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Thymic involution in pregnant mice

I. CHARACTERIZATION OF THE REMAINING THYMOCYTE SUBPOPULATIONS

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SUMMARY

Pregnancy-induced thymic atrophy was studied in mice during the course of syngeneic gestation and the post-partum period. Cortical thymocytes were greatly reduced in number as shown by the binding of fluorescein-labelled PNA. The pool of steroid-resistant (SR) medullary thymocytes appeared unchanged in pregnant mice when studied by means of a specific heteroantiserum (SRCA). Therefore, in pregnant mice, these two surface markers demonstrated that thymic atrophy was linked to steroid-sensitive (SS) cortical cell reduction. The presumed hydrocortisone resistance of the mother's remaining thymocytes is not related to a difference in the number of steroid receptors as determined by ^{3}H-dexamethasone binding.

INTRODUCTION

Thymic involution during pregnancy is a phenomenon which has been described for a long time in various mammalian species, especially mice (Persike, 1940; Pepper, 1961) and man (Hammar, 1926). It was also demonstrated that this involution was reversible during the post-partum period (Jolly & Lieure, 1930). These previous reports were based mainly on histological and morphological observations, showing that the initial involution of the thymus is apparently linked to a reduction of the thymic cortex. In the present paper we characterize the remaining thymocyte subpopulations, using cell markers specific for cortical or medullary thymocytes. As thymic involution is thought to be mediated by modifications of the hormonal environment during pregnancy (Nelson *et al.*, 1967, 1973), this type of involution was compared to steroid-induced atrophy. The concentration of steroid receptors in the pregnant mouse thymus was also studied.

MATERIALS AND METHODS

Mice. CBA/J mice (H-2^k) were used in all experiments (CSEAL, Orléans la Source, France). Six-week-old primiparous females mated with males of the same strain were used. Thymocytes were tested at various stages of pregnancy and the post-partum period. The influence of lactation was also studied, with post-partum females separated from their litters as controls.

Six-week-old virgin CBA/J females were used as controls.

Abbreviations: DM = dexamethasone, SR = steroid-resistant, SS = steroid-sensitive, n.s. = not significant, PNA = peanut agglutinin, s.e.m. = standard error to the mean, SRCA = steroid-resistant cell antiserum.

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Preparation of cell suspensions. Thymocytes were prepared in a Potter homogenizer. The cell suspension was washed at 1,400 r.p.m. for 7 min, and the pellet resuspended in Hanks' balanced salt solution (HBSS).

Study of thymic cell subpopulations

Steroid-sensitive cortical cells (SS). Cortical cells have been studied using the lectin peanut agglutinin (PNA). This lectin, purified from peanuts by affinity chromatography, is a tetramer of 100,000 daltons. In mice, PNA binds to the pool of immunoincompetent and SS thymocytes (Reisner, Linker-Israeli & Sharon, 1976; London, Berrih & Bach, 1978). Fluorescence microscopy was used to study the binding of PNA labelled with fluorescein (PNA-FITC, Pharma Industrie, Clichy, France). Thymic cells were suspended and washed in minimum essential medium supplemented with 10% heat-inactivated fetal calf serum, 0·5% BSA, 3% HEPES 1 M and 1‰ sodium azide adjusted to pH 7·2 with 1 M NaOH; 3×10^6 thymic cells in 0·1 ml of the medium were incubated with 20 μl of 2 mg/ml PNA-FITC for 30 min at 4°C. The pellet was washed three times at 1,400 r.p.m. in a refrigerated centrifuge and resuspended in a minimal amount of medium. Fluorescence was asssessed with a Leitz–Orthoplan microscope equipped for epifluorescence (FL40/1·30 Oël objective and periplan $\times 8$ eyepieces).

Steroid-resistant (SR) medullary cells. SR medullary cells were studied using a heteroantiserum raised against this subpopulation, and prepared as previously described (Papiernik & Bach, 1977). Briefly, rabbits were immunized with thymic lymphocytes of hydrocortisone-treated adult CBA mice: one i.m. injection of 40×10^6 cells and, 3 weeks later, three successive i.v. doses at 1-day intervals. Rabbits were bled 10 days after the last injection. This antiserum was absorbed extensively with thymic cortical cells isolated on Ficoll density gradients (Papiernik, Laroche & Bach, 1977). Sensitivity of pregnant and control thymic cells to this anti-SR cell antiserum (SRCA) was assessed by complement-dependent cytotoxicity: 0·05 ml thymic cells at a concentration of 6×10^6 cells/ml were incubated with SRCA (0·1 ml, 1/8 dilution upwards) and fresh guinea-pig complement (0·05 ml, 1/2 dilution) for 1 hr at 37°C. Cell viability was evaluated by the trypan blue dye exclusion test. The cytotoxic index was calculated as follows:

$$\frac{\%\text{ dead cells} - \text{control}}{100\% - \text{control}} \times 100.$$

(The control for SRCA was normal rabbit serum absorbed under the same conditions.)

