Paediatric autoimmune uveitis is associated with intraocular antibodies against Epstein–Barr virus Nuclear Antigen 1 (EBNA-1)

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Summary

Background Non-infectious uveitis is an immune-mediated disease characterized by vision-threatening inflammation within the eye. Increasing evidence indicates that microbial agents promote non-infectious uveitis, but the natural history of immune responses to pathogens in patients remains unexplored. We determined intraocular antibodies against pathogens in paediatric uveitis.

Methods We used peptide microarrays containing 3760 linear B-cell epitopes from 196 human pathogens to profile IgG levels in eye fluid biopsies and paired serum samples from 18 Dutch paediatric patients and 6 age-matched controls. We compared intensities of single epitopes and clusters based on overlapping amino acid sequence of peptides. Next-generation sequencing data was obtained to determine the *HLA-DRB1*15:01* genotype.

Findings Intraocular antibody profiles largely matched serum profiles and were characterized by high IgG against the conserved PALTAVET-motif of enterovirus family members, as well as broad epitope reactivity against *Epstein–Barr* virus (EBV). The aqueous humour of patients showed elevated levels of antibodies against peptides containing the RRPFFHPV-motif of *Epstein–Barr Virus Nuclear Antigen 1* [EBNA-1]. Antibody levels against the RRPFFHPV-motif of EBNA1 were significantly higher in individuals that carry the *HLA-DRB1*15:01* risk allele of paediatric uveitis.

Interpretation Intraocular antibodies against an immunogenic epitope of EBV showed an association with paediatric uveitis, particularly HLA-DRB1*15:01 positive uveitis, indicating a potential link between EBV-specific immune responses and autoimmune uveitis.

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Introduction

The term "uveitis" describes all forms of uveal inflammation, which remains an important cause of visual impairment worldwide.[1] The diagnostic work-up of uveitis increasingly includes anterior chamber paracentesis for identifying infectious causes by targeted PCR or meta-genomic sequencing of aqueous humour (AqH), as well as detecting pathogen-specific antibodies in intraocular fluid.[2] One in three uveitis cases can be resolved by probing for specific pathogenic agents, with the remaining cases considered "non-infectious" uveitis.

Although it remains unclear what causes non-infectious uveitis, there is unequivocal evidence for unrestrained inflammation in eye tissues. This is supported by the success of immunosuppressive and immunomodulatory therapies for controlling eye inflammation, including biologics that interrupt the signalling of inflammatory cytokines.[3–7] Other evidence is that the genetic susceptibility to noninfectious uveitis

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Research in context

Evidence before this study

Using PubMed searches conducted up to June 2024 with search terms "paediatric uveitis" AND "antibodies" OR "antibodies OR "infection," we found no prior study that conducted high-dimensional antibody epitope profiling in intraocular fluid of patients with autoimmune paediatric uveitis. Previous studies identified increased intraocular antibodies to parvovirus B19 in a small study of juvenile idiopathic arthritis-associated uveitis patients. Currently, there are no studies that have broadly assessed the intraocular antibody profiles of a cohort of children to determine whether infectious agents may contribute to paediatric autoimmune uveitis.

Added value of this study

Using high-density peptide microarrays with 3760 linear B-cell epitopes from 196 human pathogens, we analysed the intraocular and paired serum IgG antibody repertoires of 24 patients with autoimmune paediatric uveitis and age-matched children without inflammatory eye disease. While multiple common pathogen epitopes were detected in both eye fluid and serum, we found elevated IgG against peptides containing an amino acid motif present in the EBNA-1 nuclear antigen of EBV. Antibody levels against the EBV peptide motif were higher in individuals with the HLA-DRB1*15:01 risk allele, which is associated with paediatric uveitis.

Implications of all the available evidence

Our study identifies a potential association between a specific EBV peptide motif and paediatric autoimmune uveitis, especially in cases that carry risk allele HLA-DRB1*15:01. This EBV peptide motif has also been associated with HLA-DRB1*15:01-linked multiple sclerosis, suggesting the possibility of shared mechanism in these autoimmune diseases.

seems almost exclusively mediated by immune genes, especially those in the *MHC* region.[8–10] As a result, non-infectious uveitis was long thought to be driven by immune dysregulation with little microbial involvement.

More recent studies have shed new light on this. A recent immunoprofiling study identified that eye-infiltrating T-cells of patients with uveitis specifically recognize human as well as microbial peptides.[11] Also, in experimental autoimmune uveitis models, changes in gut microbial composition had an effect on activation of eye-infiltrating T-cells.[12] In these models, the pathogenic T-cells that cause eye inflammation were hypothesized to be activated by immune interactions that involved gut microbes[13] possibly by the cross-reactive recognition of microbial antigens and eye-specific antigens.[14,15] This suggests that the microbial interaction or the history of infection(s) may modify the risk of developing non-infectious uveitis. This is consistent with other autoimmune conditions that are associated with gut dysbiosis or show increased seropositivity to pathogens, such as *Epstein–Barr virus* (EBV) in multiple sclerosis (MS)[16–18] or parvovirus in juvenile idiopathic arthritis (JIA), both autoimmune conditions that may manifest as non-infectious uveitis.[19]

We do not know whether an individual's history of infections increases their chances of developing non-infectious uveitis. Deep profiling of the antibody-repertoire of patients with non-infectious uveitis may reveal a distinct natural history of microbial exposure that may increase the susceptibility to non-infectious uveitis. This may be reflected by enrichment for IgG antibodies towards distinct pathogenic species or antibody epitope motifs. Considering the intrinsically shorter exposure time that children with uveitis have to pathogens than adults, antibody profiling in children would be a more useful strategy for detecting disease-relevant alterations in non-infectious uveitis. Understanding the natural history of infectious events that underlie non-infectious uveitis could aid in the development of early interventions (e.g., vaccination) that could mitigate the effects of the disease or prevent its onset.

