

INDUCTION OF AUTOIMMUNITY IN GOOD AND POOR RESPONDER MICE WITH MOUSE THYROGLOBULIN AND LIPOPOLYSACCHARIDE*

BY PATRICIO S. ESQUIVEL,‡ NOEL R. ROSE, AND YI-CHI M. KONG

(From the Department of Immunology and Microbiology, Wayne State University, School of Medicine, Detroit, Michigan 48201)

Experimental autoimmune thyroiditis can be induced in mice using mouse thyroglobulin (MTg)¹ when it is emulsified in complete Freund's adjuvant (CFA) (1). The disease usually involves both antibody production to MTg and infiltration of the thyroid by mononuclear cells; its severity is governed by the genetic constitution of the animals (2). Whereas mice with the *H-2^k*, *H-2^a*, or *H-2^s* haplotype are good responders, developing high antibody titers to MTg and extensive cellular infiltration of the thyroid, those with the *H-2^b* or *H-2^d* haplotype are considered poor responders with lower antibody titers and little infiltration of the thyroid. It has been shown previously, using adoptive transfer experiments, that the genetic regulation is dependent upon the presence of thymus-derived (T) lymphocytes (3). These studies in poor responder mice, which have been thymectomized, irradiated, and reconstituted with T or bone marrow-derived (B) cells from good or poor responders and subsequently immunized with MTg in CFA, show that T cells are required for responsiveness to MTg. Moreover, only the transfer of T cells from good responder mice leads to infiltration of the thyroid, regardless of the source of B cells. On the other hand, using the antigen-binding assay, it has been demonstrated that normal mice have syngeneic MTg-binding B, but not T, cells (4). Also the incidence and severity of thyroiditis are decreased when B cells have been treated with labeled MTg by the antigen suicide technique. That only B cells have receptors for MTg lends support to the hypothesis that self-tolerance to MTg exists at the T-cell level (4, 5).

Bacterial lipopolysaccharide (LPS) is a mitogen for B cells (6). According to some reports, its use seems to obviate the requirement for T cells in the response to erythrocytes and hapten conjugates (6, 7). It has been shown to interfere with the induction of acquired tolerance to T-cell-dependent antigen in a manner suggesting an effect on B cells (8, 9). However, LPS is also a potent adjuvant for T-cell-dependent antigens (10) and there is accumulating evidence for an action

* Supported by U. S. Public Health Service Grants CA 16426 and CA 02357.

‡ Recipient of Fogarty Fellowship from the National Institutes of Health. Present address: Department of Experimental Medicine, Austral University, Valdivia, Chile.

¹ *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; IFA, incomplete Freund's adjuvant; LPS, bacterial lipopolysaccharide (endotoxin); MTg, mouse Tg; PBS, phosphate-buffered physiologic saline; Tg, thyroglobulin.

on T cells, as well as B cells, in several systems (11-14). Therefore, we have studied the effect of LPS on the maintenance of self-tolerance to MTg, since the T-cell-dependent responses to MTg are genetically controlled. We have found that LPS can abrogate the natural tolerance to MTg. This effect seems to require the presence of T cells and is more pronounced in good responder than poor responder mice.

Materials and Methods

Mice. Congenic strains, B10.BR (*H-2^k*) and B10.D2 (*H-2^d*), were obtained from The Jackson Laboratory, Bar Harbor, Maine. Congenitally athymic mice on a BALB/c (*H-2^d*) background, originally obtained from Dr. D. C. Shreffler, Washington University, St. Louis, Mo., were used at the ninth generation backcross. All mice were 8- to 10-wk-old females.

Antigen. Frozen mouse thyroids (Pel-Freez Biologicals, Inc., Rogers, Ark.) were processed essentially as detailed earlier (15). Briefly, the thyroids were homogenized in phosphate-buffered physiologic saline (PBS), pH 7.2, at 4°C with an Omni-Mixer (VirTis Co., Inc., Gardiner, N. Y.) at 22,000 rpm for 3 min and then extracted overnight at 4°C. An alternate method of homogenization with a Teflon-pestle tissue grinder without overnight extraction was also employed. The extract was clarified by centrifugation (15) and the MTg precipitated with 45% saturated ammonium sulfate as described earlier (16). The MTg was dissolved in 2-3 ml of PBS and dialyzed overnight. This solution was further fractionated on a Sephadex G-200 column (2.5 x 90 cm) which had been equilibrated with 0.1 M phosphate buffer, pH 7.2. The concentration of purified MTg was determined spectrophotometrically at 280 nm or by the biuret reaction (16). It was sterilized by filtration (Millipore membrane, 0.45 μ m pore size), stored at 4°C, and used within 3 wk. For immunization, 250 μ g of MTg was given with or without adjuvant on days 0 and 7.

Adjuvants. CFA was prepared as described previously (1), using 7 mg/ml of killed and dried *Mycobacterium tuberculosis*, strains C, DT, and PN (Lederle Laboratories, Pearl River, N. J.). The mycobacteria were dispersed in hexadecane (Eastman Kodak Co., Rochester, N. Y.) by sonication (Sonifier Cell Disruptor, Model W140D; Ultrasonic Industries Inc., Hicksville, N. Y.) for 30 min (17) and emulsified with MTg after the addition of Myverol (Myverol 18-98 distilled monoglycerides; Distillation Products, Division of Eastman Kodak, Rochester, N. Y.).

