Maternal T Cells in the Human Placental Villi Support an Allograft Response during Noninfectious Villitis

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Maternal T Cells in the Human Placental Villi Support an Allograft Response during Noninfectious Villitis

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During human pregnancy, proinflammatory responses in the placenta can cause severe fetal complications, including growth restriction, preterm birth, and stillbirth. Villitis of unknown etiology (VUE), an inflammatory condition characterized by the infiltration of maternal CD8⁺ T cells and fetal macrophages (Hofbauer cells) into the chorionic villi (2, 3). VUE of various grades is diagnosed in 6–34% of all third-trimester placentae (4, 5); however, pathological evaluation of placenta diagnosed with VUE compared with control and infectious villitis placentae by immunoSEQ. Immunosequencing demonstrated that VUE is driven predominantly by maternal T cell infiltration, which is significantly different from controls and infectious cases; however, these T cell clones show very little overlap between subjects. Mapping TCR clones to common viral epitopes (CMV, EBV, and influenza A) demonstrated that Ag specificity in VUE was equal to controls and significantly lower than CMV-specific clones in infectious villitis. Our data indicate VUE represents an allograft response, not an undetected infection. These observations support the development of screening methods to predict those at risk for VUE and the use of specific immunomodulatory therapies during gestation to improve outcomes in affected fetuses. The Journal of Immunology, 2020, 204: 000–000.

Villitis of unknown etiology (VUE) is a destructive inflammatory process in placental tissue initially described in 1975 (1). Lesions have since been characterized at a cellular level by infiltration of maternally derived CD8⁺ T cells and fetal macrophages (Hofbauer cells) into the chorionic villi (2, 3). VUE of various grades is diagnosed in 6–34% of all third-trimester placentae (4, 5); however, pathological evaluation of the placenta is not routinely performed in all cases, and thoroughness of histologic sampling has not historically been standardized (6), so a true incidence is difficult to ascertain. Clinical outcomes of VUE most often result in the delivery of a healthy infant, but VUE is also associated with intrauterine growth restriction (7, 8), preterm birth (9, 10), fetal/neonatal demise (7, 11), and neonatal neurocognitive impairment (12, 13). As the risk for recurrence is reported to be between 10 and 37% (2, 14), it is important to establish a diagnosis, and counsel affected women on the risks of a VUE diagnosis in a subsequent pregnancy. Specific management including treatment guidelines for pregnancies following a diagnosis of VUE are lacking and reflect limited understanding of the pathophysiology of this poorly characterized condition.

VUE may be secondary to an undetected infection during gestation or reflect an aberrant maternal immune response, resembling tissue rejection (2, 4, 5). Although some studies have reported infectious villitis cases that were misdiagnosed as VUE (15), there are many features unique to each pathology. For example, infectious villitis typically develops earlier in pregnancy, is rarely recurrent, leads to nearly all villi being abnormal, and can show a mixed inflammatory infiltrate including lymphocytes, neutrophils, and/or plasma cells (the latter especially with CMV infection), whereas VUE typically develops near term, presents as focal/patchy infiltration of lymphocytes into the terminal and stem villi, and is often recurrent (2). As the fetus is a semiallograft, maternal immunity must be tightly regulated (16, 17). Potential breakdown in tolerance of the fetal allograft and immune rejection is supported by the fact that VUE is also associated with maternal autoimmune (18, 19), neonatal alloimmune thrombocytopenia (20), and in vitro fertilization with a donor oocyte (21, 22).