In this study, we analysed the pathogen-specific antibody-repertoire of paired aqueous humour and serum by 3760-plex high-density peptide microarray in a paediatric cohort of non-infectious uveitis and controls.

Methods

Patient inclusion and sample selection

This study was approved by the Medical Ethical Committee of the University Medical Center Utrecht (TCBio 22-628/Biobank 12-514) and was conducted in accordance with the Declaration of Helsinki. All patients and/or their parents/legal guardians included in this study have given their informed consent to participate in the study.

In total, we used samples from 18 patients with paediatric uveitis (18 serum samples and 17 paired AqH samples) without established underlying infectious cause (Fig. 1A). The diagnosis was made in accordance with the *Standardization of Uveitis Nomenclature* (SUN)[20] by a paediatric uveitis specialist. All patients were referred to a paediatric rheumatologist for evaluation of underlying systemic disease. The diagnostic work-up included a thorax X-ray image, laboratory blood tests (i.e., HLA*B27 typing, antinuclear antibodies (ANA), serum angiotensin converting enzyme (ACE), Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP), *QuantiFERON*^{TM} TB test, urinary screening (for

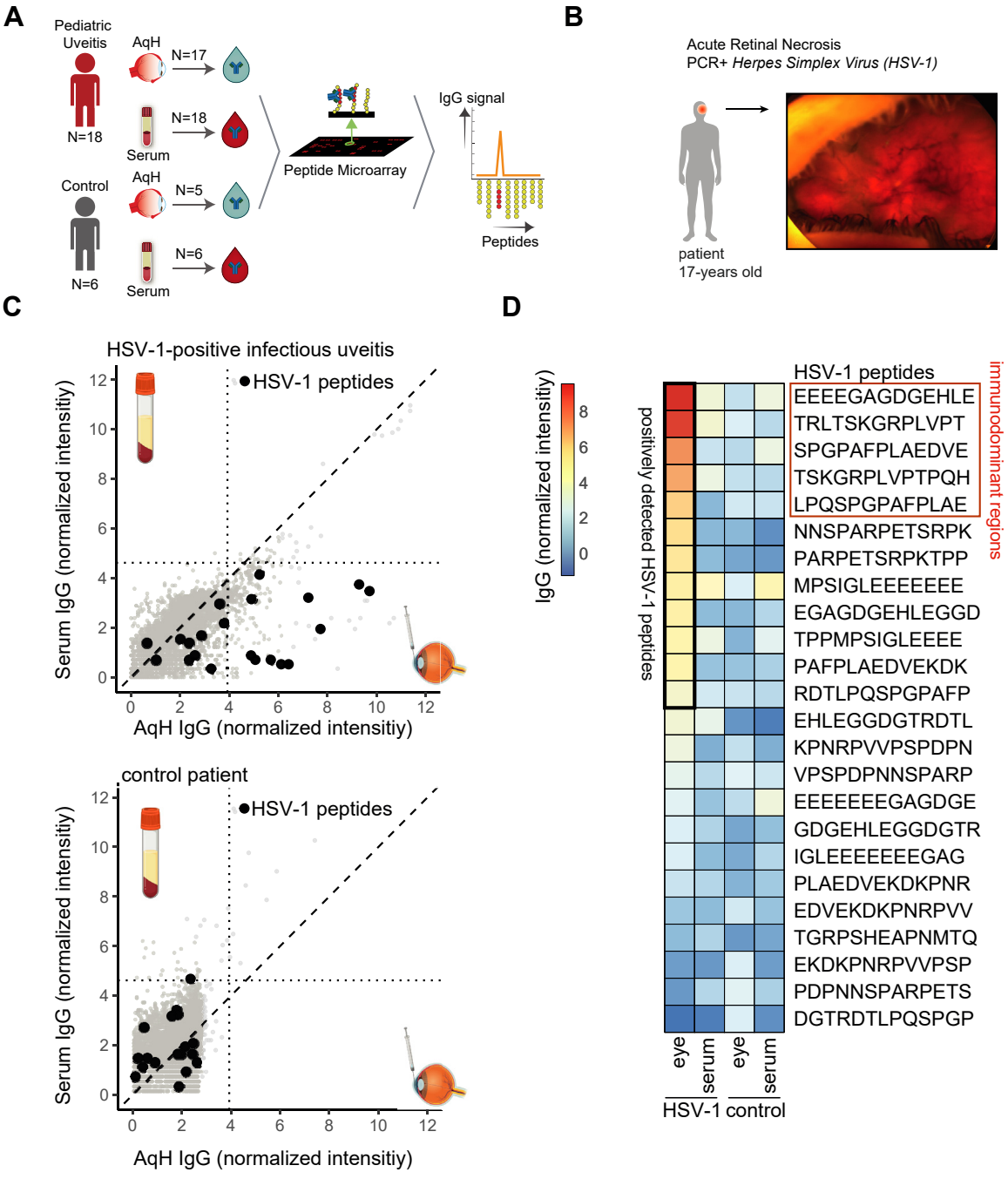

Fig. 1: **Peptide microarray analysis captures clinically relevant pathogen antibody profiles. A**) Study design. IgG antibody binding to specific epitopes was quantified in serum samples and aqueous humour (AqH) samples from paediatric uveitis patients and controls using the PEPperCHIP® Infectious Disease Epitope Microarray. **B**) A 17-year old patient with acute retinal necrosis [ARN] due to intraocular infection with HSV-1 was profiled as positive control (confirmed by PCR of AqH and local intraocular antibody production). **C**) Plot of IgG antibody intensities against 3697 linear peptides on the microarray in paired AqH and serum of the ARN patient from *b* and a non-inflammatory cataract control of 11 years old. Each (grey) dot represents the mean IgG intensity for a single peptide. Cutoff values characterizing the positive signals of the microarray are shown by the horizontal (serum) and vertical (AqH) lines. **D**) Heatmap showing the HSV-1 peptides and amino acid sequences, and their respective levels of IgG in the ARN patient and control. The 12 peptides that were positively detected in AqH are highlighted.

β2-microglobulin, total protein, creatinine) as well as serum and aqueous humour (AqH) PCR and serological tests for cytomegalovirus (CMV), varicella zoster virus (VZV), herpes simplex virus (HSV), Toxoplasmosa gondii, and rubella virus (Supplementary Table S1). Undiluted AqH samples were collected and stored at −80 °C after anterior chamber paracentesis performed during diagnostic work-up or in a later stage during surgery for complications of uveitis, such as cataract or glaucoma (Supplementary Table S1). *BD Vacutainer*^{TM} tubes were used to withdraw peripheral blood during anterior chamber paracentesis. Tubes were kept vertical for 30 min at room temperature, centrifuged at 2000*g* for 10 min at room temperature, and serum was stored directly at −80 °C. Paired AqH samples (five samples) and serum (six samples) from six age- and sex-matched children with congenital cataract or glaucoma without a history of ocular inflammation were obtained and served as paediatric controls in this study (Supplementary Table S1 and Fig. 1A). We included paired AqH and serum from a patient with confirmed local intraocular antibody production against HSV-1 and PCR for HSV-1 that served as a positive control for our antibody-profile array (Fig. 1B).

Infectious Disease Epitope Microarray

