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Lymphocyte characterization of decidua basalis spiral arteries with acute atherosis in preeclamptic and normotensive pregnancies

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ABSTRACT

Uteroplacental acute atherosis (AA) is a common spiral arterial lesion in preeclampsia, characterized by intramural foam cells, fibrinoid necrosis, and a perivascular immune cell infiltrate. A clear definition of this infiltrate is lacking. Therefore, our aim was to characterize lymphocytes in pre-defined zones regarding spiral arteries with or without AA, from preeclamptic and normotensive pregnancies.

Lymphocytes were characterized in decidua basalis samples (n = 91), previously evaluated for AA, around spiral arteries in three pre-defined zones; 1) intramural, 2) perivascular and 3) interstitial. Adjacent serial sections were immunostained to identify different T-cell populations (CD3+, CD8+, FOXP3+), and NK-cells (CD56+). CD3+CD8- T-cells were also identified. These were presumed to be largely CD4+ T-cells.

AA was associated with significantly higher intramural CD3+ cell concentrations in Zone 1, in both normotensives and preeclamptics. In preeclamptics only, this difference extended into Zone 2. Similar results were observed for CD3+CD8- cells. AA was also associated with increased intramural CD8+ concentration; however, the number of cells was low. Regulatory T-cells (FOXP3+) were generally scarce or absent in all pre-defined zones. Although intramural NK-cells (CD56+) were scarce, the intramural concentration was significantly lower in spiral arteries with AA compared to without AA in preeclamptics.

Our main finding was that CD3+CD8-FoxP3- T-cells were associated with AA. We therefore suggest that T-cells, of a non-regulatory CD4+ subtype, could be involved in the formation of spiral artery AA in the decidua basalis. Whether AA gives rise to, or is partly mediated by increased T-cell concentration around the lesions, remains to be determined.

1. Introduction

Acute atherosis (AA) of the uteroplacental spiral arteries is a pregnancy-specific lesion predominantly found in preeclampsia, also occurring in a lower proportion of uncomplicated pregnancies (Alnaes-Katjavivi et al., 2016). The mechanisms behind AA development are not established, but we have suggested that it is an inflammatory lesion caused by excessive decidual inflammation (Staff et al., 2013).

Spiral artery AA has been characterized by presence of fibrinoid necrosis in the artery media, sub-endothelial lipid-laden “foam cells”, and the presence of a perivascular infiltrate (PVI) (Robertson et al., 1976). PVI is included in many, but not all descriptions of AA, but has not been clearly defined (Hertig, 1945; Zeek and Assali, 1950; Robertson et al., 1967; Meekins et al., 1994). Most of these descriptions

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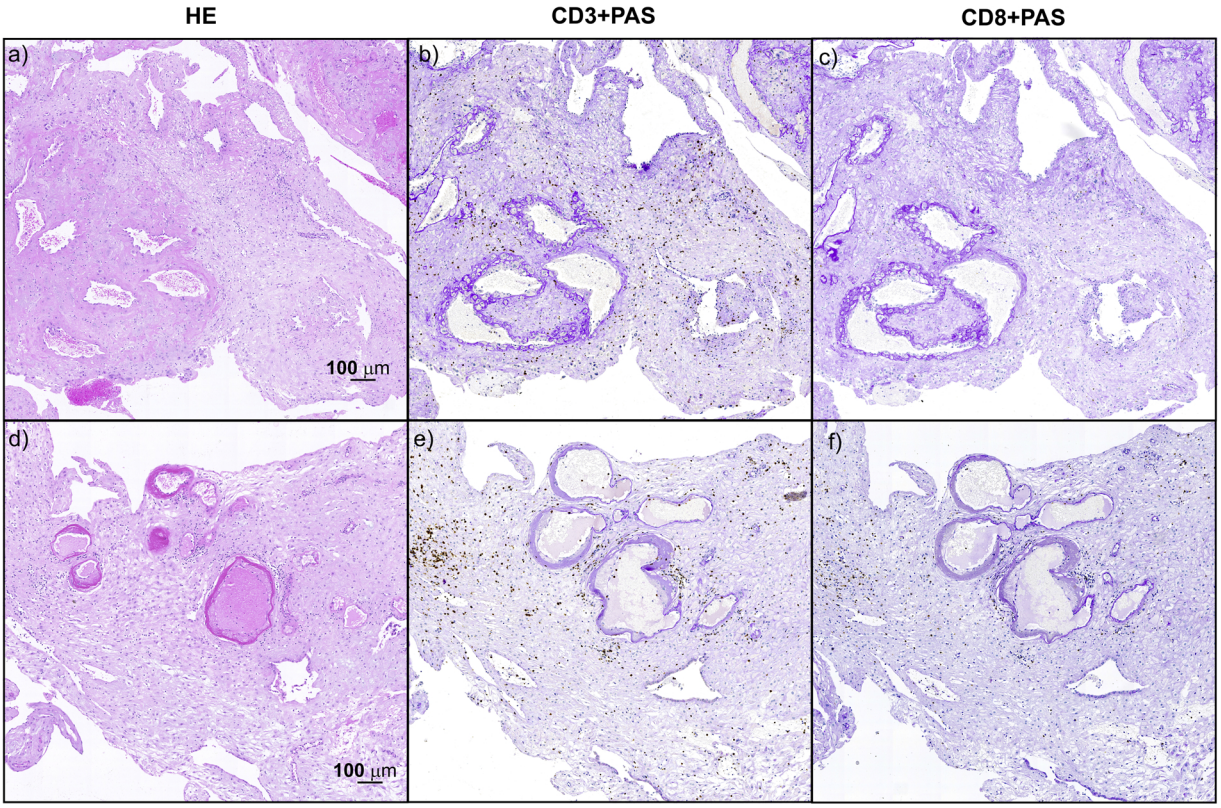

