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Characterizing Villitis of Unknown Etiology and Inflammation in Stillbirth

Hayley Derricott,* Rebecca L. Jones,* Susan L. Greenwood,* Gauri Batra,† Margaret J. Evans,‡ and Alexander E.P. Heazell*

From the Maternal and Fetal Health Research Centre, Institute of Human Development, University of Manchester, Manchester Academic Health Science Centre, Manchester; the Department of Paediatric Histopathology,† Royal Manchester Children's Hospital, Manchester; and the Department of Paediatric Histopathology,‡ Edinburgh Royal Infirmary, Edinburgh, United Kingdom*

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Address correspondence to Hayley Derricott, Ph.D., Department of Infection Biology, Liverpool Science Park IC2, 146 Brownlow Hill, Liverpool L3 5RF, United Kingdom. E-mail: hayleyderricott@live.co.uk.

Villitis of unknown etiology (VUE) is an enigmatic inflammatory condition of the placenta associated with fetal growth restriction and stillbirth. Greater understanding of this condition is essential to understand its contribution to adverse outcomes. Our aim was to identify and quantify the cells in VUE in cases of stillbirth and to characterize immune responses specific to this condition. Immunohistochemistry was performed on placentas from stillborn infants whose cause of death was recorded as VUE to identify $CD45^+$ leukocytes, $CD163^+$ macrophages, $CD4^+$ and $CD8^+$ T cells, neutrophils, and proinflammatory and anti-inflammatory cytokines. Images were quantified with HistoQuest software. $CD45^+$ leukocytes comprised 25% of cells in VUE lesions: macrophages (12%) and CD4 T cells (11%) being predominant cell types; CD8 T cells were observed in all lesions. Leukocytes and macrophages were increased throughout the placenta in stillbirths; pan-placental $CD4^+$ and $CD8^+$ T cells outside VUE lesions were increased in stillbirth with VUE. There was increased IL-2 and IL-12 and reduced IL-4 immunostaining in VUE lesions. Our results suggest VUE in stillbirth has a similar immune cell profile to live birth. Pan-placental macrophages, CD4 and CD8 T cells indicate a wider inflammatory response unrestricted to VUE lesions. The cytokine profile observed suggests a skew towards inappropriate Th1 immune responses. Full characterisation VUE lesion phenotype confirms its immunological origins and provides foundations to develop novel investigations. *(Am J Pathol 2016, 186: 952–961; http://dx.doi.org/10.1016/j.ajpath.2015.12.010)*

Stillbirth, the death of a baby after 20 weeks' gestation, places a sustained economic and emotional burden on both maternity services and the wider community. Despite significant effort, the stillbirth rate has decreased at a slower rate than neonatal or infant death.[1] In high-income countries stillbirth rates are variable. The United States and United Kingdom perform relatively poorly with rates of 1 in 160 and 1 in 212, respectively, whereas in Finland 1 in 400 pregnancies result in a stillborn infant.[2–4]

Placental dysfunction as a cause of stillbirth has increasingly become a focus of research effort with recognition that placental disorders are the cause of death in up to 65% of stillbirths.[5] However, the placental lesions associated with stillbirth are extremely varied and poorly defined, and their relation to pathologic processes leading to stillbirth is difficult to assess.[6,7] Placental lesions associated with stillbirth include those with genetic, environmental, infective, inflammatory, mechanical, metabolic, and vascular origins.[6]

Understanding of inflammatory disorders of the placenta remains relatively limited in comparison with vascular lesions. Two main inflammatory conditions have been linked to placental dysfunction in the absence of infection: villitis of unknown etiology (VUE) and chronic histiocytic intervillositis.[8] VUE is an inflammatory condition of the placenta reported to occur in up to 15% of term placentas and more frequently in pregnancies resulting in poor outcome.[9,10] The origin of VUE is unclear, although it is proposed to be maternal immune rejection of a semiallogeneic placenta.[11–13] VUE is identified and characterized by the presence of elevated numbers of fetal macrophages (Hofbauer cells) and an infiltrate

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Table 1 Antibodies and Positive Control Tissues Used for Identifying Immune Cell Populations and Cytokines in Placental Villous Tissue

