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Human CD5$^+$ B lymphocytes (B-1 cells) decrease in peripheral blood during pregnancy

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Abstract

Pregnancy is a unique immunologic state where a natural homeostasis exists between antigenically different tissues. Several earlier studies have addressed the fluctuations in the number and/or function of lymphocytes, including B cells during pregnancy, but changes within the subsets of B lymphocytes, conventional (CD5$^-$) and B-1 (CD5$^+$), have not been addressed. Here we demonstrate that the frequency of B-1 cells decreases dramatically during pregnancy, whereas the frequency of conventional B cells remains relatively constant. The missing B-1 cells return to pre-pregnancy levels 8–10 weeks after parturition. The polyreactive autoantibodies secreted by B-1 cells have been implicated in autoimmunity and immune regulation. The possible role of B-1 cells during pregnancy will be discussed in that context.

Keywords: Human pregnancy; B lymphocytes

1. Introduction

Human CD5$^+$ B cells, which are now called B-1 cells (Kantor, 1991), predominate early in B cell ontogeny (Antin et al., 1986). Greater than ninety percent of B cells in human fetal spleen and cord blood are B-1 cells (Bhat

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et al., 1992). From infancy through childhood, the percentage of B-1 cells gradually diminishes in both spleen and peripheral blood.

Stable B-1 cell adult levels, 10–45% of total B cell population and 1–8% of total peripheral blood lymphocytes (PBL), are reached in late adolescence (Gadol et al., 1986; Bhat et al., 1992). Although there is heterogeneity among unrelated individuals, the percentage of B-1 cells of total B cells within a single individual remains constant in normal adults tested repeatedly for over one year (Kipps et al., 1987; our unpublished observations).

Surprisingly, however, we find that the frequency of B-1 cells decreases during pregnancy and returns to pre-pregnancy levels at about 8–10 weeks post-partum. Conventional B ($CD5^-$) cells, in contrast, do not change.

2. Materials and methods

2.1. Cells

Heparinized peripheral blood samples were obtained from 18 pregnant and 15 non-pregnant control females aged 20–35 years, with the approval of the Committee for the Protection of Human Subjects at Stanford University. The 18 pregnant women selected for the study had normal healthy pregnancies with no complications. Of the 18, 9 were tested again 2–5 months post-partum, and of the 15 control women, one was monitored every 4–10 weeks pre-pregnancy, during pregnancy and 10 months post-partum.

The specimens were diluted 1:3 in phosphate buffered saline (PBS), and layered on a ficoll-hypaque gradient (Histopaque-1077, Sigma Diagnostics, St. Louis, MO). The mononuclear cells were washed three times in PBS, and resuspended in staining medium (RPMI with 3% FCS, 1 mM EDTA, and 10 mM HEPES) at 2.5×10^7 cells/ml. All samples were analyzed within 24 h.

2.2. Antibodies

Antibodies to the pan B cell antigen CD20 (fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)-conjugated anti-Leu-16) were obtained from Becton Dickinson Immunocytometry systems (San Jose, CA). Allophycocyanin (APC)-conjugated anti-Leu-1 (CD5, a pan T-cell antigen) was prepared and standardized for the detection of human B-1 cells ($CD5^+$ B) as previously described (Bhat et al., 1992). The gradation of expression of CD5 on human B cells makes their enumeration rather difficult. It is therefore mandatory to use a single well-standardized reagent throughout the study. The APC-conjugated reagent gave the best resolution from the six different conjugates of mouse anti-human Leu-1 (CD5), including PE- and FITC-labelled reagents, that were studied (Bhat et al., 1992).

2.3. Flow cytometry

Multi-parameter flow cytometric analysis has been described in detail

(Parks et al., 1986). In brief, 5×10^5 cells were suspended with predetermined saturating concentrations of each of the conjugated antibodies in a final volume of 125 μl, and incubated on ice for 15 min. The cells were washed and resuspended in 200 μl of staining medium and analyzed on a highly modified dual-laser FACS II (Becton Dickinson, Mountain View, CA), interfaced with a VAX 6300 computer (Digital Equipment, Maynard, MA) running FACS/desk software (Moore et al., 1986). In order to ensure reproducibility, the sorter is calibrated for each experiment with standard polystyrene microspheres (Pandex), with well defined scatter and fluorescence in all channels.