Fig. 1. Representative examples of immunohistochemistry lymphocyte staining.
Representative examples of lymphocyte staining of decidua basalis tissue sections. First row demonstrate a) H&E, b) CD3, and c) CD8 staining of tissue sections from a late-onset preeclampsia patient (gestational week > 34) without AA (nonAA artery/nonAA sample). Second row demonstrate d) H&E, e) CD3, and f) CD8 staining of tissue sections from an early-onset preeclampsia patient (gestational week < 34).

are based on simple hematoxylin and eosin staining. No explicit definition or consensus exists regarding the phenotype of the constituent PVI leukocytes. Therefore, we sought to describe the presence of lymphocytes around decidual spiral arteries in a more systematic manner by using pre-defined zones for their quantification in, and around the arteries, and specifically in relation to AA. Additionally, we sought to identify specific lymphocyte subsets.

Decidual lymphocytes are of maternal origin and are phenotypically different compared to circulating immune cells. In most previous studies, NK-cells have been emphasized as the predominant immune cells in the early stages of pregnancy, where they have essential roles in regulation of extravillous trophoblast (EVT) invasion and spiral artery remodeling (Pijnenborg et al., 2006; Cartwright et al., 2017). Towards term, the number of decidual NK-cells is significantly reduced and their role is undefined. Reduced frequency of decidual NK-cells has been reported in preeclampsia and fetal growth restriction (Williams et al., 2009a), but has not been reported in AA.

We are studying AA in third trimester decidual samples, where most decidual leukocytes are T-cells (Tilburgs et al., 2009; Williams et al., 2009b). The role of these decidual T-cells is generally unknown (Nancy and Erlebacher, 2014). They can respond to paternal/placental antigens (Sharkey et al., 2008) but could also be important to control infection (Lissauer et al., 2017). However, mice studies indicate that regulatory T-cells (Tregs) are important in implantation and feto-maternal immunotolerance (Erlebacher, 2013). Also, Tregs appear to be reduced in the circulation and at the feto-maternal interface in women with preeclampsia (Nancy and Erlebacher, 2014). The role of T-cells and Tregs in relation to AA is not known.

Here, our aim was to characterize the immune cell infiltrate present around spiral arteries with AA compared to spiral arteries without AA in preeclamptic and normotensive pregnancies, and to describe both its localization and its composition of lymphocyte subtypes.

2. Methods

2.1. Patient inclusion

Pregnant women were recruited, after written consent, as described in Supplemental Methods. Clinical characteristics of the pregnancies are presented in Supplemental Table 1. From our cohort of 237 pregnancies previously evaluated for AA in the decidua basalis collected by vacuum suctioning of the placental bed (Alnaes-Katjavivi et al., 2016), we included 91 pregnancies without diabetes and with the highest number of spiral artery sections per decidual slide. Of these, 54 had preeclampsia (27 with AA) and 37 were normotensive (10 with AA). Three pregnancies were excluded, owing to lack of spiral arteries in the tissue sections for the present study.

2.2. Acute atherosis evaluation

AA in decidua basalis was evaluated as described in Supplemental Methods and Supplemental Fig. 1. AA was defined as presence of minimum two intramural vacuolated CD68+ cells (foam cell lesion) in the wall of at least one spiral artery (Alnaes-Katjavivi et al., 2016).

2.3. Serial immunostaining of lymphocytes in decidua basalis

For each woman four 3 µm adjacent tissue sections from one decidua basalis sample were analyzed with immunohistochemistry (IHC). The sections were in continuation with those slides previously used to evaluate AA (Alnaes-Katjavivi et al., 2016). The slides were stained as follows: 1) regulatory T-cells (FOXP3: Abcam 236 A/E7 Monoclonal

mouse nuclear stain), 2) CD3+ T-cells (CD3: Roche 2GV6 Monoclonal rabbit anti-human CD3 cytoplasmic stain), 3) CD8+ T-cells (CD8: Dako Clone C8/144B Monoclonal mouse anti-human CD8 cytoplasmic domain stain), and 4) NK-cells (CD56: Cell Marque MRO-42 Monoclonal rabbit anti-human) (Figs. 1& 4). The sections were immunostained using a Ventana Benchmark XT autostainer with DAB staining, followed by Periodic Acid Schiff counterstain for identification of spiral arteries. Positive immunostaining was confirmed using human tissue as controls: tonsillar lymphatic tissue (CD3, CD8 and FoxP3), and ileum small bowel (CD56).

