Influence of Thyroidectomy and Thyroid Hormones on Adjuvant Arthritis in Rats (34482)

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(Introduced by R. Gaunt)

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Previous work has suggested that thyroid hormones potentiate, while hypothyroidism decreases, the inflammatory response of certain connective tissues of the rat to irritating substances (1–5). The present work was undertaken to establish the effects of thyroidectomy upon the adjuvant-induced arthritis in rats. The data show that thyroidectomy decreases, while thyroid hormones restore, the polyarthritic response induced by injection of *M. butyricum* adjuvant in the rat. It was also of interest to study the serum sulfhydryl–disulfide interchange reaction, as this has been found to be impaired in arthritic rats and restored to normal by treatment with anti-inflammatory agents (6).

Materials and Methods. Intact or thyroidectomized ("Tx") male Sprague-Dawley rats (Charles River CD-1) were used. Polyarthritis was induced by a single intradermal injection of 1 mg of *M. butyricum* in 0.05 ml of heavy mineral oil into the tail.

Operations were performed when the rats weighed 200 ± 10 g, and then they were held for 1 month before the start of the experiments at which time the unoperated controls weighed 300 ± 10 g and Tx rats weighed 200 ± 10 g, all being approximately the same age. The rats were fed a standard chow diet. After the injection of *M. butyricum,* the rats were divided into groups, some receiving l-thyroxine (T_4) at 5 μg/100 g body weight sc or 3, 5, 3′-triiodo-*l*-thyronine (T_3) 1 μg/100 g body weight from the day of adjuvant injection until the time of killing 21 days later. Intact, Tx, and Tx + T_4 or Tx + T_3 subgroups also received indomethacin, 0.3 mg/kg from Day 7 to Day 21. On Day 21 the rats were anesthetized with ether and were exsanguinated by heart puncture. The sulfhydryl (SH)–disulfide (SS) interchange reaction of serum was determined according to Butler *et al.* (6). Briefly, fresh serum obtained from each rat at autopsy was reacted with 5,5′-dithiobis-(2-nitrobenzoid acid) in pH 7.4 phosphate buffer, and the change in optical density (OD) between 1 and 2 min by release of 5-thio-2-nitrobenzoic acid was recorded on a Beckman DB spectrophotometer at 440 mμ. Body weights were recorded at the start of treatment (Day 7) and at the conclusion of the experiment (Day 21). Secondary arthritic lesions appeared on the extremities 10-15 days after adjuvant injection. The severity of lesions on each limb was subjectively graded three times weekly according to the following scale (6): 0 = no lesions; 1+ = detectable nodules on or swelling of paw; 2+ = moderate swelling of joint(s) and paw; 3+ = extensive swelling of joints and paw. For an overall evaluation the separate scores for each leg were added. Thus the maximal possible score for each rat was 12+ (3+ for each limb).

Results. Mean arthritic scores in intact rats rose from 0 at 11 days to 4.4 at 14 days and then plateaued between 4.5 and 5.0 until 21 days postadjuvant (Fig. 1a). When similar rats were treated with indomethacin from Day 7 to Day 21, the mean arthritic score never exceeded 2.2 (Fig. 1a). Tx rats, on the other hand, never developed severe arthritis, the maximum mean score being 2.6 on Day 21 (Fig. 1b). Indomethacin treatment did not influence the severity of arthritis at any time interval in Tx rats (Fig. 1b). On the other hand, Tx rats treated with T_4 responded to adjuvant injection with arthritic scores similar to those observed in intact rats (Fig. 1c). Treatment of such rats with indomethacin sharply reduced the severity of the arthritis (Fig. 1c). Essentially similar results were

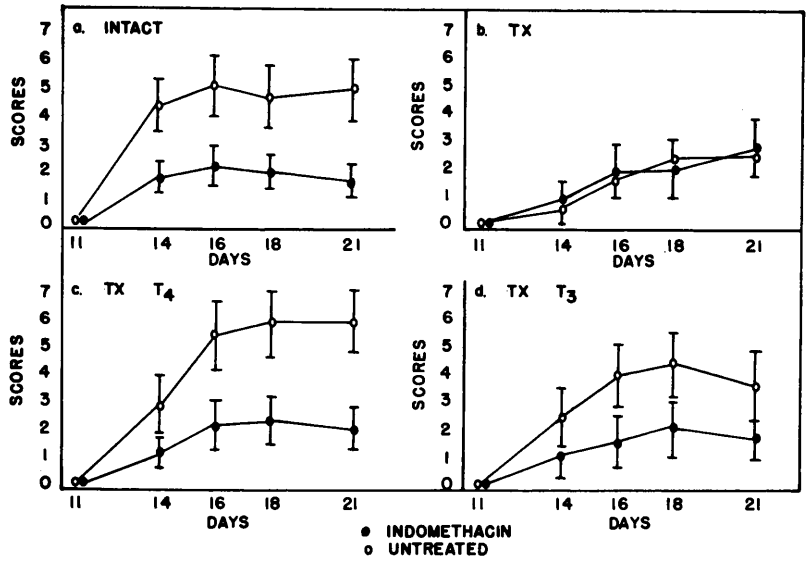

FIG. 1. Course of polyarthritis in adjuvant-treated rats. Days = days after adjuvant injection. a. Intact; b. Thyroidectomized (Tx) 30 days before adjuvant injection; c. Thyroidectomized + *l*-thyroxine, 5 μg/100 g × 21 days (Tx + T_4); d. Thyroidectomized + 3, 5, 3′-triiodo-*l*-thyronine, 1 μg/100 g × 21 days (Tx + T_3); no indomethacin (O-O); indomethacin, 0.3 mg/kg from Days 7–21 (●-●). Vertical bars indicate ± 1 standard error of the mean.

obtained in Tx rats maintained on T_3 (Fig. 1d).