Maternal T cells, specifically cytotoxic CD8⁺ cells, are not typically found in placental villi but are present in VUE (2, 3). These cells play an important role in host defense against viruses...
by recognizing non-self-antigen presented to their TCR by MHC class I molecules. Killing of target cells expressing specific Ag occurs by release of proinflammatory cytokines and cytotoxic perforin and granzyme B. The TCR is composed of two protein chains, with 90% of the T cell population expressing an α- and β-chain, and a small subset having a γ- and δ-chain. The diversity of TCRs is mostly found in the CDR3. This variability is due to somatic variable (V), diversity, and joining (J) recombination in the α/β-chain, giving T cells a diverse Ag repertoire to initiate immune responses. Profiling the T cell repertoire using single-molecule DNA sequencing and multiplex PCR methods have provided valuable insight into many diseases (23, 24). In transplant biology, TCR spectratyping has been used to monitor patients after hematopoietic stem cell transplantation and helps predict the efficacy and occurrence of graft-versus-host disease (25–27). In cancer, TCR profiling demonstrated an expansion of T cell clones in patients who responded to immunotherapy compared with those who progressed (28).

Sequencing the T cell repertoire has not been used for characterization of VUE but may provide valuable new insights into the origin and pathogenesis of this poorly understood condition. Therefore, this study defined TCR diversity in 10 placentae with VUE compared with 10 gestational, aged-matched, unaffected placentae diagnosed with VUE, with minimal overlap between patients and common viral epitopes, suggests that T cell expansion in placenta with VUE would demonstrate distinct clonal expansion, independent of known infectious causes. We hypothesized that T cells from placentae with VUE would demonstrate different clonal expansion, independent of known infectious causes. We noted that VUE-expanded clones were unique to each subject, reflecting a broad repertoire that was not pathogen specific, unlike the infectious villitis cases. Increased T cell clonality in placenta diagnosed with VUE, with minimal overlap between patients and common viral epitopes, suggests that T cell expansion is not linked to a single initiating Ag. These results further support an allograft response by maternally derived T cells in gestation during VUE.

Materials and Methods

Patient selection

This study is approved by the Mayo Clinic Institutional Review Board (16-006099) in accordance with the Declaration of Helsinki principles. Twenty-five residual placental tissue samples (formalin fixed, paraffin embedded (FFPE)) collected between 2014 and 2019 were used for this study. Ten tissues with a pathological diagnosis of high-grade VUE were selected by Amsterdam Criteria (29). These samples were matched by gestational age with control placentae. An additional five placentae diagnosed with infectious villitis (four CMV and one Toxoplasma gondii confirmed cases) were included but were not matched to VUE or control cases.

Fluorescence in situ hybridization

Six placental samples from a male fetus were used to identify infiltrating cell origin by fluorescence in situ hybridization (FISH). Slides were placed in a 90°C oven for 15 min. Slides were then deparaffinized with xylene (two times, 15 min each) at room temperature (RT), dehydrated in 100% ethanol for 5 min at RT, and placed in 10 mM citric acid (pH 6) and microwaved for 10 min. The slides were then dehydrated using an ethanol series (70, 85, and 100%) 2 min each at RT. Working solution of DXZ1/DYZ3 (Abbott Laboratories, Des Plaines, IL) was made by mixing 1 μL of concentrated DXZ1/DYZ3 probe with 9 μL of LSI/WCP Hybridization Buffer (Abbott Laboratories). Hybridization solution was applied to the target areas, coverslipped, and denatured with a Thermobrite (Abbott Laboratories) at 83°C for 5 min, and hybridized overnight in a 37°C humidified oven. Following hybridization, slides were soaked at RT twice in SSC/0.1% Nonidet P-40 (NP-40) to remove coverslips, placed in 2× SSC/0.1% NP-40 at 74°C for 2 min, and then placed into RT 2× SSC/0.1% NP-40 for 2 min. The slides were stained with DAPI (Vector Laboratories) and coverslipped. Tissue samples were scanned in their entirety, and the qualitative result was determined based on observed signal patterns by CytoVision (Leica Biosystems, Wetzlar, Germany).

