ORIGINAL ARTICLE

An Immunological Basis for Chronic Histiocytic Intervillositis in Recurrent Fetal Loss

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Problem
Chronic histiocytic intervillositis (CHIV) is a rare type of placental pathology that is associated with reproductive loss at all gestational ages. The aim of the study was to investigate the relationship between the severity of CHIV and the outcome of pregnancy and to compare the immune response between CHIV patients and controls to explore an immunological origin of CHIV.

Method of study
Microscopic slides were reviewed and scored according to a previously published grading system in 30 pregnancies of 22 CHIV patients. Partner-specific mixed lymphocyte reactions, cytotoxic T-lymphocyte precursor frequencies (CTLpf), and anti-HLA antibodies were determined in four patients and seven controls.

Results
Higher CHIV scores are associated with worse pregnancy outcome. CHIV patients demonstrated a higher CTLpf against their partner compared to non-complicated pregnancies ($P = 0.03$). The CTLpf was extremely high in 75% of the patients. Antipaternal HLA antibodies were only present in 75% of the CHIV patients compared to none of the controls ($P = 0.02$).

Conclusion
CHIV scores seem to be associated with the severity of adverse pregnancy outcome. High antipaternal cellular (T-cell) and humoral (B-cell) response to partner-specific CTLpf and the presence of anti-HLA antibodies directed to the partner suggest an immunologic origin of CHIV.

Introduction

Chronic histiocytic intervillositis (CHIV) is a rare type of placental pathology.[1] It is characterized by an intervillous infiltrate of maternal mononuclear cells. The exact incidence is unknown. One study reported an overall prevalence of 9.6 per 1000 miscarriages and in 0.6 per 1000 placentas in the second and third trimester.[2,3] Another study showed lesions in 4.4% of first trimester miscarriages.[4] CHIV is frequently associated with intervillous and perivillous fibrinoid deposition at the materno-fetal interface,

anchoring villi, and adjacent decidua.[5] This pathologic condition was initially described by Labarrere et al.[6] as massive chronic intervillositis. CHIV is a placental lesion of unknown pathogenesis associated with reproductive loss at all gestational ages.[1,2] When CHIV is found in the placenta, children were born with lower birth weight and lower crown-heel length as compared to children with only focal villitis in their placentas.[6] CHIV has a high rate of recurrence in the next pregnancy, varying between 67 and 100%.[2,3,6] A high rate of fetal loss is seen in all trimesters of pregnancy.[7] Although a non-determined placental infection cannot be ruled out completely, an immunological cause seems to be present.[2,8]

Parant et al.[1] described the correlation between infiltrating cells of mononuclear origin ($CD45^+$ and $CD68^+$) and the severity of CHIV. Semi-quantitative grading was used to classify the intensity of chronic inflammatory infiltrates and the amount of fibrinoid deposition in the intervillous space.

During pregnancy, women are tolerant to their semi-allogenic fetus. There is a change in the human immune response from a predominant Th1-type immunity to a Th2 cell type response, being protective for the fetus. Both recurrent miscarriages and CHIV are associated with a predominant Th1-like immune response.[4,9] Th1 cells mainly produce the proinflammatory cytokine IFN-γ.[10] The number of IFN-γ-producing cells is often used as marker for the number of cytotoxic T-lymphocytes (CTL). However, the actual endpoint of cytolytic activity remains the gold standard.[11] In a recent paper, we described an increased cytotoxic T-lymphocyte precursor frequency (CTLpf) directed to paternal antigens during pregnancy in patients with pre-eclampsia compared to patients without preeclampsia, while no difference was found in proliferative capacity to paternal antigens.[12] In materno–fetal alloimmunization, anti-HLA antibodies are also described. Umapathy et al.[13] reported a higher frequency of anti-HLA antibodies in women with recurrent miscarriage compared to those without. Others describe that local immunotolerance at the fetal–maternal interface during pregnancy is more likely.[14–16] Tilburgs et al.[17] suggest that local $CD8^+$ T-cell differentiation may play a crucial part in maintenance of maternal immune tolerance to the fetus during human pregnancy.

