

Allergic airway inflammation in hypothyroid rats

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Background: Hormones play a modulating role in allergic inflammation. Hyperthyroidism may increase the severity of asthma, and hypothyroidism may ameliorate coexistent asthma. The mechanisms regulating this interaction are not completely understood.

Objective: The purpose of this study was to test the hypothesis that thyroid hormones influence the development of allergic airway inflammation after antigen challenge in rats.

Methods: The experimental design included either sensitized or nonsensitized surgically thyroidectomized and sham-operated rats. Experiments were performed 50 days after surgery. Thyroidectomized rats and sham-operated controls were sensitized by subcutaneous injection of ovalbumin (OVA) and Al(OH)₃ and challenged 14 days later by OVA inhalation. Bronchoalveolar lavages were performed 24 hours after challenge.

Results: Compared with controls, thyroidectomized animals presented markedly decreased cell yields from bronchoalveolar lavage fluid after OVA challenge. The impaired response was not related to changes in the number of circulating leukocytes. Determination of antibody serum concentrations indicated that thyroidectomized rats presented a marked reduction in the level of anti-OVA IgE compared with controls, without significant differences in IgG₁ and IgG_{2a} serum concentrations. Reversal of the impaired responses was attained by 16-day treatment of hypothyroid animals with thyroxine, but not by 1- or 3-day treatment.

Conclusion: The data presented suggest that the continuing deficiency of thyroid hormones influences the development of the inflammatory component of asthma. This is due, at least in part, to a decrease in the production of IgE. (*J Allergy Clin Immunol* 1999;104:595-600.)

Key words: Asthma, allergy, IgE, thyroid, hypothyroidism, thyroxine, ovalbumin

Almost any stages of inflammation and immunologic response are affected by hormone actions. Proinflammatory and anti-inflammatory effects are recognized, and this forms the basis for the suggestion that hormones exert an integrative function on inflammation.^{1,2} Hormones and other endocrine factors play a modulating role

Abbreviations used

BAL: Bronchoalveolar lavage
OVA: Ovalbumin
T₃: Triiodothyronine
T₄: Thyroxine

in allergic inflammation, with the effects of glucocorticoids or adrenergic agents being obvious examples. Experimental findings agree with clinical evidence that striking examples of this issue are the reciprocal exclusion between asthma and diabetes mellitus,³⁻⁷ premenstrual exacerbation of asthma,⁸ and worsening of asthma and chronic urticaria by hyperthyroidism.⁹⁻¹¹ Thyroid disease and asthma, both being common conditions, occasionally occur together. Hyperthyroidism has been noted to increase the severity of asthma. Control of hyperthyroidism may relieve asthma symptoms, and the development of hypothyroidism may ameliorate coexistent asthma. Conversely, treatment of the hypothyroid state may result in worsening airway obstruction.⁹⁻¹¹ Previous publications dealing with the influence of hypothyroidism and hyperthyroidism on bronchial asthma proposed the regulation of β -receptors and mast cells by thyroid hormones as mechanisms. However, experiments failed to support these hypotheses.^{12,13} Although the pathophysiologic basis of this relationship is not known, understanding the interaction between thyroid disease and asthma is necessary for the proper management of both disorders.

The basic mechanism of asthma has long been thought to be bronchial obstruction, with bronchospasm and mucus hypersecretion playing a major role. However, there is a recent increase in the awareness of the importance of bronchial inflammation in asthma.¹⁴ Direct evidence of inflammatory cells and their mediators in the airway mucosa and lumen after allergen challenge argues for an active role of cells in bringing about inflammatory changes.¹⁴⁻¹⁶ Bronchoalveolar lavage (BAL) has been frequently used to document inflammatory cell involvement as a reflection of events at the surface of the airways and associated with the late asthmatic response.¹⁷⁻¹⁹

This study was aimed at the interaction between thyroid hormones and allergic airway inflammation or, more precisely, the effect of surgically induced hypothyroidism on the bronchoalveolar cell response to antigen inhalation. Quantitative determination of cells obtained by BAL and measurement of antibody production in sensitized hypothyroid rats revealed an impaired response

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related to antibody production that can be reversed by hormonal treatment.

METHODS

Study protocol

The experimental design included either sensitized or nonsensitized surgically thyroidectomized and sham-operated male Wistar rats that were 100 to 120 g and 4 weeks of age at the beginning of the study. Some animals underwent antigen provocation and some did not.

