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## Suppressive effect of decidual leucocytes

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**SUMMARY.** – Among Ficoll-Hypaque separated nucleated cells of the trypsin-dispersed first trimester human decidual tissue, obtained after elective termination of normal ongoing pregnancy, 38% of HLA-DR<sup>+</sup> (probably decidual macrophages), 31% of CD56<sup>+</sup>, 29% of CD2<sup>+</sup> and 10% of CD3<sup>+</sup> cells were found by indirect immunofluorescence. Decidual leucocytes, when added to 4- and 5-days mixed leucocyte reactions (MLR), caused a strong dose-dependent suppression. A similar effect of decidual leucocytes was observed when Mitomycin-C treated decidual leucocytes were added to the 5-day MLR. Decidual leucocytes caused a dose-dependent suppression of syngeneic peripheral blood lymphocyte proliferative response to phytohemmagglutinin-A (PHA). In order to identify the major cell classes of the decidua mediating the suppressive effect, decidual leucocyte fractions (adherent and nonadherent) were cocultivated as immunoregulatory cells with the peripheral blood lymphocytes stimulated by polyclonal mitogens. Both cell populations exerted a potent immunosuppressive effect on PHA induced peripheral blood lymphocyte proliferation. A similar immunosuppressive effect on peripheral blood lymphocytes, after stimulation with PHA, was also observed when CD3 depleted nonadherent decidual leucocytes were added to the culture. This cell population consists mainly of decidual granular lymphocytes – a cell population unique to the first trimester human decidual tissue.

### INTRODUCTION

Fetoplacental unit in mammals which results from allogeneic mating expresses a variety of antigens that may serve as targets for rejection by the maternal immune system. A number of mechanisms have been proposed which allow a successful implantation and development of the fetoplacental unit in a hostile environment of the maternal reproductive system (Rukavina et al 1991, Clark 1985, Wegman 1987, Chaouat 1988). Since the systemic immune responses of the mother remain basically uncompromised, for the sake of her own survival, the local immunoregulatory events at the fetomaternal interface focus most of the investigating efforts. The cells infiltrating pregnancy decidua and originating from the bone-marrow are potential candidates for mediating both specific immune responses and immunosuppression (7, 10, 18, 19).

The immunosuppressive capacity of small non-T, non-B granulated suppressor cells has been emphasized in two murine models of pregnancy with fetal resorption: 1) Mus carolli embryos transferred to Mus musculi mothers, and 2) CBA/J × DBA/2 embryos transferred to CBA/J mothers. In both models there is a deficiency of these suppressor cells (8). In human decidua macrophages, CD3<sup>+</sup>, CD8<sup>+</sup> T lymphocytes and granulated lymphocytes are all candidates for suppressor activity (3), but the role of non-leucocyte populations, such as gland epithelial cells and stromal cells, should be considered.

Here we report the results of *in vitro* immunosuppressive activity of decidual leucocytes and their fractions from early gestational human decidua on the syngeneic peripheral blood lymphocyte alloreactivity and proliferation induced by polyclonal mitogens.

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## MATERIALS AND METHODS

### Preparation of decidual leucocyte samples

Decidual tissue for preparation of single cell suspensions was obtained by curettage from elective pregnancy termination of normally progressing pregnancies at 6–9 weeks from last menstrual period. Decidual tissue was separated from fetal and chorionic products of conception and washed three times in RPMI 1640 (Institute of Immunology, Zagreb). Tissue was minced finely and exposed to trypsin digestion (0.125% Trypsin – DIFCO + EDTA) at 37°C for 90 minutes. The resulting cell suspension, free of tissue debris, was overlaid on Ficol-Hypaque and centrifugated for 20 minutes on 800 × g. The cells at the interface were collected, washed twice and resuspended in RPMI 1640 containing 5% FCS. The cells were counted and the viability was assessed by trypan blue dye exclusion and was always above 95%.