Because the exact pathogenesis of the CHIV is still unknown and an immunological origin of this rare entity is possible, we hypothesize that women with recurrent miscarriages and CHIV could have an abnormal immune response against paternal antigens. The aim of this study was twofold. First, the relationship between the severity of CHIV and outcome of pregnancy was studied. A secondary aim was to explore an immunologic origin of CHIV by comparing both the cellular (T-cell) and humoral (B-cell) immune response between patients with CHIV and other patients with recurrent fetal loss without CHIV (controls).

Material and methods

Study Population

We retrospectively collected all patients ($n = 22$), diagnosed with placental CHIV between 2000 and 2010, from the database at the Department of Pathology at the Erasmus Medical Centre in Rotterdam. Written informed consent was obtained from all patients. Microscopic slides were available from 30 of 105 pregnancies of these 22 patients. Patient characteristics and details about the outcome of all pregnancies of these patients were collected from clinical records. Birth weight percentiles were calculated using the Netherlands Perinatal Registry data.[18] Most patients with recurrent miscarriages had already been screened for abnormal parental karyotype, uterine anomaly, antiphospholipid syndrome, and thrombophilia.

CHIV Scoring

In 16 of the 22 patients one placenta was available, in four patients two, and in two patient three placentas. Microscopic slides of the 30 specimen were stained with hematoxylin and eosin (HE, Klinipath, Duiven, the Netherlands) and with anti-CD68 antibodies ($CD68^+$; Dako, Glostrup, Denmark) for the detection of the mononuclear origin of cells in the intervillous space. For a comparison of the scores based on anti-CD68 with the scores based on HE stained slides, we compared the total sum of both scores. The anti-CD68 stained slides were used for further analysis. The CHIV classification described by Parant et al.[1] was used. Additionally, we added grade 0 for the absence of CHIV because we expected that CHIV was probably not present in all specimen.

The lesions were classified as 0: absent, 1: focal (<10% of the slide), 2: moderate (10–50%), or

3: severe or massive (more than 50%). The slides were scored independently by two investigators (NMM and RK), who were blinded to all relevant clinical information and prior histological results.

Proliferative Response and Cytotoxic T-cell Response

In patients who still wish to conceive, partner-specific mixed lymphocyte reactions (MLR) and cytotoxic T-lymphocyte precursor frequency (CTLpf) were determined in peripheral blood mononuclear cells (PBMC) from women with CHIV ($n = 4$) and women who had experienced recurrent miscarriages without CHIV serving as controls ($n = 7$). The results were also compared with women with non-complicated pregnancies from our earlier report.[12]

In brief, MLR were set up in triplicate wells using 5×10^4 responder PBMC and 5×10^4 irradiated (40 Gy) partner PBMC. After 7 days, proliferation was measured by incorporation of ^{3}H-thymidine added during the last 8 hr of culture. The stimulation index (SI) was calculated by the ratio of the counts per minute obtained in the presence of antigen to the counts per minute obtained in the absence of antigen. For the CTLpf, limiting dilution cultures were performed. Briefly, 12 replicates of graded number of PBMC were titrated in 7-step double dilution starting from 5×10^4 to 781 PBMC/well in the presence of recombinant IL-2. After 7 days of culture, each well was tested for cytolytic activity of Europium-labeled target cells. After 4 hr of incubation, the supernatant was harvested, and the fluorescence of the released Europium was measured. The CTLpf was expressed as number of CTLp per million PBMC.[12,19]

Anti-HLA Antibodies

Anti-HLA antibodies in patient sera were initially detected by Lambda Antigen Tray (One Lambda, Canoga Park, CA, USA) for ELISA HLA class I and II. The sera were further tested for HLA antibody specificities by complement-dependent cytotoxicity against a panel of peripheral blood cells from 54 different donors in the absence and presence of dithiothreitol (DTT), a reducing agent that breaks down disulfide bonds of pentameric IgM, but has minimal effect when used at low concentration on IgG.[19] Specificities were verified, and, in some cases, additional specificities were found using flow cytometry or ELISA.[20] Based on the HLA antibody specificities, a value for virtual panel reactive antibodies (PRAs) was calculated (http://www.etrl.org/etrlpra/webform1.aspx).[21] This virtual PRA reflects the chance that a cross-match with a potential donor will be positive.

Only patients with positive anti-HLA antibodies were HLA typed to determine whether the antibodies were directed to the partner.

