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Undetectable serum IgA and low IgM concentration in children with congenital hypothyroidism

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Abstract

Some studies suggest thyroid hormones may regulate the human immune system. In order to evaluate the effect of thyroid hormone deficiency on antibody production, we evaluated serum IgA and IgM concentrations in 83 children with congenital hypothyroidism (CH), diagnosed by neonatal screening. Patients were compared to two healthy, age-matched control groups. Patients with permanent CH had a significantly higher frequency of undetectable IgA concentrations (thyroid agenesis, $P < 10^{-5}$; thyroid ectopy, $P = 0.013$) and lower concentrations of IgA (thyroid agenesis, $P < 10^{-6}$; thyroid ectopy, $P < 10^{-5}$; dyshormonogenesis, $P = 0.0002$) and IgM (thyroid agenesis, $P = 0.0002$; thyroid ectopy, $P < 10^{-6}$; dyshormonogenesis, $P = 0.0017$) compared to control group. No difference was observed between patients with transient hypothyroidism and controls. A significant correlation was observed between serum IgA and IgM concentrations and fT_4 levels. IgA and IgM deficiency is correlated with the severity of congenital hypothyroidism and may help to evaluate the duration and severity of thyroid hormone deficiency during prenatal life.

Keywords: Congenital hypothyroidism; Immunoglobulin; IgA; IgM; Immune system; Thyroid hormones

Introduction

Thyroid hormones are known to influence many human cells and control the development and function of many organs.

Interactions between thyroid hormones and the immune system development and function have been demonstrated in experimental studies on animals. The first studies, carried out more than 70 years ago, showed that adenohypophysis ablation caused thymic atrophy in mice [1]. Similarly, a report on humans showed that thymic development was significantly reduced and thymic tissue was atrophic in patients with sporadic congenital hypothyroidism [2]. In more recent years, several studies have clarified the role of thyroid hormones on primary and secondary lymphopoiesis [3–8].

B cell development is defective in genetically hypothyroid mice, such as dw/dw [3,9], hyt/hyt [3], and TRα−/− inbred mice [5]. Similarly, B cell lymphopoiesis, but not T cell production, is affected by thyroid hormone deficiency in hyt/hyt mice [3]. Secondary lymphocyte development, however, is normal in both dw/dw and hyt/hyt mice, and a normal humoral response to T-dependent and T-independent antigens has been demonstrated in those animals [3,10].

Although case reports have been published on the immune function in hypothyroid patients [11–14], the interaction between thyroid hormones and the immune system development and function in humans has not been completely clarified. The aim of the present study was to evaluate the role of hypothyroidism on immunoglobulin levels in children with congenital hypothyroidism at the time of diagnosis.

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Patients and methods

Patients

Eighty-three patients (35 males, 48 females, born full-term), diagnosed with congenital hypothyroidism, were included in the study. The patients were divided into four groups, according to their diagnosis. Sixteen patients had transient hypothyroidism, 17 had dyshormonogenesis, 30 had thyroid ectopy, and 20 had thyroid agenesis. Thyroid agenesis was diagnosed when a 99mTc-pertechnetate thyroid scan, performed at the time of diagnosis, showed no radionuclide uptake. Thyroid ectopy was diagnosed when a 99mTc-pertechnetate thyroid scan showed either a smaller volume of thyroid or its abnormal position. Dyshormonogenesis was diagnosed when a normal or increased uptake of 99mTc was revealed in a position that is normal for the thyroid gland [15]. Transient neonatal hypothyroidism was diagnosed in the presence of increased concentrations of thyroid stimulating hormone (TSH) with normal or decreased fT_4, both returning to normal levels within 1 month after birth and associated with normal thyroid morphology and normal 99mTc uptake [16].