Immunosequencing

Each FFPE tissue was cut into five 10-μm scrolls and DNA isolated using the AllPrep DNA/RNA FFPE Tissue Kit per manufacturer’s instruction (Qiagen, Valencia, CA). Concentration and purity of isolated DNA was determined by NanoDrop, whereas RNA quality was assessed using a Bioanalyser (Agilent Technologies, Santa Clara, CA). The extracted DNA was used for immunosequencing the TCR β-chain of T cells in placental villi using the immunoSEQ (R) Assay (Adaptive Biotechnologies, Seattle, WA). Investigators running this assay were blinded to the group allocation of the samples. Briefly, the assay amplifies rearranged CDR3 of TCRs using a bias-controlled, multiplex PCR method (23, 24). Samples were sequenced on an Illumina MiSeq platform using a 100-cycle, paired-end protocol (Illumina, San Diego, CA). The quantity of nucleated cells and T cells was determined during sequencing through the addition of reference gene primers and TCR-β-specific primers (24). Raw sequencing data were uploaded and are freely accessible through immuneACCESS (clients.adaptivebiotech.com/pub/enninga-2020-ji; DOI 10.21417/EALE2020Ji).

Calculations of richness and diversity

Diversity and richness were calculated using the Adaptive Biotechnologies immunoSEQ Analyzer Software. Heterogeneity, defined in this study as T cell richness in our sample, was estimated by the iChao1 method (30). iChao1 is a nonparametric estimator of the lower bound, or minimum number, of unique templates predicted within a subject’s repertoire at 95% confidence. Diversity scores were used to analyze highly abundant TCR clones in each sample (31). Clonality is based on Pielou evenness (J) and Shannon diversity (H) (32), which calculates a number between 0 and 1, with the higher value indicating lower diversity, or increased expansion of a single clone, in the sample. Formulas are given below, where pi is the proportion of clonotype i, b is the base of the logarithm, and S is the number of clonotypes.

\[
\text{Shannon}(H) = -\sum p_i \log_b p_i
\]

\[
\text{Pielou}(J) = \frac{H}{\log_b S}
\]

Clonality = 1 - J.

Statistical analysis

Sequencing data were analyzed using the immunoSEQ Analyzer from Adaptive Biotechnologies (http://www.adaptivebiotech.com/immunoseq). For patient demographics and continuous variables, medians and interquartile ranges were reported. Kruskal–Wallis tests were used to compare groups, and the false discovery rate was used for multiple comparisons testing by the Benjamini, Krieger, and Yekutieli method (33). For VJ and epitope mapping, means and standard deviations were reported. Group comparisons were made by ANOVA using the Benjamini–Yekutieli test to correct for multiple comparisons. Amino acid sequences and their corresponding VDJ alleles determined during sequencing were uploaded into the VDJdb browser (https://vdjdb.cdr3.net/), a curated database of TCR sequences with known Ag specificity (34). T cell clones specific for CMV, EBV, and influenza A were identified in each group using simple scoring with a confidence of 3 and substitution of 2. All analyses were two sided, and significance was defined as a p value < 0.05. Graphing and analyses were performed using GraphPad Prism 8 (GraphPad Software, San Diego, CA).

Results

Subject characteristics

Placentae were collected following delivery and underwent gross and histological evaluation. For this study, 10 VUE-diagnosed placentae were chosen and matched with 10 control placentae by gestational age at delivery. An additional five confirmed infectious villitis cases (four CMV and one T. gondii) were also included but were not matched to the control or VUE cases. Two control placentae were delivered preterm but lacked significant pathology upon examination. No differences were observed between all three groups related to body mass index, APGAR (newborn health status) scores and fetal length. Women in the cohort with infection were younger, had
smaller fetuses, and delivered preterm or suffered an intrauterine demise (n = 2). Women in the VUE cohort had significantly higher gravidity and parity than the control and infectious villitis cohorts. Women in the VUE cohort also had more prior miscarriages compared with the other cohorts, and 50% underwent a Cesarean delivery for the current pregnancy, similar to the infection cohort. These data are summarized in Table I.

