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HIGHLIGHTS

COMMENTARY

Epstein Barr virus infection and immune defense related to HLA-DR15: consequences for multiple sclerosis

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MS is a multifactorial disease in which a series of genetic and non-genetic, environmental factors plays a role in its etiology. In particular, HLA class II alleles, mainly HLADRB1*15:01 (HLA-DR15), increase the risk for this disease. Out of several environmental factors, and with regard to infections, EBV remains to be a strong candidate, and may synergize with HLA-DR15 thus increasing the risk for MS. In this issue of the *European Journal of Immunology*, Zdimerova et al. present highly interesting experimental data using EBV infection in immune-deficient mice engrafted with human immune cells, either HLA-DR15$^+$ or HLA-DRB1*04:01 (HLA DR4), here after denoted as HLA-DR15$^-$. As a result of EBV infection, the viral load and CD8$^+$ cell expansion were conspicuously higher in mice engrafted with HLA-DR15$^+$ compared to HLA-DR15$^-$ mice; and myelin basic protein specific T cells emerged in mice engrafted with HLA-DR15 bearing cells. This study sheds light on how EBV and the class II DR15 haplotype may jointly predispose and synergize in the etiology of MS.

Keywords: Epstein Barr virus · HLA · HLA-DR15 · multiple sclerosis

See accompanying article by Zdimerova et al.

Genes within the human leukocyte antigen complex (HLA) are strongly associated with MS risk. In particular, the class II and I genes are relevant, where variants of these genes encode products that present antigens to CD4$^+$ and CDD8$^+$ T lymphocytes, respectively. Class II variant HLA-DRB1*15:01 is strongly associated with the risk for MS (odds ratio ~ 3) [1–4]. I here discuss MS with regard to its genetic epidemiology and immunology, the associations to HLA class II variant and EBV in MS, and the gaps in our knowledge that the experimental study by Zdimerova et al. [5] fills in.

Despite that the strong associations of HLA class II genes to MS risk are known since decades, the basic mechanistic reasons for these associations are still not known. The functions of class II genes are to present peptides to CD4$^+$ T cells, and variations in the binding groove of HLA molecules will affect the T cell repertoire, both acting at the thymic and peripheral level. The extreme polymorphisms of class II molecules may well have been driven by pathogens, so that any single one should not escape immune control [6]. However, if so, we do not know when have such polymorphisms occurred during history, and pathogens that might have shaped the evolutionary selection remain known. Some of the variants that may have been selected against one pathogen might be poor in the immune defense against another. Thus, it is theoretically possible that certain infections might have a very good immune defense through class II-mediated CD4$^+$ activation, while, at expense of this, other infections may yield a poor, suboptimal response.

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There are no experimental models for EBV infections in rodents, severely hampering studies in relation to pathogenicity and disease mechanisms. However, the model studied by Zdimerova et al [5] is probably the closest mimicry of EBV infection to the human one which can be achieved at the moment. Through a relentless experimentation requiring thousands of immune-deficient mice, studied for many years, engrafted with human immune systems being HLA-DR15$^+$ or HLA-DR15$^-$, the authors provided convincing and very important evidence for emergence of a higher viral load in HLA-DR15$^+$ haplotype bearing immune systems when compared to those engrafted with HLA-DR15$^-$ cells. These findings provide a basis for considering a poor class II-mediated immune defense against EBV as one of the mechanisms for the HLA class II associations in MS. These findings are also important since the EBV "viral Load/replication" is difficult to study in humans. EBV infects and resides within B cells, which is not easily studied in blood samples, since the vast majority of B cells are located in lymphoid organs. In humans, one might speculate that the increased antibody levels against EBV Nuclear antigen 1 (EBNA-1) seen in MS DR15$^+$ individuals may represent an increased humoral immune response against the virus being present at higher levels, then being a "biomarker" for the viral load/replication. While the study in engrafted mice by Zdimerova et al. [5] could not assess the antibody generation, the authors found another important aspect relevant for human MS, increased expansion of CD8$^+$ T cells, consistent with the clinical manifestation observed in form of infectious mononucleosis (IM), another EBV related risk factor for MS [5]. It is interesting that the main effect seems to be mediated by the class II variants [5], and not class I genes, although these were not genotyped in the study. It is a well-established fact that CD8$^+$ T cells are the ones crucial for anti-viral defense. However, CD4$^+$ T cell help for the CD8$^+$ arm of the immunity may well be implicated. The study also provides evidence for the option of molecular mimicry, or some other mechanism for inducing CNS autoreactive T cells during EBV infection, which may be instrumental in triggering and driving the chronicity of MS [5]. The authors focused on one important autoantigen, myelin basic protein (MPB), and found reactivity to this autoantigen in infected mice. MBP is known to be able to induce CNS neuroinflammation in rodents. In total, the study [5] gives a basis for a hypothesis that a poor class II-mediated immune control of EBV may ultimately lead to MS. These results question the previous arguments for a role of EBV in MS. Very many infections have been argued to play a role in MS and most of them have been refuted as part of the MS etiology. However, EBV, and perhaps also Human Herpes virus 6A [7] are still on the scene.