Paired AqH and serum samples were profiled using the *PEPperCHIP® Infectious Disease Epitope* Microarray (*PEPperPRINT*^{TM}, Heidelberg, Germany) (Fig. 1A). This assay utilizes a peptide library consisting of 3760 linear B-cell epitopes from the *Immune Epitope Database* (IEDB) from 196 pathogens known to cause infection in humans (Supplementary Figure S1). The microarrays were stained according to the manufacturer's instructions after incubation with AqH and serum (Supplementary Table S2).

IgM antibodies to EBV viral capsid antigen (VCA)

Serum samples of the cohort were analysed for qualitative detection of IgM and IgG antibodies to EBV VCA in human serum using a chemiluminescent assay (Alinity i EBV VCA IgM and IgG, Abbott) following the manufacturer's instructions.

HLA-DRB*15:01 genotyping

The HLA-DRB*15:01 genotype of patients was obtained from a next-generation sequencing study in paediatric uveitis.[10]

Statistical analysis

Statistical analyses were performed using *R* studio (v4.1.3). For every epitope slot, we analysed the IgG signal intensities by using the spot pixel median. Next, the mean intensity was calculated for every peptide sequence (every peptide sequence occurred *in duplo* or more). Principal component analysis was performed using the *FactoMineR* v2.8,[21] and *factoextra* v1.0.7 *R* packages. Background correction and normalization of samples was done using the *normalizeVSN* function from the *Linear Models for Microarray Analysis* (*LIMMA* version 3.50.3)[22] R package (Supplementary Figure S2). We filtered out peptides with low signal intensities by inspection of the median intensity histogram using the control sample group (n = 5) and removed peptides with normalized intensity <1.8[23] (Supplementary Figure S3). After preprocessing, 3697/4348 (85%) peptides were used for subsequent analysis. We established a positive threshold for IgG binding (i.e., cutoff) at two standard deviations above the mean intensities in controls (i.e., IgG normalized intensity threshold of 3.93 in aqueous humour and 4.62 in serum). We clustered peptide sequences using the *ClustalW* method from the Multiple Sequence Alignment R package (*msa* version 1.26.0)[24] and computed the distance matrix using the *dist.alignment* function with method "identity" from the *seqinr* package (*seqinr* version 4.2.30).[25] Clusters were formed with *hclust* function with the "Ward.D2", the *cophenetic* function to calculate the cophenetic distances and *cuttree* to form the clusters (with the height chosen to include 75% of the cophenetic distance) using the *cluster* R package (cluster version 2.04)[26] (Supplementary Table S3). We conducted differential expression analysis using the *lmfit* and *eBayes* functions from *Limma,* which adjusts *P* values (Padj) with the Benjamini-Hochberg procedure. Enrichment analysis for peptides clusters was done by using the *fgseaMultilevel* formula from the *fgsea* R package (version 1.20.0)[27] using clusters with a minimal size of 5 peptides (342 clusters) for enrichment testing, and a preranked antibody profile using statistics from patient versus controls comparison (sign (log [fold change] × −log10 (*P* value)). Clusters with positive antibody signals (average expression of peptides of the cluster above the threshold in AqH or serum) and with *Padj* <0.05 in enrichment analysis were considered significantly enriched. Peptide logos were made from using *ggseqlogo* R package from multiple sequence alignments.[28] Differences in patient characteristics between patient groups was calculated using the chi-square or the Fisher test for categorical data and the Kruskal–Wallis test for continuous data (Supplementary Table S1). The full statistical analysis can be found here: https://doi.org/10.34894/RBMEUY.

