Leucocyte phagocytosis and circulating immune complexes in mothers after child birth

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Summary

Leucocyte phagocytosis and level of circulating immune complexes (CICs) were measured in pregnant women at the three trimesters of pregnancy and in mothers 24 - 48 hours after child birth to evaluate the influence of pregnancy on leucocyte functions. The mothers were divided into gestation groups depending on the ages of their pregnancies as 1st trimester, 2nd trimester and 3rd trimester. The result shows that total white blood cell count (WBC), lymphocytes (B - and T -lymphocytes) and circulating immune complexes were high in pregnant women (independent of gestational ages) and mothers 24 - 48hrs after child birth compared with non-pregnant controls. In contrast, mean percentage migration index (%M.I.), percentage candidacidal index (% C. I) and H_2O_2 production were reduced in pregnant women and mothers after child birth compared with non-pregnant controls. When test groups (pregnant women and mothers after child birth) were compared, women after child birth had least mean %C.I and %M.I while pregnant women at 1st trimester had highest mean %C. I., %M. I. and highest level of H_2O_2 production. Within the gestational groups, pregnant women at 3rd trimester had higher %M. I. compared with those in 2nd trimester while those in 2nd trimester had lower %M. I., %C. I. and H_2O_2 production compared with women at 1st trimester. Our finding shows that cell mediated immune responses vary between trimesters therefore susceptibility of pregnant women to different pathogens may vary with gestation.

Keywords: ***Phagocytosis, Leucocytes, Mothers, Babies, Immune complexes.***

Résumé

Phagocytose leucocytes et le niveau complexe immune circulant (CISs) ont été mesuré chez une femme enceinte pendant les trios trimester d'une grossesse et chez de mères 24 - 48 heures après accouchement afin d'évaluer l'influence de la grossesse sur les functions de la leucocytose. Les mères ont été divisées en groupes de gestation ça depend de l'âge de leur grossesses comme l er trimester, 2e trimestre et 3e trimester. Le résultat a indiqué que le compte du globules blanc (WBC), lymphocytes (B-et T-lymphocytes) et les complexe immune circulants étaient élevés chez des femmes enceintes (indépendent des âges gestationels) et des mères 24 - 48 heures après accouchement par rapport aux contrôles sans grossesse. Par contraste avec pourcentage moyen de l'indice de migration (% M. I.) pourcentage indice de candidacidal (% C, I) et le production H202 étaient en baisse chez des femmes enceintes et mères après accouchement par rapport aux contrôles de sans grossesses. Quand le groupe de test (femmes enceintes et des mères après accouchment) ont été comparés, des femmes après accouchement avaient le moyen le plus petit % C. 1 tandis que des femmes enceintes au cours du l er trimestre avaient le moyen le plus élevé % C. 1, % M. I et niveau le plus élevé de la production de H202. Parmi les groupes gestationnels, des femmes enceintes au cours du troisieme trimestre avaient % M. I très élevé et % C. I, en baisse par rapport aux celles du deuxième trimestre tandis que celles du 2èm trimestre avaient % M. I, % C. I, en baisse et H202 production par rapport aux femmes pendant le 1 er trimestre. Nos résultats demonstrent que les réponses immunisées cellules médiations variant d'aprés les trimestres donc la susceptibilité des femmes enceintes aux pathogènes pourrait varier avec la gestation.

Introduction

It is generally agreed that women are at greater risk of infection during pregnancy and that various inflammatory diseases particularly rheumatoid arthritis improves with pregnancy.[1] Immune-suppression during pregnancy was based on changing lymphocyte number[2] and altered polymorphonuclear leucocyte (PMNL) functions.[3] Chemotaxis, engulfing and intracellular killing are chief means by which PMNL kill invading pathogens but combination of these parameters had not been assessed in pregnancy to child birth. In the present study, we assessed leucocyte migration, mechanism of intracellular killing and circulating immune complexes in pregnant women and mothers after child birth. The outcome of the study will be supportive to medicare giving to pregnant women and mothers immediately after child birth.

Material and Methods

Subjects

A total of 139 women attending Ante-natal Clinic or delivering in the maternity wards of University College Hospital (UCH), Ibadan, Nigeria and Adeoyo State Hospital, Ibadan, Nigeria were enrolled for the study after they gave their oral consent. Pregnant women were divided into gestational age groups based on the date of last menstrual period as 1st trimester, 2nd trimester and 3rd trimester. Mothers with complicated pregnancy or delivery were excluded.