Analysis of the Data

The intra-individual difference in classification of the CHIV score between the two observers was assessed by calculating the weighted kappa value between the scores of the observers.[22] The final conclusion for the CHIV score was based upon agreement between the two investigators. Further data analysis was performed using SPSS software (version 17.0; Chicago, IL, USA). The Wilcoxon signed rank test was used to determine differences in CHIV scores between HE and CD68 staining. The Fischer's exact test was used to determine the difference in CHIV scores between groups with different outcome of pregnancy. The correlation between CHIV score and birth weight and birth weight percentiles were calculated with a Spearman's rank correlation coefficient (r_s). We also used the Fischer's exact test to determine differences in CTLpf and MLR response between CHIV patients and patients with and without pre-eclampsia and between CHIV patients and patients with recurrent miscarriage without CHIV.

Results

HE and CD68 staining (Fig. 1) was performed on placental slides from 22 patients who had 30 pregnancies, including one twin pregnancy. Patient characteristics are summarized in Table I. Only 10 of 30 pregnancies (33.3%) ended in the birth of a healthy child. Details about the outcome of the pregnancies are summarized in Table II. Among the miscarriages, there were four early miscarriages (<12 weeks of gestation) and two late miscarriages (12–16 weeks of gestation). In the clinical history of the 22 patients, we found one patient with diabetes, one with hypertension, one developed pre-eclampsia, four patients had an allergy, and four patients smoked. Eleven of the 22 patients were screened for antiphospholipid syndrome and thrombophilia. Only one patient turned out to be heterozygous for a factor V Leiden mutation.

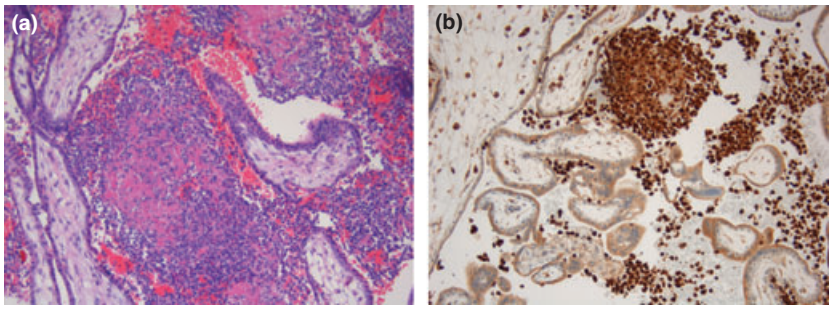

Fig. 1 Chronic histiocytic intervillositis (CHIV) with macrophages and fibrinoid depositions present in the intervillous space, observed in hematoxylin and eosin (HE) stained (a) and CD68 stained (b) microscopic slides of the placental villi of a representative case.

Table I Patient Characteristics of 22 Patients With CHIV at the Moment of The Last Pregnancy. The Characteristics Are Presented in Numbers (*n*), Mean, Standard Deviation (S.D.), and Range

Parameter	Mean (S.D.)	Range
Gravidity $n = 22$	4.5 (3.1)	1–13
Parity $n = 22$	2.3 (1.9)	0–8
Maternal age (years) $n = 22$	31.8 (4.9)	22–45
Gestational age (weeks) $n = 22$	25 + 6 (10 + 2)	8 + 0–40 + 3
Birth weight (gram) $n = 16$	1295.3 (1171.1)	55–3940
Placental weight (gram) $n = 19$	164.5 (128.6)	12–383

CHIV, Chronic histiocytic intervillositis.

The CHIV score of observer NMM and RK differed between 9 of 30 HE stained slides and 9 of 30 CD68 stained slides. The calculated weighted Kappa of these scores between both observers was 0.544. This is a reasonable measure of agreement according to the reproducibility score of Landis and Koch.[22] After combined revision of the slides, both observers reached agreement resulting in a final conclusion of CHIV score. The sum of the scores in CD68 (total: 62) is significantly higher compared to HE staining (total: 54) ($P = 0.02$). The CD68 staining was used for the classification of CHIV.[1]

