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Neonatal administration of prolactin antiserum alters the developmental pattern of T- and B-lymphocytes in the thymus and spleen of BALB/c female mice

(bromocriptine/neonatal immunomodulation/prolactin/pituitary transplantation)

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ABSTRACT **We have evaluated the effect of neonatal administration of mouse prolactin (PRL) antiserum on the developmental expression of T- and B-lymphocytes in the thymus and spleen of female BALB/c mice. Newborn female mice were injected subcutaneously with a 50-μl aliquot of PRL antiserum or normal rabbit serum on days 1, 2, and 3. On neonatal day 5, the PRL antiserum-treated group had a significantly ($P < 0.05$) increased population of cells in the thymus and the spleen that were positive for Thy-1.2 and for L3T4. Increases in Thy-1.2- and L3T4-positive cells in the thymus were detectable also on days 8 and 14 in mice that received the PRL antiserum and in mice injected with bromocriptine, a dopamine agonist that inhibits PRL release from the anterior pituitary. On neonatal days 21, 28, and 32, there were no significant differences in the percentage of cells positive for Thy-1.2, Ly-2 (formerly Lyt-2), or L3T4 antigens in the thymus. However, there were significant increases in the percentage of Thy-1.2- and L3T4-positive spleen cells in the bromocriptine-treated group at all times monitored and in the PRL antiserum-treated group except on day 14. In addition, the percentage of splenocytes that were positive for IgG was significantly increased in the PRL antiserum-treatment group on days 8–28, although not on neonatal day 32. Of tissues known to contain PRL receptors, neonatal administration of PRL antiserum or bromocriptine resulted in a significant alteration in the wet weight of spleen and liver, with no significant effect in thymus, heart, and kidney. Pituitary implants also resulted in a significant increase in both concanavalin A- and lipopolysaccharide-stimulated thymidine incorporation into murine splenic lymphocytes prepared from 45-day-old female mice. These data extend the role of PRL as an immunomodulator of adult lymphocyte function to a role in the developmental expression of T- and B-lymphocyte populations in the thymus and spleen of mice.**

A host of diverse biological effects has been ascribed to prolactin (PRL) (1–4). An area of interest in terms of the pervasive action of PRL is its effect on humoral and cell-mediated immune responses. PRL receptors have been identified on human T and B lymphocytes (5, 6), and occupation of these receptors by a lactogenic hormone results in the induction of a consistent marker of increased macromolecular synthesis, ornithine decarboxylase (6–8). Cyclosporine, the drug of choice used to produce immunosuppression in organ-transplant patients, interferes with binding of PRL to high-affinity receptors on human T and B lymphocytes (6, 9). In fact, PRL binding to human lymphocytes is totally blocked by the concentration of cyclosporine used in the management of rejection episodes in human heart-transplant patients (10). An elevation in the level of plasma PRL precedes a rejection episode in patients with heart transplants (11), further implicating PRL in the allograft rejection process.

Recent evidence supports a role for PRL in the mitogenesis of T lymphocytes. At suboptimal concentrations of Con A, the addition of rat PRL to murine splenocytes resulted in a dose-dependent increase in mitogenesis (12). Further experiments demonstrated that Con A-stimulated splenocytes make and secrete a PRL-like molecule essential for lymphocyte proliferation (13). This factor has a molecular mass of *ca.* 46 kDa, different from that of the major anterior pituitary prolactin entity in mice, which is *ca.* 24 kDa. This PRL-like substance is synthesized by Con A-stimulated lymphocytes and has a fingerprint with major similarities to mouse pituitary PRL (14).

The requirement of a PRL-like substance for mitogenesis of T lymphocytes suggested to us that manipulation of circulating PRL during a period critical for thymus and spleen development might result in an alteration in the expression of T-lymphocyte subsets. Mice were used because they have been shown to gradually develop immune responsiveness after birth; for example, splenic cultures are unable to produce significant interleukin 2 until after 20 days (15, 16). We demonstrate that treatment either with PRL antiserum or with bromocriptine, a chemical that decreases circulating PRL levels, results in a rapid and prolonged increase in the percentage of splenic T cells that express the L3T4 marker (helper T cells), suggestive of a role for PRL not only in adult cell-mediated immunity but also in immunological development.

