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European Journal of Immunology

# Letter to the Editor

[DOI: 10.1002/eji.202149179]

## HLA-DRB1*15:01 is a co-receptor for Epstein–Barr virus, linking genetic and environmental risk factors for multiple sclerosis

MS is a chronic inflammatory demyelinating disease of the CNS, which mainly affects young adults and can lead to significant physical and cognitive disability. While the precise etiology of MS is unknown, it is widely accepted that MS arises in genetically susceptible individuals through environmental triggers. The strongest genetic risk factor for MS is the human leukocyte antigen (HLA) class II allele HLA-DRB1*15:01 [1]. However, how HLA-DRB1*15:01 increases the risk of MS is unclear. The strongest environmental risk factor for MS is infection with the EBV, a B lymphotropic human $\gamma$-herpesvirus. Whereas the available data is compatible with the concept that MS is a rare complication of EBV infection, the mechanisms through which EBV exerts its role in MS are currently unknown [2]. Epidemiological studies have consistently shown that EBV and HLA-DRB1*15:01 interact in determining the risk for MS [3]. Nevertheless, the molecular mechanisms underlying the interaction of EBV and HLA-DRB1*15:01 remained elusive.

Upon EBV infection, the initial attachment of EBV to B lymphocytes is mediated by binding of the EBV surface glycoprotein gp350 to its cellular ligand CD21. Subsequently, fusion of the viral envelope with the B cell membrane requires binding of the EBV surface glycoprotein gp42 to the HLA class II molecules HLA-DR, -DQ, or –DP [4–6]. While it is therefore well known that HLA class II molecules serve as