2.4. Estimation of CD4+ T-cells

The decidua basalis tissue contained many CD4+ macrophages, consequently we estimated the CD4+ T-cell concentration based on the results from the CD3 and CD8 staining, similar to a previous publication (Stallmach et al., 1999). The CD3 pan T-cell marker stains CD3+ T-cells that are mainly either CD8+ or CD8-.The vast majority of T-cells are committed to either a CD4+ or CD8+ linage, however minor subsets (1–5%) of double negative (CD3+CD4-CD8-) cells also exist (Juvet and Zhang, 2012). As far as we are aware double negative T-cells have not been documented in the human decidua. Since the CD3 stained slide was adjacent in the sequence of slides to the slide stained with CD8 (3 µm), it allowed for estimation of a CD3+CD8- T-cells (presumptive CD4+ cell population) by subtracting the concentration of CD8+ T-cells from CD3+ T-cells, in the same tissue zone investigated.

2.5. Quantification of lymphocytes in decidua basalis

Cells were counted manually using a Motic BA-410 LED light microscope, without knowledge of the pregnancy outcome. Pictures of each spiral artery section on each slide were recorded digitally using a Moticam 2500 digital camera and Motic Images vs 2.0. Lymphocyte concentrations were determined for each spiral artery, by counting positively stained cells in pre-defined counting zones. To ensure reproducibility, two researchers independently evaluated ten percent of the tissue sections.

2.6. Pre-defined lymphocyte counting zones

We defined three specific and reproducible counting areas, named Zone 1–3 (Fig. 2). We measured the area of each zone for each artery, and calculated the lymphocyte concentrations in each zone as the number of immunopositive cells within the zone investigated, divided by the area of the zone (cells/mm^2). This enabled comparison of cell density between arteries of different sizes, from different decidua basalis samples, or different arteries within the same samples. It also enabled comparison of cell density in the three different zones. ***Zone 1,*** "*intramural*", represents the entire area of the spiral artery wall, from the endothelium to the outer limit of the smooth muscle/PAS + margin of the artery (Fig. 2). ***Zone 2***, *"perivascular"*, represents the immediate conjoining area surrounding the spiral artery wall, demarcating a "cuff" of 100 µm tissue width, from the outer limit of the artery wall (Fig. 2). In Zone 2 we examined potential co-localization of perivascular lymphocytes and AA by dividing Zone 2 into quadrants originating from the center of the artery lumen (Fig. 2a). ***Zone 3,*** "*interstitial infiltrate*", represents the area containing the largest visible concentration of T-cells (in the CD3 stained slide, Fig. 1) present in the surrounding decidual tissue continuous with the spiral artery evaluated. The three largest clusters of CD3+ cells were identified at x4 magnification, and demarcated by a counting disc (100 µm in diameter) (Fig. 2), the cells were counted at x40 magnification.

2.7. Definition of spiral artery AA categories

AA is typically "patchy" (Pijnenborg et al., 2006). When AA was identified in a sample "*AA sample*" it often contained spiral arteries with "*AA artery/AA sample*", and without AA "*nonAA artery/AA sample"*. For Zone 1 and 2, we applied this categorization to determine whether lymphocyte patterns associate with AA presence in the sample, or rather associate more specifically to the lesion of the individual artery. Samples where none of the spiral arteries had AA "*nonAA sample"* were defined as "*nonAA artery/nonAA sample*". For patient samples with more than one spiral artery in the same category, the mean lymphocyte concentration across these arteries was calculated and used in the statistical analyses.

2.8. Statistical analyses

Data were analyzed using SPSS version 22. The Fisher Exact test was used for dichotomous variables and the non-parametric Mann-Whitney test otherwise. A p-value of ≤ 0.05 was considered statistically significant.