**Total T cell composition in the placenta**

By definition, histological evaluation demonstrated increased lymphocytic infiltration of chorionic villi, with resulting fetal vascular obliteration and tissue necrosis in placentae diagnosed with VUE compared with unaffected controls by H&E staining at 100× magnification (Fig. 1A). Conversely, infectious villitis histology demonstrated at least focal plasma cell infiltration and CMV inclusions, which were absent in VUE and controls. The one case of toxoplasmosis demonstrated focal lymphoplasmacytic deciduitis and granulomas, along with Toxoplasma tachyzoites and pseudocysts confirmed by immunohistochemistry in sections of the umbilical cord. FISH probes against the X (green) and Y (red) chromosomes were consistent with previous reports that the infiltrating cells in diagnosed VUE from male placentae are of maternal origin (XX positive; Fig. 1B). Although the infectious villitis cases demonstrated XX staining, these maternal cells were observed at low levels and in small pockets indicating that, unlike VUE, this pathology is predominantly driven by cells of fetal origin. These small pockets of maternal cells in infectious villitis may coincide with the small pockets of plasma cells observed by H&E. Following nucleic acid extraction, the median amount of DNA was not significantly different between the three groups (p = 0.093; data not shown). Immunosequencing demonstrated a similar number of nucleated cells in both control and VUE tissues (69,540 versus 56,214, respectively, p = 0.1738), but significantly more nucleated cells were present in infected placentae (295,562; p = 0.0208; Fig. 1C). As expected from histological review, the median number of T cells in placentae identified by immunosequencing was higher in the VUE group compared with the control group (1340 versus 292; p = 0.0012); however, the infectious villitis cases had the highest number of T cells identified (9194), which was significantly different from control (p < 0.0001) and VUE (p = 0.0289; Fig. 1D) subjects. Finally, the ratio of T cells to all nucleated cells isolated from placental villi demonstrated a 6-fold increase in T cells in VUE compared with controls (p = 0.0011) and a 150-fold increase compared with infectious villitis (p < 0.0001; Fig. 1E). These results indicate that, unlike VUE, which is driven predominately by maternal T cells in the placenta, infectious villitis is characterized by massive fetal immune cell infiltration into the villi, involving fewer T cells.

**TCR diversity in VUE**

TCR composition in the placenta was measured and compared between groups. The average total number of productive TCR rearrangements in VUE-diagnosed placentae was significantly higher compared with controls (2060 versus 489; p = 0.002) but lower when compared with infectious villitis (11,528; p = 0.0496; Fig. 2A). This result was also observed for unique TCR sequences or sequences with a defined TCR-β CDR3 combination in VUE compared with controls (1293 versus 473; p = 0.0387) and VUE compared with infectious villitis (1293 versus 3653; p = 0.0415; Fig. 2B). Next, the unique/total CDR3 reads ratio was measured to understand TCR diversity, which is an estimate of the number of clones in the entire repertoire. A lower ratio means less diversity or an expansion of certain clones. The median ratio of VUE TCRs was 0.68, which was significantly lower than control TCRs (0.96; p = 0.001; Fig. 2C), suggesting that T cell diversity is decreased in VUE-diagnosed placentae. At the same time, the median ratio of infectious villitis TCRs was 0.50, which was not different from VUE (p = 0.06). iChao1 was used to estimate TCR richness of individual repertoires in a sample by calculating a lower bound of richness. The data demonstrated decreased richness in VUE clones compared with infection (7,724 versus 14,988; p = 0.0257); however, VUE richness was unchanged compared with control cases (7692; p = 0.7829; Fig. 2D). Finally, clonality was measured with numbers closest to 0, indicating the sample is highly polyclonal, whereas numbers closest to 1 are monoclonal. Significant expansion of T cell clones was observed in VUE cases (0.057) and infectious villitis (0.133) compared with controls (0.006; p = 0.0025 and p = 0.0274, respectively; Fig. 2E). These data indicate that T cell clones in VUE and in infectious villitis undergo clonal expansion in placental tissue compared with controls; however, the TCR repertoire of VUE T cells is less diverse compared with infectious villitis.