Sensitization to ovalbumin and antigen bronchoprovocation

Active sensitization to ovalbumin (OVA) was performed by subcutaneous injection of 0.15 mL of a sterile solution containing 100 µg of OVA and 8 mg of Al(OH)₃ in physiologic saline solution. Nonsensitized control rats received an equal volume of physiologic saline carrying the same amount of Al(OH)₃. Fourteen days thereafter, the animals underwent bronchial challenge, with OVA administered by an ultrasonic nebulizer (Pulmoclear II; Soniclear, São Paulo, Brazil) connected to a closed box (28 × 25 × 51 cm) in which the animals were kept for 2 consecutive doses. The first nebulized concentration was 100 mg of OVA in 10 mL of saline, and the second was 500 mg of OVA in 10 mL of saline; both doses were administered in a 30-minute period.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed 24 hours after OVA challenge according to techniques described in our previous studies.⁷ The animals were anesthetized with an intraperitoneal injection of 300 mg/kg chloral hydrate, and the abdominal cavity was opened for blood collection and exsanguination from the abdominal aorta. The lungs then underwent lavage by instillation of 5 mL of PBS with 1% BSA at room temperature through a polyethylene tube (1 mm in diameter) inserted into the trachea. The procedure was repeated 5 times. The lavage fluid was not used if the retrieved volume was less than 85% of the 25 mL instilled. Total cell counts were determined by using an automatic hemacytometer (CC510; CELM, São Paulo, Brazil). Differential cell counts were carried out on stained films under oil immersion microscopy. A total of 200 cells were counted and classified as neutrophils, eosinophils, or mononuclear cells on the basis of normal morphologic criteria.

Morphometric analysis

The animals were anesthetized with chloral hydrate, and the trachea was exposed and catheterized as described above. The chest wall was opened, and the lungs were removed. A portion of the left inferior lobe close to the hilum and a portion of the apical region were removed, fixed in a 4% paraformaldehyde solution in sodium phosphate buffer 0.2 mol/L (pH 7.2) for 24 hours, and then dehydrated in ethanol. Samples were embedded in paraplast embedding media (Sigma Chemical Co, St Louis, Mo), sectioned at 5 µm, and stained with hematoxylin and eosin. Lung sections were observed for the presence of mononuclear cells, neutrophils, and eosinophils in the perivascular and peribronchiolar tissue. Determinations were made in 6 randomly selected fields of lung sections by using a 10⁴ µm² total area reticle.

Induction and reversal of hypothyroidism

Hypothyroidism was induced by surgical thyroparathyroidectomy after chloral hydrate anesthesia had been achieved and 50 days before bronchial challenge. Calcium lactate (2%) was added to the drinking water after the procedure. Sham-operated animals served as controls. The effect of the surgery on the thyroid function was con-

firmed by measurements of serum triiodothyronine (T₃) and thyroxine (T₄) and determination of body weight and rectal temperature changes. The reversibility of the hypothyroidism effects on lung inflammation was assessed by treating the animals with intraperitoneal injections of L-T₄ (1 µg/100 g of body weight daily for 1, 3, or 16 days) before the experiment. Serum levels of T₃ and T₄ were measured in duplicate samples by using commercially available RIA kits (Diagnostic Products Corporation, Los Angeles, Calif).

Blood cell counts and antibody measurements

Total and differential leukocyte counts were carried out in blood samples obtained from the cut tip of the tail. Total cell counts were made in hemacytometer. Stained blood films were used for differential counts. Determinations were made immediately before challenge and 24 hours thereafter.

Antigen-specific IgG₁ and IgG_{2a} concentrations were determined by indirect ELISA. Briefly, microplates were coated overnight at 4°C with 100 µL (10 µg/mL) of OVA in borate buffer at pH 9.5. Plates were washed 5 times with PBS supplemented with 0.1% Tween-20 (Sigma) at each time of the assay. Plates were blocked with proteins from skimmed milk in PBS for 1.5 hours at 37°C. One hundred microliters of serial dilutions of serum (beginning at 1/50) were applied for 2 hours and then peroxidase-labeled mouse mAbs against rat IgG₁ or IgG_{2a} (Serotec, UK) were added for 2 hours at 37°C. Finally, 0.4 mg/mL *o*-phenylenediamine (Sigma) diluted in citrate buffer (pH 5.5) and H₂O₂ was applied. The reaction was stopped with 50 µL of H₂SO₄ 1 mol/L, and optical densities were read at 492 nm.

