PRELIMINARY COMMUNICATION

DIFFERENCES IN SURFACE-IgD OF B-LYMPHOCYTES FROM MATERNAL PERIPHERAL, MATERNAL RETROPLACENTAL AND FOETAL CORD BLOOD

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SYNOPSIS

Lymphocytes from maternal retroplacental area may be different from maternal peripheral blood. To confirm this, blood from the maternal retroplacental area, maternal cubital vein and fetal cord were collected at delivery from seven normal uncomplicated pregnancies, and the B- and T-lymphocytes were incubated with fluorescein-conjugated rabbit anti-human IgD. The fluorescence of B-lymphocytes, after correction for background T-lymphocyte fluorescence, was found to be 1.63 ± 1.69 (mean ± S.D.) per 100 lymphocytes from the retroplacental area, 3.98 ± 1.68 from peripheral blood, and 10.23 ± 3.62 from fetal cord ($p < 0.01$ to < 0.001 amongst the 3 groups).

INTRODUCTION

The majority of studies of lymphocytes in pregnant women have been carried out on peripheral blood. However, this may not reflect the situation occuring at the feto-maternal interface. It has been reported by Fuchs et al (1) that the PHA-mitogenic response of T and B lymphocytes collected from uterine blood was significantly suppressed as compared to those simultaneously collected from the peripheral blood. Uterine blood was collected from 9 women in the first trimester of pregnancy after creating a bleed in the extra-amniotic space by injection of isotonic saline through a catheter.

To our knowledge no work has been done on the surface immunoglobulins of lymphocytes collected from the retroplacental area. We have compared surface immunoglobulin D(s-IgD) in lymphocytes from the retroplacental area, with those from maternal peripheral venous blood and fetal cord blood.

MATERIAL AND METHODS:

Blood was collected from seven healthy women (5 Chinese and 2 Malay) with uncomplicated pregnancies and who had normal singleton delivery at term. The mean gravidity was 3, and the mean age was 26 years.

Blood in three women (9-18ml) was collected from the gush of blood that occurred just prior to the expulsion of the placenta. However, in four patients, this was not possible; instead the blood before clotting was drained from the placental bed after the placenta has been delivered. At the same time, 9ml each of maternal blood from cubital vein and fetal cord blood were collected. All samples were collected in heparinized plastic tubes.

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Whole lymphocytes were separated on ficoll-isopaque gradients (2).

B-lymphocytes enriched population was obtained by rosetting T cells with papain-treated sheep erythrocytes (3). 0.5 x 10^6 washed B-lymphocytes were incubated with 40ul of anti-IgD (fluorescein-conjugated rabbit anti-human IgD, Behringwerke AG) at 1:20 concentration (diluent: Medium 1640 with 20% heat inactivated fetal calf serum), at 4° C for 30 minutes. The lymphocytes were fixed in 4% formalin followed by three washes with phosphate buffered saline (PBS) and resuspended in 50% glycerol.

Staining of the lymphocytes was observed with an American Optics Microstar Series One-Ten fluorescence microscope with a halogen source using a FITC fluor cluster. Staining was considered positive only in the presence of obvious and intense fluorescence. Parallel experiments were done with T-lymphocytes from the same samples to assess the background fluorescence.

Statistical analyses were carried out with paired t-tests.

RESULTS:

B lymphocytes-enriched population which do not form rosettes with sheep RBC constituted 30.40% ± 7.69 (mean ± S.D.) of lymphocytes in maternal peripheral blood, 40.30% ± 12.13 in maternal retroplacental blood, and 38.37% ± 13.14 in fetal cord blood. The differences were not statistically significant amongst the three groups.

As shown in Table 1, staining of B-lymphocytes was found in 2.14 ± 1.35 (mean ± S.D.) per 100 lymphocytes from the retroplacental area, compared to 4.82 ± 1.38 from the maternal peripheral blood; this difference is statistically significant at 0.5% level.

Background T-lymphocyte staining for s-IgD was found to be low (Table 2), with no statistical differences amongst the three groups.

After correction for background fluorescence (Table 3), B-lymphocytes staining was still significantly lower for maternal retroplacental lymphocytes than lymphocytes from maternal peripheral and fetal cord. Contamination of retroplacental blood by maternal peripheral and fetal blood at the time of collection was minimal as evident by the results.

DISCUSSION

Functional studies of uterine lymphocyte subpopulations in response to mitogenic stimulation have been done in man and animals. The earliest report in man was by Fuchs et al (1). Yamamoto et al (4) reported marked reduction in lymphocyte reactivity of ovarian and uterine venous blood from a 43 year old multiparous woman with a corporeal myoma and cornual pregnancy at 11 week's gestation, as compared with that of basilic venous blood; reduction in reactivity was more prominent for concanavalin A (Con A) – induced lymphocyte transformation than for phytohemagglutinin (PHA) – induced lymphocyte transformation. These mitogens test T-lymphocyte reactivity (5) and add support to the importance of local lymphocyte subpopulations.