### Table I. Clinical characteristics of fetal and maternal subjects

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 10)</th>
<th>VUE (n = 10)</th>
<th>Infection (n = 5)</th>
<th>Kruskal–Wallis p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>30 (27–38)</td>
<td>33 (22–40)</td>
<td>24 (24–32)</td>
<td>0.042</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2 (1–4)</td>
<td>3.5 (2–6)</td>
<td>2 (2–3)</td>
<td>0.008</td>
</tr>
<tr>
<td>Parity</td>
<td>1 (1–3)</td>
<td>2 (1–5)</td>
<td>2 (1–2)</td>
<td>0.018</td>
</tr>
<tr>
<td>Prepregnancy BMI (kg/m²)</td>
<td>23.4 (18.8–45.2)</td>
<td>28.8 (19.8–45.2)</td>
<td>26.2 (21.5–35.8)</td>
<td>0.187</td>
</tr>
<tr>
<td>Smoking</td>
<td>2/10</td>
<td>1/10</td>
<td>1/5</td>
<td></td>
</tr>
<tr>
<td>Prior miscarriage</td>
<td>0/10</td>
<td>6/10</td>
<td>1/5</td>
<td></td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>9/10</td>
<td>5/10</td>
<td>2/5</td>
<td></td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td>0/10</td>
<td>3/10</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Fertility treatment</td>
<td>0/10</td>
<td>1/10</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td><strong>Fetal demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at birth</td>
<td>38 2/7 (34 2/7–40 1/7)</td>
<td>38 4/7 (34 3/7–39 5/7)</td>
<td>33 3/7 (27 1/7–36 1/7)</td>
<td>0.006</td>
</tr>
<tr>
<td>APGAR 1 min</td>
<td>8 (6–9)</td>
<td>8 (4–9)</td>
<td>6 (0–9)</td>
<td>0.579</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>3.4 (2.3–4.5)</td>
<td>3.0 (2.6–3.7)</td>
<td>1.4 (1.0–3.0)</td>
<td>0.014</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>50 (44.5–53.5)</td>
<td>50.5 (44.5–54.0)</td>
<td>38 (33.0–48.3)</td>
<td>0.114</td>
</tr>
<tr>
<td>Female fetus</td>
<td>4/10</td>
<td>8/10</td>
<td>2/5</td>
<td></td>
</tr>
</tbody>
</table>

Italicics indicate significance by one-way ANOVA (comparing all three groups). APGAR scores indicate newborn health status (0 = demise, 9 = excellent).

*Significant post hoc comparisons indicated by VUE versus infection.

*Significant post hoc comparisons indicated by control versus infection.

*Significant post hoc comparisons indicated by control versus VUE.

*Data are presented as medians with ranges.

BMI, body mass index.
Distribution of clones in VUE

Identifying dominant and shared T cell clones may assist with recognizing which epitopes are driving TCR expansion in VUE and infectious villitis. Therefore, the most frequently occurring rearrangements within a sample were identified, along with how much of the sample is made up of these rearrangements considering the top 100 clones in each group. Across all placentae diagnosed with VUE and infectious villitis, there was a larger distribution of top T cell clones making up the entire sample compared with controls (Fig. 3A). This suggests the TCR-β repertoire is driven largely by expanded clonotypes, which is not observed when compared with controls. Further considering only the top 15 most abundant clones, these made up 15% of the entire repertoire in VUE placentae, and 20% of the infectious T cell repertoire compared with controls (9%; \( p = 0.007 \) and \( p = 0.002 \); Fig. 3B). There was no difference in abundant clones when comparing VUE to infectious villitis (\( p = 0.085 \)). Together, this indicates that expanded T cell clones make up a larger population of the total T cell pool in VUE and infectious villitis compared with controls.