LPS from *Salmonella enteritidis*, prepared by Dr. C. D. Jeffries of our department according to the method of Ribí (18), had been tested previously for adjuvanticity (19) and mitogenicity (20). It was sterilized by filtration and, except where noted, a dose of 20 μ g was given intravenously (i.v.) 3 h after the injection of MTg on days 0 and 7.

Cell Transfer Procedure. T-cell-deficient B10.BR mice were obtained by thymectomy (21) at 5-7 wk of age followed 3-5 wk later by whole body irradiation from a Cobalt-60 source (22). The animals were maintained on chlorinated water before and after irradiation. After irradiation, they were given 2×10^7 syngeneic bone marrow cells and 2×10^7 syngeneic thymus cells i.v., each in a 0.2 ml vol. Before injection, the bone marrow cells were treated with anti- θ serum (kindly supplied by Dr. P. Campbell, National Jewish Hospital, Denver, Col.) plus agarose-adsorbed (23) guinea pig complement (Grand Island Biological Co., Grand Island, N. Y.) and washed three times.

Antibody Determination. Mice were bled from the tail artery at intervals of 7-14 days or at the termination of the experiment. Antibodies were titrated in microtiter plates using human group O erythrocytes coated with MTg by means of chromium chloride (24). In some experiments their sensitivity to 0.1 M 2-mercaptoethanol (Eastman Kodak Co.) was also determined. Antibody to LPS was determined by passive hemagglutination (25) using LPS-coated human group O erythrocytes in microtiter plates.

Histology. At intervals of 7-14 days or at the termination of the experiment, groups of mice were killed under ether anesthesia and their thyroids, livers, and kidneys removed for histologic examination. The infiltration of mononuclear cells in the thyroid was graded on a scale from 0 to 4 and expressed as pathology index (1).

Statistical Analysis. The standard error of the mean was used for calculation. The statistical significance of the differences between control and experimental groups was determined by the Student's *t* test.

Results

Effect of LPS on the Immune Response to MTg in Good Responder Mice. In good responder mice, the injection of soluble MTg induces no detectable antibody production or cellular infiltration in the thyroid, while MTg given in specially prepared CFA results in high antibody titers and severe thyroiditis (2). Using MTg alone or in CFA as controls, the capacity of LPS to abrogate self-tolerance to MTg was examined. Good responder B10.BR mice were immunized with soluble MTg followed by LPS 3 h later on days 0 and 7, a schedule known to induce autoimmune thyroiditis in mice treated with MTg in CFA. The animals were monitored weekly for antibody level and killed biweekly in groups of three to seven for histologic examination of the thyroid. Their livers and kidneys were also obtained for sectioning.

As seen in Fig. 1, the administration of MTg followed by LPS resulted in average \log_2 hemagglutinin titers approaching 14, which were as high as those observed in mice given MTg in CFA. The kinetics of antibody production were the same in both groups and all the antibodies were found to be mercaptoethanol resistant. Histologically, moderate to severe thyroiditis was observed after day 21 in mice that had received LPS as adjuvant. In other experiments, mice given LPS as adjuvant had no lesions before day 21. The pathology indices in mice that had received LPS as adjuvant were highest on day 49, as were those in mice given CFA as the adjuvant. In contrast, the injection of MTg alone did not induce the formation of detectable antibody and cellular infiltration of the thyroid; no significant difference was observed between this group and untreated, normal mice. In another experiment, control groups included mice given CFA or LPS without MTg. Neither antibody to MTg nor cellular infiltration of the thyroids was detectable in either group. In some animals receiving LPS as adjuvant, the antibody to LPS was also determined at intervals from days 10 to 28. The average \log_2 titers ranged from 1.8 to 5.6 and the antibody was mercaptoethanol sensitive.

Careful examination of the histologic sections did not reveal discernible differences in the type of cells infiltrating the thyroid of good responder mice treated with MTg and LPS or MTg in CFA. An example of severe thyroid pathology on day 49 from each group is presented in Fig. 2; a destruction of >70% of the thyroid tissue is represented in both sections. At higher magnification (Fig. 3), the mononuclear cell infiltration is clearly shown with both adjuvants. No lesions were observed in the liver and kidney sections of these animals.

Effect of LPS on the Immune Response to MTg in Poor Responder Mice. Genetic regulation of the immune response to MTg is known to occur in mice when CFA is used as adjuvant (2). To determine if such regulation also played a role in mice given LPS as adjuvant, a poor responder strain congenic to B10.BR was tested. The immunization protocol of B10.D2 mice was identical to the one used with the good responder strain. The animals were monitored weekly for changes in antibody level and sacrificed in groups of three to six for histologic examination. The results depicted in Fig. 4 show that LPS as an adjuvant was less effective than CFA in inducing high antibody levels to MTg in the poor responder strain. Antibody was first detected after 21 days compared to