Results

High density peptide array-based profiling captures clinically relevant pathogen-specific intraocular antibody profiles

We aimed to perform antibody profiling against peptide epitopes from 192 viral, bacterial, fungal, and other microbial pathogens in paediatric non-infectious uveitis (Fig. 1A). To determine whether the peptide microarray could capture disease-relevant antibody profiles, we first profiled paired AqH and serum from a 17-year-old

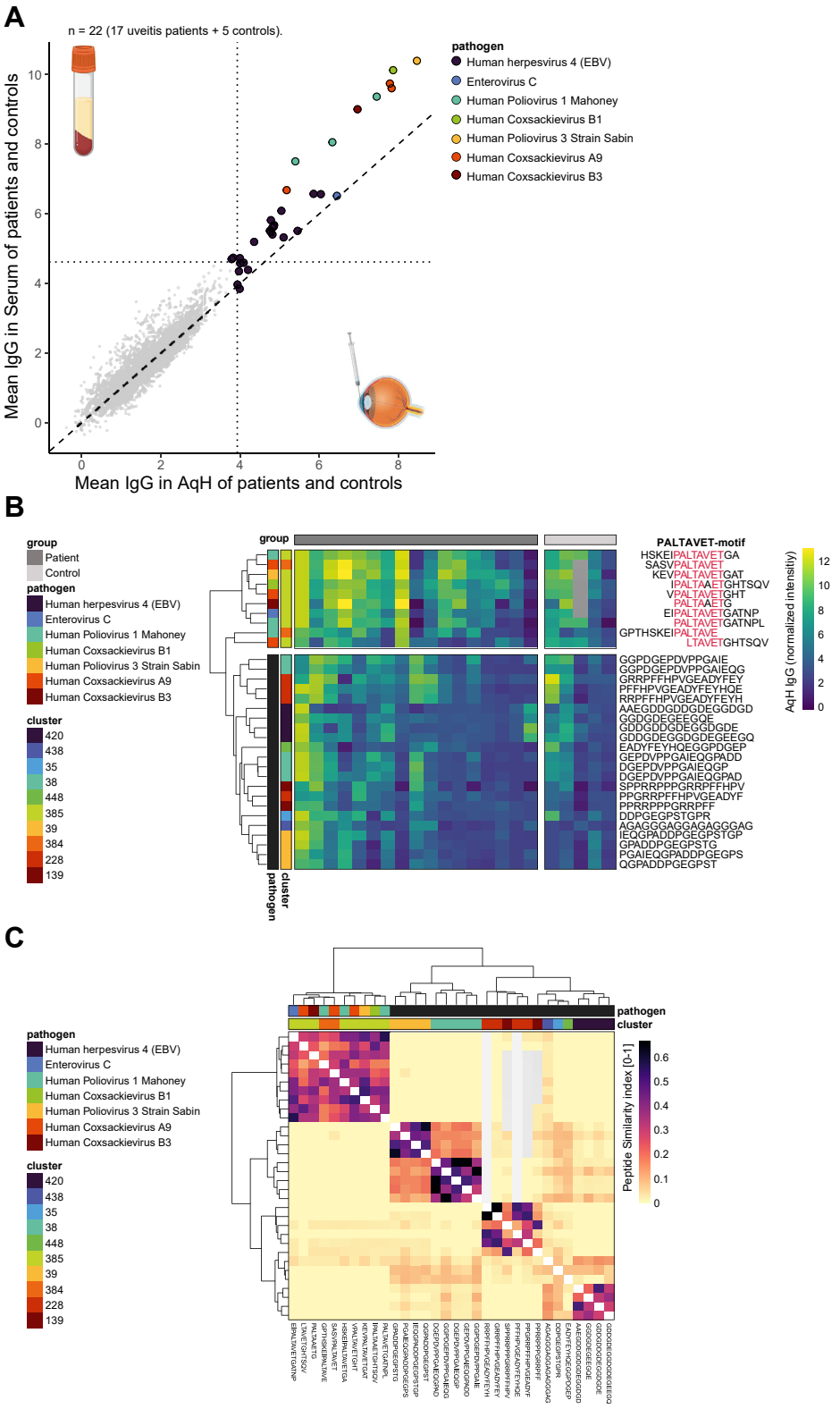

Fig. 2: **Broad intraocular antibody repertoire against EBV in the paediatric population**. A) Mean IgG antibody intensity per peptide in AqH and serum of our cohort. Cutoff values characterizing the positive signals of the microarray are shown by the horizontal (serum) and vertical (AqH) lines.

