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Presence of IgE$^+$ cells in human placenta is independent of malaria infection or chorioamnionitis

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Summary

We have shown previously that numerous IgE$^+$ macrophage-like cells are present in the villous stroma of full term placenta and that there was no difference in the amount of IgE$^+$ cells between allergic and non-allergic mothers. The presence of such an abundant number of IgE$^+$ cells in the placenta in allergic as well as non-allergic women suggests that the IgE is of some importance for a successful pregnancy outcome. Here we have investigated the IgE-pattern in 59 placentas from second and third trimesters from Sweden with different degrees of chorioamnionitis and 27 full term placentas from Ghana with and without malaria parasites. The immunohistochemical staining pattern for IgE looked similar to our previous study, with the IgE located on Hofbauer-like cells. We could not find any difference in the amount or distribution of IgE$^+$ cells between malaria-infected and non-infected placentas, nor between different degrees of chorioamnionitis. The IgE score in the placenta did not correlate with the levels of IgE in maternal serum or plasma. However, the IgE score was significantly higher in second- compared to third-trimester placentas ($P = 0{\cdot}03$). This might reflect a maturation time-point in the fetus and in the intrauterine environment during the second trimester, or it might be associated with the increased number of intrauterine fetal deaths in the second trimester.

Keywords: chorioamnionitis, human placenta, IgE, immunohistochemistry, malaria

Introduction

IgE is the least abundant antibody class in serum, with a concentration at about 150 ng/ml in healthy individuals, which is almost 100 times less than that of IgG in the circulation [1]. In allergic individuals IgE can be over 10 times the normal level, and in certain parasitic diseases the IgE concentration in serum can reach three times higher values than normal without signs of allergic disease [1]. We have shown previously that numerous IgE$^+$ macrophage-like cells are present in the villous stroma of the placenta and that there is no difference in the amount of IgE$^+$ cells in the placenta between allergic and non-allergic mothers [2]. The presence of such an abundant number of IgE$^+$ cells in the placenta in allergic as well as non-allergic women suggests that the IgE is of some importance for a successful pregnancy outcome.

Malaria is the most important parasitic infection of man and it causes at least 1 million deaths annually [3]. Pregnant women in malaria-endemic areas are more susceptible to malaria infection than non-pregnant women in the same area [4,5]. Malaria during pregnancy is associated with maternal anaemia, low birth weights, high rates of abortion and stillbirth and hypoglycaemia with consequent maternal, fetal and infant mortality [6]. Approximately 85% of individuals living in malaria-endemic areas have significantly elevated serum IgE levels [7]. Observations of higher IgE levels in severe compared to uncomplicated malaria suggest a role for IgE in the pathogenesis of the disease [7,8].

Acute chorioamnionitis, i.e. the accumulation of polymorphonuclear leucocytes in placental tissues (notably fetal membranes) is generally considered as a marker for intrauterine infection, most commonly bacterial, which can be clinical or subclinical. Further, the presence of chorioamnionitis, especially with simultaneous signs of acute vasculitis or/and funisitis (inflammation in the umbilical cord) is associated strongly with preterm labour, premature rupture of the membranes and significant perinatal morbidity and death [9,10]

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IgE shows no transplacental transfer. However, IgE has been found in amniotic fluid and it has been proposed by Jones *et al*. that this IgE is either transferred through the fetal membranes from the maternal circulation, or that it is produced by the fetus itself [11]. Jones *et al*. also showed that there is a correlation between maternal IgE level during pregnancy and amniotic IgE level [11]. This indicates that maternal IgE passes into the amniotic fluid or that factors affecting the maternal production of IgE also affect fetal production. IgE can be produced by the fetus from as early as 20 weeks' gestation, but IgE-producing cells are extremely rare at this stage [12].

The aim of this study was to evaluate the IgE status in placentas from two different settings of intrauterine infection, i.e. malaria infection and histological chorioamnionitis.