Quantification of serum IgE against OVA was done by using capture ELISA. Plates were coated with 100 µL of mouse anti-rat IgE mAb (Serotec, UK) in borate buffer. These plates were blocked with the same buffer plus 3% gelatin and 1% Tween-20 for 3 hours at 37°C. One hundred microliters of serial dilutions of plasma samples were applied for 2 hours. Finally, biotin-conjugated OVA was added at 10 µg/mL. The plates were incubated for 1 hour more, and after several washes, horseradish peroxidase-conjugated streptavidin (Sigma) was added. One hour later, the plates were washed, and the enzymatic activity was developed as described above.

Statistical analysis

All results are expressed as means ± SEM. The significance of differences between groups was assessed by using the Student *t* test or ANOVA followed by the Tukey-Kramer test. A *P* value of less than .05 was considered significant.

RESULTS

Characteristics of groups

Relative to controls, thyroidectomized animals exhibited a diminished body weight gain during the experimental period. Values were 225.0 ± 6.4 g and 72.2 ± 3.4 g, respectively (*P* < .001). Body weight gain increased to 94.2 ± 3.1 g after treatment of thyroidectomized rats with L-T₄ for 16 days. Body temperature significantly decreased from an initial value of 35.9°C ± 0.1°C before surgery to 33.7°C ± 0.2°C 50 days thereafter (*P* < .0001) in the hypothyroid group, but it increased to 35.6°C ± 0.1°C after hormone treatment. Body temperature did not vary significantly in the control group. In addition, serum T₃ and T₄ levels were greatly reduced by the thyroidectomy in comparison with control levels and returned to normal values after treatment of thyroidectomized rats

TABLE I. Inflammatory cells in BAL fluid from control and hypothyroid rats after antigen challenge

Animals	Challenge	n	Cells × 10 ⁶ /lavage			
			Total	Mononuclear cells	Neutrophils	Eosinophils
Control						
Nonsensitized	Saline	11	11.66 ± 0.57	10.81 ± 0.69	0.80 ± 0.27	0.05 ± 0.02
Nonsensitized	OVA	5	10.77 ± 1.82	9.96 ± 1.68	0.69 ± 0.24	0.12 ± 0.05
Sensitized	Saline	8	12.95 ± 1.08	11.93 ± 1.05	0.98 ± 0.21	0.04 ± 0.03
Sensitized	OVA	17	21.93 ± 1.72*	16.93 ± 1.28*	4.10 ± 0.68*	0.90 ± 0.20*
Hypothyroid						
Nonsensitized	Saline	7	5.10 ± 0.97†	4.95 ± 0.94†	0.15 ± 0.04	0
Nonsensitized	OVA	10	4.91 ± 0.62	4.37 ± 0.58	0.52 ± 0.05	0.02 ± 0.02
Sensitized	Saline	10	7.44 ± 1.13	7.01 ± 1.06	0.42 ± 0.15	0.01 ± 0.01
Sensitized	OVA	20	6.21 ± 0.67†	4.22 ± 0.48†	1.91 ± 0.40†	0.08 ± 0.03†

Animals were rendered hypothyroid by surgery 50 days before experimentation. In both control and hypothyroid groups, active sensitization against OVA was performed 14 days before antigen challenge, and BAL was performed 24 hours after challenge.

**P* < .05 compared with other values in the control group.

†*P* < .05 compared with the corresponding values in the control group.

TABLE II. Bronchoalveolar cell response to antigen challenge in treated and untreated hypothyroid rats

Animals	n	Cells × 10 ⁶ /lavage			
		Total	Mononuclear cells	Neutrophils	Eosinophils
Hypothyroid	16	6.7 ± 0.7	4.9 ± 0.5	1.5 ± 0.3	0.3 ± 0.1
Plus T ₄ (1 d)	5	4.8 ± 2.1	3.4 ± 1.8	1.2 ± 0.3	0.2 ± 0.1
Plus T ₄ (3 d)	4	8.2 ± 1.2	6.4 ± 0.9	1.2 ± 0.3	0.6 ± 0.3
Plus T ₄ (16 d)	9	19.0 ± 3.9*	11.5 ± 4.5	6.4 ± 0.9*	1.1 ± 0.3†

Animals were rendered hypothyroid by surgery 50 days before experimentation. Active sensitization against OVA was performed 14 days before antigen challenge, and BAL was performed 24 hours after challenge. L-T₄ was given by intraperitoneal injections (1 µg/100 g of body weight per day).