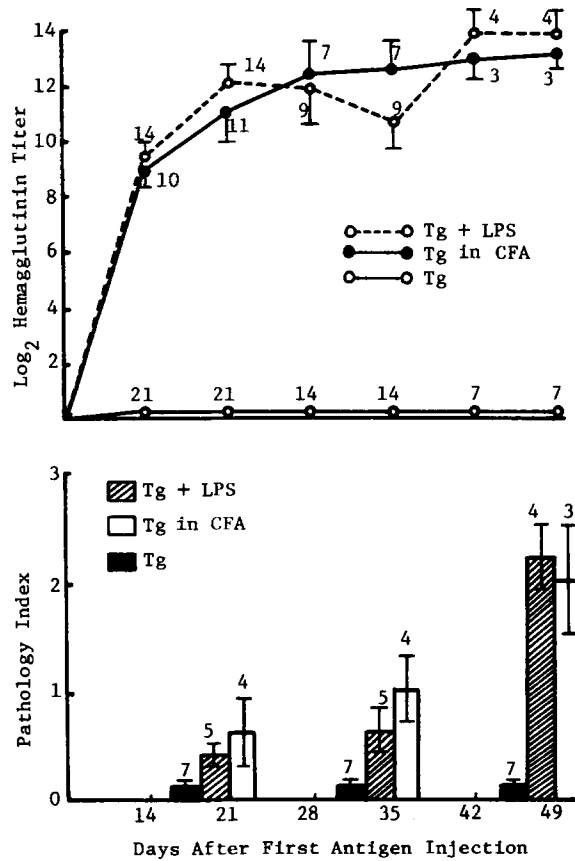
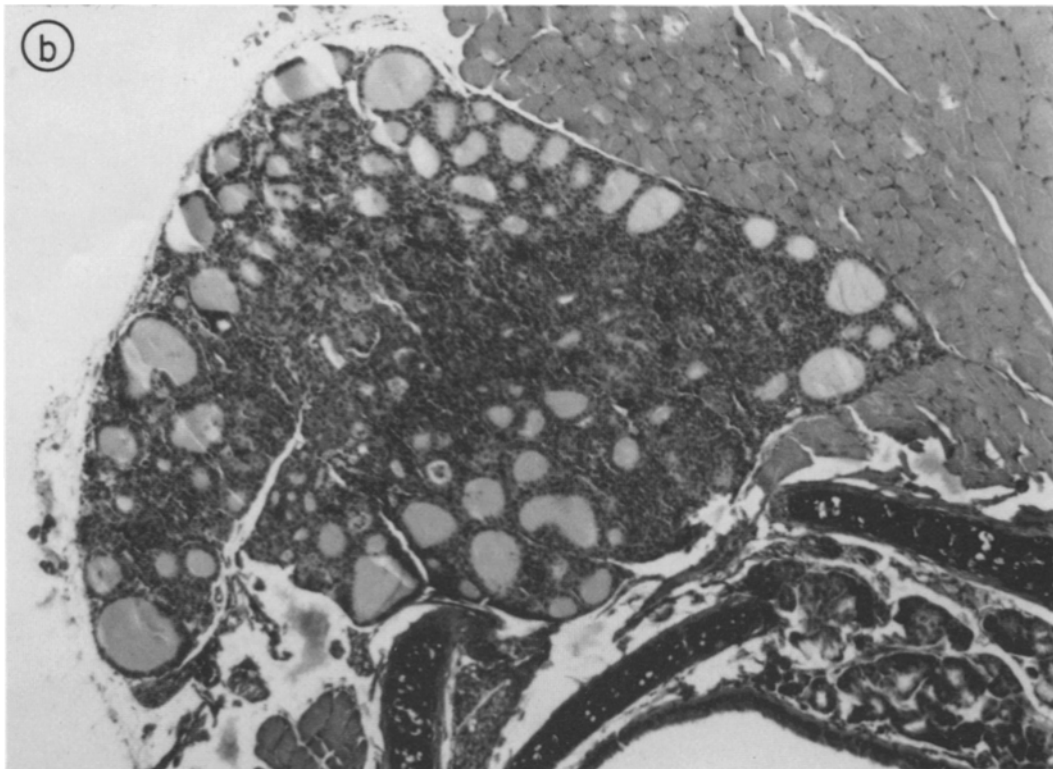
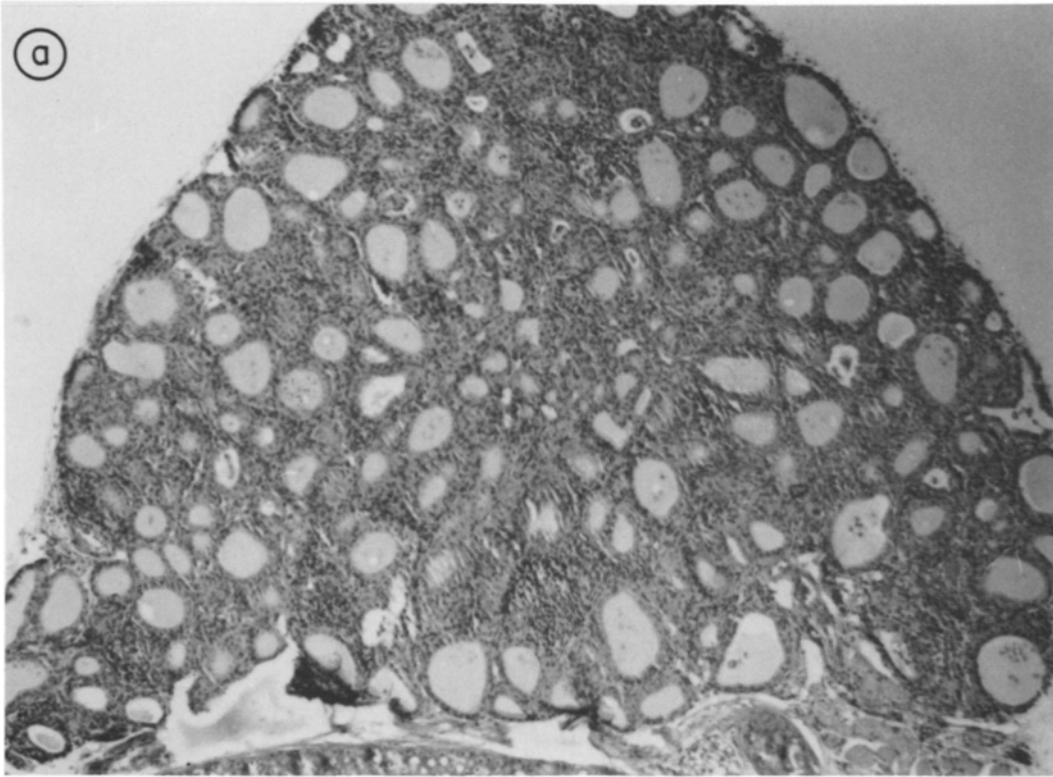