### Fractionation of the decidual leucocytes

Decidual leucocytes were placed in 50 ml plastic culture flasks (Grainer Labortechnik, Germany Nr 690160) in RPMI 1640 supplemented with 5% fetal calf serum - FCS (Gibco), L-glutamine (175 µg/l), thiamulin (100 mg/l), penicillin (500 IU/l), gentamicin (2 mg/l) and streptomycin (60 mg/l). The cells were cultured overnight at 37°C in humidified atmosphere with 5% CO<sub>2</sub> to allow adherence of monocytes and macrophages. Thereafter, the nonadherent decidual leucocytes were collected and the adherent decidual leucocytes were removed by short-term trypsin treatment. The separation of adherent cells was optically controlled under an inverse microscope. The process of trypsinization was stopped by adding 25% normal AB human serum. Cells from both nonadherent and adherent fractions were resuspended in RPMI 1640.

Some samples of nonadherent decidual leucocytes were collected and treated with anti CD3 monoclonal antibody (OKT-3, Ortho Pharmaceutical; 20 microliters of purified antibody per 1 ml of cell suspension) for 30 min at +4°C. Following this treatment, the cells were washed twice in serum-free RPMI 1640 and further incubated with guinea-pig complement for 30 min at 37°C. The dead cells were removed by Ficol-Hypaque centrifugation at 800 × g for 20 minutes. The resulting cell suspension was free of CD3<sup>+</sup> cells, as determined by indirect immunofluorescence.

### Preparation of peripheral blood leucocytes

Heparinized peripheral blood was obtained immediately before the pregnancy termination procedure initiation (syngeneic peripheral blood leucocytes). Mononuclear cells were separated by Ficol-

Hypaque centrifugation, washed three times with RPMI 1640 medium and resuspended in the same medium containing 5% FCS. The same procedure of separation was performed with the blood of unrelated donors (third party peripheral blood leucocytes).

### Immunofluorescence study

The decidual leucocytes were washed twice with PBS-EDTA containing 1/100 Na azide and 2% FCS and incubated overnight with appropriate dilutions of primary antibodies (Table 1) at +4°C. After three washings, the cells were treated with FITC-labelled secondary antibody for 30 minutes at +4°C. Control samples were labelled with secondary antibody only. After three brief consecutive centrifugation in fluorescence medium, cells were fixed in paraformaldehyde and observed for the percentage of positive membrane labelling under a fluorescence microscope (Reichert Diavar, Austria) at 180 × magnification.

### Cell proliferation assays

Decidual cells were examined for their putative immunoregulatory functions. Briefly, after the washing procedure, various numbers of decidual cells (regulatory cells) were added to the one-way mixed leucocyte reaction, with PBLs from the donors of decidual cells as responding cells, and Mitomycin-C treated third party PBLs as stimulating cells, as well as to the blastic transformation test with polyclonal mitogens of the syngeneic PBLs.

The mixed leucocytes reaction was performed by incubating a mixture of 10<sup>5</sup> peripheral blood leucocytes and various numbers of syngeneic decidual leucocytes (noted at each experiment) with 2 × 10<sup>5</sup> Mitomycin-C (Sigma Chemical Co., St. Louis) treated peripheral blood leucocytes, that were either syngeneic controls or allogeneic to the decidual leucocytes. In some experiments decidual leucocytes were also treated with Mitomycin-C. The culture conditions were similar to those already described, except that incubation was carried out for 96 hrs before the addition of <sup>3</sup>H-thymidine. Results were expressed as mean counts per minute (cpm) with standard error (SE) and by percentage of suppression.