The potential role of EBV in MS has recently been reviewed elsewhere [8]. Here, I refer to a limited set of crucial observations. IM, the clinical manifestation of EBV infection, approximately doubles the risk for MS, as shown in a meta-analysis [9]. Persons with MS have much higher anti-EBNA 1 levels, and in particular against certain fragments of EBNA 1 [10, 11]. Apparently, there is an age dependency i.e. infection during late adolescence, early adulthood is critical [12, 13]. The two aspects of EBV infection recorded, that is high EBNA-1 antibody levels and IM act partly independently and interact in their association to MS risk [14]. Recently, analysis of pre-MS samples, serologically showed that EBV infection during childhood was protective, while the risk increase occurred after the age of 18. Analysis of HHV6A serology showed an association to increase risk throughout all ages, in addition HHV6A and EBV infection interacted in their association with MS [13]. A nested case-control study demonstrated that all EBNA 1 negative individuals had serologically converted before MS onset [15, 16]. In addition, there is a potent interaction between high levels of anti-EBNA-1 antibody levels and carriage of the HLA-DR15 haplotype. Again, the study by Zdimerova et al. [5], fills in important gaps in this scenario, mainly the finding of the poor immune defense with higher viral loads in HLA-DR15$^+$ immune systems.

MS is a complex disease, meaning that a whole series of lifestyle/environmental factors and genetic predisposition, and their interactions play a role. A diverse set of factors like smoking, obesity, night shift work, brain concussion [17], organic solvents, EBV and HHV6A infection are associated to MS. They act either alone, but more so in interactions, between themselves, or with gene variants, such as carriage of the DR15 haplotype and non-carriage of the protective class I HLA A2 allele. With these genotypes and a certain environmental factor in combination odds ratios in the range of 10–20 can be reached. All these factors together lead to a substantially increased risk for MS, which in all combinations give an increased risk on top of a baseline risk, reviewed in detail here [18]. EBV has a special role, in that the infection seems to be a prerequisite to develop MS. Also, there should be a peripheral antigenic drive of autoreactive T cell responses, and EBV is a candidate for this, once again supported by the study of Zdimerova et al [5]. The synergy, and interactions, between environmental factors and gene variants tell us about common etiological and pathogenic pathways. Thus, interaction with class II genes, being instrumental in T cell responses provides evidence of immunological mechanisms for the influence of environmental factors, in view of the central role of MHC class II genes presenting antigens to CD4$^+$ T cells. This is the case for aspects of EBV infection and HLA-DR15. A limitation of the Zdimerova et al study [5], is that the basic mechanisms for the HLA-DR15 mediated less well-controlled EBV infection remain to be explained. One can consider that specific antigens/parts of EBV, crucial for the immune defense against the virus, are less well presented by the HLA-DR15 allelic molecule which then would allow a higher viral load. The critical antigens/peptides of EBV important for host immune defense remains to be defined.

The study by Zdimerova et al [5] mainly supports the concept of molecular mimicry in which parts of the virus induce cross-reactive T cell response to CNS antigens. Clearly, T cells reactive with CNS components arise upon EBV infection in humans [19]. Importantly, a very recent study documented CD4$^+$ T cell clones, which cross-reacted with peptides of EBV and autoantigens presented by both DR2a and DR2b molecules, part of the HLA-DR15 haplotype [20]. Further support comes from large-scale genetic epidemiology combined with immunology. Anoctamin 2, an ion channel was identified as an autoimmune antibody target in MS