FIG. 1. Effect of LPS on the antibody responses to MTg and thyroid pathology in good responder (B10.BR) mice. LPS was given in a dose of 20 μ g i.v. at 3 h after 250 μ g of MTg i.v. on days 0 and 7. Control groups received MTg alone or MTg incorporated in CFA. The data are represented as the mean of the indicated number of animals \pm SE.

14 days in mice given MTg in CFA. Although the antibodies detected in both groups were mercaptoethanol resistant, their concentration in mice immunized with MTg in CFA was approximately sixfold higher than in mice injected with MTg and LPS. Histologically, the use of CFA resulted in mild to moderate cellular infiltration in the thyroids of poor responder mice; the greatest pathology index found was 1.0 on day 21. No significant infiltration took place in the thyroids of mice treated with MTg and LPS.

Since MTg in the absence of LPS did not induce any detectable antibody formation, LPS administration apparently led to the abrogation of self-tolerance to MTg even in poor responder mice, although no thyroid lesions were induced. Still, compared to B10.D2 mice given CFA as adjuvant, the antibody levels were low. To determine if higher concentrations of antibody and significant thyroiditis could be induced by increasing the LPS dosage, LPS was given to B10.D2 mice on day 3 in addition to days 0 and 7. The animals were bled on days 14 and 28 and thyroid sections obtained. Control mice were given MTg or LPS alone, or MTg in CFA. As seen in Table I, this additional dose of LPS resulted in earlier

