

DECREASED LEVELS OF HELPER T CELLS

A Possible Cause of Immunodeficiency in Pregnancy

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DECREASED maternal immune responsiveness during pregnancy may partly explain the survival of the fetus as an allograft. It may also account for changes in disease activity and antibody production in several autoimmune diseases during pregnancy and in the post-partum period. Previous *in vitro* studies of cellular immunity in pregnant women have demonstrated decreased lymphocyte responses to phytohemagglutinin,¹ to mixed lymphocyte culture,¹ and to soluble antigens.² An increased incidence and severity of certain viral infections has also been observed.^{1,3} An imbalance between helper and suppressor T cells has recently been demonstrated in several autoimmune diseases^{4,5} and in immunodeficiency states,⁶ in studies using monoclonal antibodies that react against subsets of T cells. To determine whether abnormalities in T-cell subsets might help to explain the immunodeficient state in pregnancy, we used monoclonal antibodies to enumerate the proportion and absolute number of subsets of T cells and other peripheral-blood mononuclear cells from normal pregnant women, post-partum women, and newborns. We found a significant decrease in relative and absolute numbers of helper T lymphocytes (T4+ cells) throughout pregnancy. Normalization of T4+ cells occurred during the third to fifth month post partum. Our findings support previous evidence of maternal immunodeficiency during pregnancy and indicate that it may occur because of a reduction in the population of helper T cells.

METHODS

Subjects

The study groups included 76 normal pregnant women in various stages of pregnancy (eight in the first trimester, 15 in the second, 10 in the third, and 33 in labor), 13 post-partum women (eight in the first or second month and five in the third to fifth month post partum), and 15 normal newborns. Thirty-four normal adult volunteers (12 men and 22 women), who had no history of taking hormones or other medications, were used as controls for studies of cord blood. Nineteen normal, age-matched, healthy female volunteers from this group were also used as controls for the pregnant women.

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Preparation of Peripheral-Blood Mononuclear Cells

Heparinized peripheral blood (20 ml) was fractionated by the standard Ficoll-Hypaque gradient technique.

Monoclonal Antibodies

Murine monoclonal antibodies termed OKT3, OKT4, OKT8, OKM1, and OKIa (Ortho Pharmaceutical, Raritan, N.J.) were used in this study. Functionally, OKT3 defines all peripheral T cells,⁶ OKT4 defines the human helper/inducer T-cell subset,⁷ OKT8 defines the suppressor/cytotoxic T-cell subset,⁸ OKM1 defines the monocyte/null-cell population,⁹ and OKIa defines B cells, monocytes, and activated T cells.⁶

Procedures

For each subject, 1×10^6 peripheral-blood mononuclear cells per tube were pelleted in five separate tubes, resuspended in 50 μ l of the appropriate dilution of OKT3, OKT4, OKT8, OKM1, or OKIa, and incubated for 30 minutes on ice. After two washes with Ham's F-10 medium (Microbiological Associates, Walkersville, Md.) containing 10 per cent heat-inactivated fetal-calf serum (Grand Island Biological, Grand Island, N.Y.), they were incubated for 30 minutes on ice with a 1:30 dilution of a fluorescein-conjugated IgG fraction of goat antimouse IgG (heavy-chain and light-chain) (Cappel, West Chester, Pa.) that had previously been adsorbed with human IgG; they were then washed three times, fixed for 10 minutes in 4 per cent paraformaldehyde, mounted on microscopical slides, and examined under ultraviolet light by means of epifluorescence microscopy. For each slide, approximately 200 cells were counted. All analyses were performed without knowledge of the source of the specimen. Mouse IgG was used instead of monoclonal antibody to determine background staining. Uniformly less than 1 per cent of cells showed fluorescent staining with this serum.

Surface-membrane immunoglobulin-positive cells were enumerated with fluorescein-conjugated polyvalent goat antihuman immunoglobulin (Bionetics, Kensington, Md.), as previously described.⁹ Before staining, monocytes were labeled by incubation of cells in 0.02 per cent uniform latex particles in F-10 medium with 10 per cent fetal-calf serum for 60 minutes at 37°C. Latex-ingesting cells were examined by phase-contrast microscopy. Approximately 200 cells were counted on each slide.