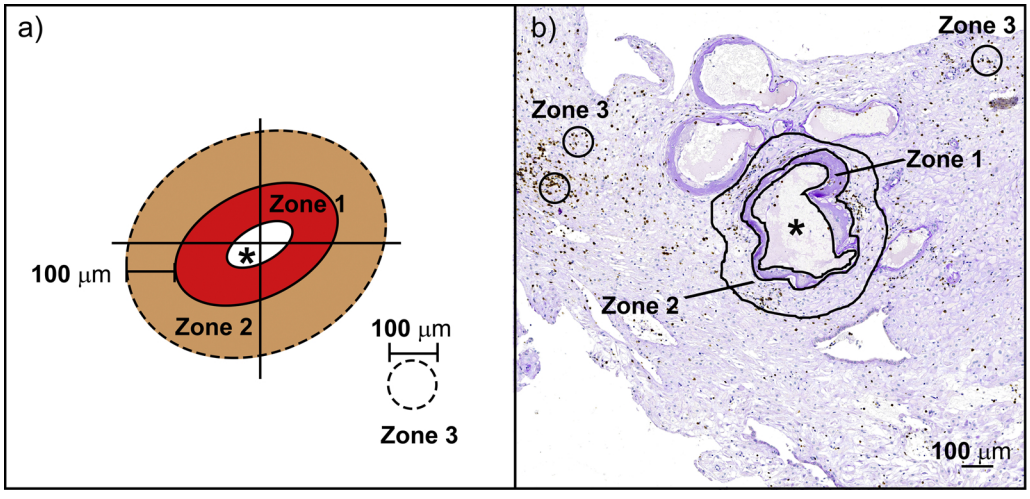

Fig. 2. Schematic illustration and examples of the pre-defined Zones for quantification of lymphocytes in and around decidua basalis spiral arteries. a) Schematic representation of a decidual spiral artery and the three different Zones used for quantification of decidual lymphocytes. Zone 1 "intramural", spiral artery wall (red with solid black perimeter). Zone 2, "perivascular", area surrounding the intramural zone. The outer perimeter is set at a depth of 100 µm from the outer margin of the artery wall (orange with interrupted black perimeter). Zone 3 "interstitial infiltrate", circle-shaped area of 100 µm in diameter in surrounding decidual tissue not directly in contact with the spiral artery but with a continuous connection to the spiral artery (circle with interrupted black perimeter). The star (*) denotes the artery lumen. b) Examples of the applied zones for a decidual spiral artery section with AA stained for CD3.

3. Results

3.1. Lymphocyte distribution across all zones

The decidua basalis samples contained a median of three spiral arteries (range 1–10). In general, scattered staining of CD3+ and CD8+ lymphocytes was observed throughout the decidual tissue. Lymphocytes were observed in all three pre-defined zones, across samples with and without AA. The highest concentration of immunopositive cells (cells/mm^2) was observed in the surrounding interstitial infiltrates (Zone 3), followed by the perivascular area (Zone 2). In Zone 1, CD3+, CD8+, and CD56+ cells were scarce or absent (Supplemental Table 2). FoxP3+ staining was scarce or absent in all zones investigated (Supplemental Table 2), both in preeclampsia and normotensive pregnancies.

3.2. Intramural T-cell distribution (Zone 1)

Although the number of cells in Zone 1 (Figs. 1 & 2) was low, there were significantly higher concentration of CD3+ cells in the artery wall in spiral arteries with AA (*AA artery/AA sample*) compared to those in samples without AA (nonAA *artery/nonAA sample)*(Fig. 3a). This was the case both in preeclamptics (Fig. 3a) and in normotensives (Supplemental Fig. 2a). In the preeclamptics, the concentration of CD3+ cells was also significantly higher in arteries with AA (*AA artery/AA sample)* than in arteries without AA (*nonAA artery/AA sample)* in samples with AA (Fig. 3a). The results were similar for CD8+ cells. The intramural CD8+ concentration was significantly higher in spiral arteries with AA (*AA artery/AA sample)* than without AA (nonAA *artery/nonAA sample),* but only in preeclamptics (Fig. 3b). There were no differences in CD8+ concentration in the normotensives (Supplemental Fig. 2b). The CD3+CD8- cells (inferred CD4+ T-cells) followed the same pattern as CD3+ cells. The CD3+CD8- concentration was significantly higher in spiral arteries with AA (*AA artery/AA sample*) compared to those in samples without AA (nonAA *artery/nonAA sample)* in both in preeclamptics (Fig. 3c) and in normotensives (Supplemental Fig. 2c). In preeclamptics, the concentration of CD3+ cells was also significantly higher in arteries with AA (*AA artery/AA sample)* than in arteries without AA (*nonAA artery/AA sample)* in samples with AA (Fig. 3c).

3.3. Perivascular T-cell distribution (Zone 2)

In Zone 2 (Figs. 1 & 2) the CD3+ and CD3+CD8- (inferred CD4+) concentration was significantly higher in preeclampsia in spiral arteries with AA *(AA artery/AA sample)* compared to spiral arteries in samples without AA (nonAA *artery/nonAA sample*) (Fig. 3d, f). There was no difference in CD8+ cells in Zone 2 in preeclampsia (Fig. 3e). In normotensives, there were no differences in CD3+, CD8+ or CD3+CD8- (inferred CD4+) cells across the spiral artery categories (Supplemental Fig. 2d-f). The T-cells in Zone 2 were distributed unevenly in clusters, between the different artery quadrants. However, we found no significant co-localization of foam cells and artery quadrants demonstrating the highest T-cell concentrations (data not shown).