Materials and methods

Tissue samples and preparation

Study samples, Ghana

Twenty-seven placentas were collected in Ghana from women who were involved in a trial of early *versus*-late supervised monthly anti-malarial treatment (SMAT II) in November 2001. All the placentas collected in Ghana were from term deliveries (Table 1). The placentas were collected from women who delivered at the Ejisu Government Hospital or from private maternity homes very close to the hospital, which made it possible to process the placentas directly after delivery. Immediately after clamping the umbilical cord, the placentas were placed into a container with sterile heparin. Tissue samples were cut around the midsection and placed in cryotubes filled with tissue Tek OCT (Sakura Finetek, *Zoeterwoude,* the Netherlands) and frozen gradiently using a gradient freezer and cryopreserved in liquid nitrogen, as described previously [13]. The collection of placentas in Ghana was approved by the ethics committee of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The Ministry of Health (MoH) Ghana gave administrative clearance.

Study samples, Sweden

Fifty-nine placentas with the histological diagnosis of acute chorioamnionitis were selected retrospectively from the archives of the Department of Pathology, Karolinska University Hospital, Huddinge, between 1999 and 2002 (Table 1). All the placentas were submitted to routine histopathological examination for clinical purposes. Three to five representative pieces were cut out, both in the periphery and central, fixed in formalin and paraffin-embedded. An additional fresh tissue sample was collected at the time of the examination, quick-frozen and stored at −20°C. The presence of chorioamnionitis was evaluated on haematoxylin and

Table 1. Demographic data on the placentas included in the study.

	Gestational age				Maternal age	Live-born infants	Intrauterine fetal death	Parity		Serum/Plasma IgE[a]		Phadiatop[b]	
	2nd Trimester		3rd Trimester		Median (range)			Median		Median (range)			
	n^c	n^c	n^c		in years	n^c	n^c	(range)	n^c	kU/l	n^c	Pos	Neg
Sweden													
CAM 1	1	1	0		33	0	1	2	1	20	1	1	0
CAM 2	20	9	11		31·5 (21–41)	8	12	0 (0–2)	12	42 (3·2–310)	17	7	10
CAM 3	38	23	15		32 (18–40)	15	23	1 (0–3)	24	27 (2–240)	29	10	19
All placentas	59	33	26		32 (18–41)	23	36	0 (0–3)	37	37 (2–310)	47	18	29
Ghana													
Malaria	8		All full term		22 (17–27)	8	0	2 (1–2)	8	360 (60–1680)	7	4	3
Non-malaria	19		All full term		23 (16–33)	19	0	2 (1–3)	19	232·5 (2–1710)	16	4	12
All placentas	27		All full term		23 (16–33)	27	0	2 (1–3)	27	267 (2–1710)	23	8	15

CAM: chorioamnionitis degree (see Materials and methods); Pos, positive; Neg, negative. [a]Plasma from 23 women from Ghana and serum from 47 women from Sweden was available for analysis of total IgE and Phadiatop as described in Material and methods. Total serum/plasma values above 122 kU/l are considered as elevated. [b]Phadiatop (Pharmacia Diagnostics AB) detects allergen-specific IgE against 11 common inhalant allergens. [c]Number of placentas.