V-J gene segments in VUE

The composition of the TCR-β V and J segments were profiled in VUE, infectious, and control placentae to identify areas of high variability that are disproportionally represented in our groups. For the V gene, 59 different alleles and 13 J gene alleles were measured. Using a Benjamini–Yekutieli correction for multiple testing, we observed differences in V gene families V2, V4, V5, V6, V7, V9, and V10 between our three groups (Fig. 5A). Interestingly, the J1 family showed increased representation in infectious villitis, whereas the J2 family was observed at a significantly lower frequency.

FIGURE 1. Basic characteristics of VUE, infectious villitis, and control placentae. (A) Representative H&E images of VUE, infectious villitis, and control placentae (original magnification \( \times 100 \)). (B) FISH-based detection of male fetal and maternal cells in the chorionic villi (X chromosome in green and Y chromosome in red). (C) Sampling depth between groups. (D) Total T cell counts in each group. (E) Ratio of T cells to all nucleated cells. Bars designate median and interquartile range (\( n = 5–10 \) per group). Boxes indicate 25th and 75th percentiles; lines indicate median, and whiskers mark the range. Data were compared by Kruskal–Wallis testing, with post hoc analysis using the Benjamini, Krieger, and Yekutieli method. *\( p \leq 0.05 \), †\( p \leq 0.05 \), ‡\( p \leq 0.05 \); control versus VUE, control versus infection, and VUE versus infection, respectively.
lower frequency in VUE and infectious villitis than in controls (Fig. 5B). Using these segments, VDJdb browser was used to query CDR3 data against Ags specific to CMV, EBV, and influenza A. T cell clones specific to these common viruses were identified in every sample in our cohort; however, the bulk of TCR clonotypes detected in VUE did not recognize known viral epitopes. As expected, Fig. 5C showed infectious CMV cases having higher median frequencies of clones recognizing CMV epitopes (0.011) compared with VUE (0.001; $p < 0.0001$). No differences were observed between CMV-specific frequencies in VUE compared with control placenta (0.0011 versus 0.0018; $p = 0.282$).

Interestingly, a significant increase in clonotype frequency was only observed when comparing control to both placental pathologies for EBV ($p < 0.0001$; Fig. 5D) and influenza A-specific epitopes ($p < 0.0001$; Fig. 5E). Thus, the frequency of T cells recognizing viral Ags in VUE was equal to the control placenta and not to what is observed in infectious villitis. Together, these data indicate that the maternally derived T cells infiltrating the placental villi during VUE do not recognize common viral Ags like their infectious counterparts, providing more evidence that VUE represents an allograft response and not an undetected infection.

**Discussion**

In normal pregnancies, T cells do not accumulate inside the fetal chorion of the placenta but are found in relatively small numbers in the maternal decidua (35). VUE is a noninfectious placental pathology characterized by the presence of high levels of maternally derived CD8+ cells in the chorionic villi; however, little is known about these T cells. This study profiled the total TCR repertoire in VUE, villitis with diagnosed infection (mainly CMV), and control placentae to characterize the specificity of these cells. As expected, T cells make up a majority of the cells infiltrating during VUE, but not during infectious villitis. Although specific T cells clones were expanded in both VUE and infectious villitis, abundant clones were not shared between the cases profiled, suggesting a unique immune response is generated in each pregnancy. Finally, Ag mapping indicated that infiltrating T cells in VUE did not recognize common viral pathogens compared with infectious villitis. This observation supports the hypothesis that VUE represents an
individually distinct allograft response, potentially resulting in fetal rejection in the third trimester or earlier, as opposed to a viral-specific response to a common epitope or group of epitopes. How a fetus escapes maternal immune rejection is of great interest to those studying prematurity and transplantation biology.