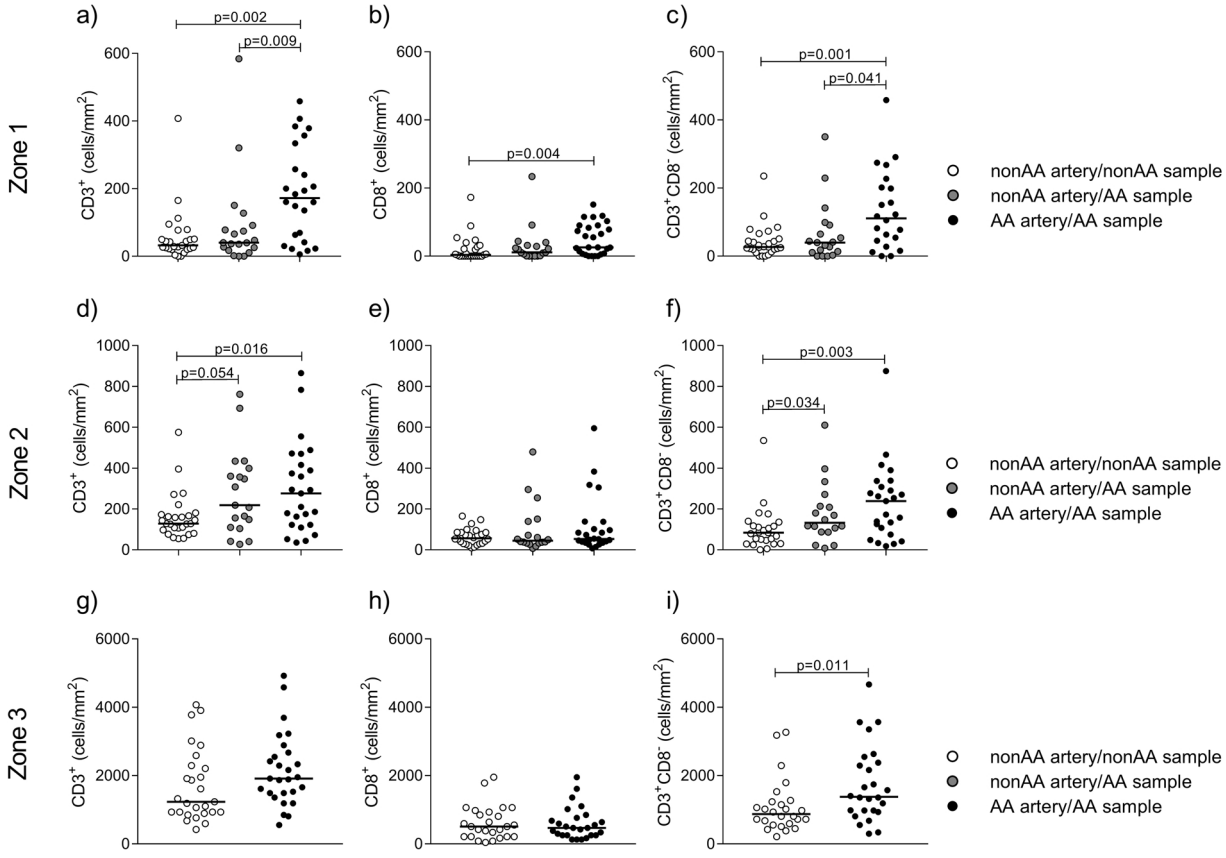

Fig. 3. T-cell concentration in the pre-defined Zones 1–3 in preeclamptic pregnancies. Dot plots of median T-cell concentrations per patient in the pre-defined counting zones. Each plot shows the T-cell concentration in three categories of spiral arteries: 1) arteries without AA in samples without AA (nonAA artery/nonAA sample, white dots), 2) arteries without AA in a sample with other arteries affected by AA (nonAA artery/AA sample, grey dots), and finally 3) arteries with AA lesions (AA artery/AA sample, black dots). The different plots show: a) CD3+ cells, b) CD8+ cells, and c) CD3+CD8- cells (inferred CD4+ cells) in Zone 1 (intramural area); d) CD3+ cells, e) CD8+ cells, and f) CD3+CD8- cells (inferred CD4+ cells) in Zone 2 (perivascular area); and g) CD3+ cells, h) CD8+ cells, and i) CD3+CD8- cells (inferred CD4+ cells) in Zone 3 (interstitial infiltrate area). The p-values were calculated using Mann Whitney U-test, a p-value < .05 was considered statistically significant.