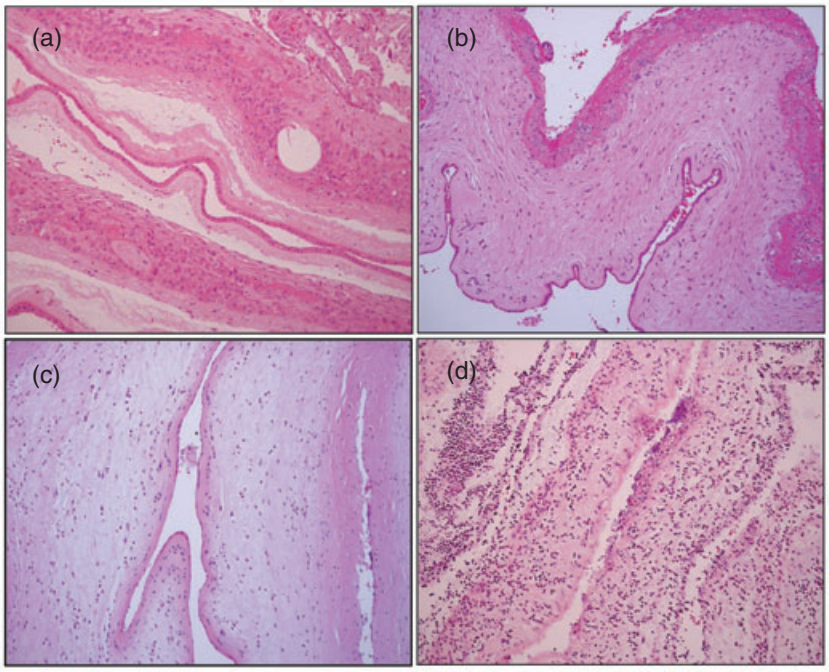

Fig. 1. Haematoxylin and eosin staining on sections from formalin-fixed placentas. (a) Normal fetal membranes without signs of inflammation; (b) grade 1 chorioamnionitis (CAM), with subchorionic inflammation and occasional granulocytes in chorion/amnion; (c) grade 2 CAM, with diffuse acute inflammatory reaction in the chorionic plate; (d) grade 3 CAM, with intense, abscess-like acute inflammation in the membranes and necrosis of amniotic epithelium. (a–d) Original magnification ×100.

eosin-stained formalin-fixed sections using a three-grade scale, as follows: grade 1: presence of polymorphonuclear leucocytes at the subchorionic plate and/or lower third of chorion; grade 2: at least two separate foci of leucocyte infiltration in chorion and amnion; and grade 3: extensive leucocyte infiltration in chorion and amnion, together with necrosis of amniotic epithelium and/or microabscess formation (Fig. 1). The presence of acute vasculitis in chorionic plate vessels or/and umbilical cord vessels or/and acute funisitis was noted. Placentas originated from cases of intrauterine fetal death ($n = 36$) as well as live-born infants ($n = 23$) (Table 1). Information about maternal age and gestational age at delivery was obtained from the clinical records. The detailed characteristics of the placentas are shown in Table 1. The study was approved by the local ethics committee at the Karolinska Institute.

Malaria parasite growth

Placentas collected in Ghana were investigated for the presence of malaria parasites. Using a 50 ml syringe and a long needle the placenta was flushed with sterile heparin in phosphate-buffered saline (PBS). The flushed blood was then collected into 50 ml tubes and centrifuged at 250 ***g*** for 10 min. This method provides contaminating fetal blood cells in less than 60% of the samples, and in those contaminated the majority contains < 6% fetal red blood cells [14]. The parasites were cultivated as described previously [15]. Briefly, the flushed erythrocytes were collected and washed three times with RPMI-1640 containing gentamycin and L-glutamine. The cells were diluted with fresh uninfected O Rh + erythrocytes and cultured in RPMI-1640 medium supplemented with 10% Albumax I (Life Technologies, Tåstrup, Denmark), 24 mM sodium bicarbonate, 2 mM HEPES buffer and 50 mg of gentamycin (Gibco, Paisley, UK) per ml. Cultures were maintained at 37°C in an atmosphere of 94% nitrogen, 5% carbon dioxide and 1% oxygen, and the culture medium was changed every other day. Thin smears were prepared from the cultures, air-dried and fixed in methanol and stained subsequently with 10% Giemsa in phosphate buffer for the determination of parasite species and percentage parasitaemia. An individual placenta was considered malaria-positive if malaria parasites were detected in the smear; on the other hand, if parasites were not detected after examining 200 oil-immersion fields of the thin smear then the blood smear was considered negative.

Analysis of plasma- or serum-IgE

Maternal plasma or serum samples collected at the time of delivery or during pregnancy were analysed for total IgE levels (Pharmacia CAP System IgE FEIA; Pharmacia Diagnostics AB, Uppsala, Sweden) and with Phadiatop (sx1 Pharmacia CAP System Phadiatop FEIA; Pharmacia Diagnostics), which detects allergen-specific IgE against 11 common inhalant allergens. The detection limit for total serum or plasma IgE was > 2 kU/l, and values below this level were set to 2 kU/l for the evaluations and statistical analysis. IgE values above 122 kU/l are considered as elevated.