Statistically significant reductions in body weight gain were observed in intact rats treated with adjuvant versus intact untreated rats ($p < .01$), Tx rats versus intact rats ($p < .001$), and in nonarthritic Tx rats versus similar Tx rats receiving T_3 or T_4 therapy ($p < .05$-$.01$, Table I). Indomethacin had no significant effect on body weight gain. Adjuvant injection did not significantly depress body weight gain in Tx rats whether or not they were treated with T_3 or T_4 (Table I).

The SH–SS interchange reaction was depressed by adjuvant injection in intact, Tx, Tx + T_4- or Tx +T_3-treated rats (Table I). Neither Tx, Tx + T_4, Tx + T_3, nor indomethacin alone influenced the SH-SS interchange reaction. However, in intact or Tx + T_3-treated arthritic rats, indomethacin restored the depressed SH-SS interchange reaction toward normal (Table I).

Discussion. As in many other situations, hypo- or hyperthyroidism induces important changes in the response to inflammatory agents or anti-inflammatory drugs (1–6). In the present case, hypothyroid rats did not develop the acute polyarthritis observed in control rats. It should be emphasized that Charles River Sprague-Dawley rats are rather resistant to adjuvant-induced arthritis and, therefore, the same results might not occur in more arthritis-prone strains (e.g., Lewis inbred). This possibility is currently under investigation. Nevertheless, the present results are clear-cut. Hypothyroidism, induced by Tx, resulted in a minimal response to injection of the standard arthritis-inducing regimen. That this deficit in response was due to hypothyroidism and not the operative procedures *per se* was proved by restoration of the polyarthritic response by injection of T_3 or T_4 in generally accepted physiological doses (*i.e.*, 1 or 5 μg/100 g/day respectively). Of particular interest was the failure of indomethacin (at a dose which significantly reduced the arthritis scores in intact rats) to inhibit arthritic responses in Tx rats. However, the identical dose of indomethacin did significantly reduce lesion formation in Tx rats treated with T_3 or T_4. Indomethacin treatment likewise restored toward normal the SH–SS interchange reaction of intact or Tx + T_3-treated rats, while not influencing

TABLE I. Influence of Thyroid Status, Indomethacin, and Adjuvant Injection on Serum SH–SS Interchange Reactions in Rats.

Group	No. rats	Adjuvant	Indomethacin	Body wt ± SE Day 7	Body wt ± SE Day 21	Change	SH–SS interchange ± SE Δ^{OD} 1–2 min at 440 mμ
Intact	15	—	—	334 ± 6.4	397 ± 9.2	+63	0.109 ± 0.004
	20	+	—	312 ± 6.4	323 ± 12.0	+11	0.038 ± 0.008
	10	—	+	323 ± 9.6	391 ± 5.8	+68	0.106 ± 0.004
	20	+	+	317 ± 6.0	350 ± 10.3	+33	0.072 ± 0.008
Tx[a]	10	—	—	213 ± 3.7	209 ± 5.3	−4	0.106 ± 0.004
	29	+	—	200 ± 4.5	202 ± 5.0	+2	0.055 ± 0.007
	10	—	+	201 ± 6.6	206 ± 7.6	+5	0.104 ± 0.005
	10	+	+	198 ± 7.3	203 ± 10.6	+5	0.069 ± 0.005
Tx + T_4[b]	10	—	—	205 ± 9.0	248 ± 12.7	+43	0.121 ± 0.004
5 μg/100 g	10	+	—	196 ± 5.0	219 ± 13.4	+23	0.057 ± 0.013
	10	—	+	207 ± 3.8	252 ± 13.8	+45	0.112 ± 0.004
	10	+	+	196 ± 3.2	217 ± 9.6	+21	0.058 ± 0.013
Tx + T_3[c]	10	—	—	196 ± 4.0	252 ± 13.9	+56	0.115 ± 0.004
1 μg/100 g	10	+	—	202 ± 7.6	260 ± 14.7	+58	0.053 ± 0.013
	10	—	+	199 ± 6.6	225 ± 19.5	+26	0.114 ± 0.004
	10	+	+	207 ± 7.6	236 ± 14.5	+29	0.091 ± 0.015

[a] Tx = thyroidectomized.
[b] T_4 = *l*-thyroxine.
[c] T_3 = *l*-triiodothyronine.

that of Tx rats. The failure of T_4 to correct the SH–SS interchange reaction has not been explained, but it may have to do with the poorer effect of T_4 versus T_3 on adrenal reactivity (7). This remains to be studied.

Present data demonstrate that body weight gain is not necessarily related to the severity of adjuvant arthritis (*i.e.*, the poorest body weight gain was recorded in Tx rats which did not develop severe arthritis. On the other hand, T_4 or T_3 therapy improved weight gain in Tx rats and yet these animals developed a more severe arthritis than did Tx controls).

Summary. The influence of Tx with or without replacement therapy (T_3 or T_4) on development of adjuvant-induced polyarthritis and its response to indomethacin therapy was studied. Tx reduced the development of arthritic lesions in response to adjuvant. The response was restored by concomitant therapy with T_3 or T_4. Indomethacin exerted an antiarthritic effect in intact or Tx + T_4- or Tx + T_3-treated rats, but had no effect on Tx rats treated with adjuvant alone. The important role of thyroid hormones in development of inflammation and its reversal by anti-inflammatory drugs is thus found to hold true in the case of a delayed inflammatory response.

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