The white-cell count and the differential count were used to calculate the number of positive cells per cubic millimeter.

Statistics

Student's two-tailed t-test was used to determine significance.

RESULTS

As shown in Figure 1 (top panel) and Table 1, the percentage and absolute number of T4+ cells were significantly decreased in normal pregnant women throughout pregnancy and during labor. The abnormalities were most obvious among the women in the third trimester (29.1 ± 6.1 per cent and 543 ± 169 cells per cubic millimeter; $P < 0.01$), as compared with the normal female controls (45.1 ± 6.1 per cent and 1073 ± 441 cells per cubic millimeter). The percentage of T4+ cells was still decreased in the first and second months post partum (34.3 ± 8.4 per cent; $P < 0.01$) and normalized during the third to fifth month post partum (39.6 ± 2.9 per cent), whereas the absolute number was already normal in the first and second months post partum (883 ± 331 cells per cubic millimeter). The percentage and absolute number of T cells (T3+ cells) (Fig. 2 and Table 1) were significantly decreased in the second and third trimesters of pregnancy. The lower

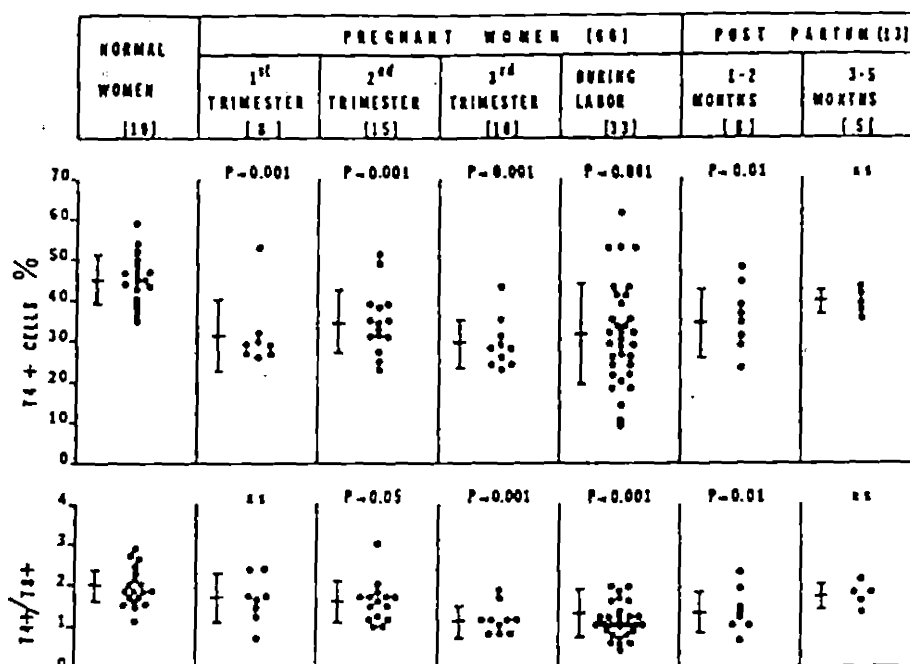


Figure 1. Mean Percentage of T4+ Cells (Helper T Cells) and the T4+/T8+ Ratio in Normal Controls, Pregnant Women and Post-Partum Women.

Bars represent S.D.; figures in brackets, the numbers of subjects; and ns, no significant difference.

values were observed in the third-trimester group (50.3 ± 4.9 per cent and 1052 ± 312 cells per cubic millimeter; $P < 0.01$), as compared with the normal female controls (68.2 ± 8.7 per cent and 1652 ± 627 cells per cubic millimeter). When the data were expressed as the T4+/T8+ ratio (Fig. 1, bottom panel), a significant difference was found in the second and third trimesters, during labor, and one to two months after delivery. The lowest ratio occurred in the third trimester (1.1 ± 0.4 ; $P < 0.001$), as compared with normal controls (2.0 ± 0.6). Normalization of the ratio took place during the third to fifth month post partum (1.7 ± 0.3). The percentage and absolute number of suppressor/cytotoxic T cells (T8+ cells) (Table 1) were not significantly different from those in normal female controls.