Immunostaining for IgE

Acetone-fixed, 7 μm thick cryostat sections were used for staining with Vector ABC-Elite Kit (Vector Laboratories,

Inc., Burlingame, CA, USA) immunohistochemical staining [2]. To block endogenous peroxidase activity, sections were treated with 0·3% H_2O_2. Sections were then incubated with normal horse serum (dilution 1/10) followed by an avidin–biotin blocking step (Vector Laboratories, Inc.). Thereafter the sections were incubated for 30 min with a primary mouse IgG_1 monoclonal anti-IgE antibody (clone B3102E8, Southern Biotechnology Associates, Inc., Burlingame, CA, USA) diluted 1/40 in 4% bovine serum albumin in PBS. This was followed by incubation with a biotin-labelled horse anti-mouse secondary antibody (Vector Laboratories, Inc.) for 30 min. The sections were then allowed to react with a preformed avidin–biotin–enzyme complex (ABC-Elite reagent, Vector Laboratories, Inc.) for an additional 30 min. The staining was developed by incubating the sections with three-amino-9-ethyl-carbazole (AEC) substrate for 15 min. Sections were counterstained with Mayer's haematoxylin. As negative controls, sections were stained with an irrelevant isotype-matched mouse IgG_1 antibody (Dako Cytomation, Glostrup, Denmark) or without any primary antibody. Placenta sections from healthy mothers included previously in another study [2] were stained in parallel as positive controls. For evaluation of the IgE staining, the number of positive cells was graded under coded conditions by three independent investigators on a scale of + to +++ (+ = few positive cells, ++ = moderate, and +++ = many positive cells) [2]. In less than 10% of the cases there was a discrepancy between different investigators. This discrepancy was never more than one scale step, and in those cases the staining was discussed a second time at the microscope until consensus was obtained and before the code was broken.

Double immunofluorescence staining

Double immunofluorescence staining was performed as described previously [2] on frozen, acetone-fixed sections of placental tissue. A mouse monoclonal (mAb) anti-CD68 antibody (clone KP1, Dako Cytomation, Glostrup, Denmark) at a dilution of 1/1000 was used, followed by a secondary goat anti-mouse antibody conjugated with Alexa Fluor 546 (dilution 1/200, Molecular Probes, Eugene, OR, USA). To visualize IgE, a fluorescein isothiocyanate (FITC)-conjugated rabbit anti-IgE antibody (Abcam, Cambridge, UK) was used at a dilution of 1/20. The sections were mounted with glycerol in PBS containing *p*-phenylenediamine to reduce the fading of staining during microscopy and analysed with a confocal laser scanning microscope (CLSM; Leica Instruments, Heidelberg, Germany). Images were acquired sequentially with green (488 nm) and red (543 nm) excitations to avoid cross-talk between channels. Red and green images were overlaid in the image processing system integrated in the Leica Confocal Software. Staining was neither observed in controls with isotype-matched antibodies, nor when omitting the primary antibodies. The numbers of double-positive cells were evaluated and compared with those observed in sections stained only for IgE or CD68.

Statistical analysis

In the Swedish placenta material, all analyses were performed using LogXact 6·3 software (Cytel Software Corporation, Cambridge, MA, USA). Due to sparse representation of cases, an exact adjacent-category logit model was used to assess the effect of stillborn/live-born, trimester and degree of chorioamnionitis on the IgE score. The outcome was expressed as the odds of having IgE score 3 instead of IgE score 2 and IgE score 2 instead of IgE score 1. This will account for the ordinal measurements on the IgE score variable.

In statistical analyses, the single placenta with chorioamnionitis degree 1 was added to the chorioamnionitis 2 group. The Mann–Whitney *U*-test corrected for ties was used to compare differences in IgE-score between malaria-infected and uninfected placentas. To evaluate the correlation between plasma or serum IgE and IgE score in the placenta, the Spearman rank-order correlation coefficient was computed. P-values $< 0{\cdot}05$ was considered significant.

Results

General pathology

Eight placentas from Ghana were considered as positive for malaria (Table 1) based on the culture of parasites from blood flushed from the placentas. Only *Plasmodium falciparum* parasites were found in the malaria-positive placentas.

The degree of chorioamnionitis of the 59 Swedish placentas was determined from formalin-fixed placenta samples stained with haematoxylin and eosin (Fig. 1). Most of the placentas had a high or moderate degree of chorioamnionitis (Table 1). Additionally, in the overwhelming majority of cases (82%) we found signs of acute vasculitis or/and funisitis, i.e. inflammation in the vessel wall and umbilical cord, respectively. It was not possible to determine with the same accuracy the degree of chorioamnionitis of the Ghana placentas due to lack of formalin-fixed samples.