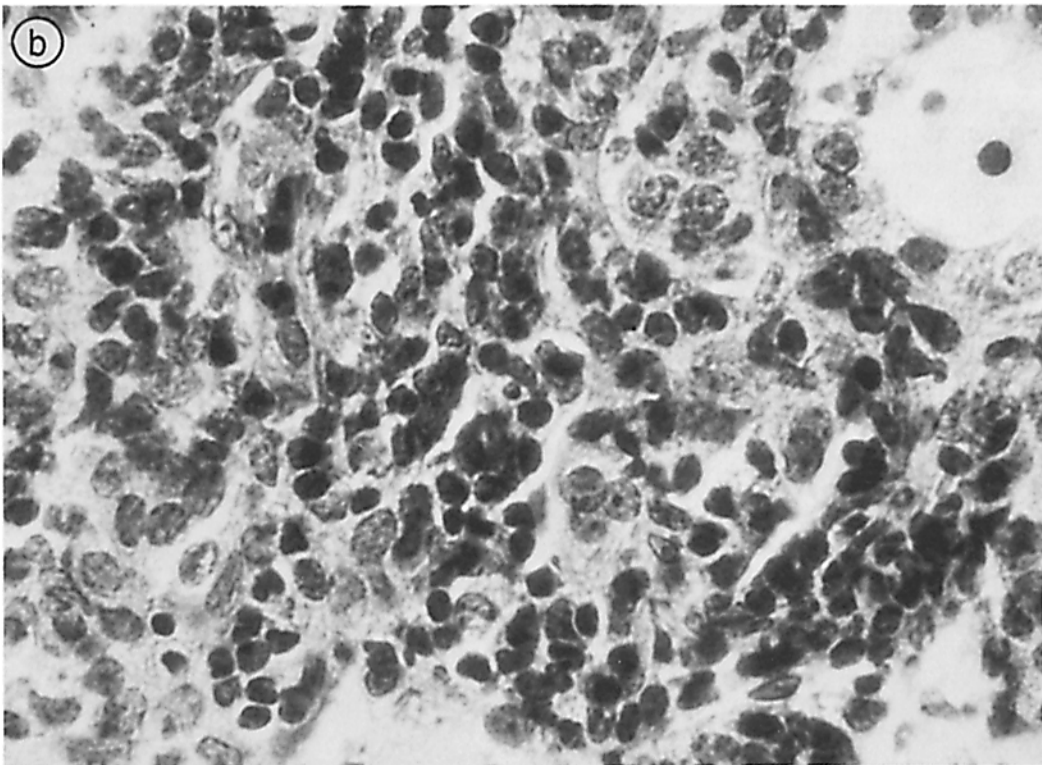
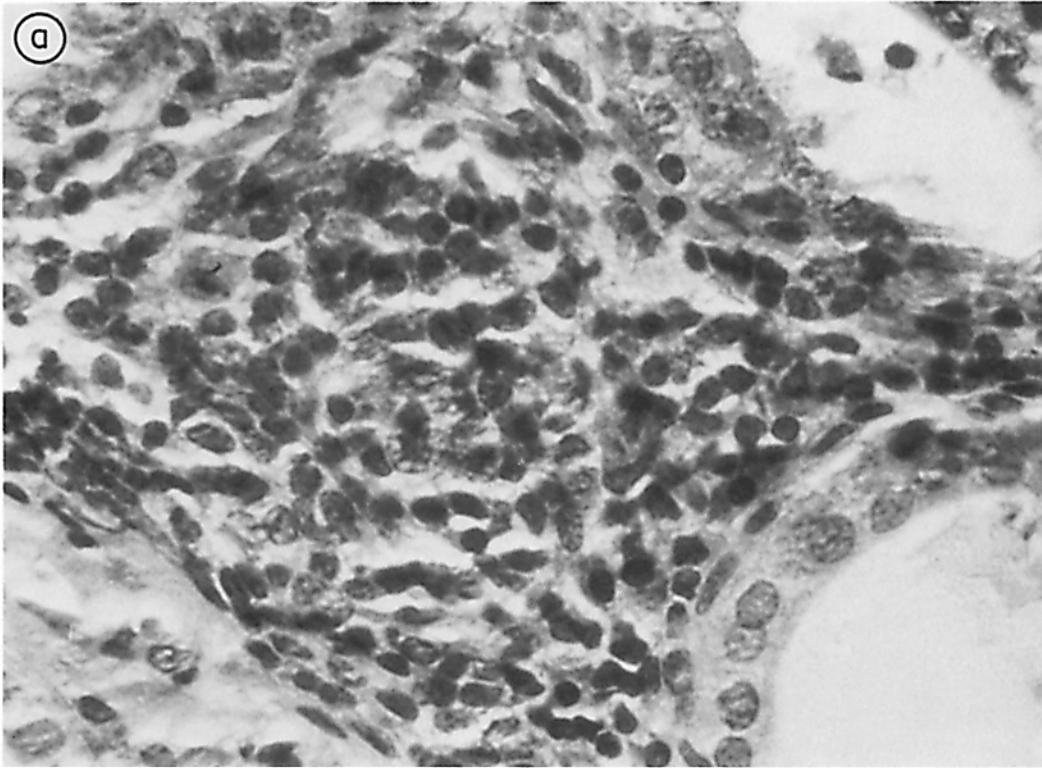


formation and higher levels of antibody to MTg, a portion of which was mercaptoethanol sensitive. In contrast, only mercaptoethanol-resistant antibody was observed in mice immunized with MTg in CFA. Despite the higher titers of antibody, little infiltration was present in the thyroid of mice given MTg and LPS (X3). Mice given MTg in CFA had still higher titers but only minimal lesions were evident in their thyroids. To monitor specific stimulation by LPS, antibody to LPS was also determined in this experiment. Both LPS-treated (X3) groups with or without MTg showed comparable antibody titers to LPS; all samples were mercaptoethanol sensitive. It is interesting that three of four animals given LPS (X3) alone had minimal thyroid lesions (0.2, 0.5 and 0.5) on day 28.

Requirement of T Cells in the Adjuvanticity of LPS. We have demonstrated thus far that genetically based differences in response to MTg between mouse strains are evident when LPS is used as adjuvant. This genetic control has previously been shown to depend upon T cells (3). As a B-cell mitogen in mice, LPS might be expected to stimulate MTg-reactive B cells directly to respond to MTg. To determine if LPS could lead to a bypass of T-cell requirement for MTg, nude mice and their heterozygous littermates were divided into groups of five to eight mice each and immunized with MTg in CFA or with soluble MTg and LPS on days 0 and 7. On day 42, their sera and thyroids were examined for evidence of response to MTg. The findings presented in Table II show that LPS was ineffective as an adjuvant in the absence of T cells; neither antibody nor infiltration in the thyroid was observed. In comparable heterozygous animals, mercaptoethanol-resistant antibodies were detected. The titers of antibody to MTg in the LPS-treated heterozygous mice were low and infiltrations of the thyroid minimal compared to higher titers and moderate infiltrations in similar animals given MTg in CFA. The results in these mice accord with those observed in B10.D2 mice and are not surprising since both BALB/c and B10.D2 strains share the same *H-2^d* (poor responder) haplotype.