MATERIALS AND METHODS

Pregnant BALB/cCRGL female mice were caged individually in plastic filter-covered (Lab Products, Maywood, NJ) cages, fed a standard pellet diet (Wayne's Lab Blox, Continental Grain, Chicago, IL), given water ad libitum, and housed at 70 ± 2°F (≈21°C) under a 12-hr light/12 hr-dark lighting schedule. Beginning within 18 hr after birth, the female offspring of these mice were given daily subcutaneous injections for 3 days of one of the following: 50 μl of rabbit anti-mouse PRL antiserum (with a titer in RIA of 1:60,000), normal rabbit serum (NRS; Antibodies, Davis, CA), bromocriptine [CB-154, Sigma; 60 μg in 25 μl of 30% (vol/vol) propylene glycol/10% (vol/vol) ethanol/60% (vol/vol)

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Abbreviations: PRL, prolactin; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; LPS, lipopolysaccharide; NRS, normal rabbit serum; mAb, monoclonal antibody.

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NaCl], or the vehicle control. Serum was stored frozen until used. On the day of birth, members of some litters received subcutaneous implants of an anterior pituitary or an equal-sized piece of muscle from the biceps femoris (as control) from 3- and 5-mo-old female BALB/c donors. The dorsal incisions were sutured with 6-0 silk and sealed with 4% (wt/vol) Parlodion (Mallinckrodt) dissolved in amyl acetate (Allied Chemical, New York) before the warmed pups were returned to their mothers. Litters were randomly assigned to treatment groups, and litter size was adjusted to about five per litter with the addition or deletion of male pups.

Preparation of Splenocytes and Thymocytes. Mice were sacrificed by cervical dislocation, the chest and abdomen were flooded with 70% ethanol, and spleen and thymus were removed aseptically and placed individually in Hanks' balanced salt solution (HBSS). Cell suspensions were prepared and counted as described (17). Monoclonal antibody (mAb) assays were conducted in 12 × 75 mm Falcon tubes. A 50-μl cell suspension of 0.8–1 × 10^6 cells in HBSS/0.1% sodium azide/1% fetal calf serum (solution A) was incubated on ice with 5 μl of anti-mouse Thy-1.2 (fluorescein conjugate), anti-mouse Ly-2 (fluorescein conjugate), anti-mouse L3T4 (phycoerythrin conjugate), or anti-mouse IgG (fluorescein conjugate). These antibodies were obtained from Becton Dickinson. After 45 min, the tubes were filled with solution A and centrifuged for 5 min at 1000 rpm. The pellet was resuspended gently in 0.5 ml of solution A, and cells were sorted with a FACS 440.

[^{3}H]Thymidine Incorporation of Con A-Stimulated Splenocytes. Cells were disaggregated in a manner identical to that for mAb assays. After the cells were counted with a hemocytometer, they were resuspended in RPMI 1640 medium supplemented with 25 mM Hepes containing 20 μg of gentamycin sulfate (Sigma) per ml, 1% Nutridoma SP (Boehringer Mannheim), and 0.5% fetal calf serum (HyClone, Logan, UT; lot 1111611; 2 ng of PRL per ml). Con A (Vector Laboratories, Burlingame, CA) was used at 250 ng/ml. Splenocytes were washed in serum-free medium (RPMI 1640/0.3% bovine serum albumin) and incubated at 37°C in 96-well round-bottomed microtiter plates with 2 × 10^5 cells per well in a total volume of 250 μl for 72 hr. At 68 hr, the cells were pulsed with 0.5 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine (6.7 μCi/mmol; ICN), harvested at 72 hr, and counted as described (17).

Statistical Determinations. Statistical differences between means were determined by the Newman–Keuls–Student test for multiple comparisons (18). Differences were considered significant at or above the 95% confidence level.