The controls were apparently healthy non-pregnant female members of staff of UCH, Nigeria. They were matched for age with the parturients. The age range was 19 - 46 years with a mean and S. D. of 30 $\pm$ 6yrs and controls on oral contraceptives were excluded.

Twenty (20)ml of venous blood sample was obtained from each of the subjects. Fifteen ml of the blood were delivered into heparinized tube to be analysed for %M.I., % C. I. and NBT dye reduction test, while 5ml was delivered into a clean bottle without anticoagulant till retraction for immediate determination of CICs.

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All methods outlined below were previously described elsewhere [4,5,18,19,20]

Total white blood cell count

Whole blood (0.1ml) was delivered into 1.9ml of Tuerk solution and mixed. With a capillary tube, an improved Neubaeur chamber was filled with diluted blood. The cells in the two diagonal square millimeters were counted using 40mm objective for total WBC number.

Preparation of blood lymphocytes from whole blood

Five (5) ml of heparinised blood was delivered into a universal bottles containing five (5)ml of medium (Hanks solution + 5iμ/ml heparin). This was mixed, carefully layered over 4ml of lymphoprep and centrifuged at 850 x G for 20 minutes. The mononuclear cells formed a visible, clear interface between the plasma and lymphoprep carefully removed and placed in a clean plastic centrifuge tube. The cells were washed 3 times with Hank-heparin solution. The lymphocyte number was determined with haemocytometer and the percentage % purity was evaluated by 0.5% trypan blue dye exclusion test.

Enumeration of T-lymphocytes

0.25ml of 4 x 10^6 lymphocytes/ml of Hanks solution was mixed with 0.25ml of 0.5% suspension of washed sheep red blood cells (SRBC). Adsorbed heat inactivated foetal calf serum (FCS, 0.1ml) was added and the mixture was incubated at 37°C for 5 minutes. This was centrifuged for 5 minutes at 100 x G to pellet the cells, and re-incubated at 4°C overnight. The % rosetting cells was determined by counting 200 lymphocytes in a haemocytometer chamber. A rosette forming lymphocyte was defined as one that bound 3 or more sheep red blood cells. The assay was performed in duplicates.

Enumeration of B-lymphocytes

The method is similar to that described for the enumeration of T-lymphocytes except that the SRBC used were sensitised with sub-agglutinating dose of guinea-pig complement. 0.25ml of 5% presensitised SRBC was mixed with 0.25ml of 4 x 10^6 lymphocytes/ml and incubated at 37°C for 45 minutes. This was centrifuged at 200 x G for 3 minutes and the % rosetting lymphocyte determined using haemocytometer.

Leucocyte migration index

Heparinised blood was mixed with equal volume of 3% dextran solution and this was allowed to stand at 37°C for 1 hr. The leucocyte rich supernatant was spun at 300 x G for 10 minutes, washed in 15% foetal calf serum and the number adjusted to 1 x 10^8 cells/L before being filled into capillary tubes. These were spun at 850 x G for 10 minutes, cut at packed cell-medium interface, placed in a migration chamber and filled with either medium or antigen-medium solution. 1 in 10 dilution of oral measles virus vaccine was used as antigen. A drop of streptomycin was added to each well and incubated at 37°C in CO_2 for 18 hrs. The migration area was projected unto transparent paper, outlined and areas measured by counting the number of small squares enclosed on a graph paper. The percentage migration index (%M.I) due to the antigen was calculated thus: % M. I. = T/C x 100 where C is the area of migration in medium and T is the area of migration in antigen solution.

Candidacidal index

A saline-wash concentrated suspension of a 24-hr culture of *C. albicans* was made in Hanks solution. This was adjusted to 5 x 10^6 cells/ml of Hanks solution and viability of the cells was confirmed to be 95% by the Trypan-blue dye-exclusion method. To a mixture of 0.25ml autologous plasma, 0.25ml Hanks solution, and 0.25ml of *Candida* suspensions was added 0.25ml of 5 x 10^6 neutrophil suspension. A similar set up was made for the control tube except that neurophil suspension was omitted. The tubes containing the mixture were incubated for 1hr with shaking at every 15 minutes. At the end of this period, 0.25ml of 2.5% sodium desoxycholate was added to each mixture to lyse neutrophils but not the *Candida.* Four (4) ml of 0.01% methylene blue was carefully removed leaving about 0.5ml to resuspend the organism. The percentages of dead *Candida* (stained cells) were determined using the improved Neubauer counting chamber.