IgE+ cells in the placenta samples

IgE+ cells were found in the villous parenchyma of all the placentas examined. The IgE+ cells were macrophage-like and located in the villous stroma (Fig. 2a–c). No staining could be observed, either when an isotype matched negative control antibody was used as primary antibody or when the primary antibody was excluded (Fig. 2d). Double immunofluorescence staining showed that these IgE+ cells were also CD68+ (Fig. 3a–c) supporting their macrophagic origin. Single stainings for CD68 and IgE showed the same pattern

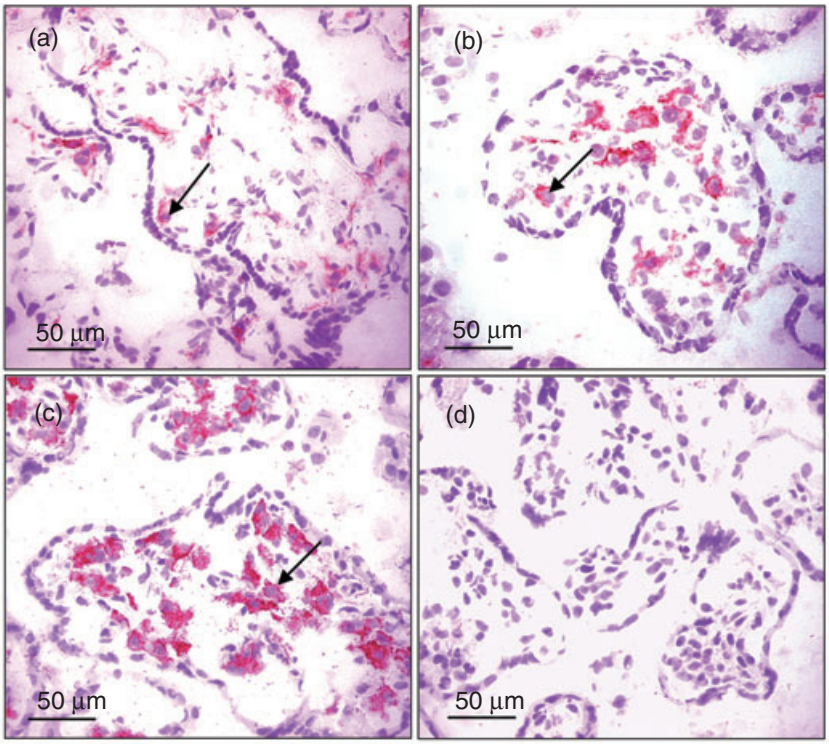

Fig. 2. Immunohistochemical staining for IgE in placenta. Representative examples of different IgE scores. (a) A third-trimester placenta with IgE score +; (b) a second-trimester placenta with IgE score ++ ; (c) a second-trimester placenta with IgE score +++ ; (d) negative control without primary antibody, second-trimester placenta. Scale bars: 50 µm.

as the double stainings. No immunoreactive cells were found among the trophoblasts.

When comparing malaria-infected and uninfected full term placentas from Ghana, no significant difference in the IgE score could be found (Table 2); nor was there any significant difference in the IgE score between different degrees of chorioamnionitis in the placentas from Sweden (Table 3). There was no correlation between the presence of vasculitis/funisitis and IgE score in the placenta (data not presented). When comparing live-born children and intrauterine fetal death, there was an indication towards a higher IgE score in intrauterine fetal death compared to live-born children ($P = 0{\cdot}06$, OR = 2·5) (Table 4). With the same comparison between the second and third trimesters, there was a significantly higher IgE score in second-trimester placentas than in third-trimester placentas ($P = 0{\cdot}03$ OR = 2·8) (Table 5).

Table 2. IgE scores in malaria-infected and uninfected placentas collected in Ghana.

	IgE score*		
	+	++	+++
Malaria	5	2	1
Non-malaria	5	11	3

*A semiquantitative scale (+ = few positive cells, ++ = moderate, and +++ = many positive cells) was used for evaluation of the IgE staining with immunohistochemistry.

Analysis of total IgE levels in maternal serum or plasma

Plasma from 23 of the women included in the Ghana study and serum from 47 of the women included in the Swedish study was analysed for total IgE (Table 1). There was no

Table 3. IgE score in placentas collected in Sweden with different degrees of chorioamnionitis.