2.4. Data and statistical analysis

During pregnancy maternal blood volume increases by an average of 40% above non-pregnant levels, but individual variations are wide. This inconsistent hemodilution made the comparison of absolute number of mononuclear cells in pregnant and non-pregnant women very difficult. We thus present our data as percent within a well defined population of mononuclear cells (total B cells, or total mononuclear cells excluding monocytes). Scatter gates were used to exclude monocytes, since monocytosis, a well defined phenomena during pregnancy, would skew the calculated percentages of B cells (Plum et al., 1978; Siegel and Gleicher, 1981; Sridama et al., 1982; Moore and Carter, 1983; Valdimarsson et al., 1983; Bailey et al., 1985). Each sample was analyzed for the presence of T and B lymphocytes within the monocyte scatter. $CD5^+/CD20^-$ cells (T cells) and $CD20^+$ cells (B cells) were rarely present within the monocyte gates, in both pregnant and non-pregnant control samples. The box in Fig. 1 shows the gates used to enumerate $CD5^+$ B cells. Statistical analysis was performed by non-parametric Kruskall-Wallis test for the cross-sectional and serial data, and by the paired one-sample Student's *t*-test for the longitudinal data set.

3. Results

The decrease in B-1 cells early in pregnancy, and the rapid return post-pregnancy, is dramatically illustrated in the serial multi-parameter FACS analysis of PBL shown in Fig. 1. This normal individual is an ideal subject for visualizing the pregnancy-related B-1 decrease, since her (non-pregnant) B-1 cell frequency (8% of total PBL and 43% of total B cells, i.e., B-1 + conventional B) is higher than the B-1 frequencies observed in most adults (1–8% of total PBL and 10–45% of total B cells). However, as the longitudinal and cross-sectional analysis presented here shows, B-1 cell frequencies decrease during pregnancy in all individuals, regardless of their frequencies of B-1 cells.