Tissue rejection by T cell priming may occur in three main ways (36). First, host T cells can be presented with foreign MHC–peptide complexes by donor APCs, known as direct recognition. Second, donor peptides can be processed and presented by host APCs to T cells, known as indirect recognition. Finally, semidirect
recognition occurs when donor MHC–peptide complexes are captured by recipient APCs. Direct recognition is believed to be the main mechanism of acute rejection after solid organ transplantation (36); therefore, the inability for maternal T cells to directly interact with fetal/placental APCs may support survival of the latter. Transplant rejection can also occur indirectly through cross-primed CD8⁺ T cells as demonstrated by AlloSkin grafts in SCID mice (37). Work using adoptive transfer of T cells into pregnant transgenic mice demonstrated that fetal recognition by maternal T cells occurs exclusively through indirect recognition (38). Organ rejection after transplantation demonstrated expansion of the TCR repertoire in blood and tissue of patients who developed T cell–mediated rejection compared with those who did not (39). T cell profiling has therefore been used to monitor patients following transplantation who are at risk for graft rejection. This method may be useful in diagnosing VUE prior to delivery. Erlebacher et al. (38) showed T cells that recognize fetal Ag have no cytotoxic function and are clonally deleted. Peripheral deletion is a (de)selection step outside the thymus that removes lymphocytes that either recognize self-antigen or are weakly primed (i.e., inhibitory costimulatory molecules and lack of inflammatory response) (40–42). During pregnancy, numerous mechanisms at the fetal–maternal interface contribute to immune tolerance toward fetal Ags (43). One could hypothesize that during VUE, maternal T cells are indirectly presented with fetal/placental peptides by maternal APCs. Alternatively, maternally derived CD8⁺ T cells migrating to the chorionic villi may be presented with fetal Ags by placental macrophages (Hofbauer cells). In both scenarios, if there are proinflammatory signals present and an absence of negative regulatory receptors, an alloreactive response may be generated resulting in fetal rejection. Our data demonstrated low frequency of T cells in VUE and control placentae recognizing Ags specific to CMV, which was increased in patients with a diagnosed CMV infection during pregnancy. In a study examining cord blood of HIV-exposed uninfected infants, HIV-specific CD8⁺ T cells were identified in 86% (6/7) of the samples (44). Clonotype frequency was 4-fold higher in this study compared with the VUE results described in this study. Additionally, we were surprised that more clonotype sharing between our CMV cases was not observed; however, data from over 600 cases of CMV-affected subjects reported that few CMV-specific clones were shared, indicating that T cell responses show high levels of specificity in each person (45, 46).

Perturbations in immunity have been described during VUE, which may regulate whether a T cell response is generated. For example, the placental and blood cell transcriptome of women with a VUE diagnosis showed a significant increase in expression of chemokines CXCL9, CXCL10, CXCL11, and CXCL13; this pattern was not observed in cases of acute chorioamnionitis (47). Soluble CXCR3, the receptor for CXCL9 and CXCL10, was found at high levels in amniotic fluid of patients with preterm labor and placental villitis (48). High levels of these chemokines may result in the trafficking of maternal T cells into placental villi. Others have profiled C4d, a component of the complement pathway associated with Ab-mediated rejection after organ transplantation (49). C4d staining was identified in over 80% of VUE cases, in syncytiotrophoblast cells, compared with control placentae (5%) (50). Higher levels of C4d deposition were also found in VUE placenta compared with placentae infected with CMV (51). In pregnancy, paternal HLA is expected to be diverse compared with the maternal HLA-type, which may have important roles in VUE and TCR diversity. We have observed significant increases in HLA class III expression and HLA mismatch between mother and child in VUE compared with control placentae (52). In a cohort of term versus preterm birth subjects, HLA-reactive Abs in maternal serum were identified at a higher rate in preterm birth, which corresponded with placental pathology (53). Although no data exist on TCR diversity in pregnancy, transplant biologists aim to use spectratyping to predict graft rejection of allogeneic HLA (25–27). As VUE is often recurrent, one could hypothesize that maternal T cells that recognize paternal HLA in the first pregnancy may more effectively mount an anti-fetal response in a subsequent pregnancy because of the generation of memory T cells.

