Hydrolytic enzyme activity in rhesus monkey placenta during early gestational malaria : histochemical studies

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Background & objectives: Early gestational malaria is found to be more fatal than late gestational infection but the pathophysiology of early gestational placenta, the maternofoetal organ responsible for maintenance of pregnancy, remains unexplored. Present study dealing with hydrolytic enzymes in early gestational placenta of rhesus monkeys during *Plasmodium cynomolgi* infection was anticipated to provide a better insight into the functional impairment of this organ during early gestational maternal malaria.

Methods: Experimental monkeys (*Macaca multtta*) at 2–2½ months of pregnancy were inoculated with *P. cynomolgi bastianelli*. After attaining first peak of parasitaemia the animals were anesthetised and placentae were collected for histochemical studies. The snap-frozen, cryostat sections were subjected to histochemical reactions for acid phosphatase and alkaline phosphatase.

Results: The placental syncytiotrophoblast showed a loss in alkaline phosphatase activity, while the trophoblast layers and phagocytic cells of the maternal blood showed increased acid phosphatase activity during early gestational malarial infection. Morphological damage to the placental tissue whenever occurred was associated with altered Alk pase activity.

Interpretation & conclusion: The altered distribution of Ac pase and Alk pase in malaria infected early gestational placenta has been discussed in the light of placental function. It could be concluded by present studies that these malaria induced changes in hydrolytic enzyme activities in monkey placenta have a direct bearing on functional and morphological integrity of the placental tissue. These changes are apparently responsible for early gestational foetal death and abortions as reported in literature

Key words   Ac pase – alk pase – malaria – placenta

The structural and functional integrity of placenta is critical to the maintenance of a normal foetal growth and development. Disruption of normal placental function might result from a variety of infections common during all stages of pregnancy. Among parasitic diseases, malaria overshadows all other infections in frequency and clinical significance and is associated with incidences of abortions, reduced mean birthweight of foetus and maternofoetal deaths[1-6]. The knowledge on potential pathology due to malaria is restricted to term human placenta[7,8] or term animal placenta[9], while early gestational malaria proves to be more fatal[10-13] but the pathophysiology of early gestational placenta in malaria remains unexplored. It is, therefore, considered worthwhile to investigate the structural and functional alterations in the placenta of a nonhuman primate model, namely rhesus monkey infected with *Plasmodium cynomolgi bastianelli*. A

part of these investigations dealing with the malfunctioning of the infected placenta at histological[12], scanning electron microscope[13] and biochemical level[11] has already been published, and the present communication deals with histochemical investigations on certain hydrolases of the malaria infected early gestational (2–2½ months) monkey placenta.

Material & Methods

Healthy pregnant rhesus monkeys (*Macaca mulatta*) of 5–6 kg body weight were procured from a local animal supplier and quarantined for one month in the Institute's animal house. Experimental protocol had prior approval from the Institutional Animals Ethics Committee. Animals were handled and used according to the guidelines of this committee. Only those monkeys which were free from tuberculosis (determined by tuberculin test and X-ray diagnosis) were included in the study. The animals were housed in a temperature (range $23 \pm 2^\circ C$), relative humidity (range 50–55%) and photoperiod (12 h of alternating light and dark periods) controlled room and maintained on monkey pellet diet (Hind Lever, Mumbai, India) supplemented with fresh fruits. Drinking water was allowed *ad libitum.*

The malarial parasite, *P. cynomolgi bastianelli* (B-strain; kind courtesy of Microbiology Division, Central Drug Research Institute, Lucknow, India) which is known to be identical to *P. vivax* in morphology and pathogenicity, was maintained in normal monkeys by serial blood passages. At 2–2½ months of pregnancy (ascertained by palpation and X-ray diagnosis) four monkeys were inoculated intravenously with 1×10^6 RBCs infected with *P. cynomolgi bastianelli*, while three monkeys were taken as control/normal. Peripheral parasitaemia in experimental monkeys was monitored by daily examination of ear vein blood samples following the standard procedure of Water Reed Army Institute, U.S.A[14], twenty-four hours after appearance of the first peak of parasitaemia. In rhesus monkey, the infection exhibits a synchronised erythrocytic cycle with two peaks of parasitaemia[15]. The

first peak, which occurs in the second week of infections, is far more higher than the second peak. The infected as well as uninfected control animals were anesthetised with pentobarbital and the placentae were collected in chilled physiological saline by the procedure published by our grourp[11-13].