There was a significant increase in the percentage of B cells at the time of labor (9.5 ± 4.9 per cent; $P < 0.05$), as compared with normal controls (7.0 ± 2.6 per cent) (Fig. 3). No significant change in the absolute number of B cells was found throughout pregnancy (Table 2).

As shown in Figure 3, the percentage of monocytes (M1+ cells or latex-ingesting cells or both) was significantly increased throughout pregnancy, with maximally increased M1+ cells at the time of labor (30.3 ± 14.0 per cent and 759 ± 415 cells per cubic millimeter), as compared with normal controls (18.4 ± 8.0 per cent and 469 ± 223 cells per cubic millimeter). The absolute number of cells bearing Ia antigen (Ia+ cells) did not significantly change, and the percentage

Table 1. Absolute Numbers of T3+, T4+, and T8+ Cells in Peripheral-Blood Mononuclear Cells from Normal Controls, Pregnant Women, and Postpartum Women.

	No. of Subjects	Mean Age (Range) yr	T3+ Cells *	T4+ Cells *	T8+ Cells *
				cells/mm ³	
Normal controls	19	28 (20-38)	1652 ± 627	1073 ± 441	568 ± 209
1st trimester	8	26 (20-29)	1209 ± 594	662 ± 203 †	546 ± 408
2d trimester	15	27 (18-34)	1231 ± 309 ‡	736 ± 232 ‡	498 ± 149
3d trimester	10	27 (21-39)	1052 ± 312 †	543 ± 169 ‡	582 ± 293
During labor	33	26 (16-38)	1375 ± 678	737 ± 352 ‡	654 ± 421
1st-2d month post partum	8	27 (19-38)	1502 ± 321	883 ± 311	620 ± 86
3d-5th month post partum	5	33 (29-36)	1731 ± 584	1087 ± 387	723 ± 321

* Means ± S.D.

† $P < 0.01$ as compared with normal controls.

‡ $P < 0.05$ as compared with normal controls.

§ $P < 0.001$ as compared with normal controls.

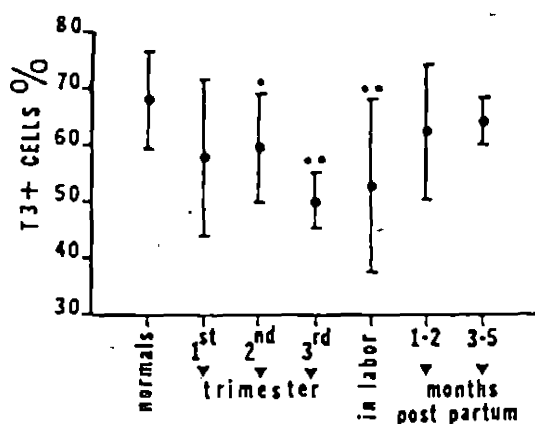


Figure 2. Mean Percentage of T3+ Cells (Total T Cells) in Normal Controls, Pregnant Women, and Post-Partum Women. One asterisk indicates $P < 0.05$, as compared with the mean value in 19 normal women; two asterisks indicate $P < 0.001$. Bars represent S.D. The numbers of subjects are the same as in Figure 1.

changed only in the third trimester (30.5 ± 5.1 per cent), as compared with normal female controls (18.3 ± 5.6 per cent) (Fig. 3 and Table 2).

Studies of cord blood showed significantly decreased percentages of T3+ cells (52.4 ± 16.1 per cent; $P < 0.01$) and T8+ cells (16.7 ± 7.6 per cent; $P < 0.01$) and significantly increased percentages of M1+ cells (29.9 ± 15.4 per cent), Ia+ cells (25.9 ± 8.1 per cent), and B cells (13.6 ± 4.4 per cent; all $P < 0.001$), as compared with normal male and female adult controls (Table 3).