RESULTS

Rapid Effect of Neonatally Administered Anti-mouse PRL Antiserum on T- and B-Lymphocyte Surface Antigens in the Thymus and Spleen. Female BALB/c mice injected on neonatal days 1, 2, and 3 with PRL antiserum had a significantly increased percentage of L3T4-positive cells on neonatal day 5 in both the thymus and the spleen compared with the control injected with NRS (Table 1). This finding was compatible with an increase in the Thy-1.2-positive cells. The percentage of Ly-2-positive cells did not differ between the two groups. Since individual thymocytes can express both Ly-2$^+$ and L3T4$^+$ phenotypes (19), these data support selective increases in singly labeled L3T4$^+$ cells. In the spleen, the percentage of cells immunostained by all of the mAbs was well below the adult levels, particularly in the case of the T-cell markers. These findings are in line with the known immaturity of the immune responsiveness of the spleen, including its inability to produce interleukin 2 (15, 16, 20).

Persistence of Splenic Alterations in mAb Expression to Maturity. Significant increases in the percentage of L3T4-positive cells were detectable in the PRL antiserum-treated group and in the bromocriptine-treated group at essentially all time points assayed (Table 2). In addition, the percentage of cells positive for the B-cell marker, IgG, was significantly decreased in both the PRL antiserum-treated group and in the group that received bromocriptine. There was no significant difference in the percentage of Ly-2-positive cells at any time monitored. In general, the increase in the percentage of Thy-1.2-positive cells corresponded to the increase in L3T4-positive cells in both the PRL- and the bromocriptine-treated groups. The mean fluorescence for IgG and L3T4 detection in the spleen was always higher in the NRS-treated group than in the anti-PRL-treated mice ($P < 0.05$).

Alteration of Thymic T-Cell Markers by PRL Manipulation Only During Neonatal Weeks 1 and 2. The percentage of L3T4-positive cells was increased in the thymus at neonatal days 5 (Table 1), 8, and 14 (Table 2). Again, these increases corresponded to a significant increase in the percentage of Thy-1.2-positive cells, with no detectable difference in the Ly-2-positive cell population.

Effect of Neonatally Administered Anti-mouse PRL or Bromocriptine on the Wet Weight of Organs Known to Possess PRL Receptors. The administration of PRL antiserum on neonatal days 1, 2, and 3 resulted in a significant increase in the net weight of the spleen and the liver on neonatal day 32 ($P < 0.05$). Thymus, heart, and kidney—other organs also known to contain PRL receptors (7, 21)—did not differ in wet weight from the organs in NRS-treated controls (Table 3). In addition, in the bromocriptine-treated group, the spleen weight was significantly increased compared with that of the controls, whereas the liver wet weight was significantly lower than that in the vehicle-treated controls ($P < 0.05$).

Enhancement of Con A and Lipopolysaccharide (LPS)-Stimulated Mitogenesis of Murine Splenic Lymphocytes by

Table 1. Effect of neonatally administered anti-mouse PRL antiserum on T- and B-lymphocyte surface antigens in the thymus and spleen of female BALB/c mice

Cells	Neonatal age, days	Treatment	Surface antigen-positive cells, %			
			Thy-1.2	Ly-2	L3T4	IgG
Thymus	5	Neonates				
		PRL antiserum	70 ± 4.8*	41 ± 5.6	67 ± 5.2*	NA
		NRS	59 ± 4.3	48 ± 4.9	50 ± 5.4	NA
		Adult female, no treatment	60 ± 5.2	53 ± 5.1	64 ± 4.7	NA
Spleen	5	Neonates				
		PRL antiserum	4.8 ± 0.2*	1.0 ± 0.1	6.4 ± 0.5*	20 ± 2.3
		NRS	2.1 ± 0.1	0.8 ± 0.2	2.9 ± 0.2	16 ± 1.1
		Adult female, no treatment	41 ± 3.8	13 ± 0.9	23 ± 1.7	44 ± 4.3

A 50-μl aliquot of either PRL antiserum or NRS was injected subcutaneously into newborns on days 1, 2, and 3. See text for details. Each value represents the mean ± SEM of at least four organ preparations. In each preparation, 104 cells were analyzed for mAb immunofluorescence on a FACS 440. NA = not applicable.
*Data differ from controls (NRS), $P < 0.05$.