After extraction of placental tissue all the animals were treated with antimalarials and rehabilitated till they tested negative for parasitaemia following the rehabilitation procedure of Institutional Primate House.

Thin slices of placental tissue were soaked in 5% polyvinyl alcohol at $0–4^\circ C$ for 5 min, snap-frozen in Freon-22 cooled by liquid nitrogen and cryostat sections (8 μm) were cut at $–25^\circ C$. A minimum of five section sets each consisting of eight sections (with an interval distance of 40 μm between each section) were prepared from each monkey placenta. The section sets were obtained by random sampling of almost the entire thickness of the tissue slice. The sections mounted on clean glass slides were air dried and used immediately for different histochemical reactions.

Acid phosphatase (acid phosphomonoesterase, EC 3.1.3.2; Ac pase) was demonstrated by a modified Gomori's metal salt procedure[16] and alkaline phosphatase (alkaline phosphomonoesterase, EC 3.1.3.1; Alk pase) was localised by simultaneous azocoupling (substituted naphthol) method as described by Pearse[17]. Appropriate controls for the incubations were also run by omitting the substrates from the respective media. To ensure maximum resolution of the histochemical reaction products for microscopy and to obtain optimum contrast in photomicrography, counterstaining was omitted in all the preparations.

Results

Parasitaemia levels in the four *P. cynomolgi* infected monkeys selected for the present study are given in Table 1. Peak parasitaemia (range 5.1–7.1%) was observed on Day 11 post-inoculation. After attaining peak parasitaemia several changes were observed in

Table 1. Peripheral parasitaemia in the pregnant monkeys infected with *P. cynomolgi bastianelli*
(Inoculum : 1×10^6 parasites)

Monkey	Parasitaemia (%)							
	Days post-inoculation							
	5	6	7	8	9	10	11	12
3129	0.12	0.15	0.25	0.3	1.2	2.65	5.75*	1.25
3132	0	0.10	0.10	0.25	0.3	2.00	5.10*	3.00
3133	0.03	0.10	0.25	0.6	1.8	2.30	6.95*	4.00
3140	0.04	0.20	0.60	1.5	1.3	4.60	7.10*	5.20

*Values indicate peak parasitaemia.

histological, scanning electron microscopical and bio-chemical observations of malaria infected monkey placenta (already published data[11-13]), while hydrolytic enzymes were localised as follows:

Ac pase

Lysosomal localisation of Ac pase activity was seen in all the cellular elements of the normal placenta and in the macrophages of the maternal circulation (Fig. 1).

In the placenta of malaria infected animals the tropho-blast and the macrophages and polymorphs in the villi and surrounded maternal blood showed intense Ac pase activity (Figs. 2a and b). However, this intensity
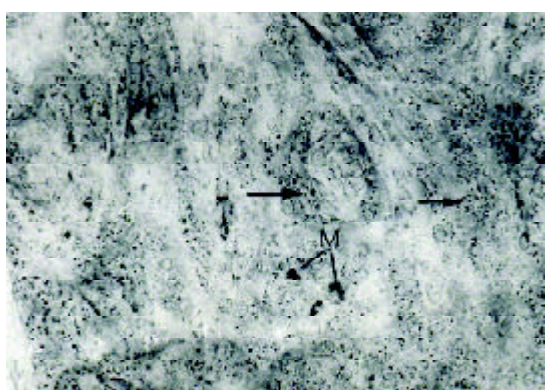
(a)

(b)