DISCUSSION

Several studies have demonstrated decreased cell-mediated immune responses in normal pregnant women, as measured by in vitro lymphocyte transformation.^{1,2} However, evaluations of the percentage and absolute number of peripheral T lymphocytes in pregnant women have yielded conflicting findings, including no change,¹⁰ a decrease,¹¹ and an increase.¹² This discrepancy in total T-lymphocyte enumeration may be due to variability in erythrocyte-rosetting methods. Moreover, recent evidence indicates that not all cells that are capable of forming erythrocyte rosettes are thymus-processed cells.¹³ In this study, we used a monoclonal antibody⁶ that specifically reacts against almost all T cells (OKT3) and found a significant decrease in the percentage and absolute number of T lymphocytes (T3+ cells) in the second and third trimesters of pregnancy. This decrease in total T cells may be explained by a decrease in the proportion and absolute number of helper T cells (T4+ cells) throughout pregnancy. Our finding supports previous evidence of decreased cell-mediated immune responsiveness during pregnancy, and this mechanism may be important in nonrejection of the fetal allograft by the mother. Decreased immune responsiveness may operate in conjunction with other possible mechanisms,

including the local mechanical barrier,¹⁴ blocking antibodies,¹⁴ and soluble suppressor factors from the fetus¹⁵ — all leading to a successful outcome of pregnancy.

Several autoimmune diseases have been associated with low numbers of suppressor T cells (T8+ cells) and a high ratio of helper cells to suppressor cells. If patients with these disorders become pregnant, a de-

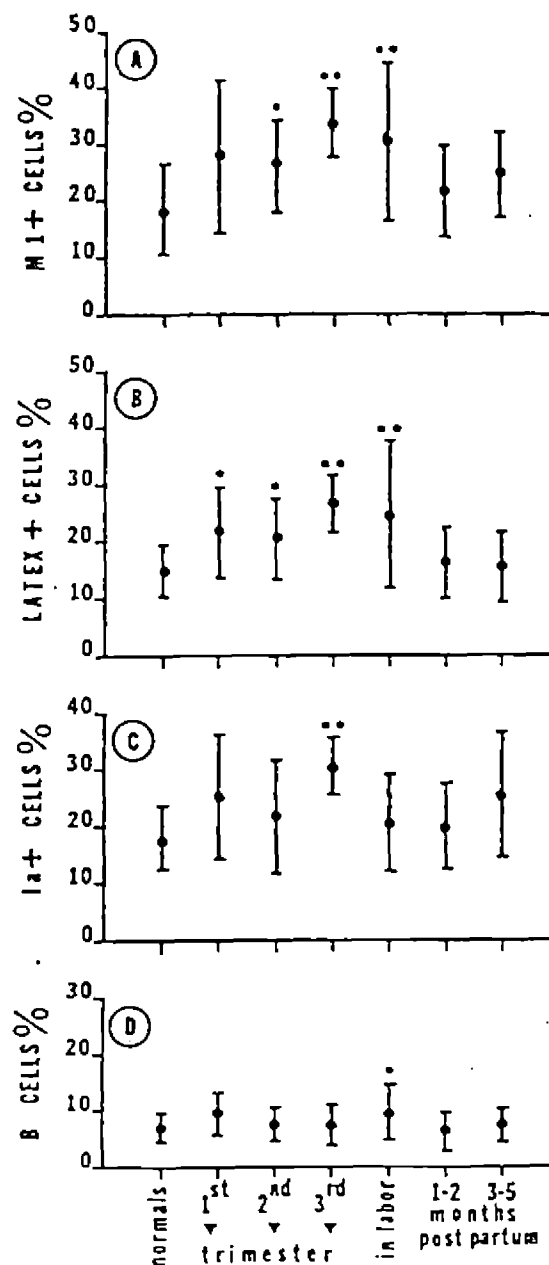


Figure 3. Mean Percentage of M1+ Cells (Panel A), Latex-Ingesting Cells (B), Ia+ Cells (C), and B Cells (D) in Normal Controls, Pregnant Women, and Post-Partum Women.