Table 2. Developmental effects of neonatally administered anti-mouse PRL antiserum and the dopamine agonist bromocriptine on T- and B-lymphocyte surface antigens in the thymus and spleen of female BALB/c mice

Cells	Neonatal age, days	Treatment	Surface antigen-positive cells, %			
			Thy-1.2	Ly-2	L3T4	IgG
Thymus	32	PRL antiserum	85 ± 8	48 ± 2	41 ± 2	NA
		NRS	84 ± 6	45 ± 3	42 ± 2	NA
		Bromocriptine	83 ± 6	51 ± 2	40 ± 2	NA
	28	PRL antiserum	85 ± 6	48 ± 4	42 ± 3	NA
		NRS	82 ± 5	45 ± 3	42 ± 4	NA
		Bromocriptine	84 ± 7	47 ± 4	43 ± 4	NA
	21	PRL antiserum	84 ± 8	55 ± 3	40 ± 3	NA
		NRS	71 ± 7	51 ± 4	37 ± 3	NA
		Bromocriptine	82 ± 6	52 ± 4	42 ± 4	NA
	14	PRL antiserum	70 ± 4**	54 ± 5	45 ± 5**	NA
		NRS	86 ± 6	50 ± 5	58 ± 5	NA
		Bromocriptine	75 ± 3**	52 ± 6	41 ± 3**	NA
	8	PRL antiserum	69 ± 3**	47 ± 4	55 ± 4**	NA
		NRS	52 ± 4	40 ± 5	42 ± 3	NA
		Bromocriptine	73 ± 6**	45 ± 4	53 ± 5*	NA
Spleen	32	PRL antiserum	33 ± 1**	11 ± 0.8	27 ± 2**	34 ± 3
		NRS	26 ± 1	11 ± 0.5	20 ± 1	42 ± 2
		Bromocriptine	35 ± 1**	12 ± 1.1	29 ± 3**	35 ± 2
	28	PRL antiserum	25 ± 1**	8 ± 0.7	16 ± 0.8**	28 ± 2**
		NRS	19 ± 1	7 ± 0.9	10 ± 0.5	40 ± 2
		Bromocriptine	26 ± 1**	9 ± 1.0	15 ± 0.6**	32 ± 2*
	21	PRL antiserum	12 ± 0.5*	4 ± 0.2	9 ± 0.5*	25 ± 2**
		NRS	10 ± 0.6	5 ± 0.3	7 ± 0.4	38 ± 2
		Bromocriptine	16 ± 0.7**	4 ± 0.3	11 ± 0.8**	23 ± 2**
	14	PRL antiserum	11 ± 0.9*	3 ± 0.2	7 ± 0.4	23 ± 2**
		NRS	8 ± 0.6	3 ± 0.2	5 ± 0.4	30 ± 4
		Bromocriptine	14 ± 0.9**	3 ± 0.2	9 ± 0.6**	24 ± 3*
	8	PRL antiserum	7 ± 0.5*	2 ± 0.3	6 ± 0.3*	20 ± 1*
		NRS	5 ± 0.5	2 ± 0.2	4 ± 0.2	31 ± 4
		Bromocriptine	8 ± 0.7*	2 ± 0.2	7 ± 0.4*	24 ± 2

Adult female thymus or spleen was assayed with each experiment to assure the reproducibility of the mAb binding procedure. The values were averaged and subjected to SEM evaluation ($n = 9$). Adult cell populations run with each assay demonstrated the reproducible nature of the assay, and the values are reported in Table 1. A 50-μl aliquot of PRL antiserum or NRS was injected subcutaneously into newborns on days 1, 2, and 3. See text for details. Bromocriptine (25 μl; 0.1 mg) was injected on neonatal days 1, 2, and 3. Each value represents the mean ± SEM of at least three organ preparations for which 104 cells were scanned for mAb immunofluorescence on a FACS 440. NA, not applicable.
*Data differ from controls (NRS), $P < 0.05$.
**$P < 0.01$.