Fig. 1: Cryostat section of a normal placenta showing localisation of acid phosphatase in trophoblast cells (arrow). Macrophages (M) of maternal blood also show the activity [× 350].
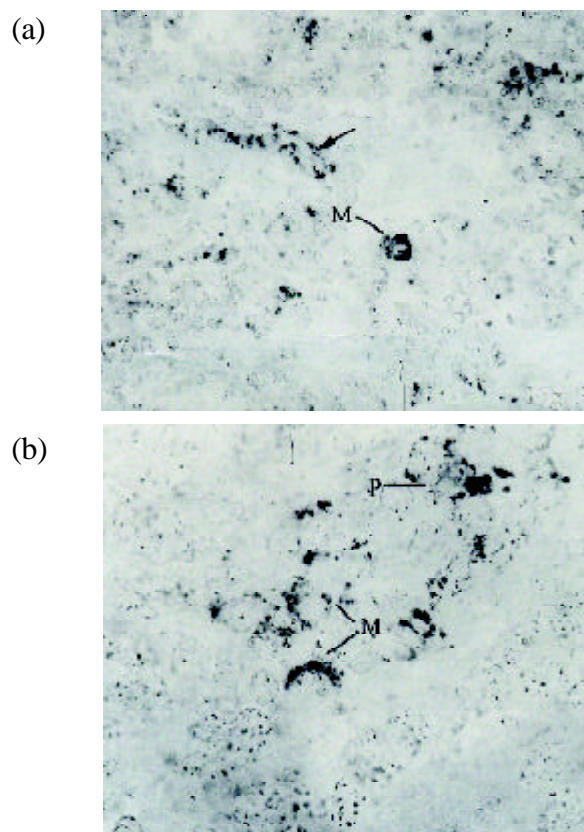
Fig. 2a: Cryostat section of malaria-infected placenta. 'Foci' of cells in trophoblast layer showing increased acid phosphatase activity (arrow). An enzyme rich macrophage (M) is also seen in intervillous space [× 350].

Fig. 2b: High magnification of another section of malaria-infected placenta showing intense acid phosphatase activity in maternal macrophages (M) and polymorphs (P) [× 870].

was not found uniformly distributed throughout the placenta but rather was restricted to groups of cells (foci). These 'foci' were more abundant in damaged trophoblast cells than in intact villi.

Alk pase

In normal placenta Alk pase activity was characteristically localised in the 'brush border' of trophoblast layer, while the stroma seemed generally devoid of the activity except for the phagocytic cells in it. No enzyme activity could be observed in the cytotrophoblast layer but the adventitial layer of blood vessels and foetal capillary endothelium showed a strong activity (Fig. 3).

In malaria infected placenta the enzyme activity in the 'brush border' of most villi became extremely weak. However, despite the reduction of enzyme activity the morphological integrity of these villi appeared unimpaired (Fig. 4a). On the other hand, in the villi which showed morphological damage, the loss in the Alk pase activity was almost complete (Fig. 4b).

Discussion

It is well-known that rhesus placenta, like that of human beings, is a typical haemochorial type[18-21]. In

the present study, it is demonstrated that the placental tissue in rhesus monkey exhibits changes in the activity patterns of acid phosphatase and alkaline phosphatase during malaria infection and that these changes may represent functional alterations either preceding or running concurrently with morphological damage in the placenta.

The localisation pattern of acid phosphatase in different cellular elements of rhesus monkey placenta is similar to that reported in human[22]. The characteristic discrete particulate localisation of the enzyme obtained

(a)

(b)