To increase the probability of detecting an adjuvant effect of LPS on B cells, T-cell-deficient good responder mice were prepared. B10.BR mice were thymectomized, irradiated, and reconstituted with syngeneic bone marrow cells (treated with anti- θ serum and complement) alone or together with thymus cells. They were then immunized on days 0 and 7 as before and killed on day 21. In addition to removing the blood and thyroid, tissues of the mediastinum were obtained for histologic examination. The few animals with thymus remnants were eliminated from consideration. The results of one typical experiment are presented in Table III. It can be seen that mice that had received both bone marrow and thymus cells and LPS or CFA as adjuvant exhibited antibody to MTg in high titers; marked infiltration with pathology indices of 1-3 was observed in 10 of 11 animals. Little difference in the degree of cellular infiltration was observed between the two groups. In contrast, mice that had received bone marrow cells only showed neither detectable antibody nor significant thyroid infiltration.

FIG. 2. Cellular infiltration in the thyroid of good responder (B10.BR) mice at 49 days after immunization with MTg using as adjuvant (a) LPS or (b) CFA. In both sections, the low magnification ($\times 40$) shows a grade 3 infiltration involving a large portion of the gland. See legend of Fig. 1 for immunization protocol.



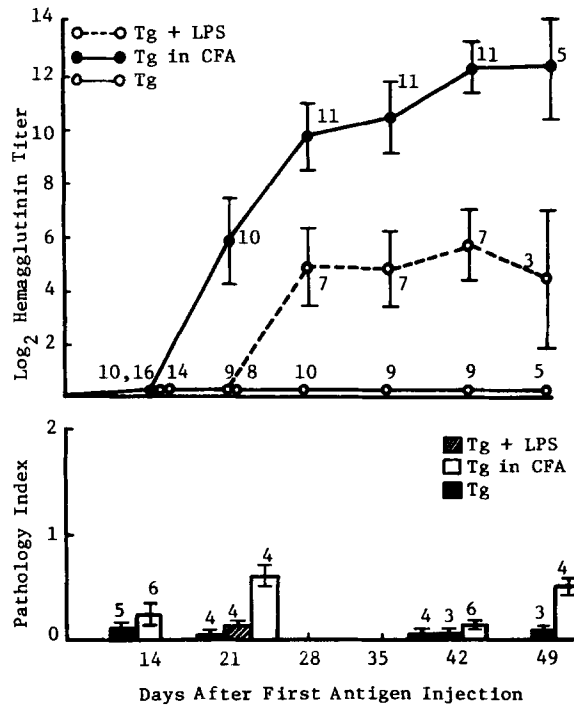


FIG. 4. Effect of LPS on the antibody response to MTg and thyroid pathology in poor responder (B10.D2) mice. See legend of Fig. 1 for immunization protocol.

Thus, in both good and poor responder mice, the abrogation of self-tolerance to MTg by LPS apparently requires the presence of T cells.

Discussion

These investigations reaffirm previous observations that mice fail to respond to soluble MTg, showing that the antigen was not significantly altered during extraction and preparation. However, LPS and CFA can abrogate this self-tolerance. Several explanations have been advanced in the past for the ability of CFA to overcome the natural tolerance of mice to MTg. One is that macrophages and other granulomatous cells, attracted by the acid-fast organisms and the water-in-oil emulsion, partially digest MTg and render it antigenic, as proteolytic enzymes can do in vitro (26). The acid-fast component, combined with a depot injection, may favor cell-mediated rather than humoral immunity. Also, the emulsifying process by itself may alter MTg. Such explanations cannot account for the adjuvant action of LPS. Although many other agents, including incomplete Freund's adjuvant (IFA) (27), aluminum salts (27), silica (27), alhydrogel (28), and even latex particles (N. R. Rose and C. J. van Oss, unpublished observations), can act with homologous thyroglobulin (Tg) in producing anti-

FIG. 3. Cellular infiltration in the thyroid of good responder (B10.BR) mice at 49 days after immunization with MTg using as adjuvant (a) LPS or (b) CFA. In this higher magnification ($\times 400$) of Fig. 2, an abundance of infiltrating lymphocytes and macrophages is seen in both sections.

TABLE I
Effect of Increasing the Dosage of LPS on Immune Response to MTg in Poor Responder B10.D2 Mice

Immunization*	Day	Mean log ₂ anti-MTg titer [‡] ± SE		Mean log ₂ anti-LPS titer ± SE	Mean pathology index [‡] ± SE
		- Mercaptoethanol	+ Mercaptoethanol		
MTg + LPS (X3)	14	9.7 ± 0.4	7.0 ± 1.5	0.7 ± 0.3	0.1 ± 0.0
	28	7.2 ± 1.2	3.5 ± 2.2	2.2 ± 0.6	0.3 ± 0.2
MTg + LPS (X2)	21	<1.0			0.1 ± 0.0
	42	5.9 ± 1.5			0.0
MTg in CFA	14	13.8 ± 1.8	13.8 ± 1.8		0.5 ± 0.1
	28	15.5 ± 0.5	15.5 ± 0.5		0.5 ± 0.2
MTg	14	<1.0			0.0
	28	<1.0			0.0
LPS (X3)	14	<1.0		2.0 ± 0.9	0.0
	28	<1.0		2.0 ± 1.1	0.4 ± 0.1

* 250 µg of Tg was given on days 0 and 7 either subcutaneously in CFA or i.v. in a soluble form; 20 µg of LPS was injected i.v. on days 0, 3, and 7 (X3) or on days 0 and 7 (X2).