There are some limitations to interpreting our study results. First, as the paraffin blocks used contained small amounts of decidual tissue, some of the captured T cells, especially in control placentae, may have been decidual and not infiltrating T cells. Alternatively, fetal T cells may also be included in our analysis, especially in infectious villitis; however, these numbers in the villous capillary vessels of VUE placenta likely would be small, as lymphocyte populations are predominately of maternal origin (54). Second, the current method cannot determine whether the TCR sequences

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**Table II. Most abundant TCR rearrangements in each cohort**

<table>
<thead>
<tr>
<th>Amino Acid Sequence</th>
<th>No. of Subjects</th>
<th>Amino Acid Sequence</th>
<th>No. of Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>VUE</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
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<td></td>
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<td>3</td>
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<td>5</td>
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<td>5</td>
<td></td>
</tr>
<tr>
<td>CASSIDRGKDEQFF</td>
<td>3.67</td>
<td>GDRGRNTIYF</td>
<td>4.95</td>
</tr>
<tr>
<td>CASSQGPEQFFFF</td>
<td>3.18</td>
<td>CASSLGQPEQFFFF</td>
<td>4.94</td>
</tr>
<tr>
<td>CASSPDTYNTAEFF</td>
<td>2.49</td>
<td>CASSLAGGPDTEAFF</td>
<td>3.30</td>
</tr>
<tr>
<td>CASSSDRAETQYF</td>
<td>2.36</td>
<td>CASSLRWASYNQOEF</td>
<td>2.84</td>
</tr>
<tr>
<td>CASSNTRDTQYF</td>
<td>2.09</td>
<td>CASSAVGMPQHF</td>
<td>2.44</td>
</tr>
</tbody>
</table>

**Table III. Most commonly shared TCR rearrangements in each cohort**

<table>
<thead>
<tr>
<th>Amino Acid Sequence</th>
<th>Productive Frequency (%)</th>
<th>No. of Subjects</th>
<th>Amino Acid Sequence</th>
<th>Productive Frequency (%)</th>
<th>No. of Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>VUE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>CASSLQNTAETQF</td>
<td>0.57</td>
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<tr>
<td>2</td>
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<td>0.38</td>
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<tr>
<td>3</td>
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<tr>
<td>4</td>
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<td>2.09</td>
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<td></td>
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<tr>
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<td>CASSQDOETQYF</td>
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<tr>
<td>Infection</td>
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<td>VUE</td>
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<td></td>
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<tr>
<td>1</td>
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<td>2</td>
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<tr>
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<tr>
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</tr>
<tr>
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<td>CAWTGGGYTYF</td>
<td>0.042</td>
<td>3</td>
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</table>
assumed the majority of the cells profiled are CD8+ cytotoxic regulatory T cells (reviewed in Ref. 55). For VUE placentae, we compared CD4+ Th cells in VUE versus infection, respectively. T cells, as the presence of CD4+ Th cells in VUE is reported to be low (2). Finally, γδ–T cells have also been described at low levels in the decidua, especially during early pregnancy (56). The data presented in this study did not include γδ-TCRs.

Despite these limitations, the current study does provide new details on the abundance and extent of the T cell compartment in both VUE and normal human placentae. The lack of shared TCR clones between sequenced VUE placentae provides further support for an alloimmune reaction; whether this reaction is maternal or fetal in origin is important to determine. Although VUE occurs commonly, the pathogenesis of this condition is unknown. Clear guidelines for management in initial and subsequent pregnancies to improve outcomes are lacking. Better characterization of the T cell repertoire and stimuli prompting potential migration into placental villi during VUE is fundamental not only to reproductive biologists, but may also provide insights into tissue rejection, which is important for the field of transplant biology. Further research in this field may not only support the development of specific immunomodulatory therapies during gestation to improve outcomes in affected fetuses, but could also have broad implications for bone marrow and solid organ transplantation, as well as immuno-oncology.

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Disclosures
P.R. has a financial interest in Adaptive Biotechnologies. The other authors have no financial conflicts of interest.

References