Degree of chorioamnionitis	IgE score*		
	+	++	+++
1			1
2	3	9	8
3	4	23	11

*A semiquantitative scale (+ = few positive cells, ++ = moderate, and + ++ = many positive cells) was used for evaluation of the IgE staining with immunohistochemistry.

Table 4. IgE scores in placentas collected in Sweden from live-born and stillborn children/aborted fetuses.

	IgE score*		
	+	++	+++
Live-born	4	15	4
Stillborn/aborted†	3	17	16

*A semiquantitative scale (+ = few positive cells, ++ = moderate, and + ++ = many positive cells) was used for evaluation of the IgE staining with immunohistochemistry. †Stillborn/aborted is defined as aborted fetuses or children born with Apgar score 0-0-0.

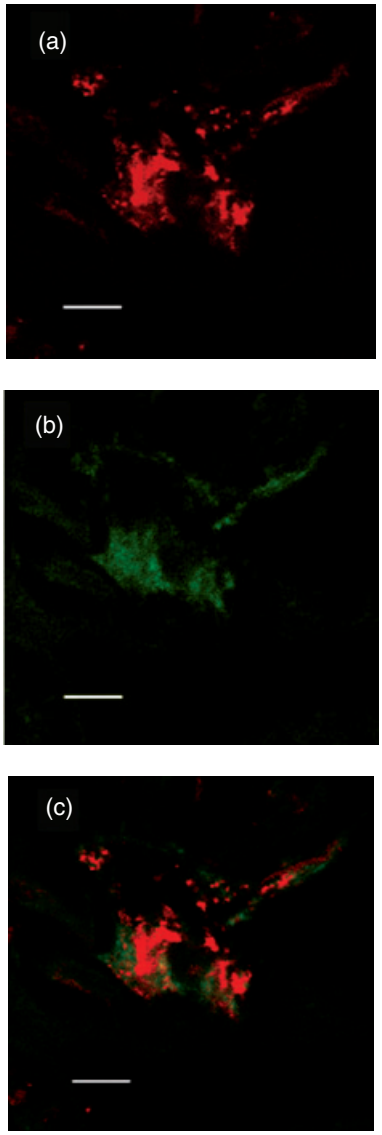

Fig. 3. Double immunofluorescence staining for IgE and CD68. Acetone-fixed cryostat sections of placenta were double-stained with a fluorescein isothiocyanate-conjugated rabbit anti-IgE antibody and an unconjugated mouse anti-CD68 antibody (clone KP1) followed by an Alexa Fluor 546 conjugated secondary goat anti-mouse antibody. The sections were analysed with a confocal laser scanning microscope. (a–c) Double-staining of a placental section from a full term placental biopsy showing a $CD68^+$ (a), IgE^+ (b) and $CD68^+IgE^+$ (c) cell in the villous stroma. Scale bars: 4 μm.

correlation between these levels and the IgE score in the placenta. Eighty-six per cent of the malaria-positive mothers from Ghana had elevated plasma IgE levels and 57% were Phadiatop-positive. Of the malaria-negative mothers, 69% had elevated plasma IgE levels and 25% were Phadiatop-positive (Table 1). In the Swedish material, 23% of the mothers had increased levels of serum IgE and 38% were Phadiatop-positive (Table 1).

Discussion

We have shown previously the presence of IgE^+ cells in the villous stroma of full term placenta without any difference in the number of IgE^+ cells between allergic and non-allergic mothers [2]. We then hypothesized that the IgE in the placenta might have a protective role during pregnancy. Therefore, we have investigated here pathological placenta material obtained from spontaneous abortions and different infections, including malaria, to see if the IgE-pattern in these placenta specimens varied.

Our malaria material includes placentas from mothers in Ghana likely to be exposed to malaria and parasites leading to increased serum IgE levels. Maternal malaria during pregnancy leads usually to massive infestation of the placenta with *P. falciparum*-infected erythrocytes [16]. These infected erythrocytes adhere to several different receptors in the placenta, mainly on the syncytiotrophoblast [17]. The amount and distribution of IgE^+ cells in our study did not differ between the malaria-infected and -uninfected placentas and is in agreement with our previous study [2]. Our present observation is, however, in disagreement with a previous study by Maeno *et al.* [18], who detected IgE immune complexes on the syncytiotrophoblast cells and in fetal blood vessels in placenta from women infected with *P. falciparum*. They used a rabbit anti-human IgE serum which had not been absorbed against human serum, and as a negative control they used normal rabbit serum. There might be differences in antibody specificity between our mouse monoclonal antibody and the rabbit serum used by this group. In addition, we have observed that staining placental tissue with rabbit anti-serum that has not been absorbed against human serum is associated with relatively high background staining. This could make the results from Maeno *et al.* [18] difficult to interpret.