[‡] Mean of four to eight mice per group; the lowest dilution tested in hemagglutination was 1:2.

bodies to Tg in various species, only LPS adjuvant and CFA have been shown to induce concomitant thyroid lesions in mice.

Since soluble MTg, injected in conjunction with LPS, induced an autoimmune response, these experiments further demonstrate that self-tolerance can be terminated by native Tg of the same species. It does not require postulating the involvement of cross-reactive T cells which can be stimulated by heterologous (4), haptened (29), or emulsified (1-3) Tg. We are thus able to implicate the participation of MTg-reactive T as well as B cells.

The effectiveness of LPS as adjuvant for MTg is dependent upon the *H-2* haplotype of the animal, as shown by the use of congenic strains. In B10.BR (*H-2^k*) mice, the high levels of antibody to MTg and the extensive mononuclear cell infiltration in the thyroid of the LPS-treated group were comparable in quantity and quality to those observed in mice immunized with MTg in CFA (Figs. 1-3). In B10.D2 (*H-2^d*) mice, LPS promoted the development of antibody to MTg without concomitant cellular infiltration in the thyroid (Fig. 4). This genetic restriction according to the *H-2* haplotype follows the pattern for good and poor responder strains classified previously using MTg in CFA (2) and indicates that the putative *Ir-Tg* gene codes for the responsiveness to MTg rather than susceptibility to CFA.

In mice, response to MTg is T-cell dependent. By adoptive transfer studies, Vladutiu and Rose (3) showed that the *H-2*-linked genetic regulation of responsiveness to MTg, with subsequent thyroiditis, was dependent upon T cells. The genetic involvement of B cells, if present at all, played a minor role. Since these T-cell-based genetic differences in responsiveness to MTg are clearly evident with LPS, it appears most likely that LPS promotes the development of thyroid-

TABLE II
T-Cell Requirement for the Adjuvant Action of LPS on Immune Response to MTg in Poor Responder (BALB/c) Nude Mice

Genotype	Immunization*	Mean log ₂ hemagglutinin titer [‡] ± SE	Pathology index [‡]					
			Mean ± SE	No. mice showing				
				≤0.5	1	2	3	4
nu/nu	MTg + LPS	<1.0	0.0	6	—	—	—	—
	MTg in CFA	<1.0	0.0	8	—	—	—	—
+/nu	MTg + LPS	8.5 ± 2.6	0.1 ± 0.1	5	—	—	—	—
	MTg in CFA	13.1 ± 0.7	1.5 ± 0.4	1	4	1	—	1

* 250 μg of MTg was given on days 0 and 7 either subcutaneously in CFA or i. v. in a soluble form followed by 50 μg of LPS i. v. 3 h later.

[‡] Obtained on day 42; the lowest dilution tested in hemagglutination was 1:2.

itis in good responder mice by utilizing T cells, rather than by circumventing the need for T cells (7, 8) and directly stimulating MTg-binding B cells (4). If we postulate that LPS activates MTg-reactive B cells, either directly as has been reported for foreign protein and haptenized antigens (30–33) or by way of macrophages (6), we would also have to suppose a genetic regulation of the response to MTg in the B cells.

Our data in thymectomized and irradiated good responder mice showed that T cells were required for both the production of antibody to MTg and thyroiditis (Table III); no difference was observed between mice treated with CFA or LPS as adjuvant in this regard. The requirement of T cells for LPS adjuvanticity was also found in poor responder mice where antibody, but little or no disease, was induced. Thus, athymic BALB/c (*H-2^d*) mice failed to produce antibody to MTg, whereas their heterozygous littermates produced mercaptoethanol-resistant antibody when LPS was given as adjuvant (Table II). That T cells are required in demonstrating the adjuvant effect of LPS to several T-cell-dependent foreign antigens (12, 13, 34) and homologous antigen (35) has also been reported by other workers.

In poor responder mice the antibody titers to MTg are generally lower than those in good responder mice. The difference in antibody production to MTg cannot be attributed to low responsiveness to LPS in poor responder mice, since LPS is mitogenic for spleen cells of both B10.BR and B10.D2 mice (20). Furthermore, when we compared the antibody titer to LPS in both strains, no significant difference could be found.

To see if the titer of antibody was the main determining factor for the development of thyroid lesions, poor responder mice were given an additional dose of LPS in an effort to increase their antibody levels to MTg. Although antibody appeared earlier and reached higher titers, there was no evidence of greater thyroid infiltration (Table I). In addition, poor responder mice with very high titers of antibody (e.g. Table I, day 28 in the MTg-in-CFA group) showed only minimal infiltration, indicating that the levels of circulating antibody per se do not determine the establishment of thyroid lesions. Thus, MTg-reactive B cells in poor responder mice can be made to respond vigorously without bringing

TABLE III
T-Cell Requirement for the Adjuvant Action of LPS on Immune Response to MTg in Good Responder B10.BR Mice