patient with acute retinal necrosis due to infection with Herpes Simplex Virus (HSV-1), confirmed by diagnostic PCR and ELISA and a control patient without inflammatory eye disease (Fig. 1B and C). Microarray profiling of the remainder AqH of the diagnostic anterior chamber paracentesis of the HSV-1-positive patient specifically detected high intraocular IgG against 4 peptides of HSV-1, while no HSV-1 peptides were positively detected in the control sample (Fig. 1C and D). The top 4 peptides of HSV-1 all contain key immunodominant epitopes of the glycoprotein G of HSV-1,[29] which supports that our approach is effective in detecting relevant antibody epitopes.

Broad antibody repertoire against EBV in the paediatric population

Next, we profiled AqH and paired serum samples of 17 patients and 5 controls (Fig. 2A, Supplementary Table S1). Overall, we detected 32 peptides with positive IgG levels in either AqH or serum. The IgG binding profiles for these peptides showed relatively comparable patterns between AqH and serum in our cohort (Spearman rho = 0.92) (Fig. 2A). We detected high IgG signals in approximately half of the samples in AqH and serum against epitopes from the Enterovirus family (e.g., Polioviruses, Coxsackievirus and Enterovirus C), which exhibit high peptide sequence similarity due to a shared partial sequence known as the PALTAVET-motif (peptide clusters 384 and 385: Fig. 2B and C), which is consistent with high antibody titres against this motif in ~50% of the population, due to routine vaccination against polio.[30–34]

Furthermore, we noted positive IgG levels in AqH and serum against multiple peptide clusters from the EBV nuclear antigen 1 (EBNA-1) of *Human Herpesvirus 4* (more commonly known as EBV) (Fig. 2A). Several peptides were only positively detected in AqH or serum (Fig. 2A). In total, we detected 22 unique peptides (20 in AqH and 16 in serum) across 8 peptide clusters with diverse IgG binding patterns across samples, indicating broad antibody responses against EBV in children (Fig. 2B and C). Additional serological testing of available leftover serum samples (22/24 samples) for EBV VCA IgM revealed that none of the patients or controls had a primary EBV infection (Supplementary Table S1).

Antibodies recognizing a motif in EBV nuclear antigen 1 are locally enriched in paediatric uveitis

Analysis of individual peptides in AqH or serum showed no significant differences between patients and controls after correction for multiple testing (*Padj* >0.05, Supplementary Table S3). The microarray contains many overlapping peptide sequences which allows us to test for differences in IgG levels that are reactive towards epitopes shared by multiple peptides and fine map the peptide motifs. To achieve this, we assessed the enrichment of peptide clusters (clusters with ≥5 peptides per cluster, n = 342 clusters tested) which share sequence similarity in AqH and serum of patients versus controls. In total, there were 5 peptide clusters of which the average expression levels were above the threshold of detection (Fig. 3A). These included two clusters with the PALTAVET-motif (clusters 384 and 385) and 4 clusters with peptides from different regions of EBNA-1 (clusters 35, 38, 39 and 228).). Cross referencing the data from the enrichment analysis for all clusters revealed that cluster 228 was elevated within the AqH of patients (Normalized Enrichment Score (NES) = 1.74, with Benjamini-Hochberg correction for multiple comparisons, *Padj* = 0.042, Fig. 3A and Supplementary Table S3). In some patients, the signal intensity for the peptide 228 cluster surpassed the signal detected for the vaccine-driven PALTAVET-motif signature.

Despite positive detection in serum, the peptide cluster was not statistically enriched in serum of patients (cluster 228 in serum, NES = 1.25, *Padj* = 0.53) (Fig. 3B). Cluster 228 exhibited high internal peptide sequence similarity indicating an antibody response driven by a shared EBV epitope (Fig. 3C). Considering this, we noted that peptides from cluster 228 also clustered together with two positively detected peptides from cluster 139 in our samples, indicating a possible shared epitope between cluster 228 and these two peptides from cluster 139 (Fig. 3C and D). Investigation of peptide sequences of cluster 228 revealed that the motif of cluster 228 indeed partly overlapped with cluster 139 and map to the same region in EBNA-1, but were distinct from the motifs of clusters 35, 38, and 39 that map to a downstream immunodominant region of EBNA-1 (Fig. 3C). The average expression of cluster 139 was below the threshold in AqH, which was caused by low signals for peptides with sequences that were more upstream from peptides of cluster 228 (Fig. 3C). Intriguingly, the signal of cluster 228 was driven by a subgroup of patients that also exhibited increased levels of IgG for the two peptides from cluster 139 and supported antibody responses against the same EBNA-1 epitope (Fig. 3D). By comparing the sequence of the positively detected peptides of cluster 228 and 139 in patients (n = 8 peptides), we determined the antigenic determinant (or minimal epitope) as the RRPFFHPV-motif [402-409] in EBNA1 (Fig. 3D). Separately testing

Positively detected peptides are colour-coded. B) Heatmap showing the IgG antibody intensities in the AqH against the detected peptides in patients and controls. Peptides are annotated by pathogen species and peptide cluster. A dendrogram indicates the average distance between the peptides of the clusters. C) Heatmap of the amino acid sequence similarity of the peptides in a and b according to peptide similarity index from little similarity (0) to identical sequence (1). The dendrograms indicate Ward's minimum variance between peptides of the clusters.