CHIV was absent in 10% (3/30) of the slides, focal in 13.3% (4/30), moderate in 36.7% (11/30), and severe/massive in 40.0% (12/30) of the slides. A higher CHIV score was associated with a shorter duration of pregnancy ($r_s = -0.37$, $P = 0.04$). CHIV score 3 was significantly more often seen in miscarriages (MISC; 4/6; 66.7%; $P = 0.008$), intrauterine fetal death (IUFD; 4/6; 66.7%; $P = 0.008$), and neonatal death (NND; 3/7; 42.9%; $P = 0.05$), while none of the pregnancies resulting in a living child had a CHIV score 3 (Fig. 2). Birth weight was available in 21 of 30 cases, and percentiles could be calculated in 15 cases with CHIV being present. Lower birth weight ($r_s = -0.53$, $P = 0.01$; Table III) and a tendency to lower birth weight percentiles ($r_s = -0.49$, $P = 0.07$) were seen in patients with higher CHIV scores.

Neither statistical significant correlation was observed between CHIV score and number of previous pregnancies ($r_s = -0.01$, $P = 0.94$) nor between CHIV score and the number of previous miscarriages ($r_s = 0.35$, $P = 0.06$).

Abnormal Immune Response in CHIV Patients

At the moment of the study, only four patients were still visiting our outpatient clinic because of a strong wish to conceive.

Patient A

Patient A is a 37-year-old woman with a history of three pregnancies without having living children. Her first pregnancy was terminated at 23 weeks because of extreme growth retardation, and CHIV was found in the placenta. Her second pregnancy, a dichorionic twin pregnancy, resulted in fetal death at 10 and 13 weeks, respectively. Both fetuses had already growth retardation at the time of fetal death, and again, CHIV was found in both placentas. The third pregnancy of this patient ended in spontaneous miscarriage after fetal death at 10 weeks, and placental tissue was not available for pathologic examination. Recently, this patient became mother of a healthy twin. Pregnancy was achieved by *in vitro* fertilization with the gametes of the patient and her husband and was successfully carried until term by a surrogate mother.

Patient B

Patient B is a 32-year-old woman with 15 pregnancies in the obstetric history and no living children. Her first pregnancy was a premature delivery at

Table II Morphology of the Outcomes of the 30 Studied Pregnancies. IUFD is Intrauterine Fetal Death, NND is Neonatal Death, TOP is Termination of Pregnancy, and Alive are Children Who Survived

	Outcome					
	Term	Immature	Premature	Miscarriage	TOP	Total
IUFD	0	4	2	0	0	6
NND	0	5	2	0	0	7
Miscarriage	0	0	0	6	0	6
TOP	0	0	0	0	1	1
Alive	3	1	6	0	0	10
Total	3	10	10	6	1	30

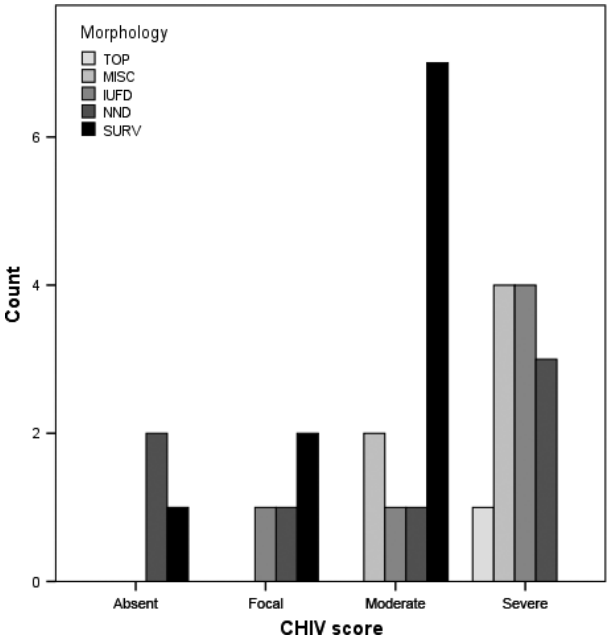

Fig. 2 Pregnancy outcome ($n = 30$) in relation to CHIV score. TOP is the termination of pregnancy, MISC are miscarriages, IUFD is intrauterine fetal death, NND is neonatal fetal death, and SURV children who survived. White bars, CHIV score: absent; gray bars, CHIV score: focal; dark gray bars, CHIV score: moderate; black bars, CHIV score: severe.