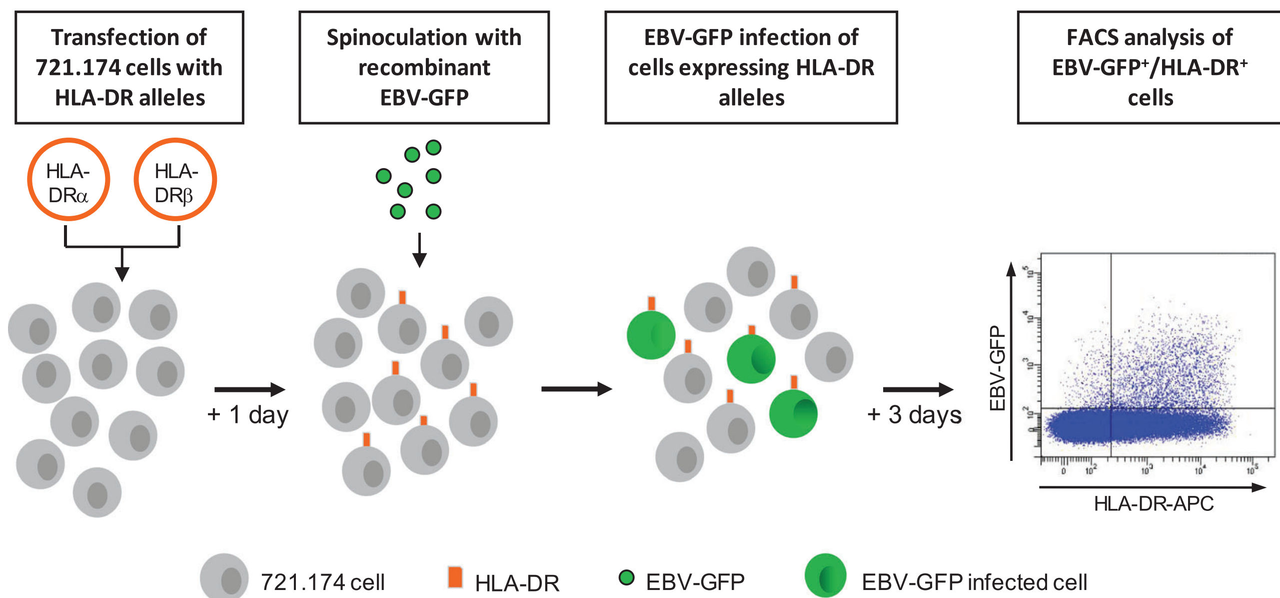


**Figure 1.** Synopsis of the experimental strategy. The CD21-positive, human leukocyte antigen (HLA) class II-negative lymphoblastoid cell line 721.174 was co-transfected with plasmids encoding the HLA-DR $\alpha$-chain HLA-DRA*01:01 and the HLA-DR $\beta$-chain HLA-DRB1*01:01 or HLA-DRB1*15:01. After 1 day, the transfected cells were infected by spinoculation with recombinant EBV containing the gene for GFP (EBV-GFP). Three days later, the percentage of EBV-GFP infected among HLA-DRB1 transfected cells was determined by flow cytometry.

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coreceptors for EBV, it was hitherto unknown whether this extends to HLA-DRB1*15:01.

To investigate whether HLA-DRB1*15:01 acts as a coreceptor for EBV, we transfected the CD21-positive, HLA class II-negative human B lymphoblastoid cell line 721.174, which is normally resistant to infection with EBV, with an HLA-DRB1*15:01 plasmid and subsequently infected the transfected cells with recombinant EBV containing the gene for GFP (EBV-GFP). The number of EBV-GFP infected among HLA-DRB1*15:01 expressing cells was then determined by flow cytometry (Fig. 1; see Supporting Information for details of the methodology). Untransfected 721.174 cells and 721.174 cells transfected with an empty vector did not express HLA-DR molecules and were not infectable with EBV. In contrast, transfection with HLA-DRB1*15:01 resulted in the expression of HLA-DRB1*15:01 on 721.174 cells, which rendered 721.174 cells infectable with EBV (Fig. 2A). While transfection with HLA-DRB1*01:01, an HLA-DRB1 allele previously found to be protective against MS [7], likewise rendered 721.174 cells infectable with EBV, the percentage of EBV infected cells was higher in HLA-DRB1*15:01 as compared to HLA-DRB1*01:01 transfected cells ($p = 0.002$, Fig. 2A, B).

This work provides proof of principle that HLA-DRB1*15:01 acts as a co-receptor for EBV on a B cell line. While this complies well with previous observations demonstrating that HLA-DR is a co-receptor for EBV entry into B cells [4–6], the present findings specifically show that this extends to the MS associated HLA-DR allele HLA-DRB1*15:01. Thus, our study suggests a mechanistic link between the most important genetic and environmental risk factors for MS. It also evokes the intriguing hypothesis that one mechanism underlying the association of HLA-DRB1*15:01 and MS could be the function of HLA-DRB1*15:01 as a co-receptor for EBV.

Although only two HLA-DRB1 alleles were studied in the present work, our findings suggest that different HLA-DRB1 alleles may differ in their ability to confer