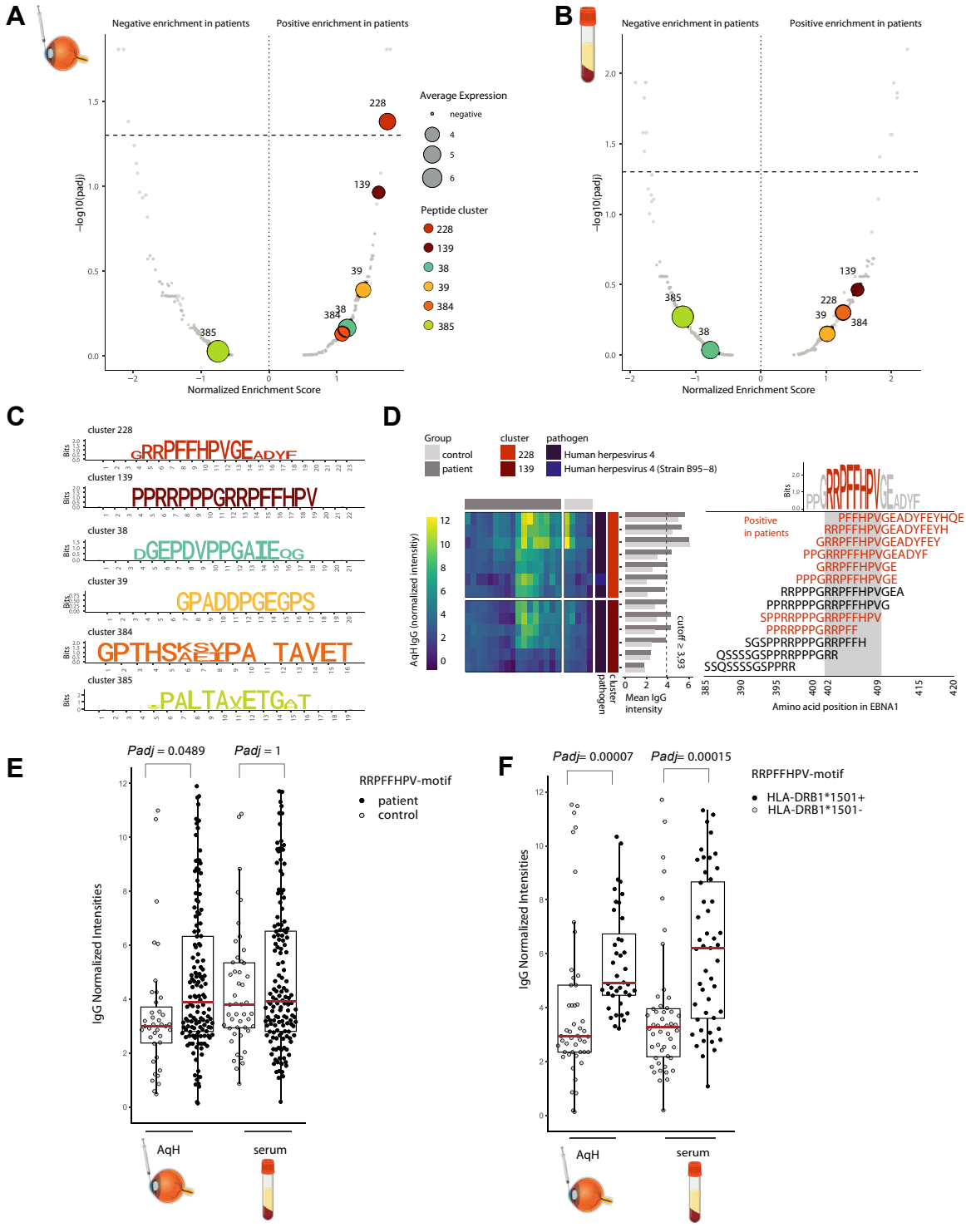

Fig. 3: **Intraocular antibodies to EBNA1 402-409 are enriched in patients with paediatric uveitis**. **A**) Peptides cluster enrichment analysis in aqueous humour (AqH) and **B**) in serum. Clusters with an average expression below the threshold for detection have been marked as "negative" (grey). NES = Normalized Enrichment Score for 342 clusters used in the enrichment analyses. The horizontal dotted line indicates the threshold for statistical significance (−log10 (Padj)). **C**) Peptide motifs of clusters identified in *a* and *b*. Peptides detected in patient AqH (>cutoff value) were used to generate motifs. **D**) Heatmap with IgG intensities for all peptides from clusters 228 and 139 in AqH of patients and controls. Peptides are annotated by cluster and pathogen. The average IgG intensities in AqH for peptides in patients and controls is shown in barplots.

the IgG intensities of the 8 peptides with the RRPFFHPV-motif of EBNA-1 in the serum and the AqH revealed this motif was significantly increased in the AqH of patients versus controls (Wilcoxon signed-rank test with Benjamini- Hochberg correction for multiple comparisons, *Padj* = 0.048) (Fig. 3E), but not in serum (*Padj* = 1).