30 weeks of gestation. The baby died because of hydrocephaly and associated congenital anomalies. After this pregnancy, she experienced 11 early miscarriages (<12 weeks) and three late miscarriages (12–16 weeks). CHIV was found in the placenta of an intrauterine fetal death at 15 weeks and also in the placenta of her last miscarriage at 16 weeks of gestation. Placentas of the other pregnancies were not available for pathological examination.

Table III Relations Between CHIV Score (0–3) and Birth Weight (gram) in 30 Pregnancies. 0: Absent CHIV, 1: Focal (<10% of the Slide), 2: moderate (10–50%), or 3: Severe or Massive (more than 50%)

		Birth weight (gram)		
Score	N	Mean	S.D.	Range
0	2	1595	2093	115–3075
1	4	1590	720	875–2310
2	9	1371	1171	80–3940
3	6	291	209	55–580
Total	21	1125	1076	55–3940

CHIV, Chronic histiocytic intervillositis.

Patient C

Patient C is a 35-year-old woman with 10 pregnancies in the obstetric history and three living children. She had two living children, and she experienced one early miscarriage with her first husband. With her new husband, she experienced two early miscarriages: one ectopic pregnancy and one late miscarriage. Their fifth pregnancy ended in the birth of a healthy daughter. After this healthy child, she experienced two late miscarriages again. CHIV was found in the placenta of one of the two late miscarriages. Placentas of the other pregnancies were not available for pathological examination.

Patient D

Patient D is a 33-year-old woman with a history of four pregnancies. Her first pregnancy ended in a term delivery of a healthy daughter. All three pregnancies thereafter were characterized by premature rupture of the membranes at 21, 18, and 14 weeks,

respectively. The second child was born at 31 weeks with lung hypoplasia but survived. The other two pregnancies were terminated at 19 and 15 weeks. CHIV with CD68 and CD3 positive cells was present in all three placentas.

Peripheral blood from these four women was obtained. In PBMC, we determined the proliferative and cytolytic response to paternal antigens. Anti-HLA antibodies were determined in plasma. Seven women with recurrent miscarriages without signs of CHIV served as controls.

CHIV patients had comparable proliferative responses to their partners compared to controls [median and range: 78 (6–173) versus 9 (2–123) $P = 0.16$]. The partner-specific MLR response from CHIV patients was comparable with women without pre-eclampsia from our earlier report.[12]

The CTLpf directed to paternal antigens was found to be extremely high (>400/10^6 PBMC) in three of four patients with CHIV (Fig. 3). Only one of the seven control women with multiple miscarriages without signs of CHIV had high partner-specific CTLpf ($P = 0.09$, Fischer's Exact Test). We found significantly higher partner-directed CTLpf in CHIV women compared with women with non-complicated pregnancies [695 (66–895) versus. 67 (9–235); $P = 0.03$] (Fig. 3).[12]

In three of four CHIV patients, anti-HLA antibodies were found compared to none of seven controls ($P = 0.02$). All three CHIV patients had partner-directed anti-HLA class I IgG antibodies, and one of these three patients also had circulating anti-HLA class II IgG antibodies directed to her partner.

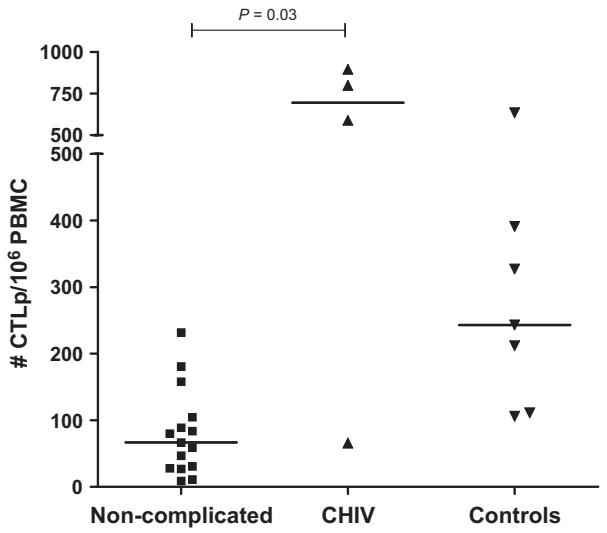

Fig. 3 Cytotoxic T-lymphocyte precursor frequency (CTLpf) against the partner was measured in peripheral blood mononuclear cells (PBMC) from women with non-complicated pregnancies,[12] from women with signs of CHIV during pregnancy (CHIV), and from women who had experienced recurrent miscarriages without CHIV (controls).