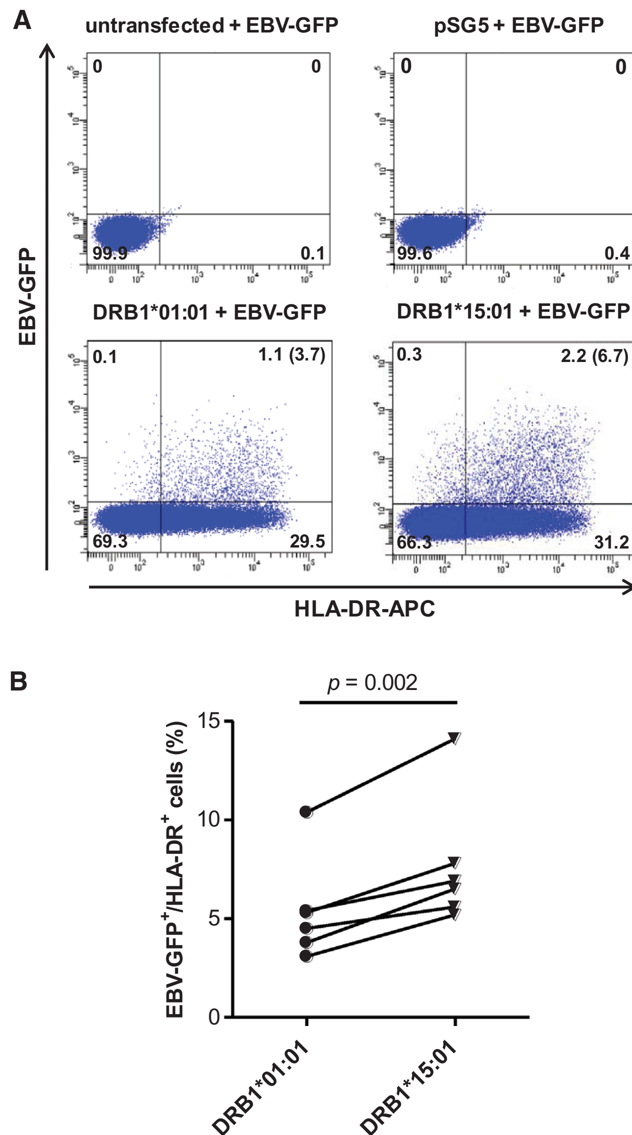


**Figure 2.** Infection of 721.174 cells transfected with HLA-DRB1 alleles with EBV-GFP. (A) 721.174 cells were either not transfected (untransfected) or transfected with an empty vector (pSG5) or HLA-DRB1*01:01 or HLA-DRB1*15:01 and subsequently infected with EBV-GFP. The percentage of cells in each quadrant is indicated. The percentage of EBV-GFP infected among all cells in the lower and upper right quadrant is indicated in brackets in the upper right quadrant. Flow cytometry dotplots show one experiment representative of $n = 6$ independent experiments with similar results. (B) Percentage of EBV-GFP infected cells among 721.174 cells transfected with HLA-DRB1*01:01 or HLA-DRB1*15:01. The figure shows data from $n = 6$ independent experiments with each symbol representing the mean of two technical replicates. Values from the same experiment are connected by a line. Statistical significance of differences was assessed by paired $t$-test.

susceptibility to EBV infection, as previously shown for different HLA-DQ alleles [8]. Importantly, the site of EBV gp42 binding to HLA-DRB1 is the β1-chain of HLA-DRB1, that is, the highly polymorphic chain of HLA-DRB1 involved in forming the peptide-binding groove [4], which additionally supports HLA-DRB1 allele-specific differences in mediating EBV infection. The most likely explanation for the higher percentage of EBV-GFP infected cells among HLA-DRB1*15:01 as compared to HLA-DRB1*01:01 transfected cells therefore is that HLA-DRB1*15:01 acts as a more efficient co-receptor for EBV. Differential survival of EBV-GFP infected HLA-DRB1*15:01 and HLA-DRB1*01:01 transfected cells appears to be an unlikely

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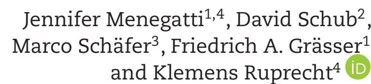
alternative explanation, as EBV-GFP infection is not expected to substantially influence survival of 721.174 cells, which are already EBV transformed and thus immortalized B lymphoblastoid cells. Furthermore, as the percentages of HLA-DRB1*15:01 and HLA-DRB1*01:01 transfected cells and the fluorescence intensities of HLA-DRB1*15:01 and HLA-DRB1*01:01 transfected cells were similar in each experiment, different surface expression of HLA-DRB1*15:01 and HLA-DRB1*01:01 is also unlikely to account for the observed differences.