However, related studies with animal models showed conflicting results. In the mouse, Anderson (6) reported that lymphocytes collected from lymph nodes that drained the fetal implantation site were not significantly different from lymphocytes collected at distal sites; the lymphocytes were stimulated in vitro with PHA, Con A and Escherichia coli lipopolysacharide (LPS). In the ewe, Miyasaka and McCullagh (7) reported that maternal lymphocytes returning from the uterine circulation were not functional different from lymphocytes in the general circulation, as indicated by mixed-lymphocyte cultures and stimulation with Con A and LPS. The absence of functional lymphocyte difference in

TABLE 1: B-LYMPHOCYTES STAINED WITH ANTI-IgD ANTISERUM IN VARIOUS BLOOD SAMPLES

Subjects	Maternal cubital			Maternal retroplacental			Fetal Cord		
	Total counted	No stained	%	Total counted	No stained	%	Total counted	No stained	%
1	206	9	4.37	226	4	1.77	262	29	11.07
2	239	12	5.02	243	5	2.06	221	20	9.05
3	130	7	5.38	185	2	1.08	170	17	10.00
4	173	7	4.05	252	6	2.38	188	29	15.43
5	193	5	2.59	179	3	1.68	177	26	14.69
6	345	18	5.22	293	3	1.02	254	45	17.72
7	266	16	7.08	300	15	5.00	241	20	8.30
Total	1512	74		1678	38		1513	186	
Mean per 100 lymphocytes @	4.82			2.14			12.32		
± Standard deviation	±1.38			±1.35			±3.61		

@ $P < 0.005$ between maternal cubital and fetal cord B-lymphocytes; and between maternal cubital and maternal retroplacental B-lymphocytes.
$P < 0.001$ between maternal retroplacental and fetal cord B-lymphocytes.

TABLE 2: T-LYMPHOCYTES STAINED WITH ANTI-IgD ANTISERUM IN VARIOUS BLOOD SAMPLES

Subjects	Maternal cubital			Maternal retroplacental			Fetal Cord		
	Total counted	No stained	%	Total counted	No stained	%	Total counted	No stained	%
1	26	0	0	98	0	0	17	0	0
2	96	3	3.1	101	2	2.0	83	4	4.8
3	36	0	0	44	0	0	30	0	0
4	86	0	0	61	0	0	99	1	1.0
5	103	1	0.97	118	1	0.84	101	0	0
6	112	1	0.89	134	1	0.75	112	10	8.93
7	115	1	0.87	99	0	0	128	0	0
Total	574	6		655	4		570	15	
Mean per 100 lymphocytes	0.83 n.s.			0.51 n.s.			2.10 n.s.		
± Standard deviation	±1.10			±0.76			±3.48		

n.s. = Differences not statistically significant amongst the 3 groups

TABLE 3: B-LYMPHOCYTES STAINED WITH ANTI-IgD ANTISERUM AFTER CORRECTION FOR BACKGROUND FLOURESCENCE

Subjects	Maternal cubital (%)	Maternal retroplacental (%)	Fetal Cord (%)
1	4.37	1.77	11.07
2	1.92	0.06	4.35
3	5.38	1.08	10.00
4	4.05	2.38	14.43
5	1.62	0.84	14.69
6	4.33	0.27	8.79
7	6.21	5.00	8.30
Mean per 100 lymphocytes @	3.98	1.63	10.23
± Standard deviation	±1.68	±1.69	±3.62

@ $P < 0.01$ between maternal cubital and fetal cord B-lymphocytes; $P < 0.005$ between maternal cubital and maternal retroplacental B-lymphocytes; $P < 0.001$ between maternal retroplacental and fetal cord B-lymphocytes.

lymphocytes draining the uterus may be due to regeneration of s-IgD which would occur in a short period of time after its removal from the trophoblast. In Anderson's study, this occurred while at the regional lymph nodes, and could account for the germinal aplasia in such lymph nodes as reported by Nelson and Hall (8) in the human. In Miyasaka and McCullagh's study, the lymphocytes were cryopreserved, with regeneration of surface-immunoglobulins occurring post-thaw; it may be necessary for trophoblast to be present during the regeneration for maintenance of local responses of such lymphocytes, such as diminished s-IgD.

Our results show that the B-lymphocytes from the retroplacental area have a decrease in s-IgD staining when compared to B-lymphocytes collected simultaneously from the peripheral blood. It has been suggested by Cambier et al (9) that loss of s-IgD from mature B lymphocytes is directly correlated to an increase in susceptibility to the induction of tolerance. This loss of s-IgD may be due to proteolytic enzymes (10) or to induction by trophoblast, either by direct contact or by substances secreted from it. Further work on the role of the fibrinolytic system in relation to possible immunotolerance or immunosuppression in lymphocytes is being done in our laboratory.

CONCLUSION:

We emphasise the importance of studies on retroplacental lymphocytes in order to understand better why the fetus is accepted as an allograft; this source of blood has previously been ignored. Our study shows that lymphocytes from the maternal retroplacental area have diminished s-IgD at term as compared with maternal peripheral blood. This finding complements other reports in man of diminished reactivity of lymphocytes collected from the local circulation of the uterus.

More work needs to be done in order to determine the role and properties of retroplacental lymphocytes.

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