The biochemical severity of congenital hypothyroidism was assessed from the first quantitative thyroxine (fT_4) and thyroid-stimulating hormone (TSH) measurements, before initiation of thyroid replacement therapy. Since thyroid ectopy and thyroid agenesis are usually diagnosed at an earlier age than transient hypothyroidism and dyshormonogenesis because of the severity of the symptoms, two different control groups, matched for sex and age, were enrolled in the study. Control group 1 included 102 healthy controls, sex- and age-matched with the transient hypothyroidism and dyshormonogenesis group. Control group 2 included 49 healthy controls, sex- and age-matched with the thyroid ectopy and thyroid agenesis group. All the control children underwent a review of their medical history, a physical examination and a nutritional assessment, as well as biochemical and endocrine examinations. The main features of the patient and control groups (sex and age at diagnosis, and type of feeding) and the diagnosis are reported in Table 1.

The ethics committee of Meyer Children's Hospital approved the study; informed written consent was obtained from the parents of all subjects.

Laboratory methods

Free-T_4 and TSH serum levels were determined by immunometric assays (Immulite™ 2000 Third generation, DPC Diagnostic Products Corporation, Los Angeles, CA, USA). Within- and between-run coefficients of variation were less than 12.5% for TSH, and were less than 7.5% for fT_4. The normal range of fT_4 and TSH serum concentrations were 0.8–1.9 ng/dl and 0.4–4 μUI/ml.

Table 1
Main features of patients and controls included in the study

	Patients (n)	Sex (M or F)	Age at diagnosis, mean ± SD (days)	Type of feeding (breast- or bottle-feeding)
Transient hypothyroidism	16	10M, 6F	33.2 ± 10.5	13 breast, 3 bottle
Dyshormonogenesis	17	10M, 7F	33.7 ± 18.6	14 breast, 3 bottle
Thyroid ectopy	30	11M, 19F	22.6 ± 6.4	27 breast, 3 bottle
Thyroid agenesis	20	4M, 16F	25.0 ± 7.2	15 breast, 5 bottle
Total	83	35M, 48F	27.5 ± 11.8	69 breast, 14 bottle
Control group 1	102	56M, 46F	36.8 ± 15.5	80 breast, 22 bottle
Control group 2	49	21M, 28F	22.7 ± 7.1	37 breast, 12 bottle

Immunoglobulin concentrations were measured by ELISA (IgA-human-ELISA quantitation kit and IgM-human-ELISA quantitation kit, Vinci Biochem, Vinci, Italy). Values lower than 10 mg/dl for IgA and lower than 20 mg/dl for IgM were retested by radial immunodiffusion technique (The Binding Site, Birmingham UK; lower limit of detection: 0.8 mg/dl for IgA and 0.7 mg/dl for IgM), using serum dilution when appropriate. IgA concentrations were considered undetectable when they were <0.8 mg/dl in two separate assays. The intra- and inter-assay coefficients of variation for IgA and IgM concentrations were less than 2.1 and 2.7%, respectively. CD19+ B cells, as well as B cells bearing surface membrane IgM/IgD [SmIg(D+M+)], were enumerated by flow cytometry using fluorescein or phycoerythrin-conjugated monoclonal antibodies (Becton Dickinson, San José, CA, USA). Flow cytometry was performed on a Becton-Dickinson FACScan (Becton-Dickinson, San Jose, CA, USA) immediately after cell staining.

Differences between means (analyzed by the Student's t test for unpaired samples), differences in frequencies (evaluated by χ^2 test or Fisher's exact probabilities, as appropriate), and correlation analyses (Pearson) were measured using an SPSSX statistical package (SPSS Inc., Chicago, IL). For analysis of correlation, TSH values over 100 μUI/ml were considered as 100. For the same purposes, fT_4 values less than 0.1 ng/dl were considered as 0.1 and IgA values less than 0.8 mg/dl were considered as 0.8. Two-tailed P values were determined and P values < 0.05 were considered significant.

Results

No abnormalities were found in the medical history, the physical examination, the nutritional assessment, and the biochemical or endocrine examination within the control groups.

No differences were found in the sex distribution and mean age between any group of patients and their control group. Similarly, no differences in type of feeding were found between any of the four groups of patients and their control group. Within the control groups, no difference was found in serum IgA and IgM concentration between males and females.