Binding studies of glucocorticoid receptors in thymocytes

After adjustment of volume to give 10^7 cells/ml, 0·3 ml of cell suspension was added to 0·3 ml of ^{3}H-dexamethasone (^{3}H-DM; 23–28 Ci/nmol) solution in 1·5-ml conical plastic vials. The final concentration of radioactive steroid ranged from 3×10^{-9} to 10^{-7} M. Samples were incubated at 37°C for 20 min with continuous shaking. To separate the bound steroid from that remaining free following the incubation period, the samples were centrifuged for 15 sec in an Eppendorf 3200 centrifuge, supernatants were discarded and the cells were resuspended in 0·3 ml of steroid-free, ice-cold medium A. This buffered solution, pH 7·4, contained 133 mM Na^+, 6 mM K^+ 1 mM Ca^{++}, 1 mM Mg^{++}, 134 mM Cl^-, 6 mM $H_2PO_4^-$, 5 mM Tris–HCl and 5 mM glucose. Aliquots of 0·2 ml were then filtered through Whatmann GF/A filters and washed three times with 5 ml of ice-cold buffer A. The radioactivity collected on the filters was counted by liquid scintillation spectrometry. In each experiment, cells were incubated with ^{3}H-DM alone or with ^{3}H-DM plus 5×10^{-5} M non-radioactive DM to determine the non-specific binding of the tracer (this non-specific binding represents 20–30% of the total binding at 10^{-7} M tritiated dexamethasone).

RESULTS

Evolution of total thymic population during gestation and the post-partum period

Lymphoid cells in the thymus were counted at different stages of pregnancy and at regular intervals during the post-partum period until 40 days post-parturition (Fig. 1). In primiparous pregnant

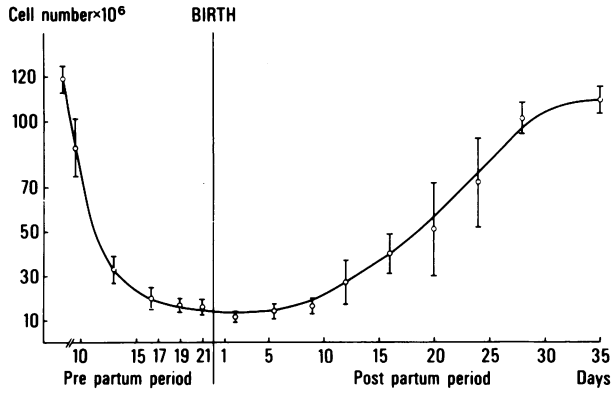

Fig. 1. Number of cells per thymus during the course of pregnancy and in the post-partum period.

mice, thymic atrophy began before the 10th day of gestation and reached its maximum (90%) during the first week of the post-partum period. This thymic atrophy lasted for 3 weeks with complete recovery near the end of the first month of the post-partum period. No influence of lactation was observed as this atrophy was the same if the mothers were separated from their litters at birth. Virgin female mice were used as controls.

Study of thymic subpopulations

The number of cortical thymocytes was evaluated by studying the binding of fluorescein-conjugated PNA to thymic cells (Table 1). The number of PNA^+ cells was greatly reduced during the pre- and post-partum periods, showing that the thymic involution was due essentially to a reduction in the number of cortical cells. This reduction begins in the pre-partum period and reaches its peak in the immediate post-partum period.

Control thymus contained $68{\cdot}7 \pm 6{\cdot}4 \times 10^6$ PNA^+ cells per thymus, this number being reduced to $9{\cdot}5 \pm 3{\cdot}4 \times 10^6$ during days 12–20 of gestation. This reduction in the number of PNA^+ cells was greatest ($2{\cdot}8 \pm 0{\cdot}7 \times 10^6$ cells per thymus) in the immediate post-partum period and the recovery of this cortical population was effected by the end of the first month.