In the individual shown in Fig. 1, the percentage of B-1 cells among total

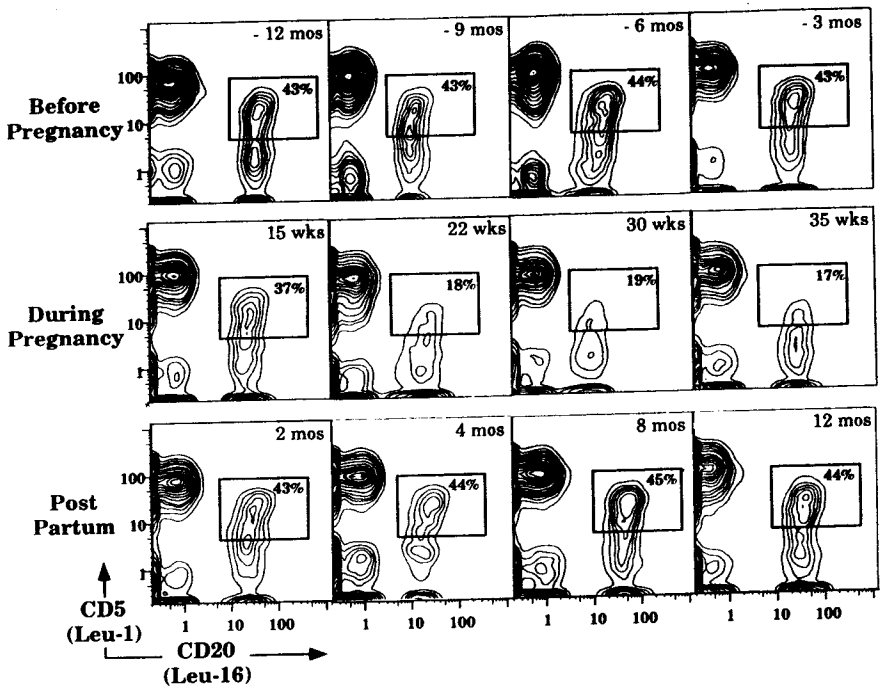

Fig. 1. Frequency of B-1 cells decreases during pregnancy ($P = 0.005$, Student's t-test) and returns to pre-pregnancy levels as first tested eight weeks post-partum. Immunofluorescence profiles of mononuclear cells stained with anti-Leu-1 (CD5, a pan T cell marker), and anti-Leu-16 (CD20, a pan B cell marker) are shown. FACS contours plots in the top panel are from serial pre-pregnancy analysis, the middle panel during pregnancy and the bottom panel from post-partum analysis of a single individual. Numbers on the top right corner are the months before and after pregnancy and the weeks of gestation when the samples were analyzed. The percentage of B-1 cells of total B lymphocytes ($CD20^+$ cells only) is shown in the boxes used as gates for the calculation. Similar gates were used for the calculation of B-1 cells presented in Table 1 and Fig. 2. Changes in the frequency of conventional B cells pre-pregnancy, during pregnancy, and post-partum were not statistically significant.

B cells gradually dropped from 43 to 18% in the first 15–20 weeks of pregnancy, and was maintained at this level through the rest of the pregnancy. However, by eight weeks post-partum, the B-1 frequency in this individual had already returned to the pre-pregnancy level. Furthermore, it remained stable at this level when tested every month for over one year post-partum (Fig. 1), and remained at that level when tested again 2 years post-partum (data not shown).

A cross-sectional analysis of 18 pregnant (11–38 weeks gestation) and 15 non-pregnant age-matched women also demonstrates that the frequency of

Table 1
B-1 cells decrease during pregnancy (cross-sectional analysis)

	Percentage in PBL mean (range)				% B-1 Cells of total B cells mean (range)
	$CD5^+$ B cells	$CD5^-$ B cells	$CD5^+$ and $CD5^-$ B cells	T cells	
Pregnant ($n = 18$)	1.1 (0.07–3.4)	7 (2.5–12.7)	8.2 (3.2–13.6)	76 (63–83)	14.6 (1.5–32.7)
Control ($n = 15$)	2.7 (0.7–8.8)	7.7 (4–13.7)	10.5 (5.2–20.4)	72 (50–87)	23 (10.2–43.1)
P*	0.03	NS	NS	NS	0.03

*Kruskall-Wallis test.

B-1 cells decreases significantly during pregnancy ($P = 0.03$, Kruskall-Wallis test, Table 1) while the frequency of conventional B cells and total B cells (B-1 + conventional B) does not change ($P > 0.05$). The failure to detect a significant change in total B cells despite the decrease in B-1 cells during pregnancy is due to the higher frequencies of conventional B cells in PBL of most individuals (on an average 4–10 times higher than B-1 cells). Because of this offside ratio, changes in frequencies of B-1 cells usually have little impact on the frequencies of total B cells.

Results of our cross-sectional analysis are in agreement with earlier studies that also report no significant change in total B cells during pregnancy (Dodson et al., 1977; Birkeland and Kristoffersen, 1979; Sridama et al., 1982; Castilla et al., 1989). However, two studies do report a statistically significant decrease in total B cells in a cross-sectional analysis of pregnant and age-matched control women (Valdimarsson et al., 1983; Bailey et al., 1985). These findings could be explained if the population sampled in the latter studies happened to be composed of individuals in whom B-1 cells represented a larger fraction of total B cells. This potential source of variation within experiments, introduced by sampling methods, dictates the necessity for obtaining longitudinal data.