The frequency of undetectable serum IgA was significantly higher in patients with thyroid agenesis (12/20, 60%) and thyroid ectopy (7/30, 23.3%), compared to their control group (2/49, 4.1%), while no difference was found between serum IgA concentrations of patients with dyshormonogenesis (2/17, 11.8%) or transient hypothyroidism (0/16) and their controls (3/102, 2.9%). Data are shown in Table 2. Compared to control group 1, the frequency of undetectable serum IgA was higher, and the serum IgA concentrations were lower, as expected, in control group 2 (younger controls), but this difference did not reach statistical significance. Serum IgA concentrations were significantly lower in patients with thyroid agenesis or thyroid ectopy or dyshormonogenesis compared to their control groups. No difference was found between serum IgA concentrations in patients with transient hypothyroidism and their control. Data are shown in Table 2. IgM concentrations were significantly lower in patients with thyroid agenesis, thyroid ectopy, and dyshormonogenesis, compared to their control groups, while no difference was found between IgM concentrations of patients with transient hypothyroidism and their control group (Table 2). In evaluating all groups of patients, a correlation was found between fT_4 levels and serum IgA concentrations ($r = 0.74$; $P < 10^{-5}$) and between fT_4 and serum IgM concentrations ($r = 0.52$; $P < 10^{-5}$). A negative correlation was found between IgA concentrations and TSH ($r = -0.56$, $P < 10^{-5}$) and between IgM concentrations and TSH ($r = -0.46$ $P < 10^{-5}$). The percentage of CD19+ and SmIg(D+M+) B cells was 17.9 (range 9–29) and 16.3 (range 8–24), respectively, in the 83 patients included in the study [normal value 6–25 % for both CD19+ and SmIg(D+M+) cells].

Discussion

Increasing evidence shows that the immune system operates in a coordinated way with the neuroendocrine system and that immune cells can be influenced by hormones, neurotransmitters, and neuropeptides [17,18].

The present study shows that children with permanent congenital hypothyroidism (thyroid agenesis, thyroid ectopy, dyshormonogenesis) have a defect in immunoglobulin production. The frequency of undetectable serum IgA concentrations is significantly higher in patients with these disorders than in controls and the defect is more common in patients with the most severe hypothyroidism. Similarly, serum IgM concentrations are significantly lower in patients with hypothyroidism and there is a strong correlation between the concentrations of IgA or IgM and the concentrations of fT_4. The IgG level was not considered as a reliable parameter for evaluation of antibody production in the neonate because neonatal serum IgG concentrations reflect the transplacental passage of IgG from the mother in the third trimester, rather than the neonatal antibody synthesis. In contrast to infants with persistent hypothyroidism, the frequency of undetectable serum IgA concentrations in patients with neonatal transitory hypothyroidism is identical to that found in healthy controls. Furthermore, IgM concentrations are also normal in transitory hypothyroidism patients. These data suggest that transitory hypothyroidism has no effect on B cell development and/or function. These findings differ from data obtained in animal studies that demonstrate that transitory propylthiouracil-induced hypothyroidism in the earliest phases of development causes transient changes in thymic T cell development in immune cell sub-populations and delayed B cell development. However, immunoglobulin concentrations were not reported in those studies [19].

It is not easy to speculate whether the effect of fT_4 deficiency acts directly on B cell function or if it is dependent on other factors. In humans, thyroid hormones exert immunomodulatory activities and the thymus is one of their target organs [20]. Thyroid hormone receptors are

Table 2
IgA and IgM serum concentrations in hypothyroidism patients and control groups

Diagnosis	IgM levels (mg/dl), mean ± SD	*P*	Frequency of undetectable IgA[a], *n* (%)	*P*	IgA levels (mg/dl), mean ± SD	*P*
Transient hypothyroidism	40.4 ± 15.7	ns	0/16 (0)	0.64	7.2 ± 7.4	ns
Dyshormonogenesis	28.6 ± 9.9	0.0017	2/17 (11.8)	0.14	4.5 ± 2.6	0.0002
Control group 1	41.6 ± 15.4	–	3/102 (2.9)	–	11.1 ± 7.0	–
Thyroid ectopy	23.2 ± 5.9	$<10^{-6}$	7/30 (23.3)	0.013	2.6 ± 2.4	$<10^{-5}$
Thyroid agenesis	22.1 ± 6.9	0.0002	12/20 (60.0)	$<10^{-5}$	0.9 ± 0.2	$<10^{-6}$
Control group 2	38.8 ± 15.2	–	2/49 (4.1)	–	9.2 ± 6.9	–

ns = Not significant.
SD = standard deviation.
[a] Undetectable IgA was defined as IgA < 0.8 mg/dl.