SR cells were studied by SRCA in a complement-dependent cytotoxicity test (Table 2). The percentage of SRCA-sensitive cells increased in the pre-partum period ($10{\cdot}9 \pm 2{\cdot}3$) and this enhancement was highly significant in the early post-partum period ($28{\cdot}1 \pm 4{\cdot}3$). These results are correlated with the unmasking of a small SR population after reduction of the cortical cell number (Papiernik & Bach, 1977). At the time of maximum thymic atrophy, the pool of SRCA-positive cells

Table 1. PNA binding cells in the thymus of pregnant mice and of untreated or hydrocortisone-treated virgin mice. Strong reduction in PNA-positive cells occurs early in pregnancy but is reversible in the late post-partum period

	Pregnant mice				Virgin mice	
		Days post-partum				
	Pre-partum days 12–20	0–3	5–17	29–38	Untreated	Hydrocortisone-treated
PNA^+ cells*	$9{\cdot}5 \pm 3{\cdot}4$	$2{\cdot}8 \pm 0{\cdot}7$	$33{\cdot}8 \pm 3{\cdot}1$	$67{\cdot}8 \pm 9{\cdot}7$	$69{\cdot}7 \pm 6{\cdot}4$	$0{\cdot}3 \pm 0{\cdot}1$
P value†	$<0{\cdot}001$	$<0{\cdot}001$	$<0{\cdot}01$	n.s.		

* Results are absolute number of PNA-positive cells per thymus ($\times 10^{-6}$).

† Statistical analysis was performed to compare the number of PNA-positive cells in pregnant and untreated virgin mice.

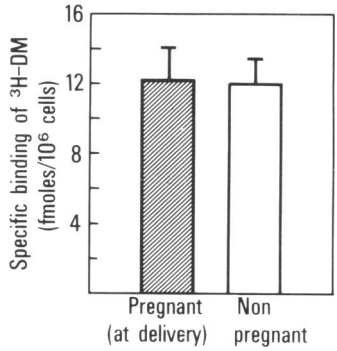

Fig. 2. Specific binding of ^{3}H-DM in thymocytes isolated from pregnant and virgin CBA mice. Cells (10^7 cells/ml) were incubated at 37°C for 20 min in the presence of ^{3}H-DM (concentrations ranging from 3×10^{-9} M to 10^{-7} M). Parallel incubations in the presence of unlabelled DM were made to determine non-specific binding at each concentration of ^{3}H-DM. The results, calculated from Scatchard plots, are expressed in fmol/10^6 cells (mean values ±s.e.m.)

in pregnant mice was not significantly different from the pool of SRCA-positive cells in virgin mice after hydrocortisone treatment. Thus, the percentage of SR cells was the same in pregnant mice during maximal thymic atrophy and in virgin mice during hydrocortisone treatment. After thymic recovery, the percentage of SR cells was also the same in the two groups of mice (Table 2).

Steroid receptors in thymocytes of pregnant mice (Fig. 2)

Specific binding of ^{3}H-DM was determined in thymocytes isolated from pregnant mice at parturition and virgin adult female mice. As shown in Fig. 2, no significant variations in the number of steroid-binding sites per cell could be observed in pregnant mice, the steroid receptor concentration being 12·2±3·3 fmol/10^6 cells in pregnant mice as compared to 12·1±5·0 in virgin mice. These values correspond to about 7,000 sites per cell, a result in good agreement with those previously published (Duval, Dausse & Dardenne, 1976). The affinities of these binding sites for the tracer, determined from the slope of the Scatchard plots (Scatchard, 1949), were similar in pregnant and virgin mice ($2{\cdot}13 \times 10^{-8}$ M, $n=5$, and $2{\cdot}28 \times 10^{-8}$ M, $n=13$ respectively).

DISCUSSION

There is a major reversible physiological atrophy of the maternal thymus during gestation. Since this thymic regression is the same whether mating is syngeneic or allogeneic (Maroni & de Sousa,

Table 2. SRCA sensitivity of thymocytes from pregnant and virgin mice

	Pregnant mice†			Virgin mice†	
		Days post-partum			
	Pre-partum days 10–16	1–7	16–22	Untreated	Hydrocortisone-treated
SRCA$^+$ (%)*	10·9±2·3	28·1±4·3	5·6±0·4	6·8±1·0	36·7±3·2
P value‡	n.s.	<0·001	n.s.		