Discussion

In our study, CHIV was found to be associated with a high fetal loss rate. These findings are comparable with other studies.[1,3,7] Although the data in our retrospective study are not complete, our results are indicative for an immunologic origin of CHIV. CHIV was not associated with other diseases like diabetes, hypertension, and pre-eclampsia. Several studies have shown that HLA antibodies develop during progressing pregnancy[23] and can therefore be found more often in both normal women and women with recurrent miscarriage who have previously given birth.[24] Nielsen et al.[25] showed before that the presence of HLA antibodies in recurrent miscarriage patients is associated with a reduced chance of a live birth.

We demonstrated that the CHIV classification for microscopic scoring is a reliable technique. We observed a reasonable measure of agreement between investigators. The specimens were scored by an experienced and an inexperienced investigator. Therefore, the kappa value might have been even higher when the scoring would have been done by experienced investigators only. Higher score in CD68 stained microscopic slides as compared to HE staining was found. Therefore, we conclude that in case of unexplained (recurrent) fetal loss, CHIV should be part of the differential diagnosis, and microscopic examination of the materno–fetal interface after immunohistochemical staining for CD68 positive cells is of additional value for the diagnosis and scoring of CHIV.

We used staining with anti-CD68 antibodies, because CD68 is specific for macrophages and has proven to be useful in the diagnosis of CHIV, as a significant difference was found between the counts of CD68 positive cells in cases and controls. Although this does not rule out an unrecognized infectious process completely, an infectious origin is most unlikely. Infections are only seen incidentally in a single miscarriage, and there is no evidence that infections are involved in the origin of recurrent miscarriages.[26,27] To our opinion, the scoring system of Parant et al.[1] was best defined and the easiest system to use. In our study, a high CHIV score was also related to more severe adverse pregnancy outcome. High CHIV scores were associated with earlier fetal

loss, and live births were only observed in patients with low CHIV scores. Boyd and Redline[2] define CHIV as monomorphic infiltration of the placental intervillous space by cells identifiable as belonging to the mononuclear phagocyte lineage (histiocytes) by morphologic criteria. They graded CHIV on a 1–3 qualitative scale for the number of histiocytes and the amount of fibrinoid material in the intervillous space, but they did not specify when a score 1, 2, or 3 was given. In another article, Redline defines two grades, focal or diffuse chronic villitis.[28]

CHIV also occurs in primigravidas, which tells us that the abnormal immune response may develop in early pregnancy, probably as a reaction to paternal antigens on cytotrophoblast cells in the maternal circulation or even before pregnancy as a result of maternal contact with paternal sperm cells.

Our limited data on CTLpf and MLR in four patients who still visit our outpatient clinic suggest an immunologic underlying cause of CHIV, based on a mismatch between donor (fetal–paternal antigens) and graft recipient (mother). Nevertheless, the CTLpf is a very robust and reproducible assay.[29] Some studies reported that patients benefit from prophylactic treatment with prednisone 20–40 mg/day in combination with aspirin (100–160 mg/day) starting from the beginning of pregnancy, while others conclude that there is no statistical significant difference in live birth rate between treated and non-treated patients.[1,3,8,30] Further studies are needed to assess the efficiency of such treatment and to confirm the immunologic origin of this entity.

One of the limits of this study is the retrospective design of our study with possible biases caused by, for example, referral policy to our tertiary center of only cases with recurrent pregnancy loss. The disease is very rare, and therefore, the number of patients is small, especially of patients who still visit our outpatient clinic.

In conclusion, CHIV is associated with adverse pregnancy outcome, and CHIV scores seem to be associated with the severity of adverse pregnancy outcome. High antipaternal cellular (T-cell) and humoral (B-cell) response to partner-specific CTLpf and the presence of anti-HLA antibodies directed to the partner suggest an immunologic origin of CHIV.

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