Hormonal Control of Intestinal Fc Receptor Gene Expression and Immunoglobulin Transport in Suckling Rats

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Abstract

Hormonal control of immunoglobulin (Ig) absorption and of intestinal Fc receptor mRNA expression were investigated in rats to assess its potential role in the normal postsuckling inhibition of this transport system. Corticosterone and L-thyroxine therapy caused premature inhibition of the absorption of orally administered murine monoclonal antibody and of Fc receptor mRNA expression in a dose- and time-dependent manner. Low-dose corticosterone had no effect on Fc receptor mRNA synthesis after 3 d but decreased Ig transport fivefold after 7 d. High dose corticosterone resulted in a threefold reduction in Fc receptor after 3 d, and there was almost complete inhibition (> 30-fold) of transport and of Fc receptor transcript levels after 7 d. Similarly, 7 d of high-dose thyroxine decreased both serum Ig transport and Fc receptor (> 30-fold). However, adrenalectomy did not prevent the normal post-suckling declines in Ig transport or receptor synthesis. This study demonstrates that exogenous corticosteroids and thyroxine hormone inhibit Ig transport and steady-state duodenal Fc receptor mRNA levels in suckling rats. Endogenous adrenal steroids however, do not appear to be entirely responsible for the age-dependent decline in this transport system. (*J. Clin. Invest.* 1993. 91:2844–2849.) Key words: absorption • ontogeny • thyroxine • corticosterone • immunity

Introduction

Immunologic memory is an essential aspect of the immune system that is obtained only with exposure to various antigens (1). Since germ lines do not encode distinct antigenic memory, developing mammals are particularly prone to infection and must rely on "maternal memory" for protection. Many mammals obtain their protective arsenal by passive acquisition of Ig (2, 3).

Developing mammals receive antibodies either in utero or postnatally. The humoral immune system of the newborn rat is primarily acquired postnatally, and is almost entirely of maternal origin (4). While small amounts of Ig are transferred in utero, newborn rats are severely hypogammaglobulinemic and rely on transport of IgG from maternal milk. Through a process called transcytosis, IgG is transported by a specific intestinal Fc receptor (FcRn) from luminal to basolateral membrane of the enterocyte, where the antibody-receptor complex dissociates, and immunoglobulins enter the circulation (5–7).

We have previously demonstrated that orally administered murine monoclonal antibodies (mAb) retain their biologic activity and are rapidly and efficiently absorbed into the systemic circulation of suckling rats via a Fc-dependent mechanism (8). We have also shown that the Fc receptor mRNA is expressed in a proximal to distal distribution in the small intestine which ceases at the time of weaning (Martín, M. G., S. V. Wu, and J. H. Walsh, manuscript submitted for publication) (9, 10). These data demonstrate that the disappearance of IgG transport at weaning is controlled at a transcriptional level with repression of FcRn mRNA steady state levels. The factors that regulate the developmental expression of this gene remain unelucidated. Investigating the role of corticosteroids and thyroxine is an obvious first step since these central "hard-wired" hormones have been shown to regulate numerous intestinal phenotypes (11–13).

The objective of this study was to determine the role of exogenous and endogenous corticosteroid and thyroid hormone on the induction and repression of antibody absorption, and to assess their effects on the transcriptional expression of the intestinal Fc receptor.

Methods

Animal and hormonal protocol. Pregnant Sprague Dawley rats (Harland Sprague Dawley, San Diego, CA) were used for all experiments, and the day of birth was assigned as day-zero of life. Following hormonal or surgical manipulations, all pups were allowed to nurse freely until the morning of monoclonal antibody (mAb) administration. Pups at various ages were separated from their dams for 1 h prior to the oral administration of either anti-somatostatin (CURE S.6), or anti-keyhole limpet hemocyanin (KLH) monoclonal antibodies (14). In these experiments 1 mg of mAb was mixed with SIMILAC® formula (Ross Laboratories, Columbus, OH), to a final volume of 300 μl and delivered orally over 1 min with a blunt-end 24-gauge syringe. In all experiments euthanasia was performed 6 h later by carbon dioxide administration. All serum and proximal duodenal samples were stored at −70°C.

Corticosterone acetate (Sigma Chemical Co., St. Louis, MO) was delivered according to previous reports and separated into either low- or high-dose groups (15). Low and high-dose pups were given 10 or 100 μg/g body wt of corticosterone, respectively, injected on alternate days beginning on day 7, and studied on either day 10 or 15 of life. L-thyroxine (Sigma Chemical Co.) diluted in 0.3 M NaOH (1 mg/1 ml) was injected subcutaneously in one of two doses; 0.1 or 0.4 μg/mg body wt daily beginning on day 7 and animals were studied on day 10 and 15 (16). Age-matched controls received an equal volume of vehicle.