Fig. 4a: Malaria-infected placenta showing uniform loss in alkaline phosphatase activity of the syncytiotrophoblastic brush border (B). The foetal capillary endothelium (E) shows normal enzyme activity [× 140].
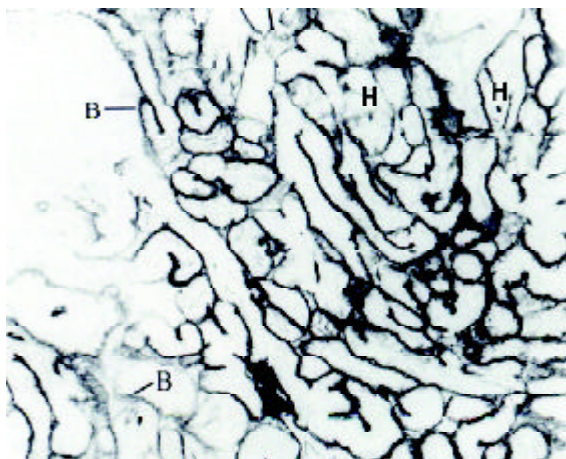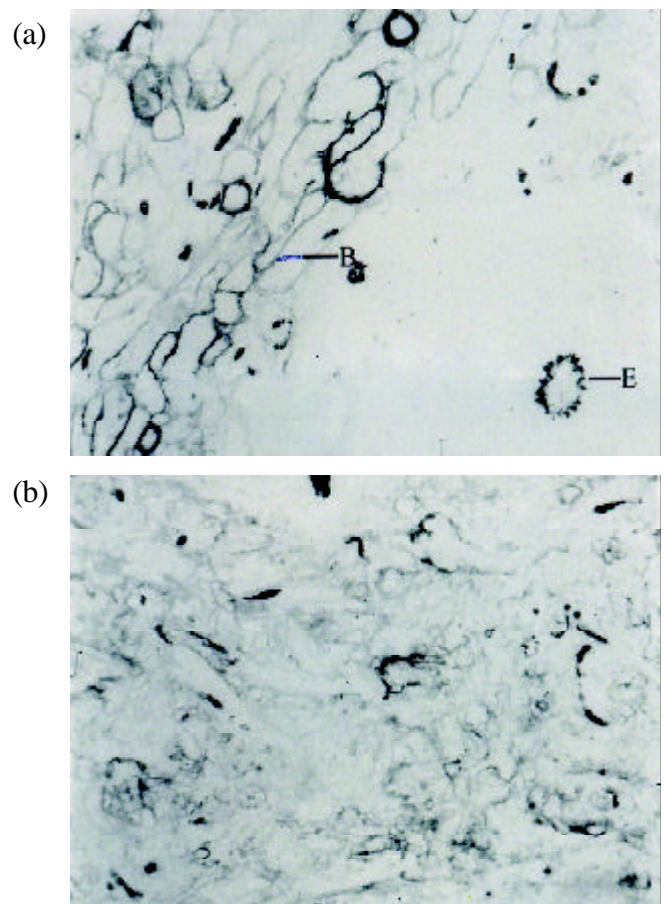
Fig. 3: Cryostat section of normal placenta showing localisation of alkaline phosphatase activity in the brush border of syncytiotrophoblast layer (B) and Hofbauer cells (H) [× 140].

Fig. 4b: Cryostat section of infected placenta showing decreased alkaline phosphatase activity and morphological damage of placenta cells [× 140].

in the trophoblast layers indicates that in these cells acid phosphatase is predominantly lysosomal and is uniformly distributed. In malaria infected monkeys several foci in trophoblast layer became strongly positive for the enzyme. This altered enzyme activity appeared to be due to focal damage to the cells and findings are in support of our earlier histological and biochemical observations. Further, the cells of these foci showed at electron microsocope level, an abundance of dense bodies (unpublished observations). It is thus evident that cellular autophagy, which is known to be mediated by lysosomes[23,24] was in progress in these foci. Although the pathogenesis of the focal cellular damage in placenta cannot be directly inferred from the present study, but it may be a consequence of hypoxia which has also been shown to enhance the lysosomal activity in trophoblast[25]. Alternatively the migrant malaria pigment-laden macrophages found within the villi[12] could bring about the damage or certain macrophage derived mediators might be involved in tissue damage in malaria[26].

Macrophages and polymorphonuclear leucocytes are phagocytic cells and phagocytosis in these cells is mediated by several hydrolytic and oxidative enzymes. The presence of several macrophages showing intense acid phosphatase activity as well as malarial-pigment in the placenta suggests active phagocytosis of the parasitised erythrocytes by these cells.

Alkaline phosphatase has been shown to play an important but ill-defined role in transmembrane transfer mechanism as the enzyme is found primarily in cell membrane where active transport processes take place[27]. The distribution of this enzyme on the syncytial surface and cytoplasm, foetal vascular aventitia and capillary endothelium of normal monkey placenta also provides indication that the enzyme might participate in the transport of substances across the membranes. Similar view was earlier put forward by Wislocki and Padykula[22] who pointed out that many substances and metabolites may depend on phosphatases for their transfer across cellular boundaries. In the present study the overall decrease in the alka-line phosphatase activity in malaria-infected monkey placenta indicates an impairment in the cellular transport mechanism. In contrast to the close relationship observed between patchy increase in acid phosphatase and the focal morphological damage in the placental tissue, the distribution of alkaline phosphatase was more or less uniformly changed. This possibly indicates early alteration in the enzyme activity before tissue damage.

Conclusion

In conclusion, the present histochemical study reveals that malaria infection during early gestation induces changes in hydrolytic enzyme activities in the monkey placenta which may have a direct bearing on the functional and morphological integrity of the placental tissue. These changes are apparently responsible for the early gestational foetal death and abortions reported earlier[11-13] in infected monkeys.

Acknowledgement

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