Decidual leucocytes and their non-adherent and adherent cell-fractions as well as CD3 depleted nonadherent decidual leucocytes were added separately as immunoregulatory cells to syngeneic PBLs stimulated by polyclonal mitogens (PHA, Con-A and PWM). The assays were performed in flat-bottomed 96 well plates (Goliass, Ljubljana, Slo). Each well contained 10<sup>5</sup> decidual leucocytes in 250 µl RPMI 1640 supplemented with 20% normal pooled

male AB serum and PHA 90 µg/ml (The Wellcome Research Laboratories, England) or 100 µg/ml Con-A (Pharmacia Fine Chemicals, Sweden) or PWM (GIBCO, Grand Island, N.Y.) in a final dilution of 1:500. All tests were performed in triplicates on cocultures of decidual and peripheral blood leucocytes (the number of cells per well is noted for each experiment). Cells were incubated for 72 hrs at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. At the end of this incubation period, 1 µCi of <sup>3</sup>H thymidine (Amersham, UK) per well was added and the incubation was continued for another 18 hrs. The thymidine uptake was measured with a β counter (Tracor Analytic, Delta 300) and the results expressed as mean count per minute (cpm) with standard error as well as by percentage of suppression.

The percent of suppression was calculated using two variables, the expected value, which was cpm of PBL, and the obtained value, which was calculated by subtracting the cpm of decidual leucocytes or their fractions from the cpm value of PBL and decidual leucocyte (or their fractions) cocultures.

The percent of suppression was calculated by the following formula: % SUPPRESSION = (expected cpm – obtained cpm) × 100 / expected cpm

The results were statistically analyzed by standard computer t- test.

## RESULTS

### Characterization of decidual leucocytes in cell suspension

In the decidual cell suspension obtained by enzymatic dispersion from decidual tissue after elective termination of pregnancy, the major cell class appears to be HLA-DR<sup>+</sup> cells (Table 1). After removing the adherent fraction, the percentage of the remaining HLA-DR<sup>+</sup> cells in nonadherent fraction was reduced to 4% (±3%) and remained unchanged in CD3 depleted nonadherent decidual leucocytes fraction. Simultaneously, the percentage of HLA-DR<sup>+</sup> cells in the fraction of adherent cells increased from 38% to 63% (not shown). CD3<sup>+</sup> cells were found in 10% of decidual leucocytes, their percentage being slightly higher among nonadherent decidual leucocytes. The proportions of CD56<sup>+</sup> cells and CD2<sup>+</sup> cells are very similar among decidual leucocytes. It should be noted that about 80% of nonadherent decidual leucocytes had the morphology of large granular lymphocytes, but only 44% expressed the CD56 antigen. After depletion of CD3<sup>+</sup> cells, the cells bearing this antigen could not be found by indirect immunofluorescence, while the percentage of CD2 and CD56 positive cells slightly increased.

TABLE 1

First trimester decidual leucocytes determined by indirect immunofluorescence

Monoclonal antibodies	Antigen	Specificity	CD3 depleted		
			Decidual leucocytes* (n=10)	Nonadherent decidual leucocytes* (n=8)	nonadherent decidual leucocytes* (n=6)
OKT 3	CD 3	Mature T lymphocytes	10 ± 2	12 ± 5	0 ± 0
OKT 11	CD 2	Leucocyte function antigen 3	29 ± 4	37 ± 5	39 ± 6
NKH-1	CD 56	NK-cells	31 ± 5	44 ± 2	48 ± 4
OKB-7	CD 21	B-lymphocytes	6 ± 5	7 ± 5	7 ± 6
Anti-Class II	HLA-DR	Nonpolymorphic determinant of HLA-DR molecule	38 ± 10	4 ± 3	4 ± 3

\* = percentage of positive cells ± standard deviation

TABLE 2

Suppression of peripheral blood leucocytes (PBL) alloreactivity by first trimester decidual leucocytes in 5 day mixed leucocytes reactions (n=11)