To assess the role of endogenous corticosterone on the natural decline in Ig absorption at weaning, bilateral adrenalectomy was per-

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formed at 10 d of age. Adrenals were removed through a single dorsal incision under halothane anesthesia, and sham-operated littermates served as controls. The excised adrenals were examined to assess the integrity of the capsule and the complete removal of the gland. Pups were weaned on day 18 and studied at 23 d of age. Subcutaneous *d*-aldosterone (Sigma) (0.08 pg/g body wt) was administered daily (17), and all pups were allowed free access to 0.9% NaCl drinking water.

Murine monoclonal antibody synthesis and measurement. CURE S.6 and KLH monoclonal antibodies (IgG_1) were produced at the CURE/UCLA Digestive Disease Center and were previously demonstrated to be absorbed after enteral administration via a dose-, time-, and Fc-dependent mechanism in suckling rats (8). Antibody was obtained by standard mouse ascites technique and purified by protein A sepharose (Pharmacia, Uppsala, Sweden), equilibrated with 0.15 M glycine/3 M NaCl, pH 8.9 and eluted with 0.1 M citrate, pH 3.0. Eluted Ig was placed in 0.05 M PBS, pH 7.4, with the use of Spectra/Por Stirred Cell and a 100 K Ultra Filter Disc (Spectrum Medical Supplies, Los Angeles, CA).

Murine immunoglobulin was measured in rat serum utilizing an ELISA system (18, 19). Monoclonal antibody standard (1:100) and serum samples (20 μl) were applied to microtiter plates coated with goat anti-murine Fc antibody (Sigma Chemical Co.), and serially diluted. Rabbit anti-goat antibody conjugated with horseradish-peroxidase (Sigma Chemical Co.) (1:2,000) was applied followed by a mixture of *o*-phenylenediamine (Sigma Chemical Co.) and H_2O_2 diluted in 0.1 M citrate, pH 5.0. The reaction was terminated at 3 min with 1.8 M H_2SO_4. Absorbance was measured at 492 nm, with a Titertek® Multiscan MCC/340 MK II ELISA reader (Flow Labs, McClean, VA).

Measurement of Fc Receptor mRNA expression. RNA was isolated from duodenal samples using a modification of the guanidine isothiocyanate method of Chomczynski (20). Tissue homogenized with extraction buffer (4 M guanidine isothiocyanate, 0.1 M sodium acetate, 10 mM Tris·HCl, pH 7.4, 1 mM EDTA), was centrifuged at 12,000 *g* in 220 μl of chloroform/isoamylalcohol (24:1) and phenol at 4°C for 15 min. RNA was precipitated with 2-propanol and washed multiple times with 80% ethanol before suspending in tris-EDTA, pH 7.4.

Northern and dot blot analyses were performed according to previously described methods from our laboratory (21). 20 μg of total RNA (A_{260}) was denatured by the addition of 17% deionized formamide (Fisher Scientific, Fair Lawn, NJ), 20% formaldehyde (J. T. Baker Inc., Phillipsburg, NJ), and 3-[*n*-morpholino] propanesulfonic acid (Sigma Chemical Co.) and incubation at 65°C for 15 min. RNA was chromatographed on a 1.2% formaldehyde gel, then transferred to Hybond membrane (Amersham Corp., Arlington Hts., IL) with a transblotter (Bio-Rad Laboratories, Richmond, CA).

Dot blots were performed by serially diluting 10, 5, and 2.5 μg of total RNA in duplicate to Hybond membranes (Amersham Corp.) with a 96 well Manifold II-dot blot apparatus (Schleicher and Schuell Inc., Keene, NH). Denaturing was performed with a 1:1 mixture of 4.6 M formaldehyde and 20× saturated sodium citrate, incubated at 65°C for 15 min. All membranes were air dried, baked in a vacuum oven at 80°C for 2 h, and UV-illuminated for 5 min. Nonspecific background was estimated using an equivalent quantity of rat liver RNA, and the consistency of loading was assessed by rehybridizing with 18S ribosomal oligo-deoxynucleotide (5′ CGG CAT GTA TTA GCT CTA GAA TTA CCA CAG 3′) labeled by standard 5′ end-labeling technique (22).

FcRn cDNA was produced from the duodenum of a 13-day-old rat, and PCR was performed utilizing primers (5′(5′AGG CCA TCA GGA CCC TGG AG 3′) and 3′ (5′GAG CTC CGG GTA GAA GGA 3′)) designed according to the published sequence (23). The 402 base pair PCR-product was subcloned into the SmaI site of pSK(−) and sequenced by Sequenase (Boehringer Mannheim, Indianapolis, IN) method. The FcRn insert was released, chromatographed, eluted and labeled by standard random primer technique (21). Nuctrap Push Column (Stratagene, San Diego, CA) was used to purify labeled template.