Antigen	Antigen retrieval	Supplier	Working concentration (μg/mL)	Ab type	Control tissue
CD45	Sodium citrate	Dako	0.5	M mAb	Tonsil
CD163	Sodium citrate	AbD Serotec	10	M mAb	Tonsil
CD4 (clone 4B12)	Tris-EDTA or sodium citrate	Dako	4.6	M mAb	Tonsil
CD8	Tris-EDTA	Dako	1.6	M mAb	Tonsil
NE	None	Dako	1.1	M mAb	PTLI
IL-2	Sodium citrate	Novus Biologicals	4.5	R pAb	Tonsil
IL-4	Sodium citrate	Thermo Scientific	2.5	R pAb	Tonsil
IL-6	Sodium citrate	Novus Biologicals	9.6	M mAb	Tonsil
IL-10	None	Abcam	5	M mAb	Liver (Kupffer cells)
IL-12	Sodium citrate	Sigma-Aldrich	2.5	R pAb	Trophoblast
TGF-β	Sodium citrate	Novus Biologicals	10	M mAb	Tonsil

M mAb, mouse monoclonal antibody; NE, neutrophil elastase; PTLI, preterm labor with infection; R pAb, rabbit polyclonal antibody; TGF, transforming growth factor.

of maternal T lymphocytes in the villous stroma. Areas of the placenta not affected by VUE lesions display apparently normal structural characteristics.[9] The cell profile of VUE has previously been characterized in both appropriately grown and growth-restricted live born infants.[12,14–16] Conflicting reports state either $CD4^+$ or $CD8^+$ T cells are the most common lymphocyte type in VUE lesions.[12,14,17,18] The subtype of $CD4^+$ cells (Th1/Th2) has not been examined; therefore, the type of immune response in VUE remains to be elucidated.

A thorough characterization of VUE has yet to be completed in stillbirth; it is not understood why it may cause fetal demise. In addition, the cytokine profile in VUE has not been described in either live births or stillbirths. Characterization of the cell types present and their cytokine profile will provide a greater understanding of the pathophysiology of VUE. We hypothesized that the immune cells in VUE lesions in stillbirth would be comparable with those described in live birth and that evidence of widespread inflammatory changes would be detectable in the placentas of stillborn infants with VUE. In this immunomorphologic study we aimed to accurately quantify the type and number of immune cells present in VUE lesions, assess leukocyte infiltration across the placenta, and analyze the expression of a range of proinflammatory and anti-inflammatory cytokines with the use of immunohistochemical approaches, coupled with an unbiased sensitive image analysis method. We compared leukocyte infiltration and cytokine expression across the placenta to ascertain whether this was altered in dysfunctional placentas from stillbirth with and without VUE and fetal growth restriction (FGR) compared with healthy controls.

Materials and Methods

Study Population

Placental tissues from pregnancies resulting in stillbirth in which a contributory cause of death was recorded as VUE ($n = 12$) or FGR ($n = 12$) were obtained from the Pediatric Histopathology departments of Manchester Royal Children's Hospital and Edinburgh Royal Infirmary. Samples from stillborn growth-restricted infants had no evidence of VUE lesions recorded on postmortem examination. Consent for tissue use in research was obtained at the time of consent for postmortem; their use in this project was approved by Humber Bridge Research Ethics Committee (Ref. 13/YH/0176). Live born infants ($n = 11$) with an individualized birth weight centile less than the fifth were considered FGR infants; placental tissue was obtained from the Maternal and Fetal Health Research Group Biobank, University of Manchester (North West REC 08/H1010/55). Matched placental samples from women with uncomplicated pregnancies ($n = 12$) were selected from the Maternal and Fetal Health Research Group

Table 2 Demographic Information for the Women Included in the Study

Characteristic	Stillbirth with VUE ($n = 12$)	Stillbirth with FGR ($n = 12$)	Live birth with FGR ($n = 11$)	Normal pregnancy ($n = 12$)	P
Maternal age (years)	32 (21–36)	23.5 (19–42)*	32 (16–35)	35 (25–42)	0.019
Gestational age (weeks)	39 (20–41)	35.21 (28–38)	37.8 (27.7–42.1)	33.5 (30.0–41.7)	0.098
Gravidity	3 (1–7) ($n = 7$)	1 (1–4)	1 (1–9)	2 (1–18)	0.298
Parity	1 (0–3) ($n = 7$)	0 (0–2)	0 (0–3)	1 (0–3)	0.118
Birthweight (g)	NA	1061 (385–2000)***	2246 (747–3060)**	3430 (2900–3840)	<0.001
IBC	23 (3–90) ($n = 7$)	NA	3 (0–10)***	45 (12–93)	<0.001

Results are presented as median (range).

*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared with normal pregnancy.

FGR, fetal growth restriction; IBC, individualized birth weight centile; NA, not available; VUE, Villitis of unknown etiology.