One asterisk indicates $P < 0.05$, as compared with the mean value in 19 normal women; two asterisks indicate $P < 0.01$. Bars represent S.D. The numbers of subjects are the same as in Table 2.

Table 2. Absolute Numbers of M1+ Cells, Monocytes (Latex-Ingesting Cells), Ia+ Cells, and B Cells in Peripheral-Blood Mononuclear Cells from Normal Controls, Pregnant Women, and Post-Partum Women.

	No. of Subjects	M1+ Cells *	LATEX-INGESTING CELLS *	Ia+ CELLS *	B CELLS *
		cells/mm ³			
Normal controls	13	469 ± 223	375 ± 154	461 ± 200	165 ± 46
1st trimester	8	660 ± 296	491 ± 237	559 ± 143	188 ± 140
2d trimester	15	549 ± 236	426 ± 186	461 ± 232	155 ± 81
3d trimester	10	692 ± 255 †	552 ± 197 †	620 ± 148	159 ± 118
During labor	33	759 ± 415 †	688 ± 489 †	547 ± 211	185 ± 98
1st-2d month post partum	8	676 ± 245	476 ± 218	557 ± 282	189 ± 131
3d-5th month post partum	5	646 ± 247	362 ± 90	690 ± 408	191 ± 81

*Means ± S.D.

†P<0.05 as compared with normal controls.

crease in helper T cells during pregnancy may lead to a lower helper/suppressor ratio and consequently to lower antibody production. This phenomenon may explain the finding of decreased autoantibody production during pregnancy in autoimmune thyroid diseases¹⁶ and systemic lupus erythematosus¹⁷ and may also explain the favorable course and clinical improvement in some cases of systemic lupus erythematosus,¹⁸ rheumatoid arthritis,¹⁹ and Graves' disease²⁰ during pregnancy. However, exacerbation and progression of systemic lupus erythematosus during pregnancy has also been observed.²¹ This may reflect the natural course of systemic lupus erythematosus and a failure of the altered immune status during pregnancy to modify that course. A return to pre-pregnancy levels of helper T cells in the post-partum period may explain the post-partum exacerbation and rebound of autoantibody production in autoimmune thyroid diseases^{16,20} and systemic lupus erythematosus.^{18,21}

Increased rates of abortion and fetal loss have been observed in pregnant women with systemic lupus erythematosus¹⁸ or Graves' disease.²² These findings may be explained in part by a decrease in T8+ cells in these patients,^{4,5} which could lead to higher helper/suppressor ratios than are present in normal pregnant women; a low helper/suppressor ratio is probably important in the maintenance of normal pregnancy. However, other factors may also contribute to the high abortion rate among these patients — for example, uremia, vasculitis, and the hyperthyroid state.

A decrease in T4+ cells may be a consequence of the hormonal changes associated with pregnancy. Human chorionic gonadotropin, estrogen, progesterone, corticosteroids, α -fetoprotein, prolactin, and α -globulin have been shown to have immunosuppressive effects during in vitro studies.¹⁴

Our studies of B cells confirmed the previous finding that there is no change in the absolute numbers of B cells in pregnancy.¹⁰ The increase in the percentage of monocytes that we noted during pregnancy may have been partly due to the decrease in the proportion of T cells. However, there was also an increase in the absolute number of monocytes during pregnancy. The in-

crease in the percentage of Ia+ cells in the third trimester may have been due to the increase in the percentage of monocytes.

Recent studies of subpopulations of T cells by methods identifying Fc receptors²³ demonstrated increased T γ cells and decreased T μ cells (which were thought to be suppressor and helper T cells, respectively) in normal pregnant women. However, several studies have demonstrated that T γ and T μ cells are not homogeneous populations of suppressor and helper T cells.^{12,24} A high proportion of T γ cells react with OKM1, lack T-cell markers (the T3 antigen),²⁴ and have high natural-killer activity.¹³ Our findings suggest that the immunodeficient state in pregnancy is probably due primarily to a decrease in helper T cells (T4+ cells).