Membranes were bathed in hybridization solution (50% deionized formamide, 5× SSC, 5× Denhardt's, 0.1% SDS and 200 μg/ml salmon sperm DNA) with random prime and oligonucleotide blots incubated at 42 and 37°C, respectively. Membranes were prehybridized for 24 h then bathed in fresh solution containing 10^6 cpm/ml of label for 24 h. Membranes were washed twice, for 15 min, at the specified solution and temperature. FcRn blots were washed in 2× SSC/0.1% SDS at 25°C, and in 1× SSC/0.1% SDS at 42°C. A final wash was performed at 50°C in 0.1× SSC/0.1% SDS. 18S blot washes were similar, save a final rinsed at 42°C with 0.1× SSC/0.1% SDS. Membranes were exposed to XAR film (Eastman Kodak Co., Rochester, NY) for 1–2 d at −70°C, and a Jandel Image Analysis System (Jandel Scientific, Corte Madera, CA) was used to assess the density of FcRn and 18S mRNA signal.

Statistics. All conclusions are derived from two-sample (unpaired) *t* tests and data are expressed as mean±SE.

Results

Effect of either corticosteroids or thyroxine on immunoglobulin transport. In this set of experiments, we determined the effects of exogenous corticosterone or L-thyroxine on Ig transport. Rats that received either low or high dose corticosterone for 7 d

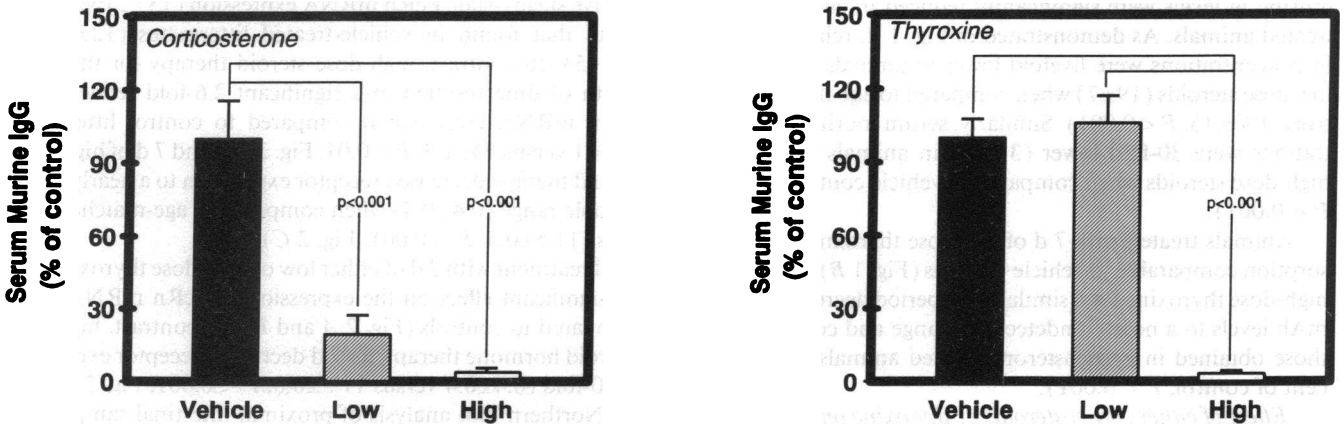

Figure 1. Effects of 7 d of high and low-dose corticosterone and thyroxine on relative serum murine mAb concentration. High (*striped bar*) and low-dose (*white bar*) corticosterone acetate (*A*) or L-thyroxine (*B*) therapy was initiated on day 7, and 1 wk later rats were fasted for 1 h prior to the oral administration of 1 mg of murine mAb. Murine Ig levels are expressed in relative concentrations with controls (*black bar*) reaching serum values of 62±9.3 μg/ml. The figures represent two different experiments with each group containing 6–9 animals/group and data expressed as mean±SE.