* Cytotoxic index expressed in per cent.

† Mean value ±s.e.m. for seven experiments.

‡ Percentage of SRCA-sensitive cells is significantly higher in thymocytes from pregnant mice than in those of untreated virgin mice ($P<0{\cdot}001$) 1–7 days post-partum; however, it is not significantly different from the percentage of SRCA-sensitive cells in hydrocortisone-treated mice.

1973), this phenomenon seems to be independent of immunization against paternal histocompatibility antigens, and can be considered as a non-specific phenomenon.

The reduction in number of thymocytes during gestation (Fig. 1) is in agreement with previous studies (Baines, Pross & Millar, 1977; Anderson, 1978). The thymic involution occurs early during gestation (around the 10th day), reaching its greatest level about parturition, and is progressively reversible. The cell number is normal around the end of the first month post-partum.

The question is to know which of the two main thymocyte subpopulations is affected: the cortical thymocytes, the medullary thymocytes, or both?

Two surface markers allow us to specify the mode of the thymic involution which has been studied with histological methods elsewhere (Persike, 1940; Pepper, 1961; Ito & Hoshino, 1962; Maroni & de Sousa, 1973; Millar, Mills & Baines, 1973). Indeed, histological observations performed on several mammalians, particularly in mice, guinea-pig and rats (Persike, 1940; Pepper, 1961; Ito & Hoshino, 1962), have demonstrated an important atrophy of the cortical zone of the thymus, while the medullary area remains unaffected. The use of PNA labelling enabled us to confirm that thymic atrophy is linked to SS cortical cell reduction, as PNA has been shown to label essentially cortical cells. The decrease in PNA^+ cell number begins in the pre-partum period, is maximal immediately post-partum, and recovers by the end of the 4th week after delivery (Table 1).

Previous studies have shown that SRCA (anti-steroid-resistant cell antiserum) is a reliable marker of thymic medullary SR lymphocytes (Papiernik & Bach, 1977). Using this antiserum, we can see that a large proportion of the lymphoid cells remaining in the maternal gestational thymus after cortex reduction are SR, since up to 28% of the unseparated thymocyte (Table 2) populations are killed by SRCA and complement during the first week of pregnancy. This percentage is not significantly different from the one found in hydrocortisone-treated virgin mice.

This raises the question as to whether the atrophy of the thymus during pregnancy can be considered as a hydrocortisone-like effect or not. It has been shown that *in vivo* treatment with high doses of glucocorticoids (Dougherty & White, 1945; Ishidate & Metcalf, 1963; Dougherty *et al.*, 1964) or sex steroids (oestrogens, progesterone) or chorionic gonadotrophins (Dougherty, 1952; Nelson *et al.*, 1967) leads to involution in the thymus which is even more strongly evident when associated synergistically, whereas castration leads to hypertrophy of the thymus (Eidinger & Garrett, 1972). As there are important modifications in the hormonal environment during pregnancy, the thymic involution may be related to a hormonal phenomenon.

Several observations, made in cell lines derived from lymphoid tissues, suggest that a correlation may exist between the number of cell glucocorticoid receptors and the sensitivity of cells to steroids (Rosenau *et al.*, 1972; Lippman *et al.*, 1973), hydrocortisone-sensitive cells binding more glucocorticoid than hydrocortisone-resistant cells. As the involution of the thymus of pregnant mice is due mainly to the reduction of the cortical subpopulation of thymocytes, whereas the medullary subpopulation is preserved, we tried to determine if the supposed hydrocortisone resistance of the mother's remaining thymocytes was related to a difference in the quantity of steroid receptors. We compared the steroid-binding ability of maternal thymocytes during pregnancy with the binding by control virgin thymocytes. The number of thymocyte glucocorticoid receptors appeared to be identical in virgin and pregnant mice. In terms of glucocorticoid receptors, the remaining thymocytes of the mother (that are considered as SR cells) cannot be differentiated from the whole thymocyte population of virgin control mice. These results confirm previous studies demonstrating that hydrocortisone resistance may not be explained by a defect of cytoplasmic glucocorticoid receptors in mice (Duval *et al.*, 1976). Thus the surface and cytoplasmic markers studied in this paper do not allow us to differentiate the thymic involution during pregnancy and the thymic atrophy induced by the injection of exogenous steroids. Therefore, it would be of great interest to determine if the gestation and the exogenous steroid administration induce the same functional changes in the thymus.

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