Cells transferred*	Immunization†	Log ₂ hemagglutinin titer		Pathology index				
		Mean ± SE	No. positive§/ total	Mean ± SE	No. mice showing			
					≤0.5	1	2	3
Bone marrow	MTg	<1.0	0/3	0.0	3	—	—	—
	MTg + LPS	<1.0	0/5	0.0	5	—	—	—
	MTg in CFA	<1.0	0/5	0.2 ± 0.1	5	—	—	—
	None	<1.0	0/4	0.0	—	—	—	—
Bone marrow + thymus	MTg	<1.0	0/3	0.2 ± 0.2	3	—	—	—
	MTg + LPS	9.8 ± 2.2	5/6	1.1 ± 0.2	1	4	1	—
	MTg in CFA	14.3 ± 0.6	5/5	1.8 ± 0.4	—	2	2	1

* Mice were thymectomized at 7 wk, exposed to 950 R at 11 wk, and reconstituted within 8 h.

† 250 µg of MTg was given on days 0 and 7 either subcutaneously in CFA or i.v. in a soluble form; 20 µg of LPS was injected i.v. 3 h later. The animals were immunized 4 wk after reconstitution and killed 3 wk later.

§ A log₂ titer of 1 or above was considered positive; the antibodies were mercaptoethanol resistant.

about the infiltration of effector cells in the thyroid. Further evidence that antibody level by itself does not determine the presence or absence of thyroid lesions can be inferred from an experiment in which CFA and IFA were compared directly as adjuvants in good responder (B10.BR) mice (P. S. Esquivel, unpublished observations). Antibody levels were equivalent but only mice given MTg in CFA had lesions. The antibodies were indistinguishable by passive hemagglutination and mercaptoethanol reduction.

Therefore, it is not a question merely of reaching a certain threshold of antibody to induce lesions. It may be that CFA, IFA, and LPS, as adjuvants, induce different antibody classes or subclasses. The antibodies may differ in affinity, or in ability to activate the complement system or to cooperate with a population of "killer" cells (36). Another possibility to explain the low incidence of thyroiditis even in the presence of high antibody levels is selective suppressor cell function. Such suppressors must interfere with development of lesions without necessarily impeding antibody synthesis. But further study is required to examine these possibilities.

Although there is increasing evidence that LPS acts on T cells as well as B cells, the mechanism of its adjuvant action on T cells is unknown. There are at least two possibilities. One is that it stimulates T cells directly or via macrophages to release nonspecific helper factors that activate MTg-reactive B cells. The other possibility for LPS action is that it activates (directly or indirectly) specific MTg-reactive T cells in the presence of MTg. This hypothesis better explains the *H-2*-linked differences between good and poor responder strains.

The absence of naturally occurring self-reactive T cells (4) has been suggested to explain self-tolerance (4, 5). More recently, T cells reactive with encephalitogenic protein (37) and membrane antigens (38) have been demonstrated. These autoreactive T cells may be held in check by suppressor cells or their soluble products (39), or by low levels of circulating antigen as has been postulated for Tg (5, 40). Our finding that T cells are the direct or indirect target of LPS adjuvant effect implies that T cells reactive to unaltered MTg are present in the mouse. More direct evidence supporting this view is provided by recent studies

showing that MTg is antigenic in good responder mice when given in conjunction with polyadenylic-polyuridylic acid (41), an adjuvant with known effect on T cells (42).

Summary

The administration of soluble mouse thyroglobulin (MTg) in conjunction with bacterial lipopolysaccharide (LPS) led to the termination of natural tolerance to MTg in mice. The extent of autoimmunity correlated with responsiveness to MTg, previously shown by the injection of MTg in complete Freund's adjuvant (CFA) to be dependent upon the *H-2* haplotype. In good responder B10.BR (*H-2^k*) mice given MTg either with LPS or in CFA, high antibody levels to MTg and extensive mononuclear cell infiltration in the thyroid were observed. In contrast, congenic poor responder B10.D2 (*H-2^d*) mice given MTg plus LPS showed low levels of antibody to MTg, compared to those receiving MTg in CFA, and insignificant cellular infiltration of the thyroid. In no instance did autoimmunity develop in either good or poor responder strain given MTg, LPS, or CFA alone although LPS was antigenic in both of these congenic strains.

Since the genetic difference in responsiveness to MTg is known to be T-cell based, the involvement of T cells in LPS-treated mice was suspected. This was further ascertained by the use of athymic poor responder (BALB/c) mice and thymectomized, irradiated, and bone marrow-reconstituted B10.BR mice. Antibodies to MTg were detected only in heterozygous (*nu/+*) mice and good responder mice reconstituted with both thymus and bone marrow cells. In addition, significant cellular infiltration in the thyroid occurred only in fully reconstituted good responder mice. Thus, the adjuvant effect of LPS on responsiveness to MTg required T cells. Since unmodified MTg and LPS abrogated self-tolerance to MTg, the need for cross-reactive T cells could be excluded. These observations suggest the presence of self-reactive T cells.

Note Added in Proof. After the submission of this manuscript, we received the *Eur. J. Immunol.* containing the article by D. B. Ness et al. (6:650, 1976). These workers also described the requirement of T cells for the adjuvant effect of LPS on the IgG response to (T, G)-A-L in both high and low responder mice.

Received for publication 8 December 1976.

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