Type of cells	Mean cpm ± SE	% SUPPRESSION (mean ± SE)
R	1,220 ± 142	
R × S	28,135 ± 1,902	
R × S × D <sub>1</sub> (10 × 10 <sup>4</sup> )	10,143 ± 2,332	53.5 ± 12.1
R × S × D <sub>2</sub> (5 × 10 <sup>4</sup> )	11,429 ± 2,471	49.3 ± 13.2
R × S × D <sub>3</sub> (2.5 × 10 <sup>4</sup> )	13,864 ± 2,130	40.3 ± 12.1
R × S × D <sub>4</sub> (1.25 × 10 <sup>4</sup> )	14,710 ± 2,362	37.6 ± 11.2
R × S × D <sub>5</sub> (0.625 × 10 <sup>4</sup> )	16,173 ± 1,928	36.9 ± 9.4

### LEGEND

R (responder) = 1 × 10<sup>5</sup> peripheral blood lymphocytes

S (stimulator) = 2 × 10<sup>5</sup> third party PBL treated with Mitomycin-C

D<sub>1</sub> – D<sub>5</sub> = untreated decidual leucocytes in various concentrations

R × S × D = cpm<sub>(R×S×D)</sub> – cpm<sub>(D×S)</sub>

### Functional studies

Ficol-Hypaque separated nucleated cells of the trypsin dispersed decidual tissue (decidual leucocytes) were added to the 4- and 5-day one-way mixed leucocyte reaction using syngeneic PBLs as responding cells, and to the syngeneic peripheral blood leucocyte cultures stimulated with polyclonal mitogens. In both assays the addition of decidual leucocytes caused a strong dose-dependent sup-

pression of lymphocyte proliferation. Untreated decidual leucocytes added to the 4- and 5-day MLR (Table 2 and Table 3) exerted a potent immunosuppressive effect in a dose-dependent manner. Similar observations were made in another series of ex-

TABLE 3

*Suppression of peripheral blood leucocytes (PBL) alloreactivity by first trimester decidual leucocytes in 4 day mixed leucocytes reactions (n=7)*

Type of cells	Mean cpm $\pm$ SE	% SUPPRESSION (mean $\pm$ SE)
R	1,310 $\pm$ 208	
R $\times$ S	20,582 $\pm$ 1,990	
R $\times$ S $\times$ D <sub>1</sub> (10 $\times$ 10 <sup>4</sup> )	8,538 $\pm$ 1,322	59.2 $\pm$ 3.8
R $\times$ S $\times$ D <sub>2</sub> (5 $\times$ 10 <sup>4</sup> )	13,717 $\pm$ 1,588	33.3 $\pm$ 5.1
R $\times$ S $\times$ D <sub>3</sub> (2.5 $\times$ 10 <sup>4</sup> )	14,438 $\pm$ 1,821	30.1 $\pm$ 6.5
R $\times$ S $\times$ D <sub>4</sub> (1.25 $\times$ 10 <sup>4</sup> )	15,006 $\pm$ 3,063	26.4 $\pm$ 6.8
R $\times$ S $\times$ D <sub>5</sub> (0.6 $\times$ 10 <sup>4</sup> )	15,822 $\pm$ 1,419	21.8 $\pm$ 6.0

## LEGEND

R (responder) = 1  $\times$  10<sup>5</sup> peripheral blood lymphocytes

S (stimulator) = 2  $\times$  10<sup>5</sup> third party PBL treated with Mitomycin-C

D<sub>1</sub> - D<sub>5</sub> = untreated decidual leucocytes in various concentrations

R  $\times$  S  $\times$  D = cpm<sub>(R $\times$ S $\times$ D)</sub> - cpm<sub>(D $\times$ S)</sub>

TABLE 5

*Suppressive effect of first trimester decidual leucocytes in blastic transformation tests (n=10)*