Demonstrations of high suppressive activity of neonatal lymphocytes during in vitro studies led to the hypothesis that suppressor factors from the fetus might cause immunodeficiency in pregnancy. Our finding of decreased T8+ cells in the cord blood is in agreement with previous studies²⁵ but argues against that hypothesis. Evidence that T4+ and T8+ cells in cord blood are functionally different from adult T cells²⁵

Table 3. Percentages of Various Cell Types in Mononuclear Cells from Cord Blood of 15 Normal Newborns (Eight Boys and Seven Girls) and from Peripheral Blood of 34 Normal Adult Controls (12 Men and 22 Women).*

CELL TYPE	ADULT CONTROLS	CORD-BLOOD SAMPLES
T3+ (%)	65.4 ± 8.6	52.4 ± 16.1 †
T4+ (%)	43.0 ± 7.5	43.5 ± 15.9
T8+ (%)	24.2 ± 5.4	16.7 ± 7.6 †
T4+/T8+ ratio	1.9 ± 0.6	3.2 ± 2.2 ‡
M1+ (%)	19.5 ± 8.1	29.9 ± 15.4 §
Latex-ingesting (%)	14.6 ± 4.0	18.7 ± 18.5
Ia+ (%)	18.5 ± 6.8	25.9 ± 8.1 §
B cells (%)	7.3 ± 4.0	13.6 ± 4.4 §

*Means ± S.D.

†P<0.01 as compared with adult controls.

‡P<0.05 as compared with adult controls.

§P<0.001 as compared with adult controls.

makes a full interpretation of the results of the cord-blood study impossible.

In conclusion, our finding of decreased helper T cells in normal pregnancy supports the evidence of immunodeficiency in pregnant women and may indicate one mechanism allowing survival of the fetal allograft during normal pregnancy.

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INTRAMUSCULAR OR INTRALIPOMATOUS INJECTIONS?

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VIEWING routine computerized-tomography (CT) scans of the pelvis suggests that in a North American population the majority of injections intended to be intramuscular are actually delivered into fat.

To confirm or refute this impression we studied the thickness of gluteal fat in a hospital population. We found that few female patients and under 15 per cent of male patients do in fact receive an intramuscular injection when a needle of the usual size is inserted into the buttocks. The implications of this finding are discussed below.

METHODS

To learn where the needle is introduced in intramuscular injections, we observed both student and graduate nurses. The classic area of the upper outer quadrant of the buttock was used in all instances by both groups. In simulated injections in which a ball-point pen was used to demarcate the injection site, 12 student nurses scattered their injections on the upper quadrant over a 4.5-cm circle when the patient lay prone and over a 5.6-cm circle when the patient was in an oblique decubitus position. Although the epicenter was the same distance from the iliac crest when the patient was in either of these positions, it lay 3 cm posteriorly when the patient was in a semioblique position. In adult patients who were about to have CT scanning of the pelvis, graduate senior nurses taped a lead shot to the skin to mark the position where they would give the injection. These shots were placed in the same demarcated area at levels varying from the lower border of the fifth lumbar vertebra to the lower part of the second sacral segment. It was found that the thickness of the pre-muscular fat did not vary significantly either vertically or transversely over the likely injection sites selected by both student and graduate nurses. We measured the distance between the skin and muscle at these levels and found negligible differences in the thickness of the layer of fat, although the muscle bulk diminished as the level selected approached the level of the iliac crest.

The gluteal-fat thickness was then measured on the left side of 213 adults (both inpatients and outpatients) scheduled for a CT examination that included the pelvis. We assumed that this sample hospital population could be considered representative of all patients receiving intramuscular injections. The total of pre-muscular fat — both superficial and deep — to the deep fascia at the S1 level was measured in the 213 adults, and body weight was recorded in 123.

RESULTS

At any given weight women had a mean gluteal-fat thickness nearly 2.5 cm greater than men, confirming the obvious fact that women are more steatopygous (Fig. 1). The depth of fat increased until the fourth decade of life and leveled off or diminished thereafter in the overall series of 213 adults.

The needle generally used for intramuscular injections in our hospital is 3.5 cm long. Observation has

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