**P* < .001 compared with values in the other groups.

†*P* < .05 compared with values in untreated and 1-day treated hypothyroid group.

TABLE III. Morphometric analysis of lung tissue sections from sensitized rats after antigen challenge

Animals	Cells/10 ⁴ µm ²					
	Perivascular			Peribronchiolar		
	Mononuclear cells	Neutrophils	Eosinophils	Mononuclear cells	Neutrophils	Eosinophils
Control	42.50 ± 2.30	74.00 ± 3.70	5.44 ± 1.30	41.35 ± 2.25	74.92 ± 4.61	6.23 ± 1.40
Hypothyroid	26.76 ± 1.30*	60.27 ± 2.55†	1.22 ± 0.24‡	27.08 ± 1.35*	59.88 ± 3.64†	1.58 ± 0.28‡
Hypothyroid plus T ₄ (16 d)	48.82 ± 1.91	77.93 ± 1.77	4.62 ± 1.31	46.46 ± 1.65	75.92 ± 2.68	5.29 ± 1.33

Animals were rendered hypothyroid by surgery 50 days before experimentation. Active sensitization against OVA was performed 14 days before antigen challenge, and BAL was performed 24 hours after challenge. L-T₄ was given by intraperitoneal injections (1 µg/100 g of body weight per day). Determinations were made in 6 randomly selected fields of lung sections (2 samples for 3 animals in each group).

**P* < .001, †*P* < .01, ‡*P* < .05 compared with corresponding values in the other groups.

with L-T₄. Values for serum T₃ were 85.9 ± 7.4, 32.3 ± 4.5, and 77.9 ± 7.3 ng/dL in control (n = 14), thyroidectomized (n = 20), and thyroidectomized animals treated with L-T₄ (n = 10), respectively (*P* < .01). Values for serum T₄ were 3.4 ± 0.3, 0.4 ± 0.2, and 5.8 ± 0.2 µg/dL in control (n = 14), thyroidectomized (n = 20), and thyroidectomized animals treated with L-T₄ (n = 10), respectively (*P* < .001).

Hypothyroidism and bronchoalveolar cell response

Compared with saline, OVA challenge significantly increased the cell yield in BAL fluid of control animals.

Neutrophil, eosinophil, and mononuclear cell counts were elevated, with the total cell number being approximately twice baseline values (Table I). In contrast, animals rendered hypothyroid by surgery showed no significant increase of leukocyte counts in BAL fluid 24 hours after OVA bronchial challenge. Furthermore, baseline counts of total and mononuclear cells were lowered by hypothyroidism compared with the control group (Table I). Inhalation of OVA by nonsensitized animals did not significantly change BAL fluid cell yield in either group.

Whereas treatment with L-T₄ for 1 or 3 days before antigen challenge did not restore the capacity of thy-