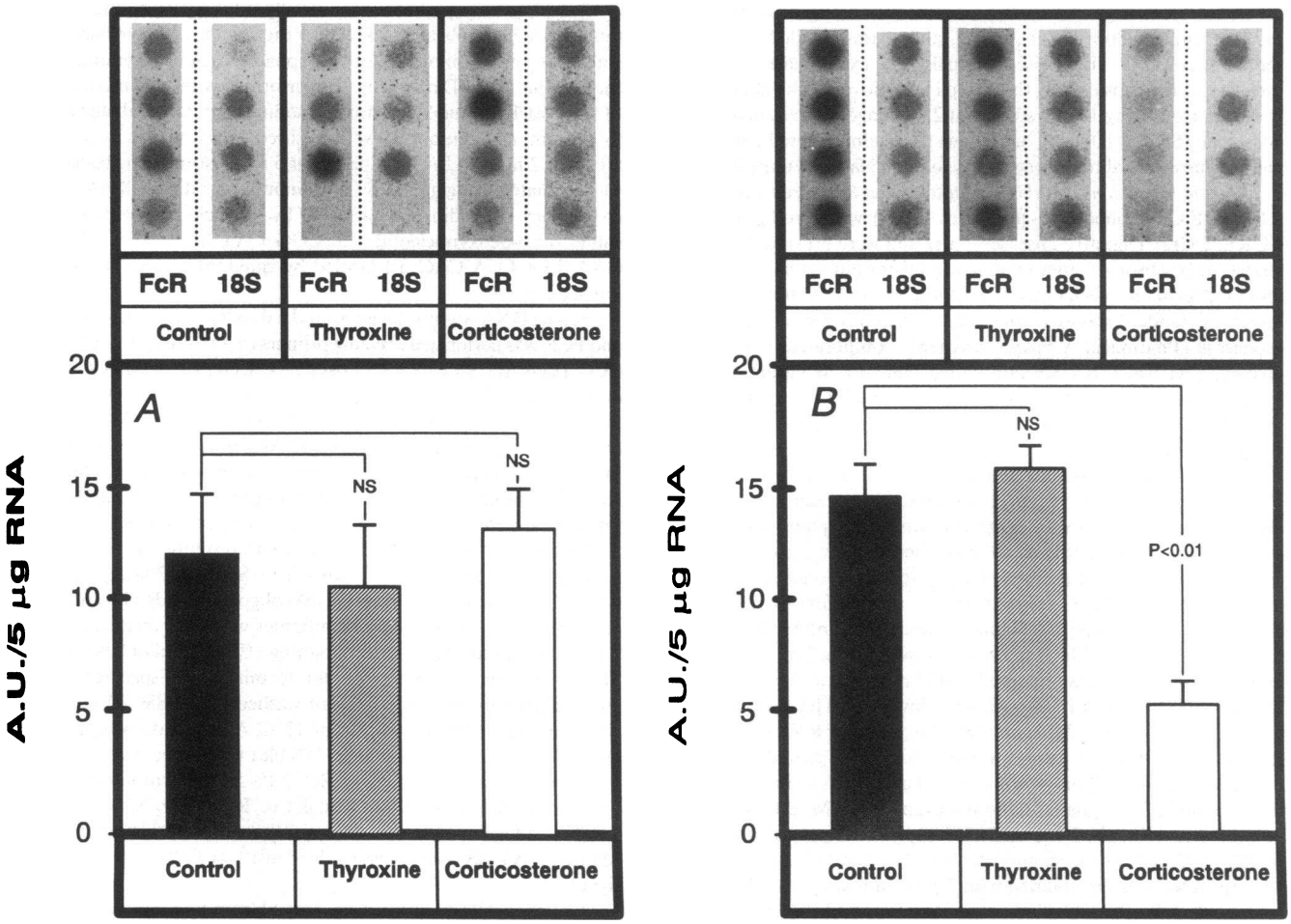

Figure 2. Dot blot analysis of various doses of corticosterone and thyroxine on duodenal FcRn mRNA expression in 15- and 10-day-old rats. Low and high-dose hormones, (*A*) and (*B*), respectively, were administered for 3 d, and RNA from proximal duodenum was hybridized with FcRn and 18S probes. (*C*) represents 7 d of high-dose therapy with either hormone, and as with all displayed blots, represents 5 µg of total RNA hybridized with both FcRn and 18S probe. Immediately below each blot, the data pertaining to the animals are displayed. Data are expressed as arbitrary units (A.U.) and mean±SE, with between 4 and 9 animals/group.

were examined for their ability to absorb enterally delivered murine mAb. 6 h after administration of 1 mg of mAb, serum murine Ig levels were significantly reduced in corticosterone-treated animals. As demonstrated in Fig. 1 *A*, relative murine Ig concentrations were fivefold lower in animals treated with low dose steroids (19±7) when compared to age-matched controls (100±15, $P < 0.001$). Similarly, serum murine Ig concentrations were 30-fold lower (3±0.2) in animals treated with high-dose steroids when compared to vehicle controls (100±7, $P < 0.001$).

Animals treated with 7 d of low-dose thyroxine had Ig absorption comparable to vehicle controls (Fig. 1 *B*). In contrast, high-dose thyroxine for a similar time period decreased murine mAb levels to a nearly undetectable range and comparable to those obtained in corticosterone treated animals (3.1±1 percent of control, $P < 0.001$).

Effect of either corticosteroids or thyroxine on intestinal Fc receptor mRNA expression. In order to assess if the inhibition of Ig uptake occurs at the transcriptional level, steady-state levels of FcRn mRNA expression were examined by dot blot analysis. Dot blot analysis of FcRn mRNA expression was performed on duodenal samples isolated from animals treated with low and high dose corticosterone or thyroxine. Fig. 2 *A* demonstrates that with 3 d of low dose corticosterone therapy, relative steady-state FcRn mRNA expression (13±2) was similar to that found in vehicle-treated littermates (12±2.5, $P > 0.05$). In contrast, high dose steroid therapy for the same length of time resulted in a significant 2.6-fold reduction of FcRn mRNA levels when compared to control littermates (5.3±1 versus 14±1.3, $P < 0.01$, Fig. 2 *B*), and 7 d of high-dose steroid therapy decreased receptor expression to a nearly undetectable range (0.4±0.4) when compared to age-matched controls (11.5±0.5, $P < 0.001$, Fig. 2 *C*).