Type of cells (Number of cells per well)	POLYCLONAL MITOGENS		
	PHA	Con-A	PWM
PBL (1 $\times$ 10 <sup>5</sup> )	114,156 $\pm$ 16,432	90,439 $\pm$ 16,460	53,238 $\pm$ 5,603
PBL (1 $\times$ 10 <sup>5</sup> ) + DL <sub>1</sub> (1 $\times$ 10 <sup>5</sup> )	77,449 $\pm$ 16,797	69,499 $\pm$ 12,531	39,588 $\pm$ 4,171
% SUPPRESSION*	40.6 $\pm$ 9.2	28.7 $\pm$ 11.7	22.6 $\pm$ 6.1
PBL (1 $\times$ 10 <sup>5</sup> ) + DL <sub>2</sub> (5 $\times$ 10 <sup>4</sup> )	79,087 $\pm$ 11,349	66,737 $\pm$ 12,845	45,644 $\pm$ 5,428
% SUPPRESSION*	32.5 $\pm$ 4.1	30.5 $\pm$ 10.5	20.2 $\pm$ 8.3
PBL (1 $\times$ 10 <sup>5</sup> ) + DL <sub>3</sub> (0.6 $\times$ 10 <sup>4</sup> )	88,113 $\pm$ 10,478	59,420 $\pm$ 11,316	43,067 $\pm$ 2,525
% SUPPRESSION*	24.6 $\pm$ 8.5	34.1 $\pm$ 6.4	18.5 $\pm$ 3.7

## LEGEND

PBL = peripheral blood lymphocytes

DL = first trimester decidual leucocytes

PBL+DL = mean count per minute  $\pm$  standard error

\* = mean  $\pm$  standard error

% SUPPRESSION = (expected - obtained)  $\times$  100/expected

TABLE 4

*Suppression of peripheral blood leucocytes (PBL) alloreactivity by Mitomycin-C treated first trimester decidual leucocytes in 5 day mixed leucocytes reactions (n=5)*

Type of cells	Mean cpm $\pm$ SE	% SUPPRESSION (mean $\pm$ SE)
R	803 $\pm$ 213	
R $\times$ S	24,974 $\pm$ 4,275	
R $\times$ S $\times$ D <sub>1</sub> (10 $\times$ 10 <sup>4</sup> )	10,210 $\pm$ 3,454	63.1 $\pm$ 12.1
R $\times$ S $\times$ D <sub>2</sub> (5 $\times$ 10 <sup>4</sup> )	12,791 $\pm$ 3,846	55.9 $\pm$ 13.5
R $\times$ S $\times$ D <sub>3</sub> (2.5 $\times$ 10 <sup>4</sup> )	15,923 $\pm$ 3,341	36.1 $\pm$ 10.8
R $\times$ S $\times$ D <sub>4</sub> (1.25 $\times$ 10 <sup>4</sup> )	16,355 $\pm$ 2,202	32.2 $\pm$ 5.5
R $\times$ S $\times$ D <sub>5</sub> (0.6 $\times$ 10 <sup>4</sup> )	22,835 $\pm$ 1,477	11.3 $\pm$ 9.9

## LEGEND

R (responder) = 1  $\times$  10<sup>5</sup> peripheral blood lymphocytes

S (stimulator) = 2  $\times$  10<sup>5</sup> third party PBL treated with Mitomycin-C

D<sub>1</sub> - D<sub>5</sub> = untreated decidual leucocytes in various concentrations

R  $\times$  S  $\times$  D = cpm<sub>(R $\times$ S $\times$ D)</sub> - cpm<sub>(D $\times$ S)</sub>

TABLE 7

*Suppressive effect of CD3 depleted first trimester nonadherent decidual leucocytes (DL) stimulated by PHA (n=6)*

Type of cells (Number of cell per well = 1 $\times$ 10 <sup>5</sup> )	PHA
Peripheral blood lymphocytes (PBL)	82,568 $\pm$ 1,390*
PBL + Nonadherent decidual leucocytes	40,245 $\pm$ 13,387*
% SUPPRESSION	52.6 $\pm$ 15.0
PBL + CD3 depleted nonadherent DL	47,398 $\pm$ 11,323*
% SUPPRESSION	43.4 $\pm$ 13.0