Increased immune responses against the RRPFFHPV-motif of EBNA-1 is strongly implicated in the autoimmune disease multiple sclerosis,[35,36] and has been shown to be increased in HLA-DRB1*15:01+ donors. Since we previously associated *HLA-DRB1*15:01* with paediatric uveitis,[10] we could obtain the HLA-DRB1*15:01 genotype data for patients in this cohort that were previously genotyped by next-generation sequencing[10] (available for n = 12 patients, n = 6 HLA-DRB1*15:01 positive and n = 6 HLA-DRB1*15:01 negative). This analysis revealed a significantly increased antibody level against the RRPFFHPV-motif in HLA-DRB1*15:01+ donors in both the AqH and serum (*Padj* = 0.00007 and *Padj* = 0.0002 respectively) (Fig. 3F).

Discussion

Our study determined the antibody profiles of children with uveitis against nearly 200 human pathogens and found that one specific epitope in a highly immunogenic region of EBV transcription factor EBNA-1 was elevated in paediatric autoimmune uveitis.

A high overall IgG signal was seen against multiple different sequences of EBNA-1, a multifunctional EBV specific viral protein, essential for EBV replication and viral DNA maintenance and a key antigen for adaptive immunity. It is estimated that 90% of adults are seropositive for EBV by the time they reach adulthood.[37–40] Despite a lower seroprevalence in children, which varies with age and socioeconomic background, it is still well above 50% and can present with mild symptoms.[40,41] This is in line with our data, which also showed that approximately half of the children exhibit strong IgG signals to EBV peptides.

In some studies, EBV has been speculated as a driver of disease mechanisms of non-infectious uveitis, although direct evidence has yet to be established.[42–46] More importantly, EBV is of interest because it has been implicated as a trigger in several autoimmune diseases, such as SLE and MS.[39] Specifically, the role of EBV has been most widely examined for multiple sclerosis (MS) where seropositivity for EBV has been linked to the manifestation of MS.[16] Reactivation of EBV, characterized by polyclonal B cells responding to multiple epitopes of EBV antigens,[47] has been shown to contribute to the development of autoimmune diseases.[48] However, serum level VCA IgM which increases during primary infection or reactivation,[49–51] was found to be negative within our cohort. More notably, by fine mapping of antibody epitopes using peptide clusters, we associated one particular epitope on EBNA1, peptide motif 'RRPFFHPV' (EBNA1 AA402-409), with paediatric uveitis. This suggests a persistent IgG response against a specific EBNA1 epitope after EBV infection is implicated in the pathogenesis of non-infectious uveitis.

Interestingly, this peptide motif has been observed in the studies of multiple sclerosis (MS), an autoimmune condition where epidemiological evidence suggests a more than 30 times of risk after EBV infection.[52–56] In our study, the highest IgG signal against this RRPFFHPV -motif was seen in non-anterior (intermediate and panuveitis) uveitis patients and was found to be associated with HLA-DRB1*1501 carriers. Notably, all these patients had a history of papillitis, papilloedema and/or increased thickness of the RNFL implying inflammatory involvement of the optic nerve, also an important symptom of MS in the form of optic neuritis.[57] Also, *HLA-DRB1*15:01* is a key risk allele for intermediate uveitis, which is also the primary MHC allele associated with MS.[10,58,59] Given the clinical and genetic relationship between (specifically intermediate) uveitis and MS, the intraocular elevation of antibodies against this epitope is significant and may provide an explanation for the longstanding association between MS and uveitis.[57,60]

The disease mechanisms that relate EBNA1 epitopes in MS involve close mimicry with autoantigens.[61] The in our study identified RRPFFHPV-motif overlaps with the RRPFF-epitope linked to adaptive immune responses towards the autoantigen alpha-crystallin B (CRYAB).[36,53]

CRYAB has been identified as autoantigen in MS and as an important myelin antigen for T-cells.[62] CRYAB is a major component of the human eye well expressed in the lens, neural retina, retinal pigment epithelium of the eye.[63] Serum antibodies to CRYAB have been associated with uveitis[64–66] and in mouse models, which supports the idea that antibodies directed against CRYAB play a role in uveitis. CRYAB inhibits eye

The dashed line indicates the cutoff value for AqH. Peptide sequences with a positive detection in patients are highlighted in red and were used to generate the peptide motif. **E**) IgG antibody signal intensities for 8 peptides from *d* with the RRPFFHPV-motif (EBNA1 AA402-409) in AqH and serum of patients (AqH = 17 samples, serum = 18 samples) and controls (AqH = 5 samples, serum = 6 samples) (*Padj* from Wilcoxon signed-rank test with Benjamini- Hochberg correction for multiple comparisons). **F**) IgG antibody signal intensities for 8 peptides from *d* with the RRPFFHPV-motif (EBNA1 AA402-409) in AqH and serum of HLA-DRB1*15:01 positive (AqH = 5 samples, serum = 6 samples) and HLA-DRB1*15:01 negative (AqH = 6 samples, serum = 6 samples) uveitis patients (*Padj* from Wilcoxon signed-rank test with Benjamini- Hochberg correction for multiple comparisons).

inflammation via microglia, which trigger neuroinflammation in autoimmunity of the eye.[67–69] Collectively, our results make it tempting to suggest that prior EBV infection increases the likelihood of autoimmunity towards CRYAB in non-infectious uveitis.