Our chorioamnionitis material represents both full term pregnancies and spontaneous abortions. There were no significant differences in the amount or localization of the IgE^+ cells between the different degrees of chorioamnionitis. Our finding of IgE^+ cells primarily in the villous stroma is in agreement with our previous observation, where we detected IgE on macrophage-like cells in the villous stroma on placentas from healthy and allergic mothers [2]. These IgE^+ cells were confirmed to be placental macrophages, i.e. Hofbauer

Table 5. IgE score in placentas from different trimesters collected in Sweden.

	IgE score*		
	+	++	+++
2nd trimester	1	18	14
3rd trimester	6	14	6

*A semiquantitative scale (+ = few positive cells, ++ = moderate, and +++ = many positive cells) was used for evaluation of the IgE staining with immunohistochemistry.

cells, by positive staining for human leucocyte antigen-D related (HLA-DR) and CD14 [2]. Here, we show that the IgE^+ cells are also positive for the macrophage marker CD68 [19] (Fig. 3a–c), further supporting their macrophage phenotype. The amount and location of IgE in the placentas in our present study does not seem to be influenced by the presence of *P. falciparum* parasites or chorioamnionitis. However, when comparing the amount of IgE^+ cells between the second and third trimesters, we saw a significant decrease from second to third trimesters. We could also see an indication towards a higher IgE score when the infants were stillborn or there was a spontaneous abortion. As a high number of the infants in the second trimester were stillborn/aborted, compared to the third trimester where most infants survived, it is hard to say which of these factors affect the amount of IgE^+ cells. The higher amount of IgE^+ cells in the second- compared to the third-trimester placentas is not likely to be explained by a higher amount of Hofbauer cells at this time-point, as both immunological data and electron microscopic studies show the persistence of Hofbauer cells in the placental stroma throughout pregnancy [20].

Even though the allergic mothers in our previous study [2] had significantly higher serum IgE levels than the non-allergic mothers, no difference was found in the IgE score in the placenta. In this study we could still not find any correlation between serum/plasma IgE levels and the IgE score in the placenta, although the IgE levels in some of the women from Ghana are above 1000 kU/l. This could indicate that the IgE does not come from the mother, or at least it is not transported passively into the placenta.

The finding of numerous IgE^+ cells in the placenta raises questions regarding the origin of this IgE and possible receptors for IgE. Previous results have shown that IgE in the placenta is not bound to the high-affinity receptor FcåRIα, nor to the low-affinity receptor CD23 [2], indicating that the IgE is bound to some other receptor. It is believed widely that IgG is the only immunoglobulin isotype that can cross the placental barrier [21], although it has been speculated that maternal IgE could cross the fetal membranes into the amniotic fluid [11]. Another possibility is that the IgE is produced by the fetus itself. In our previous study [2] we could not find any evidence for IgE production locally in the placenta, but others have shown that IgE can be produced by fetal B cells from as early as 20 weeks' gestation [12]. Another hypothesis is that the IgE crosses the placenta via IgG/IgE complexes. This type of complex has been detected in cord blood as well as in the maternal circulation (our own unpublished observations). There are some studies suggesting transfer of certain maternal immune complexes across the placenta, for example complexes of IgG and hepatitis B antigen [22] or animal insulin [23].

In conclusion, we have shown that IgE is present on macrophage-like cells in the villous stroma of placentas from malaria-infected and non-infected women and in placentas with chorioamnionitis. There was no difference in the amount or distribution of IgE^+ cells between malaria-infected and non-infected placentas, nor between different degrees of chorioamnionitis. However, the IgE score was significantly higher in second- compared to third-trimester placentas. This might reflect a maturation time-point in the fetus and in the intrauterine environment during the second trimester, or it might be associated with the increased number of intrauterine fetal deaths in the second trimester.

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