## LEGEND

\* = mean cpm  $\pm$  standard error

% SUPPRESSION = mean percent  $\pm$  standard error

## DISCUSSION

Immunoregulatory potential of decidual cells and leucocyte subpopulations residing in decidua (macrophages and lymphocytes) has been proven in various experimental models. Skin allografts implanted into uterine horns of hormonally (estrogen and progesterone) pretreated rats (pseudopregnancy) or in the uteri of pregnant females, have had a much longer survival time than allografts placed at nonuterine sites or in the uterine horns of nonpregnant rats (1). The intensity of systemic immunity following intrauterine allograft rejection was much lower compared to the sensitization after skin allografts were transplanted orthotopically (21). Following this concept, major investigative effort is being focused on the materno-fetal interface, namely, on the immunocompetent cells in pregnant endometrium and fetally derived trophoblast. The decidua has to account for a dual function, the immune tolerance to the conceptus on one hand, and local immune response to infectious organisms on the other, for which an intact maternal systemic immune function is necessary. The cell composition of the decidua is adapted to fulfill both functions. Besides decidual cells, macrophages, large granular lymphocytes or endometrial stromal granulocytes and nongranulate lymphocytes are also found (4, 15). In immunofluorescence analysis of the cell content of curettage specimens of trypsin dispersed decidual tissue obtained from the first trimester human pregnancy, we found a predominance of macrophages and CD56<sup>+</sup> cells. The percentage of CD4<sup>+</sup> and CD8<sup>+</sup> cells exceeded the percentage of CD3<sup>+</sup> cells (15) (Table 1). This finding suggests that decidua derived cells, other than CD3<sup>+</sup> cells, can readily express CD4 and/or CD8 antigens. The expression of alpha/alpha homodimer of CD 8 antigen on NK-cells as well as on some macrophages is well documented (23).

periments, where prior to being added to the 5-day MLR, decidual leucocytes were treated with Mitomycin-C in order to eliminate the proliferative response of decidual leucocytes (Table 4). This way treated decidual leucocytes caused a statistically significant ( $p < 0.05$ ) suppression of alloreactivity response only when added in higher concentrations.

Similarly, dose dependent suppressive effects were obtained when decidual leucocytes were added to the blastic transformation test in case of stimulator by PHA (Table 5). In cultures stimulated by ConA and PWM, lower immunosuppression was observed.

In order to identify the major cell classes of the decidua mediating the suppressive effect, the decidual cell suspension had been cultivated overnight and two classes of cells - adherent and nonadherent - were obtained. Both cell populations, when added to the peripheral blood leucocytes in cell ration 1:1, exerted a potent immunosuppressive effect on PHA induced PBL proliferation (Table 6). There was no statistically significant difference in the suppressive effect of nonadherent and adherent decidual leucocytes.

TABLE 6

*Suppressive effect of first trimester decidual leucocytes fraction in blastic transformation tests (n=14)*

Type of cells (Number of cell per well = 1 $\times$ 10 <sup>5</sup> )	PHA
Peripheral blood lymphocytes (PBL)	105,846 $\pm$ 5,828*
PBL + Nonadherent decidual leucocytes	70,693 $\pm$ 7,125*
% SUPPRESSION	45.6 $\pm$ 6.0
PBL + Adherent decidual leucocytes	52,287 $\pm$ 5,617*
% SUPPRESSION	52.2 $\pm$ 3.4

## LEGEND

\* = mean cpm  $\pm$  standard error

% SUPPRESSION = mean percent  $\pm$  standard error

Suspensions of CD3 depleted decidual leucocytes contained CD2<sup>+</sup> and CD56<sup>+</sup> cells in higher proportions, as proven by indirect immunofluorescence (Table 1). The addition of these cells to the syngeneic PBLs stimulated by PHA caused a strong immunosuppression of the proliferative response, comparable to the suppressive effects of both unfractionated (decidual leucocytes) and nonadherent nondepleted decidual leucocytes (Table 7).

In the present investigation it was found that decidual leucocytes (identified on the basis of morphology and surface antigens) represent an important cell class in the early gestational human decidua, capable of abrogating syngeneic peripheral blood leucocyte alloreactivity as well as proliferative response to polyclonal mitogens (PHA; Con-A and PWM).