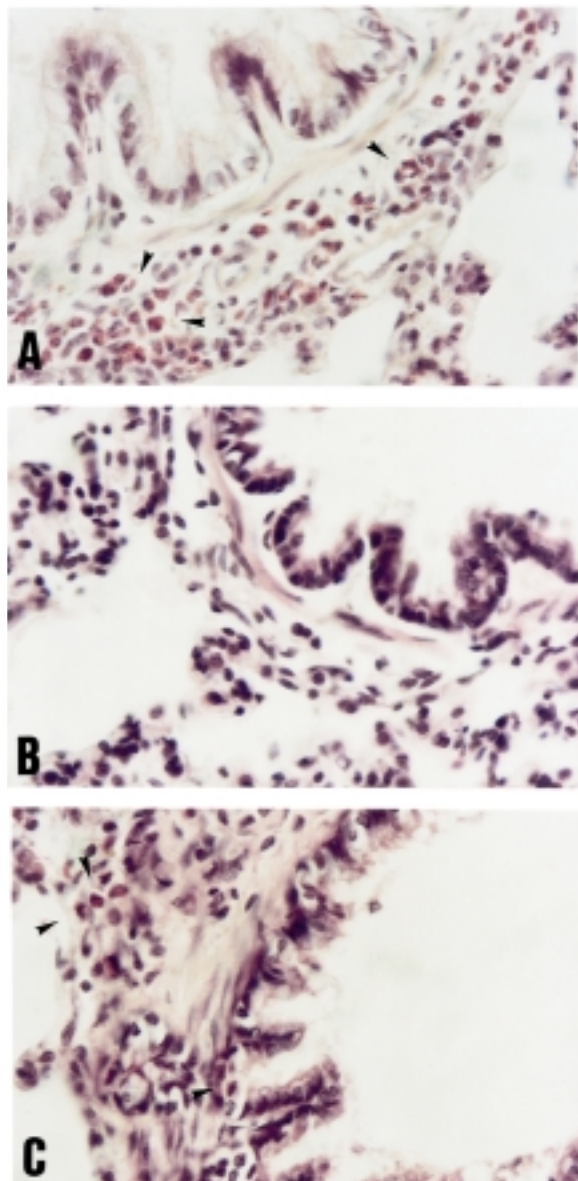


FIG 1. Microphotographs of lung tissue after OVA challenge. Lung sections (5 μ m) were stained with hematoxylin and eosin. A, Control rat; B, hypothyroid rat; C, hypothyroid rat treated with L-T₄ for 16 days. Presence of eosinophils (arrowheads) in the surrounding airway tissue was widely observed in both A and C (original magnification, $\times 400$).

roidectomized animals to respond to OVA, treatment for 16 days started before sensitization caused a 3-fold increase in the BAL cell number because of neutrophil and eosinophil migration. Mononuclear cell numbers did not significantly change (Table II). Similar results were observed when lung tissue sections were observed for the presence of inflammatory cells close to the vessels and in the surrounding airway tissue. Relative to controls, the number of neutrophils, eosinophils, and mononuclear cells were markedly reduced in thyroidectomized animals. Values returned to control levels after treatment of

hypothyroid rats with L-T₄. Results are summarized in Table III. Representative sections of these preparations are presented in Fig 1.

Blood leukocyte counts

Blood leukocyte counts performed immediately before and 24 hours after OVA challenge in sham-operated and thyroidectomized animals showed that under basal conditions total and differential counts were equivalent in both groups and that similar changes occurred during the 24-hour interval after challenge. A 1.5-fold increase in total counts was observed from $12,421 \pm 823$ cells/mm³ before challenge to $19,539 \pm 1196$ cells/mm³ after challenge in control rats ($P < .01$) and from $13,492 \pm 482$ cells/mm³ to $19,977 \pm 1547$ cells/mm³ in thyroidectomized rats ($P < .001$) because of changes in the number of mononuclear and polymorphonuclear leukocytes. The comparison between hypothyroid and control groups did not reveal significant differences at any time point for any cell type. Accordingly, the reduced cell yield in BAL fluid observed in thyroidectomized animals cannot be attributed to specific changes in the number of circulating leukocytes.

Hypothyroidism and antibody production

Both control and hypothyroid rats exhibited anti-OVA IgG₁ and IgG_{2a} serum levels of the same magnitude after sensitization with OVA. However, relative to controls, thyroidectomized animals exhibited only 55% of anti-OVA IgE serum concentration. Treatment of the animals with L-T₄ completely restored the levels of anti-OVA IgE in the serum (Fig 2). The correlation-regression analysis between values of cell yield in the BAL fluid and values of IgE concentration in the serum of the animals is depicted in Fig 3.

DISCUSSION

This study was designed to explore the relationship between the continuing deficiency of thyroid hormones, as observed in hypothyroidism, and the cellular response to OVA inhalation in sensitized rats. Several animal species have been used successfully as models of human asthma and have yielded important clues to the mechanisms of allergic airway inflammation. Among these species, we find different rodents, including the Wistar rat, that produce high IgE antibody titers to active immunization and develop airway inflammation after subsequent allergen challenge, as previously described, which stresses the validity of this model.⁷

Results presented in this study suggest that IgE production may be one of the steps in the inflammatory allergic cascade that is influenced by the deficiency of T₃ and T₄. The suggestion is supported by the following observations. (1) Relative to controls, the number of leukocytes recovered from the BAL fluid of thyroidectomized animals after challenge with OVA was 70% reduced because of a decrease in the number of mononuclear cells, neutrophils, and eosinophils. (2) This leuko-

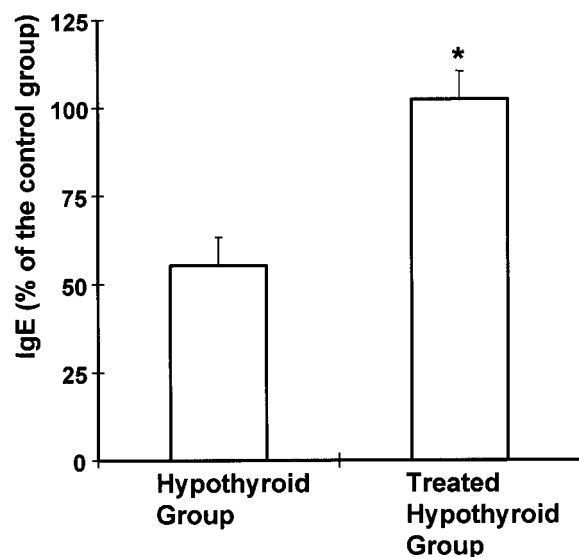


FIG 2. Thyroidectomized animals were treated with L-T₄ injections for 16 days. **P* < .02 compared with hypothyroid group.