present on lymphocytes and studies in mice have demonstrated that thyroid hormones have a direct role in B cell development and that they are required for normal B cell production in the bone marrow [3,4]. In the present study, no decrease in the percentage of CD19+ or SmIg(D+M+) B cells was demonstrated in any of the groups of patients studied. The defect of immunoglobulin production, therefore, does not seem to be due to a reduction of antibody-producing cells but to a functional defect.

On the other hand, it cannot be excluded that immunoglobulin production defect in hypothyroidism patients is related to the high levels of TSH present in these patients. Thyroid-stimulating hormone has a variety of immune-regulating cytokine-like activities that can influence T cell development in the thymus and intestine, and affect humoral and cell-mediated responses of peripheral lymphocytes [21]. Many hematopoietic cells in the bone marrow produce TSH and express the TSH receptor, as do subsets of dendritic cells, monocytes, and lymphocytes in the spleen and lymph nodes [21].

Another mechanism could be hypothesized: the action of the TSH and thyroid hormone could be mediated by other hormones or neurotransmitters. For example, thyroid hormones have a permissive action on the adrenergic function and patients with untreated hypothyroidism have a down-regulation of the adrenergic receptor [22–24]. Adrenergic stimulation increases antibody production by B cells [18]. Therefore, the effect of hypothyroidism on IgA and IgM synthesis might be mediated by adrenergic down-regulation. Experiments performed on animals demonstrate that both beta-adrenergic receptor expression and IgM production are increased in hyperthyroid mice and decreased in hypothyroid mice after immunization and that IgM production is related to regulation of beta-adrenergic receptors on immune cells [25]. The low levels of IgA and IgM found in patients with congenital hypothyroidism did not result in an increased number or severity of infections. It may be hypothesized that the normal values of serum IgG concentrations, due to the transplacental passage of that isotype of immunoglobulin, are a sufficient help against infections in these patients during the first months of life. The follow-up of patients included in the present study, ongoing in our clinic, will clarify whether low serum IgM or IgA concentrations are associated with a suboptimal response to immunization.

Patients with untreated congenital hypothyroidism usually have severe neurological and growth impairment [26], and even patients who are treated promptly may have neurological damage and mild motor and neuropsychological impairment [26].

Many parameters (fT_4 level at birth, TSH level at birth, age at diagnosis, age of treatment start, initial thyroid replacement therapy dosage, bone age at birth, electroencephalogram at birth) have been reported to be prognostic factors for growth or neuropsychological development [27–31]. Some of these features have also been used as indicators of the severity of prenatal thyroid hormone deficiency. However, none of these parameters accurately reflect the severity or age at onset of hormone deficiency during fetal life.

The present study shows that patients with permanent congenital hypothyroidism have lower concentrations of IgM than controls and an extremely high frequency of undetectable serum IgA concentrations. IgM serum concentrations are directly related to the severity of hormone deficiency and the lowest values of IgM can be found in those patients (thyroid agenesis, thyroid ectopy) in which the defects have their onset earlier during prenatal life. Therefore, the presence of normal levels of IgM or IgA in an infant with hypothyroidism could be considered as a positive prognostic factor, suggesting a less severe deficiency of thyroid hormones or a later age of onset during fetal life. Long-term follow-up of patients included in the present study will clarify whether serum IgM or IgA concentrations also predict the prognosis for neurological development or growth of congenital hypothyroidism patients. Preliminary data suggest that serum IgA and IgM concentrations gradually increase after L-thyroxine therapy has started. Further studies are also necessary to clarify the mechanisms underlying the thyroid control of antibody production in congenital hypothyroidism.

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