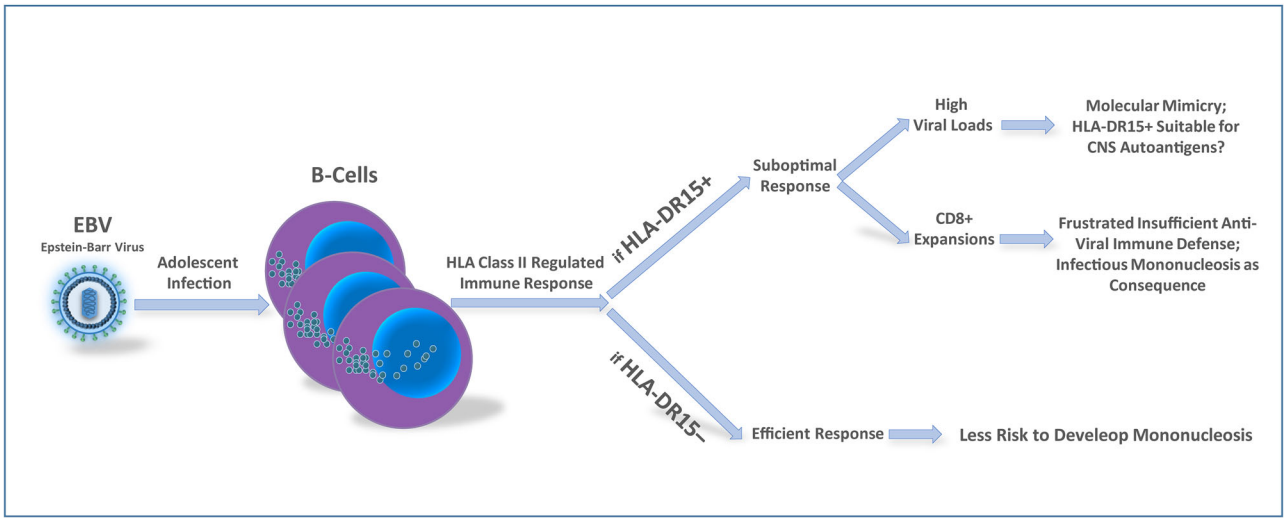

Figure 1. Hypothetical scenario: EBV and HLA lass II variants in MS. EBV infection of B cells may provoke an immune response in which HLA class II alleles are central in presenting EBV antigens to $CD4^+$ T cells, which ultimately is important in the viral defense through $CD8^+$ cells and antibodies. HLA-DR15 can provide a poor class II-mediated activation of the $CD4^+$ response, with ensuing higher viral load, higher anti-EBNA-1 antibody levels, and expansion of $CD8^+$ T cells, which may be less effective in the immune defense. In addition, there are prospects for activation of autoimmunity against CNS autoantigens, (art-work: Mohsen Khademi)

[21]. Antibodies cross-reactive with EBV, were selective for MS. The occurrence of these antibodies, increased the association to MS, providing strong evidence for their etiological/pathogenic role in the disease [22] (Fig. 1).

The study by Zdimerova et al. [5] fills in one of the most important gaps in the understanding of EBV, HLA-DR15, and MS by experimentally demonstrating that this class II variant most probably gives a poor immune defense against the infection with ensuing higher viral loads. In addition, despite a more vigorous $CD8^+$ expansion, in turn, dependent on an ineffective $CD4^+$ class II HLA-$DR15^+$ mediated response, the immune defense against the virus is likely to be less effective. The study also suggests that auto-reactivity to one potential autoantigen: myelin basic protein is promoted. So, hereby, one aspect of the threefold increased risk MS among persons carrying DR15 is potentially explained. However, this does not exclude other, additional, and mutually non-exclusive mechanisms for the HLA class II gene variant influence on MS. Thus, certain class II variants might also be particularly good at presenting autoantigens from particular organs such as the CNS in MS, and tentatively the HLA-DR15 haplotype may belong to this category of antigen-presenting molecules. Such a scenario is very clear in EAE, in which certain myelin antigen peptides are encephalitogenic depending on particular rodent class II molecule variants, reviewed in [23].

In conclusion, a further piece of evidence suggesting how HLA-DR15 may predispose to MS in relation to EBV is at hand. This reinforces thoughts of attempts for therapy or prevention. One option to eliminate EBV viral reservoir is the highly effective anti-CD20 treatment in MS [24], extensively used today. In addition, EBV vaccination at a young age could be another solution. However, as a cautionary note, suboptimal vaccination might drive EBV infection into a higher age group with increased risks for MS, which may be above the age of 18 [13]. Perhaps more promising in the short perspective is the development of EBV selective antiviral drugs with a prospect to halt a persistent systemic antigenic drive from EBV [25].

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Abbreviations: **EBNA-1**: EBV Nuclear antigen 1 · **HLA**: human leukocyte antigen · **IM**: infectious mononucleosis

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