Pituitary Implants. Studies of the response of murine splenic lymphocytes to the T- and B-cell mitogens Con A and LPS, respectively, were conducted after the response was known to have matured at neonatal day 45. Only mice with pituitary implants that had been in place since neonatal day 1, presumably possessing increased levels of circulating PRL, exhibited significantly increased responses to both Con A and LPS (Table 4). The controls received an implant of an equal-sized fragment of biceps femoris muscle. Transplants were taken from 3- and 5-mo-old female BALB/c donors. The increase in radiolabeled thymidine incorporation in the pituitary-implant group (60,530 ± 3,203 cpm) was 160% that of the muscle-implant group (38,124 ± 3,438; $P < 0.05$). This effect is similar to that reported for PRL-exposed murine splenocytes stimulated with a suboptimal concentration of Con A (12).

DISCUSSION

Immune responses now known to involve prolactin action include contact sensitivity skin reaction to dinitrochlorobenzene (22, 23), antibody formation to sheep erythrocytes and to bacterial LPS (22, 23), adjuvant arthritis (23–25), experimental allergic encephalitis (23), graft-versus-host reaction (26, 27), allograft rejection process (11, 12, 26), lymphocyte mitogenesis (11, 12, 27, 28), natural killer cell activity (29), cyclosporine immunosuppressive action (5, 6, 27, 30), and macrophage activation (31, 32). The earliest endocrine

Table 3. Effect of neonatally administered anti-mouse PRL antiserum and bromocriptine on organ wet weights at neonatal day 32 in female BALB/c mice

Treatment	Organ weight, g				
	Spleen	Thymus	Heart	Liver	Kidneys
PRL antiserum	0.13 ± 0.007*	0.14 ± 0.005	0.11 ± 0.01	1.16 ± 0.009*	0.27 ± 0.02
NRS	0.10 ± 0.005	0.12 ± 0.009	0.11 ± 0.01	1.10 ± 0.010	0.24 ± 0.02
Bromocriptine	0.12 ± 0.007*	0.13 ± 0.006	0.10 ± 0.01	0.96 ± 0.009*	0.22 ± 0.02
Vehicle only	0.09 ± 0.008	0.13 ± 0.005	0.10 ± 0.01	1.09 ± 0.009	0.23 ± 0.02

Each value represents the mean ± SEM of at least four separate organs.
*Data differ from the appropriate control, $P < 0.05$.

Table 4. Effect of various techniques to manipulate PRL levels on [^{3}H]thymidine incorporation into murine splenic lymphocytes stimulated with Con A or LPS

Treatment	*n*	Response, cpm of [^{3}H]thymidine incorporated		
		Con A	LPS	Vehicle controls
Pituitary implant	7	60,530 ± 3203*	54,258 ± 3814*	1952 ± 327
Muscle implant	6	38,124 ± 3438	37,844 ± 1069	2065 ± 40
PRL antiserum	7	42,385 ± 2373	40,932 ± 3977	2624 ± 664
NRS	7	41,394 ± 1000	36,011 ± 2613	1981 ± 125
Bromocriptine	4	41,262 ± 1959	39,258 ± 151	2476 ± 706

Each value represents the mean ± SEM.
*$P < 0.05$ compared with controls receiving muscle implant or any other group. See text for details.

change detected after exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a potent dioxin found in Agent Orange, is a significant depression in serum PRL levels within 4 hr (33) and a later attenuation of receptor coupling of PRL to physiological events in tissues containing PRL receptors (33). One of the consistent effects of TCDD toxicity is a suppression of both cell-mediated and humoral immunity, again effects that could now be attributed to the effect of TCDD on PRL levels and PRL receptor activity. Recently, the rapid effect of TCDD on PRL levels in the serum has been traced to the ability of TCDD to increase the steady-state level of dopamine in the median eminence (34).

Thus, the data presented in this paper extend the role of PRL as an immunomodulator of adult lymphocyte function to include participation in the developmental expression of T- and B-lymphocyte populations in the thymus and spleen of mice. Whether these developmental differences result in altered immune responses remains to be determined. The effect of long-term increase of PRL level demonstrated herein coincides with the effect of PRL on Con A-stimulated mitogenesis *in vitro* (12), further supporting the physiological relevance of these findings. The extensive evidence of PRL action on the mammalian immune system mandates further studies of its alterations in known immunodeficient states.

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