Nitroblue tetrazolium (NBT) dye reduction index

For stimulated NBT procedure, 50μl of NBT solution was transferred to a vial. 25μl heparinized blood and 25μl of stimulant solution (non-viable bacterial extracts) were added. This was mixed gently, incubated at 37°C for 10 minutes and for further 10 minutes at room temperature. Thick smear of the mixture was prepared, air dried for 10minutes, treated with undiluted Wright stain for 15 seconds, diluted Wright stain (1:1) for 30 seconds before rinsed in water and air-dried. 100 neutrophils were counted under oil immersion objective and neutrophils showing dark formazan deposits were recorded as positive. % stimulated NBT was calculated as neutrophils with dark formazan deposit (positive) / total neutrophil count.

Determination of circulating immune complexes

PEG 6000 solution was added to serum in borate buffer to give a final concentration of 3.7% PEG and 1 in 3 dilution of serum. After incubation at room temperature, the immune complex concentrations were measured at 450nm wave length using spectrophotometer.

Statistical method

Data generated in this study were compared using ANOVA

Results

The mean ages of all subjects considered for this study were similar, thus eliminating the influence of age on immune parameters (Table 1). Table 1 also show that total WBC count, %T - and %B- lymphocytes were high during pregnancy and child birth compared with the controls. %M. I, %C. I and stimulated NBT reduction were depressed during pregnancy and child birth compared with the controls. When test mothers were compared, women at child birth had least %M. I. while pregnant women in 1st trimester had highest %M. I. Moreover, mothers at birth had least %C. I. while pregnant women in 1st trimester highest %C.I. Those in 3rd trimester

Table 1 Comparison of ages and WBC (total and differential) counts in parturient mothers and non pregnant controls.

	Controls (n = 43)	1st trimester (n = 30)	2nd trimester (n = 26)	3rd trimester (n = 22)	At child birth (n = 48)	F-, p-values
Ages	30 ± 6.9	27 ± 6.3	29 ± 4	30 ± 1.2	31 ± 7.0	0.8, 0.96
Total WBC (x10⁹/L)	6.9 ± 2.3	12.5 ± 5.2*	10.3 ± 6.0*	11.4 ± 4.3*	10.3 ± 4.3*	8.25, 0.00
% T - cell	58 ± 10	76 ± 11	69 ± 9*	60 ± 12*	66 ± 18*	7.61, 0.00
%B - cell	19 ± 5	21 ± 3	23 ± 4*	21 ± 2	23 ± 3*	10.96, 0.00

p<0.05 is significant

** Significantly different from the controls.*

Table 2 %M. I., %C. I. % stimulated NBT dye reduction and CIC in parturients compared with the controls

	Controls (n = 43)	1st trimester (n = 30)	2nd trimester (n = 26)	3rd trimester (n = 22)	At child birth (n = 48)	F-, p-values
%M.I.	67 ± 11	60 ± 12	44 ± 11*	58 ± 16*	37 ± 11*	17.9, 0.00
%C.I.	68 ± 18	66 ± 12	56 ± 11*	46 ± 18*	42 ± 14*	10.0, 0.00
NBT	88 ± 28	72 ± 20*	57 ± 16*	52 ± 18*	54 ± 16*	12.6, 0.00
ClC(mg/dL)	6.2 ± 4	10 ± 4*	18 ± 10	19 ± 9	14 ± 6*	29.3, 0.00

p < 0.05 is significant

** Significantly different from the controls.*

had least stimulated NBT reduction index while those in 1st trimester had highest NBT reduction index (Table 2). The level of CICs were high in the test mothers compared with the controls. Pregnant women in 3rd trimester had highest CIC level followed by those in 2nd trimester and least in 1st trimester (Table 2).

Discussion

The resuls of this study shows that pregnancy is immunologically dynamic particularly when the parameters in the 1st and 3rd trimesters are compared. The %B-cell was highest in the 3rd trimester and least in 1st trimester while %T-cell was highest in 1st trimester and least in 3rd trimester. This shows that early pregnancy favours cell-mediated immunity while late pregnancy favours humoral mediated immunity. This is in support of a previous observation that immune status during early pregnancy is dominated by Th 1 cytokines (inducers of cell mediated immunity) but this is replaced by Th 2 cytokines (inducer of humoral mediated immunity) afterwards.[5] The implication is that at early stage of pregnancy, women will be resistant to intracellular pathogens and other pathogens that are susceptible to leucocyte attack. On the other hand, at late stage of gestation, women will be resistant to pathogens and toxins that are readily resolved by antibodies. Our observations may form the basis for previously observed[6,7] increased susceptibility of pregnant women to *Plasmodium* and *Leishmania* parasites as gestation increases.