A suppression of MLR by the human decidua has been reported in the study of Golander et al. (13), which was not designated to establish the identity of the suppressor cells. Daya et al. (10) identified suppressor cells isolated from the late first trimester pregnancy decidua as a small cell class which suppressed Con-A induced proliferation of maternal peripheral blood leucocytes. Parhar et al. (19) showed that irradiated, Ficoll-Hypaque-separated nucleated cells of the collagenase-dispersed early gestational decidua as well as their plastic-adherent and nonadherent fractions caused a strong dose-dependent suppression of the 3-, 4-, and 5-day mixed leucocyte reaction. The major class of mediator molecules was identified as prostaglandin E<sub>2</sub> (PGE<sub>2</sub>).

In our study two cell classes, adherent and nonadherent decidual leucocytes, had a similar immunosuppressive effect to proliferative response of peripheral blood leucocytes stimulated by polyclonal mitogens. In the adherent cell fraction, two thirds of the cells were HLA-DR<sup>+</sup> i.e. most of them were macrophages. Therefore, the major immunosuppressive effect in vitro could be due to the secretion of prostaglandin E<sub>2</sub>, since it was shown that the suppressive effect could be abrogated by prostaglandin synthesis inhibition (9).

Nonadherent decidual leucocytes (decidual lymphocytes) contained 12% of CD3<sup>+</sup> cells, 44% of CD56<sup>+</sup> cells and only 4% of HLA-DR<sup>+</sup> cells. In order to identify the major cell class responsible for immunosuppression mediated by nonadherent cells, CD3<sup>+</sup> cells were depleted from the suspension of decidual lymphocytes. The resulting cell suspension, free of CD3<sup>+</sup> lymphocytes and containing 48% of CD56<sup>+</sup> cells, and only 4% of HLA-DR<sup>+</sup> cells (determined by indirect immunofluorescence) was capable of suppressing PBL proliferative response to polyclonal mitogens in a degree comparable to that of unseparated decidual leucocytes. The observed immunosuppressive effect of CD3<sup>+</sup> depleted decidual lymphocyte exerted *in vitro* suggests that CD56<sup>+</sup> cells, the most numerous among various immunocompetent cells infiltrating decidual tissue at fetomaternal interface, could also be responsible for this phenomenon. Since CD56<sup>+</sup> decidual lymphocytes exert a certain degree of natural killer activity in vitro (14, 16) the complexity of functions of decidual granular lymphocytes remains to be further clarified.

The composition of cells obtained from the decidual tissue by various enzyme dispersal techniques is thought to be of utmost importance.

Immunohistochemical studies of human first trimester pregnancy decidua biopsy specimens (5) reveal a similar, although not identical range of various cell populations to the one detected by immunofluorescence technique following trypsin digestion and Ficoll-Hypaque density gradient centrifugation applied in our experiments, and can therefore represent a model comparable to the events taking place *in situ*. However, there is a striking difference in the overall percentage of bone marrow derived cells found in frozen sections of intact decidual tissue (32%) versus more than 75% of cells in enzymatically dispersed decidual cell suspensions (20).

Our results suggest an important immunoregulatory role of both adherent and nonadherent decidual leucocyte fractions from the early gestational human decidua. By allowing the adherence of the adherent decidual leucocytes and eliminating CD3 positive cells with anti CD3 antibody and complement treatment, a highly purified population of decidual lymphocytes (CD2<sup>+</sup>, CD56<sup>+</sup>) can be obtained. Both untreated and CD3 depleted nonadherent decidual leucocytes exert comparable immunosuppressive effect upon the proliferative response of syngeneic PBLs stimulated by PHA. Accordingly, immunosuppressive effects in proliferation assays reported here could be at least partly attributed to the cell population unique to the first trimester human decidual tissue – decidual granular lymphocytes.

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