Treatment with 3 d of either low or high dose thyroxine had no significant effect on the expression of FcRn mRNA when compared to controls (Fig. 2 *A* and *B*). In contrast, high dose thyroid hormone therapy for 7 d decreased receptor expression > 30-fold (0.4±0.4 versus 11.5±0.6, $P < 0.001$, Fig. 2 *C*).

Northern blot analysis of proximal intestinal samples isolated from animals treated with 7 d of either high-dose corticosteroids or L-thyroxine therapy is displayed in Fig. 3. The intensity of the 1.7 kb FcRn specific-transcript in treated animals was significantly less than in age-matched controls. These data suggest that exogenous corticosteroids and thyroxine have par-

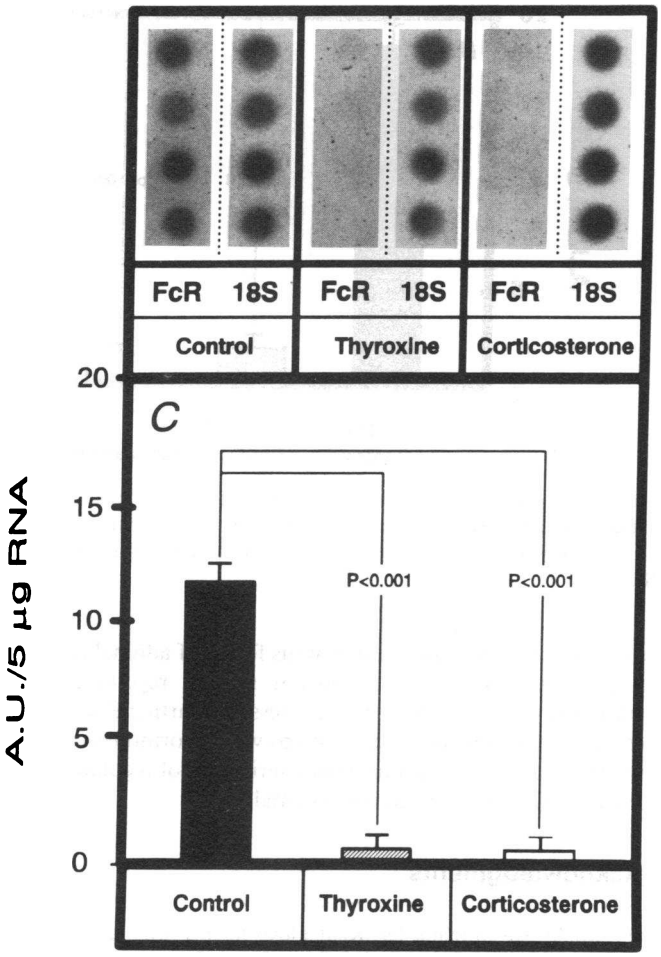

Figure 2 (Continued)

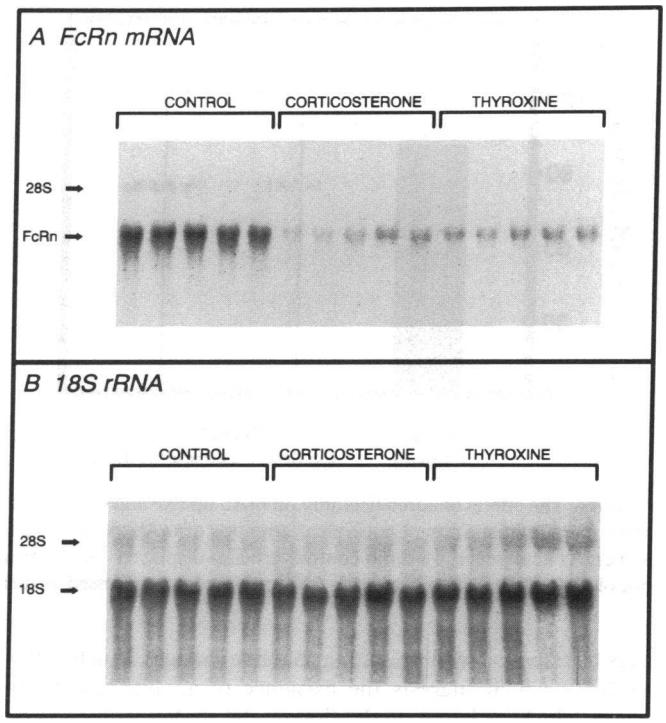

Figure 3. Northern blot analysis of FcRn mRNA expression in 15-d-old rats following high-dose corticosterone and thyroxine therapy. In this experiment, each lane represents mRNA isolated from the duodenum of a single 15-d-old animal. Animals were grouped into either vehicle, thyroxine, or corticosterone treatment. The upper image represents the 1.7 kb specific-transcript of the intestinal Fc receptor. The lower panel is an 18S ribosomal RNA autoradiogram displaying similar quantities of total RNA levels per lane.

allel effects on Ig transport and FcRn expression that are dose and time dependent.