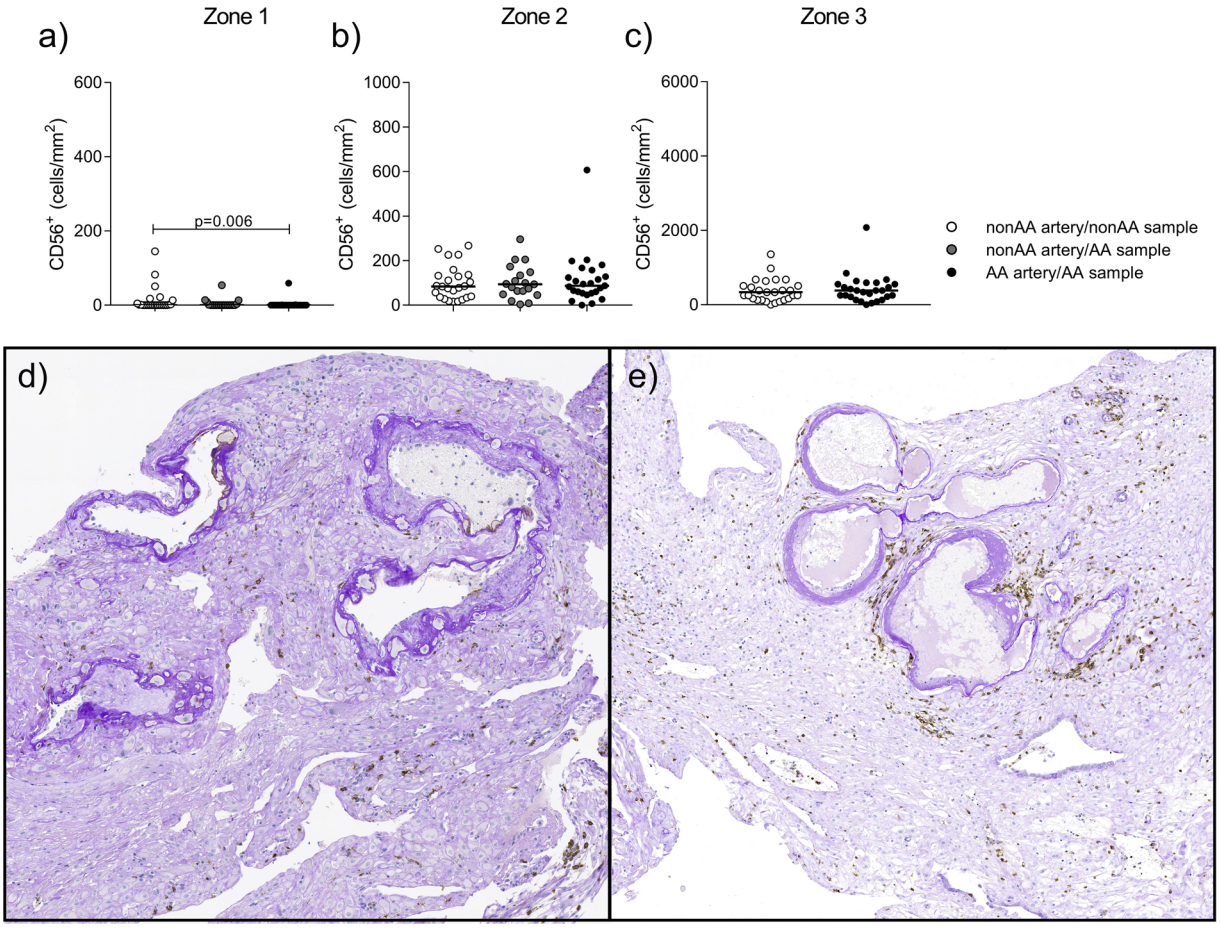

Fig. 4. NK-cell concentration in the pre-defined counting Zones 1–3 in preeclamptic pregnancies. Dot plots of median NK-cell concentrations per patient in the pre-defined counting zones. Each plot shows the NK-cell in three categories of spiral arteries: 1) arteries without AA in samples without AA (nonAA artery/nonAA sample, white dots), 2) arteries without AA in a sample with other arteries affected by AA (nonAA artery/AA sample, grey dots), and finally 3) arteries with AA lesions (AA artery/AA sample, black dots). The different plots show: a) CD56+ cells in Zone 1(intramural area), b) CD56+ cells in Zone 2 (perivascular area), and c) CD56+ cells in Zone 3 (interstitial infiltrate area). The p-values were calculated using Mann Whitney U-test, a p-value < .05 was considered statistically significant. d) Representative serial staining of CD56 in decidual tissue sections from a late-onset preeclampsia patient (nonAA artery/nonAA sample). e) Representative staining of CD56 in decidual tissue sections from an early-onset preeclampsia patient (AA artery/AA sample).

3.4. Interstitial T-cell distribution (Zone 3)

For Zone 3 (Figs. 1 & 2), the concentration of CD3+CD8- cells (inferred CD4+) in samples with AA was higher than in samples from women without AA in preeclampsia (Fig. 3i). A similar trend (but not significant) was observed for CD3+ cells in preeclampsia (Fig. 3g). None of the T-cell markers were significantly changed for AA in Zone 3 in normotensives (Supplemental Fig. 2g-i).

3.5. NK-cell distribution across all zones

CD56+ NK-cells Figs. 1 & 4 d-e) were less abundant than CD3+ cells in all zones (Supplemental Table 2), with the highest concentration of NK-cells in Zone 3. In Zone 1 there was a significantly lower concentration of cells in arteries with AA as compared to arteries without AA, however the NK-cells were generally scarce or absent in this zone (Fig. 4a). In Zone 2 and 3, the NK cell concentration was not significantly different between samples with AA compared to without AA in either preeclamptics (Fig. 4a-c) or normotensives (Supplemental Fig. 3a-c).

3.6. Preeclamptic vs. normotensive pregnancies

We found no significant differences for any of the lymphocyte markers assessed (CD3, CD8, FOXP3 and CD56) when comparing preeclamptic and normotensive pregnancies in Zone 2 and 3 (Supplemental Fig. 4).

4. Discussion

A perivascular infiltrate (PVI) has been considered a classical characteristic of uteroplacental AA lesions. However, its definition and characterization is lacking. Decidua basalis contains many different maternal immune cells and the composition of these cells change during the course of pregnancy. Lymphocytes are always present in the tissue surrounding the spiral arteries, and the presence of perivascular leukocytes is not a unique feature of AA (Robertson et al., 1967; Stevens et al., 2012; Alnaes-Katjavivi et al., 2016). Here, we characterized the lymphocyte concentration in pre-defined zones without considering whether the cells should be termed “PVI” or not, and found a general significant increase in T-cell concentration around spiral arteries with AA. We rarely observed dense clusters of perivascular lymphocytes around spiral arteries with or without AA. Therefore, the term “PVI” may not be useful as a mandatory prerequisite for identifying AA in decidua basalis spiral arteries. However, knowledge about the phenotype of the lymphocytes present could improve understanding of AA lesions, and their relation to pregnancy complications such as preeclampsia.