We thus obtained longitudinal data for 9 of the 18 pregnant women included in our cross-sectional analysis. There was a statistically significant decrease in the frequency of B-1 cells of total lymphocytes and of total B cells in each of the nine individuals during pregnancy compared to their post-partum levels ($P = 0.001$, one-sample Student's t-test, Fig. 2). In contrast to B-1 cells, frequencies of conventional B cells did not change significantly dur-

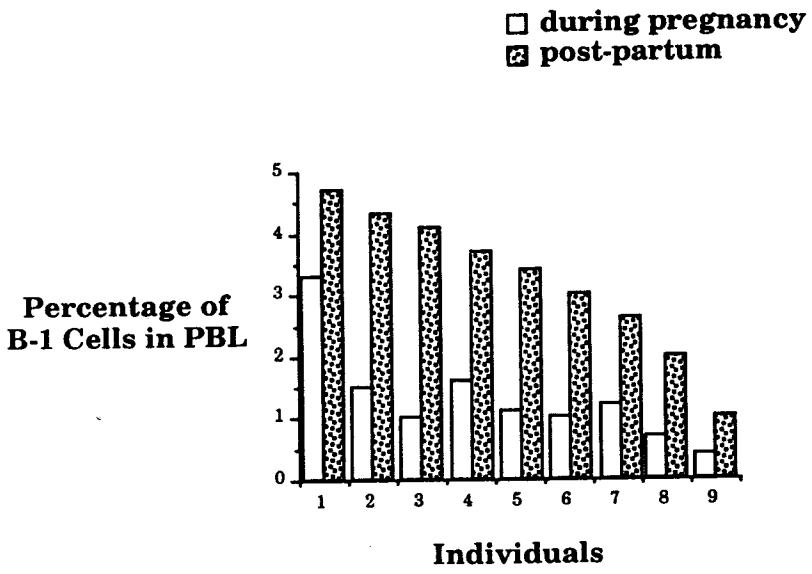

Fig. 2. Percentage of B-1 cells in PBL decreases during pregnancy in all nine pregnant individuals studied ($P = 0.001$, one sample Student's t-test). The gestational ages of the pregnant women ranged from 22 to 36 weeks, and post-partum analysis was obtained between 2 to 5 months.

ing or after pregnancy in any of the nine women tested ($P > 0.05$, one-sample Student's t-test, data not shown).

Of the nine, three individuals were tested 4, 15, 30, 60 and 90 days post-partum, to more closely determine when B-1 frequencies return to adult levels. In all three individuals, the frequency of B-1 cells did not increase until 8–10 weeks post-partum (data not shown). Another three of the nine individuals were also analyzed 1 year post-partum. All three showed B-1 frequencies similar to the frequencies obtained 2–5 months post pregnancy (data not shown), i.e., their post-partum frequencies reflected the stable adult levels.

4. Discussion

This is the first study demonstrating pregnancy-related changes in subsets of mature human B lymphocytes. A recent study in mice has demonstrated that although mature B cells in murine spleen do not change, pregnancy and estrogen-treatment leads to a selective reduction of B cell precursors in the pregnant marrow (Medina et al., 1993). Our preliminary analysis of pregnant

mice concurs with the above study, demonstrating no changes in B-1 or conventional B lymphocytes in murine spleen or peritoneal cavity (data not shown). This failure to detect changes in mature B cells in mice may be due to the much shorter gestational period (19–21 days) compared to humans (280 days), since the reported lifespans of B cells in mouse and man are not very different (Mota, 1981; Freitas and Rocha, 1993).

B-1 cells are the main producers of the polyreactive autoantibodies that are readily detected in the sera of healthy individuals (Digherio et al., 1986). In fact, an earlier study in mice has demonstrated an increase in the production of autoantibodies by estrogen-treated B-1 cells, but again no change in the numbers of B-1 cells (Ahmed et al., 1989). Thus, B-1 cells may be important cellular targets for the immune modulation mediated by sex hormones (Talal, 1989).

It has been suggested that natural autoantibodies produced by B-1 cells may maintain the dynamics of the immune response through a network of idiotypic and anti-idiotypic interactions (Holmberg and Coutinho, 1985). It is thus tempting to speculate that the decrease in mature circulating B-1 cells during human pregnancy may be one of the mechanisms responsible for maintaining the immunological balance involved in preventing rejection of the fetal allograft. Further studies, such as prolonged exposure of B cells to pregnancy-related hormones, and its effect on plasma cell formation and immunoglobulin/cytokine production may help in understanding the role of B-1 cells in reproductive immunology.

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