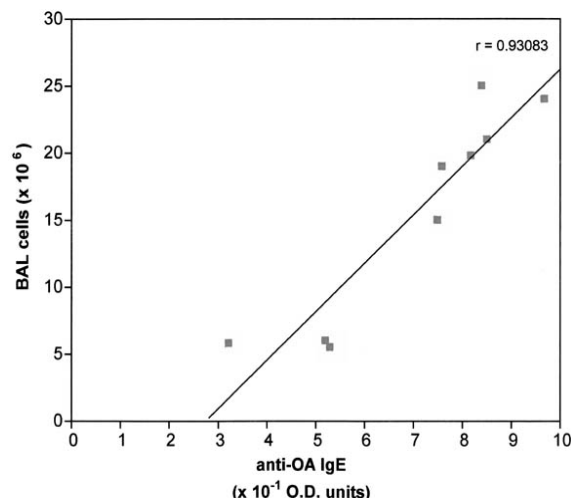


FIG 3. Correlation-regression analysis between serum IgE levels and number of cells in BAL fluid. Both euthyroid and hypothyroid rats are represented in the graph. Correlation coefficient (*r*) is indicated.

cyte reduction was accompanied by a marked reduction in the concentration of IgE in the serum of these animals. (3) Both the cellular response to antigen inhalation and levels of serum IgE were completely restored after treatment of hypothyroid rats with T₄, which was started before sensitization. Treatment with the hormone over a shorter period of time (1 or 3 days) was ineffective. In fact, there is a good correlation (*r* = 0.93083) between values of cell yield in the BAL fluid and values of IgE concentration in the serum of the animals. In addition, the impaired response was unrelated to changes in the number of circulating leukocytes. Control and hypothyroid animals had comparable blood leukocyte counts under basal conditions and after challenge.

Rat anaphylactic antibodies are considered to be either of the reaginic type or associated with IgG-type globulin.^{20,21} Because the immunization procedure used in this study induces considerably higher IgE than IgG titers, this may imply that the anaphylactic response elicited in these animals was predominately mediated by reaginic-type antibodies.⁷ Allergen-specific IgE antibodies play an important role in mediating allergic reactions. They bind to high-affinity IgE receptors on mast cells and basophils. Binding of allergens to this receptor-bound IgE results in receptor activation that triggers the mast cell to release soluble mediators, such as histamine, leukotrienes, prostaglandins, and proteases, which cause allergic reactions in various target organs, including the eyes, intestines, skin, and respiratory tract. The relevance of IgE in allergic inflammation is also demonstrated by studies in human allergy, which show that blocking IgE improves inflammatory parameters.²²⁻²⁴ Therefore the suggestion is that thyroid hormones are capable of controlling the asthmatic inflammation through the production of IgE.

The production of IgE by allergen-specific B lymphocytes (plasma cells) is tightly regulated by a complex array of cytokines secreted from allergen-specific T lymphocytes and possibly from other cell types, such as mast cells.^{25,26} Thyroidectomy reduces the response of lymphocytes to both PHA and pokeweed mitogen. Thyroid hormone (T₃) administration to thyroidectomized animals restores the blastogenic response of the lymphocytes.²⁷ Because these cells are known to have receptors for T₃ and T₄,²⁸ the proinflammatory effect of thyroid hormones in the allergic inflammation might therefore be either on some T-cell subsets that might be enhancing B-cell function or may be a direct effect on B cells. This is a subject for further investigation. Also, changes in the cytokine profile and defects in leukocyte-endothelial interactions should be investigated to provide a better understanding of inflammatory allergic responses, including asthma, in the hypothyroid state.

In conclusion, data presented suggest that production of IgE is one of the events in allergic airway inflammation that is regulated by T₃ and T₄, which represents a new insight for a better understanding of the relationship between thyroid hormones and the asthmatic reaction.

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