Effect of adrenalectomy on immunoglobulin transport and intestinal Fc receptor mRNA expression. Adrenalectomy studies were performed to assess the role of the endogenous surge of corticosterone at weaning on the Ig transport process. Fig. 4 *A* reveals that only minimal amounts of murine Ig were detected in sera of adrenalectomized (1.0±0.1) and control 23-d-old animals (1.1±0.2), which were substantially lower than in 15-d-old suckling controls (100±4, $P < 0.001$).

Dot blot analyses of steady-state FcRn mRNA expression from the duodenum of 15- and 23-d-old groups were performed. As predicted from the Ig uptake data, FcRn mRNA expression in the duodenums of adrenalectomized and sham-operated littermates was comparably lower than in 15-d-old controls ($P < 0.001$, Fig. 4 *B*).

Discussion

In this study we examined the effects of exogenous and endogenous corticosterone and thyroxine upon murine Ig uptake and intestinal Fc receptor mRNA expression. We have found that Fc receptor mRNA is initially expressed after birth and abruptly declines at the time of weaning (Martín, M. G., S. V. Wu, and J. H. Walsh, manuscript submitted for publication). Moreover, murine mAb absorption mirrors the decline in receptor transcript. To delineate some of the factors responsible for controlling this developmental pattern of expression, we examined the role of corticosteroids and thyroxine on the IgG transport system.

Immunoglobulin uptake and receptor expression data indicated that exogenous corticosterone and thyroid hormone precociously repress transport in a dose- and time-dependent fashion. Treatment for 3 d with high dose corticosterone or for 7 d with low-dose corticosterone resulted in a partial decline in transport, while maximal inhibition was reached with 7 d of therapy. High dose thyroid hormone therapy for 7 d was required to inhibit transport significantly. While others have shown that corticosteroids (24, 25) and thyroxine (26) decrease immunoglobulin uptake, the dose and time-dependency of IgG absorption and FcRn mRNA expression have not previously been examined.

In humans, where IgG transfer occurs primarily in utero, it is unlikely that intestinal IgG transport occurs after 48 h of life, when acid and pancreatic enzyme secretion are nearly fully mature (27). Numerous data, however, suggest that intestinal Ig absorption may occur in fetal, premature, and full-term newborns (< 48 h). Simister has reported a similar size p51 protein in human fetal intestine (28).

Diamond proposed a "hard-wired" model to identify factors responsible for regulating intestinal adaptation (11). The model suggests that either hard-wired or environmental stimuli

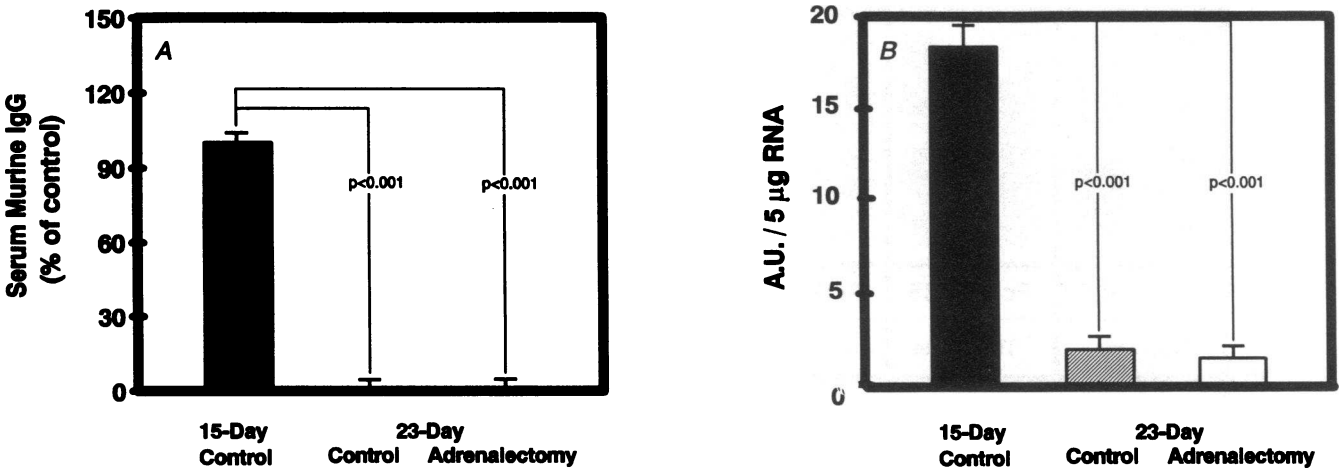

Figure 4. The effects of adrenalectomy on mAb uptake and duodenal FcRn mRNA expression in 23-d-old rats. (*A*) Serum IgG was measured 6 h after oral administration of equivalent amounts of mAb to 23-d-old littermates that underwent either adrenalectomy or sham-operation 13 d earlier. As a reference, 15-d-old controls are also displayed. (*B*) Represents duodenal FcRn mRNA expression from similar animals assessed by dot blot analysis. Data represent 5–6 animals and are expressed as mean±SE.