Cell mediated immunity (leucocyte engulfing and generation of reactive O_2) which is important in the defence against intracellular parasites was highly depressed in 3rd trimester and at birth. The depressed cellular immunity may also be protective to foetal rejection. Predominant humoral immunity experienced at late stage of pregnancy explained depressed leucocyte migration during 2nd and 3rd- trimesters of pregnancy and in women after child birth. Gleicher et al[8] showed that a specific IgG fraction was responsible for the activation of cells involved in leucocyte migration and that in excess of these antibodies and its corresponding antigens, inhibition of leucocyes migration was observed. Thus reduced leucocyte migration at late pregnancy and at childbirth might have been caused by a state of antibody excess since over production of IgG had been demonstrated in mothers at birth due to influence of pregnancy.[9] The implication is that beneficial antibodies capable of preventing foetal rejection but protective against pathogens are produced during pregnancy.

Mechanisms other than IgG such as complement components, interleukins and lymphokines are also involved in the migration process. Leucocyte migration inhibitory factor (LMIF) is a lymphokine which prevents random migration of leucocytes away from point of infection or inflammation, therefore in vitro estimation of LMIF correlates with in vivo state of delayed hypersensitivity of lymphocyte donor.[5] Depressed leucocyte migration might indicate increased secretion of LMIF by T-lymphocytes which were higher in the peripheral blood of pregnant women and mothers at child birth than non-pregnant controls. The reason for rise in T- and B- lymphocytes of test subjects might be a relative lack of inhibitory factor which masks the surface membrane receptors for E- or EAC - rosette in plasma of parturients. Inhibitory substances to E- or EAC- rosette (i.e. T- or B- lymphocyte count respectively) were detected and characterized in other conditions and some healthy individuals.[19,20] There was a report of increase in lymphoid population of para-aortic lymph nodes during allogeneic pregnancy.[11]

Pregnancy is a state of natural transplantation in which foetus is not recognised as foreign in the mother. There has been much interest in suppression of cellular immunity during pregnancy[10] and this is thought to be the possible basis for foetal acceptance. PMNL are involved in hyperacute graft rejection[13] and it seems plausible that they, too, would be suppressed during pregnancy. Indeed, the present study supported this concept since we demonstrated depressed engulfing and reduced killing ability of PMNL.

Phagocytic ability of PMNL represented by candidacidal index was depressed in mothers during pregnancy and child birth but El-Maallem and Fletcher[14] reported normal phagocytosis during pregnancy. Also, there is a discrepancy between the present finding of reduced intracellular killing as shown by low stimulated NBT index and the report that chemiluscence or free oxygen radical are increased during pregnancy.[3] However, our result is in line with that of Persellin and Leidfarth[18] who showed that phagocytosis is inhibited by undefined pregnancy serum factor so as to prevent adverse pregnancy reaction as in preemclapsia. The end-stage of

pre-emclapsia involves increased phagocytic activity leading to excessive free radical generation and tissue injury, paralleled by platelet activation and coagulation derangement.[2, 15] Reduced candidacidal index and intracellular killing observed in mothers during pregnancy and at child birth shows that abnormalities in leucocyte functions occurred at both receptor (binding) and post receptor (generation of H_2O_2) levels.

Increased number of total WBC, T-lymphocytes, B-lymphocytes, reduced %M. I., NBT reduction index and candidacidal index observed in test subjects are similar to changes in parasitic infections or chronic granulomatous disease[5,14,16], therefore suggesting a common underlying mechanisms. Although, reduced leucocyte phagocytosis may contribute to susceptibility of pregnant women to pathogens, it also explains the observed amelioration of rheumatoid arthritis in pregnancy since rheumatoid arthritis is a disease in which the synovial damage is stimulated by the over activity of PMNL[14]. CICs were high in pregnant women particulary in 2nd - and 3rd - trimester compared with the controls. This supports the findings of Clare-Michele et al[19] that advanced pregnancies lead to production of anti-H-Y antibodies against H-Y minor histocompatibility antigens of the foetus, thus combination of these antigens and antibodies in successive pregnancy coupled with inadequate removal from circulation by phagocytes will lead to increased formation of immune complexes as observed in the present study. The findings of this study supports susceptibility of pregnant women to infections particularly those at the late stage of pregnancy.

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