We found that in pregnancies affected by preeclampsia, AA in spiral arteries was associated with higher concentrations of CD3+ cells in both intramural and perivascular zones compared to spiral arteries without AA. However, the cell counts in the intramural area (Zone 1) were low and therefore we propose that T-cells in the perivascular area (Zone 2) may be more important in the context of AA development. More specifically, this increase in CD3+ cells in the perivascular zone was due to an increase in the CD3+CD8- subset, which we use as a surrogate measure of CD4+ cells. These cells were also FOXP3-. In addition, CD4+ T-cells were increased in interstitial infiltrates (Zone 3) in preeclampsia samples with AA. This increase in perivascular and interstitial T-cells could indicate a localized decidual response to the AA affected artery. We found that decidual NK-cells were few in all three zones investigated, with a lower intramural concentration in AA in our 3rd trimester samples. However, we cannot exclude a role for decidual NK-cells at an earlier point in the development of AA.

Stevens et al (2012) observed that a perivascular infiltrate was present more often in arteries with AA, however not distinguishing between decidua basalis and parietalis (Stevens et al., 2012). In a large study of over 14,000 pregnancies, Kim et al (2015) used the presence of PVI in their AA definition, without defining PVI (Kim et al., 2015). This could explain their low frequency of AA as compared to our cohort (Alnaes-Katjavivi et al., 2016). In these studies, only H&E staining was used, and hence they cannot distinguish the types of immune cells present. Hecht et al (2016) studied decidua parietalis tissue from six patients with preeclampsia, identifying with CD45 (pan-lymphocyte marker) small lymphocyte aggregation around arteries with AA, however without comparing to arteries without AA (Hecht et al., 2016). Interestingly, these lymphocytes also stained for nitrotyrosine, a marker of oxidative injury (Baltajian et al., 2014).

Our results could indicate that some AA lesions are associated with an adaptive immune response that involves CD4 helper T-cells. However, as our samples were collected at the time of delivery, when AA is well established, we cannot determine whether the higher concentrations of CD3+CD8- cells (inferred CD4+ T-cells) are a cause or a consequence of AA. CD4+ T-cells consist of several subtypes with different inflammatory and suppressive properties, and exist in several differentiation stages. However, the T-cells we observed probably do not have a regulatory phenotype since FOXP3+ staining was almost absent in our samples. Based on the markers used in our study, we cannot conclude regarding the function or the differentiation stage of the CD4+ cells that we observed. However, recent studies show that a majority of decidual T-cells are highly differentiated effector and memory cells (Feyaerts et al., 2017; Lissauer et al., 2017). This means that they are experienced antigen-specific T-cells, that probably have been in contact with fetal antigens (Powell et al., 2017). Decidual T-cells have a unique transcriptional profile with high expression of genes involved in inflammatory interferon signaling (Powell et al., 2017). However, the number of T-cells we observe are few, and do not imply a massive tissue inflammatory reaction.

We previously reported a lack of intramural (Zone 1) EVTs in spiral arteries with AA lesions (Alnaes-Katjavivi et al., 2016). The absence of intramural EVTs could influence the activation and signaling of nearby lymphocytes. Interaction of decidual NK-cells with EVTs regulate their secretion of cytokines and angiogenic factors, besides cytotoxicity, and is an important part of the placentation process (Cartwright et al., 2017). However, AA is also found in basal arteries of the decidua parietalis where there is no trophoblast invasion (Pijnenborg et al., 2006). Trophoblasts appear to be able to regulate the induction of Tregs in the decidua by direct interaction with T-cells and by secreted factors (Svensson-Arvelund et al., 2015). Here, we found that Tregs, defined as FOXP3+ cells, were scarce in all the three zones investigated. Hence, we speculate that simultaneous increase in T-cells and lack of EVTs in the spiral arteries, could potentially lead to local decidual inflammation that is not regulated by EVTs or Tregs. This in turn could contribute to foam cell development and AA formation.

We recently found that a combination of maternal activating KIR haplotype (KIR-B) and fetal HLA-C2 genes was associated with AA in preeclampsia (Johnsen et al., 2018). In the decidua KIR receptors are predominantly expressed by NK-cells, but a subset of T-cells can also express KIRs (Tilburgs et al., 2009). These results indicate a role for decidual NK-cells and possibly T-cells in AA. Based on our findings here we find increased support for the involvement of T-cells in AA etiology.

A limitation of our study is that the results are based on IHC staining of formalin-fixed-paraffin-embedded tissue, which limits the number of markers used. Conversely, IHC has the advantage of showing the specific location of the cells in connection with spiral arteries. The indirect estimation of CD4+ T-cells is likely less accurate than direct counting of stained cells, and might be an overestimation as there could be CD3+ cells present that are neither CD8+ nor CD4+, such as NKT-cells or other 'double-negative' subsets. However, we believe that any under- or overestimation of the CD4+ cells would be the same regardless of AA, and therefore not affect our conclusions.