Our current data do not allow drawing any conclusions on whether differences in the amount of EBV infection mediated by different HLA-DRB1 alleles may translate into the different risk of MS associated with such alleles. However, future studies systematically analyzing the ability of various HLA-DRB1 alleles, which have been associated with increased or decreased risk of MS, to act as co-receptors for EBV on B cells appear warranted.

Unlike in our experimental system, in which only one HLA-DRB1 allele was expressed on 721.174 cells, human B cells express two alleles of HLA-DR, -DQ, and -DP each, with all of these potentially contributing to the net susceptibility to EBV infection [6]. Dissection of the effects of particular HLA-DR alleles, and of the potential interaction of HLA-DR with HLA-DQ and HLA-DP alleles, on EBV infection of human B cells therefore appears challenging and will require large and systematic efforts. Nevertheless, that HLA-DR is involved in EBV infection of primary human B cells has been shown by inhibition of EBV infection of (T cell depleted) human peripheral blood mononuclear cells by a monoclonal antibody to the HLA-DR β-chain [5]. Furthermore, a role for HLA-DRB1*15:01 in modulating EBV infection in vivo is strongly suggested by a recent study showing that mice engrafted with HLA-DRB1*15:01$^{+}$ human immune cells have a higher viral load and an increased CD8$^{+}$ T cell response upon EBV infection than mice engrafted with HLA-DRB1*15:01$^{-}$ human immune cells [9].

Altogether, this work provides proof of principle that HLA-DRB1*15:01 acts as a co-receptor for EBV on a B cell line, linking the strongest genetic and environmental risk factors for MS. Future studies should clarify the precise consequences of this molecular interaction.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**Acknowledgements:** This work is dedicated to the memory of Nikolaus Mueller-Lantzsch †, former head of the Institute for Virology, Saarland University Hospital, Homburg, Germany. The authors like to thank Richard Longnecker for providing 721.174 cells and HLA-DRA*01:01 and HLA-DRB1*01:01 plasmids and Wolfgang Hammerschmidt for providing 293 2089 cells, as well as Ruth Nord for excellent technical assistance. This work was supported by a research grant from Novartis Pharma GmbH to KR and Stiftung Charité (BIH Clinical Fellow Program) to KR.

Open access funding enabled and organized by Projekt DEAL.

**Conflicts of interest:** J.M., D.S., M.S., and F.G. report no commercial or financial conflicts of interest. K.R. received research support from Novartis Pharma, Merck Serono, German Ministry of Education and Research, European Union (821283-2), Stiftung Charité and Arthur Arnstein Foundation; received speaker honoraria from Bayer and travel grants from Guthy Jackson Charitable Foundation.

**Data availability statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Peer review:** The peer review history for this article is available at https://publons.com/publon/10.1002/eji.202149179

## References

1 **Sawcer, S. et al.**, *Nature* 2011. **476**: 214–219.

2 **Ruprecht, K.**, *Expert Rev. Clin. Immunol.* 2020: 1–15.

3 **Jacobs, B. M. et al.**, *Mult. Scler.* 2020: 1352458520907901.

4 **Spriggs, M. K. et al.**, *J. Virol.* 1996. **70**: 5557–5563.

5 **Li, Q. et al.**, *J. Virol.* 1997. **71**: 4657–4662.

6 **Haan, K M. et al.**, *J. Virol.* 2000. **74**: 2451–2454.

7 **Ramagopalan, S. V. et al.**, *PLoS Genet.* 2007. **3**: 1607–1613.

8 **Haan, K. M. and Longnecker, R.**, *Proc Natl Acad Sci U S A* 2000. **97**: 9252–9257.

9 **Zdimerova, H. et al.**, *Eur. J. Immunol.* 2020.

Keywords: Epstein-Barr virus • HLA-DRB1*15:01 • human leukocyte antigen • multiple sclerosis • receptor

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Received: 12/1/2021
Revised: 3/4/2021
Accepted: 19/5/2021
Accepted article online: 21/5/2021

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