may be responsible for initiating adaptive changes. Much of the evidence which suggests the existence of an intrinsic hard-wired "clock" is based on developmental studies with corticosteroids and thyroxine, the best studied endocrine regulators of gastrointestinal adaptation (12). In the rat, thyroxine synthesis increases shortly after birth, reaching maximum concentrations at the end of the first week of life (26), while corticosterone levels peak at the time of weaning (29). Both hormones have been shown to accelerate the maturation of gastric acid (30) and pepsin secretion (31), salivary and pancreatic amylase and peptidase synthesis (27), and jejunal sucrase-isomaltase and lactase production (32).

While others have found that dietary (i.e., luminal Ig) and hormonal manipulations may alter the exact timing of a specific gastrointestinal phenotype, functional adaptation at weaning appears to be preprogrammed. Adrenalectomy was ineffective at counteracting the normal decline in absorption at weaning, suggesting that the induction of corticosteroids at weaning is not solely responsible for terminating the transport process. While exogenous hormones have been shown to alter various intestinal phenotypes, adrenalectomy usually delay, but never prevent, the eventual ontogenic alteration (12, 33). More specifically, adrenalectomy does not alter either the exact timing or the eventual level of sucrase expression in sucklings, but appears to decelerate an intrinsic program that induces enzyme synthesis (34). These data suggested the presence of an intrinsic clock, which allows age-specific phenotypes to "bend but never break" their pre-programmed patterns of expression.

A working model which encompasses these findings is that the intrinsic clock may represent a network of transcriptional factor(s) which, when acting as *trans*-acting factors, would function to inhibit gene expression in suckling animals (i.e., FcRn) and induce adult phenotypic characteristics. While thyroxine and corticosterone have been shown to bind *cis*-acting elements (i.e., AP_1), they may alter transcription either in *cis* or in *trans* through this proposed network of developmental transcriptional factors. Alternatively, as with other genes, other negative regulatory elements may be essential in inhibiting the expression of the receptor. These data demonstrate that the Fc receptor gene is responsive to exogenous corticosteroids and thyroid hormone, while endogenous forms of adrenal corticosteroids do not play a significant role in regulating programmed patterns of decreased expression. Further elucidation of the exact molecular mechanism by which hormones regulate intestinal adaptation awaits characterization of regulatory elements of the pertinent developmental genes.

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References

1. Apodaca, G., M. Bomsel, J. Arden, P. P. Breitfeld, K. Tang, and K. E. Mostov. 1991. The polymeric immunoglobulin receptor: A model protein to study transcytosis. *J. Clin. Invest.* 87:1877–1882.

2. Kraehenbuhl, J. P., and M. A. Campiche. 1969. Early stages of intestinal absorption of specific antibodies in the newborn. *J. Cell Biol.* 42:345–365.

3. Porter, P. 1979. Absorptive immunization of the neonate by breast factors. *In* Immunology of Breast Milk. P. L. Ogra and D. Dayton, editors. Raven Press, New York. 197–206.

4. Mackenzie, N. M. 1991. Transport of maternally derived immunoglobulin across the intestinal epithelium. *In* Ontogeny of the Immune System of the Gut. T. T. MacDonald, editor. CRC Press, Boca Raton, FL. 69–81.

5. Mostov, K. E., and N. E. Simister. 1985. Transcytosis. *Cell.* 43:389–390.

6. Rodewald, R., and D. R. Abrahamson. 1982. Receptor-mediated transport of IgG across the intestinal epithelium of the neonatal rat. *Ciba Found. Symp.* 209–232.

7. Rodewald, R. 1976. pH-dependent binding of immunoglobulins to intestinal cells of the neonatal rat. *J. Cell Biol.* 71:666–669.

8. Martin, M. G., S. V. Wu, G. Ohning, and J. H. Walsh. 1990. Oral somatostatin monoclonal antibody induces rapid increase in plasma gastrin and gastrin mRNA in suckling rats. *Gastroenterology.* 98(5):A422. (Abstr.)

9. Rodewald, R. 1973. Intestinal transport of antibodies in the newborn rat. *J. Cell Biol.* 58:189–211.

10. Rodewald, R., and J. P. Kraehenbuhl. 1984. Receptor-mediated transport of IgG. *J. Cell Biol.* 99:159s–164s.

11. Diamond, J. M. 1986. Hard-wired local triggering of intestinal enzyme expression. *Nature (Lond.)*. 324:408.

12. Henning, S. J., and L. L. Leeper. 1982. Coordinate loss of glucocorticoid responsiveness by intestinal enzymes during postnatal development. *Am. J. Physiol.* 242:G89–G94.