The cause of the spiral artery PVI is still unknown. However, here we give a more detailed description of its composition and location in and around spiral arteries in the decidua basalis. We conclude that AA is associated with an increased concentration of T-cells (inferred CD4+) in the intramural, perivascular and possibly the interstitial zones in pregnancies with preeclampsia. This increase in T-cells coincides with a lack of intramural EVTs in the spiral arteries (Alnaes-Katjavivi et al., 2016). We speculate that this leads to a lack of interaction between fetal and maternal cells that would be important for regulation of immunological processes in decidua basalis. We suggest that this could be one of several factors contributing to the formation of AA.

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Conflict of interest statement

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jri.2019.03.003.

References

Alnaes-Katjavivi, P., et al., 2016. Acute atherosis in vacuum suction biopsies of decidua basalis: an evidence based research definition. Placenta 37, 26–33.
Baltajian, K., et al., 2014. Placental lesions of vascular insufficiency are associated with anti-angiogenic state in women with preeclampsia. Hypertens. Pregnancy 33, 427–439.
Cartwright, J.E., et al., 2017. The role of decidual NK cells in pregnancies with impaired vascular remodelling. J. Reprod. Immunol. 119, 81–84.
Erlebacher, A., 2013. Mechanisms of t cell tolerance towards the allogeneic fetus. Nat.

Rev. Immunol. 13, 23–33.
Feyaerts, D., et al., 2017. Human uterine lymphocytes acquire a more experienced and tolerogenic phenotype during pregnancy. Sci. Rep. 7, 2884.
Hecht, J.L., et al., 2016. Revisiting decidual vasculopathy. Placenta 42, 37–43.
Hertig, A.T., 1945. Vacular pathology in hypertensive albuminuric toxemias of pregnancy. Clinics 4, 602–614.
Johnsen, G.M., et al., 2018. The combination of maternal KIR-B and fetal HLA-C2 is associated with decidua basalis acute atherosis in pregnancies with preeclampsia. J. Reprod. Immunol. 129, 23–29.
Juvet, S.C., Zhang, L., 2012. Double negative regulatory T cells in transplantation and autoimmunity: recent progress and future directions. J. Mol. Cell Biol. 4, 48–58.
Kim, Y.M., et al., 2015. The frequency of acute atherosis in normal pregnancy and preterm labor, preeclampsia, small-for-gestational age, fetal death and midtrimester spontaneous abortion. J. Matern. Fetal Neonatal Med. 28, 2001–2009.
Lissauer, D., et al., 2017. Maternal effector T cells within decidua: The adaptive immune response to pregnancy? Placenta 60, 140–144.
Meekins, J.W., et al., 1994. Immunohistochemical detection of lipoprotein(a) in the wall of placental bed spiral arteries in normal and severe preeclamptic pregnancies. Placenta 15, 511–524.
Nancy, P., Erlebacher, A., 2014. T cell behavior at the maternal-fetal interface. Int. J. Dev. Biol. 58, 189–198.
Pijnenborg, R., et al., 2006. The uterine spiral arteries in human pregnancy: facts and controversies. Placenta 27, 939–958.
Powell, R.M., et al., 2017. Decidual T cells exhibit a highly differentiated phenotype and demonstrate potential fetal specificity and a strong transcriptional response to ifn. J. Immunol. 199, 3406–3417.
Robertson, W.B., et al., 1967. The pathological response of the vessels of the placental bed to hypertensive pregnancy. J. Pathol. Bacteriol. 93, 581–592.
Robertson, W.B., et al., 1976. Maternal uterine vascular lesions in the hypertensive complications of pregnancy. Perspect. Nephrol. Hypertens. 5, 115–127.
Sharkey, A.M., et al., 2008. Killer Ig-like receptor expression in uterine NK cells is biased toward recognition of HLA-C and alters with gestational age. J. Immunol. 181, 39–46.
Staff, A.C., et al., 2013. Review: Preeclampsia, acute atherosis of the spiral arteries and future cardiovascular disease: Two new hypotheses. Placenta 34 (Suppl), S73–8.
Stallmach, T., et al., 1999. Aberrant positioning of trophoblast and lymphocytes in the feto-maternal interface with pre-eclampsia. Virchows Arch. 434, 207–211.
Stevens, D.U., et al., 2012. Decidual vasculopathy and adverse perinatal outcome in preeclamptic pregnancy. Placenta 33, 630–633.
Svensson-Arvelund, J., et al., 2015. The human fetal placenta promotes tolerance against the semiallogeneic fetus by inducing regulatory T cells and homeostatic M2 macrophages. J. Immunol. 194, 1534–1544.
Tilburgs, T., et al., 2009. Expression of NK cell receptors on decidual T cells in human pregnancy. J. Reprod. Immunol. 80, 22–32.
Williams, P.J., et al., 2009a. Altered decidual leucocyte populations in the placental bed in pre-eclampsia and foetal growth restriction: a comparison with late normal pregnancy. Reproduction (Cambridge, England) 138, 177–184.
Williams, P.J., et al., 2009b. Decidual leucocyte populations in early to late gestation normal human pregnancy. J. Reprod. Immunol. 82, 24–31.
Zeek, P.M., Assali, N.S., 1950. Vascular changes in the decidua associated with eclamptogenic toxemia of pregnancy. Am. J. Clin. Pathol. 20, 1099–1109.