A better understanding of the driving force behind B cell activation in uveitis and the role of EBV could facilitate the development of more targeted therapeutic approaches.

Several studies have shown that vitamin D3 supplementation in humans reduces anti-EBNA1 antibodies levels to the region involving the RRPFFHPV-motif without affecting other antibodies,[70–72] most profoundly in young individuals with HLA-DRB1*1501.[73] Large cohort studies identified low vitamin D levels as a risk factor for non-infectious uveitis and supplementation with vitamin D has been associated with less disease activity.[74,75] Vitamin D supplementation may be an effective strategy to reduce anti-EBNA1 antibodies and potential cross-reactive reactions that result in inflammation of the eye.

Also, studies have investigated targeted prophylactic vaccinations against EBV.[76] Although, at this time, no clinically approved vaccines against EBV infection are available yet, currently, two vaccines by Moderna and the NIH are being investigated in a Phase I clinical trial in addition to some successes seen in mice models.[77,78] A vaccine that could prevent EBV antibody formation against EBNA1 could potentially prevent the cascade of B cell and T cell autoactivation.

Limitations

The following limitations should be considered when interpreting our study. The number of patients included in this study is limited due to the rarity of uveitis and the sparsity of aqueous humour samples. As the study design included a modest sample size of paired serum and AqH samples from paediatric patients and controls, statistical power was limited when testing thousands of peptides individually. However, we were able to cluster multiple epitopes on the microarray together based on shared peptide homology and therefore shared antibody binding profile to improve power. Our filtering strategies, including the requirement that the mean expression levels of peptides in a cluster should be above the detection threshold, also resulted in 'independent' validations of putative epitopes of interest (under the assumption that peptides with the same epitope should show similar expression patterns). While this method was beneficial for detecting changes in high IgG levels in EBV, the variation in IgG signals towards peptides between samples and the small sample size may have hampered the detection of changes in relatively lower expressed IgG against other relevant pathogens in uveitis. A follow-up study should expand the sample size and ideally include adult non-infectious uveitis patients as well. The microarray we used for our measurements contains linear B-cell epitopes and unequal representations of pathogens, as well as a non-exhaustive library of potential epitopes of interest for uveitis. Future studies should use phage-display technologies for deep profiling of pathogen-specific antibodies in non-infectious uveitis to identify other relevant and perhaps disease-specific antibody changes.

In this study, as the focus was broad pathogenic antibody profiling, we did not test antibody reactivity to autoantigens such as CRYAB and only speculated on possible cross-reactivity based on findings in literature. In future studies we could use suspension bead technology with N-terminally biotinylated recombinant peptides of alpha-crystallin B to demonstrate antibody reactivity against CRYAB and pretreat the aqueous humour samples with $EBNA1^{401–420}$ to further examine cross-reactivity between the elevated peptide motif we found and autoantigen CRYAB.[36] In combination with platforms that profile other autoantigens, such technologies could determine if uveitis patients with antibodies towards the EBNA1 motif also cross-react with ocular autoantigens. This will also help us understand how cases with antibodies to the EBNA1 motif develop uveitis, since high EBV signals were also detected in some controls. For further understanding of the role of infectious history in immune dysregulation in uveitis, other factors that influence immunity, such as genetic disposition (i.e., HLA alleles), should be considered as well.

Conclusions

We identified elevated antibodies against an epitope of EBNA1 of EBV (AA 402-406) in the AqH of patients with paediatric uveitis, showing an association with HLA-DRB1*1501, which has also been linked to CNS autoimmunity.

Contributors

J.H.: Investigation, Methodology, Formal analysis, Visualization, Writing—original draft; L.J.B.: Methodology, Writing—review and editing; P.A.W.J.F.S.: Resources, Writing—review and editing; J.D.F.G.: Resources, Writing—review and editing; J.H.B.: Conceptualization, Funding acquisition, Methodology, Supervision, Visualization, Writing—review and editing; J.J.W.K.: Conceptualization, Funding acquisition, Formal analysis, Methodology, Supervision, Visualization, Writing—review and editing. All authors read and approved the final version of the manuscript. The authors J.H. and J.J.W.K. have accessed and verified the underlying data.

Data sharing statement

All relevant data files and the statistical analysis plan are available at https://doi.org/10.34894/RBMEUY.

Declaration of interests

J.H., L.J.B., P.A.W.J.F.S., J.D.F.G., J.H.B., J.J.W.K. all declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.105681.

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