 to primary CD4+ T cells. Exogenous EBV gB, which in an MS patient might originate from EBV virions or from dying lytic B cells, can be endocytosed and processed by APCs and presented on HLA-DRB1*15:01. This line of evidence follows the classical pathway of antigen presentation by APCs to CD4+ T cells on HLA class II.

B cells are highly effective APCs for their cognate antigens, which are endocytosed and presented on HLA class II upon B cell receptor (BCR) binding. In lytic EBV-reprogrammed B cells, however, gB is expressed inside the cell and channeled into the endoplasmic reticulum (ER) and further toward the secretory pathway. Intracellular proteins are usually presented on HLA class I to cytotoxic CD8+ T cells. However, cross-presentation between HLA class I and II has been described for certain viral antigens (18). In the context of EBV gB, cross-presentation would imply that antigen presentation by the EBV-transformed B cell is independent of the cell's BCR. This B cell could then receive CD4+ T cell help, become activated, and secrete antibodies. The CD4+ T cell would also become licensed to activate other immune cells, including B cells, cytotoxic CD8+ T cells, and innate immune cells (Fig. 1). To investigate cross-presentation, Drosu et al. (3) expressed gB in SUDHL4 cells and primary HLA-DRB1*15:01 B cells, and measured CD4+ T cell activation. Interestingly, protein trafficking into the rough ER was necessary for gB to be cross-presented on HLA-DRB1*15:01 and to activate CD4+ T cells. Several other EBV secretory and membrane proteins were tested in the same way, of which only gH elicited similar T cell responses. While CD4+ T cell activation by gB and gH is clearly dependent on HLA-DRB1*15:01, it is not specific to MS patients. The observed CD4+ T cell responses to cross-presented gB and gH, were in contrast to broader CD4+ T cell responses against all lytic EBV proteins, induced with protein

lysate from lytic EBV+ B cell lines exogenously added to the cell culture, which was independent of HLA-DRB1*15:01 and also independent of the diagnosis of MS. In summary, the study shows that presentation of gB and gH is uniquely facilitated by HLA-DRB1*15:01, and that these antigens can be cross-presented in B cells and elicit CD4+ T cell responses. Translation of gB and gH occurs during the lytic or abortive lytic phase of an EBV infection. The described mechanism has several potential implications for autoimmunity, including the potential for feedback activation of the presenting B cell independent of its BCR, and misguided activation of other B cells, CD8+ T cells, and innate immune cells (Fig. 1).

Cross-presentation of intracellular antigens on HLA class II might be a relevant phenomenon that is underappreciated in autoimmunity. The mechanism is particularly interesting in B cells, as they are highly effective APCs and fundamentally important for autoimmune pathology. It enables presentation of antigens that are unrelated to the specificity of the cell's BCR. Cross-presentation on HLA class II has been described for other secretory viral proteins with signal peptides for the ER and endosomal or lysosomal compartments (18). However, the mechanism of loading antigenic peptides onto HLA class II in the ER is incompletely understood. In the ER, the binding groove is still occupied by the invariant chain (CLIP peptide), which is released only upon interaction with HLA-DM in the endolysosome. It stands to reason that exquisite binding affinity of gB and gH antigens would facilitate an exchange of the invariant chain in the ER. The current study does not present additional mechanistic details, and it would be tempting to speculate if the observed effect is directly mediated by the virus or by a B cell-specific mechanism, i.e., an antiviral defense mechanism. Furthermore, it raises the question whether similar cross-presentation on HLA class II could be observed in other EBV-associated autoimmune diseases.

Drosu et al. (3) draw notable parallels between MS and celiac disease (CD), which is associated with HLA-DQ2 and HLA-DQ8, and the exogenous driver of inflammation is gluten. Differing from gluten-specific B cells in CD, B cells in MS might not need BCRs specific to EBV gB and gH, as cross-presentation of EBV proteins could provide the direct mechanistic basis for their presentation on HLA-DRB1*15:01. If the lesson from CD can be extended to MS, it suggests that withdrawing the exogenous driver of the disease fully abrogates the ongoing autoimmune response. This indicates that efficient therapeutic strategies to eradicate EBV—once developed—will provide a highly promising and potentially fundamental therapeutic approach for MS patients.

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