13. Yeh, K. Y., M. Yeh, and P. R. Holt. 1989. Differential effects of thyroxine and cortisone on jejunal sucrase expression in suckling rats. *Am. J. Physiol.* 256:G604–G612.

14. Wong, H. C., J. H. Walsh, H. Yang, Y. Tache, and A. Buchan. 1990. A monoclonal antibody to somatostatin with potent in vivo immunoneutralizing activity. *Peptides.* 11:707–712.

15. Tseng, C. C., K. L. Schmidt, and L. R. Johnson. 1987. Hormonal effects on development of the secretory apparatus of chief cells. *Am. J. Physiol.* 253:G274–G283.

16. Lakshmanan, J., J. Padbury, T. Macaso, D. Wang, U. Beri, and D. A. Fisher. 1986. Involvement of developing sympathetic nervous system in thyroxine-mediated submandibular gland nerve growth factor and epidermal growth factor responses. *Pediatr. Res.* 20:232–236.

17. Schroeder, R. J., and S. J. Henning. 1989. Roles of plasma clearance and corticosteroid-binding globulin in the developmental increase in circulating corticosterone in infant rats. *Endocrinology.* 124:2612–2618.

18. Macy, E., M. Kemeny, and A. Saxon. 1988. Enhanced ELISA: how to measure less than 10 picograms of a specific immunoglobulin protein (immunoglobulin) in less than 8 hours. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 2:3003–3009.

19. Stevens, F. 1987. Modification of an ELISA-based procedure for affinity determination:correction necessary for use with bivalent antibody. *Mol. Immunol.* 10:1055–1060.

20. Chomczynski, P., and N. Sacci. 1987. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol extraction. *Anal. Biochem.* 162:156–159.

21. Wu, S. V., A. Giraud, M. Mogard, K. Sumii, and J. H. Walsh. 1990. Effects of inhibition of gastric secretion on antral gastrin and somatostatin gene expression in rats. *Am. J. Physiol.* 258:21:G788–G793.

22. Clements, J. A., B. A. Matheson, D. R. Wines, J. M. Brady, R. J. MacDonald, and J. W. Funder. 1988. Androgen dependence of specific kallikrein gene family members expressed in rat prostate. *J. Biol. Chem.* 263:16132–16137.

23. Simister, N. E., and K. E. Mostov. 1989. An Fc receptor structurally related to MHC class I antigens. *Nature (Lond.)*. 337:184–187.

24. Halliday, R. 1959. The effect of steroid hormones on the absorption of antibody by the young rat. *J. Endocrinol.* 18:56–66.

25. Morris, B., and R. Morris. 1976. The effects of corticosterone and cortisone on the uptake of polyvinyl pyrrolidone and the transmission of immunoglobulin G by the small intestine in young rats. *J. Physiol. (Lond.)*. 254:389–403.

26. Fisher, D. A. 1977. Ontogenesis of hypothalamic-pituitary-thyroid function in man, sheep, and rats. *R. Prog. Horm. Res.* 33:59–116.

27. Lu, R. B., K. Chaichanwatanakul, C. H. Lin, E. Lebenthal, and P. C. Lee. 1988. Thyroxine effects of exocrine pancreatic development in rats. *Am. J. Physiol.* 254:G315–G321.

28. Israel, E. J., N. Simister, E. Freiberg, R. Hendren, and W. A. Walker. 1988. Immunoglobulin G binding site on the human fetal intestine. *Clin. Res.* 36:806A. (Abstr.)

29. Henning, S. J. 1978. Plasma concentration of tatal and free corticosterone during development in the rat. *Am. J. Physiol.* 235:E451–E456.

30. Tseng, C. C., K. L. Schmidt, and L. R. Johnson. 1987. Hormonal effects on development of the secretory apparatus of parietal cells. *Am. J. Physiol.* 253:G284–G289.

31. Furihata, C. T., T. Kawachi, and T. Sugimura. 1972. Premature induction of pepsinogen in developing rat gastric mucosa by hormones. *Biochem. Biophys. Res. Commun.* 47:705–711.

32. Yeh, K. Y., M. Yeh, and P. R. Holt. 1991. Thyroxine and cortisone cooperate to modulate postnatal intestinal enzyme differentiation in the rat. *Am. J. Physiol.* 260(23):G371–G378.

33. Yeh, K. Y., and F. Moog. 1974. Intestinal lactase activity in the suckling rat: influence of hypophysectomy and thyroidectomy. *Science (Wash. DC)*. 182:77–79.

34. Henning, S. J. 1987. Functional development of the gastrointestinal tract. *In* Physiology of the Gastrointestinal Tract. L. R. Johnson, editor. Raven Press, New York. 285–300.