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Potentiation of humoral immune response and activation of NF-κB pathway in lymphocytes in experimentally induced hyperthyroid rats

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Abstract

This study explored the effect of hyperthyroid state on humoral immune response and NF-κB signaling in lymphocytes. Male Wistar rats were treated with L-thyroxin for four weeks. Animals were immunized with sheep red blood cells (SRBC) after three weeks of L-thyroxin treatment. After one week of immunization, serum anti-SRBC titer was measured and NF-κB signaling was studied in lymphocytes by Western blot analysis of p-IKB-α, IKB-α, and p65. These results were compared with that of control rats. Antibody response and density of p-IKB-α and p65 were significantly higher in L-thyroxin treated rats in comparison to controls. The antibody response was found to have significant correlation with density of p-IKB-α and p65. Our results indicate that NF-κB signaling pathway in lymphocytes is activated in hyperthyroid state which might play a role in potentiation of antibody response.

Keywords: NF-κB; Hyperthyroid; Antibody response; Lymphocyte; IKB-α; p65

1. Introduction

Immune response is regulated by various hormones including thyroid hormones. The effect of thyroid hormones on humoral immune response has remained unclear. There are reports which shows that humoral immune response is enhanced by thyroid hormone treatment [1–3] and thyroidectomy suppresses immune response [3]. Contradictory reports are also available in the literature indicating either a suppressed antibody response with thyroid hormone treatment [4,5] or an enhanced humoral immune response with anti-thyroid drug treatment [6]. There is also report to show that thyroid hormones do not have any effect on antibody response [7]. Hence, defining the effect of thyroid hormones (e.g., L-thyroxin) on immune response is important. It is difficult to elucidate the effect of thyroid hormones on immune response in healthy euthyroid state because intricate interactions and modulations of many hormones and factors are involved in its regulation.

Hyperthyroidism is a common health problem all over world. Many hyperthyroid conditions in human are autoimmune in nature. A potentiation or inhibition of humoral immune response by thyroid hormones thus can contribute to the pathological process by aggravating or suppressing the autoimmune state. The direction of change in humoral immune response in hyperthyroid state can elucidate the effect of thyroid hormones on humoral immunity. An optimum immune response is crucial for good health because a hypo-responsive state of immune system can increase the susceptibility to infections and development of cancer and a hyper-responsiveness may lead to hypersensitivity and autoimmunity.

The nuclear factor (NF)-κB pathway is responsible for the expression of a wide variety of genes that are involved in the control of the immune and inflammatory response [8–11]. In unstimulated cells, the NF-κB proteins are predominantly localized in the cytoplasm and are associated with a family of inhibitory proteins known as IκB.

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In response to stimuli, the cytoplasmic NF-κB/IκB complex is activated by phosphorylation on conserved serine residues of IκB. This process activates NF-κB, which is then translocated to the nucleus and binds to its cognate DNA-binding site in the promoter regions of specific genes bringing its effect. The p65–p50 heterodimer was defined as classical NF-κB binding form. This is reviewed by Hayden and Ghosh [12] and others [9–11].

NF-κB plays an important role in lymphocyte functions. In B-cells, NF-κB is involved in transcription of both heavy chain and light chain of immunoglobulins [13,14], gene assembly [15], lymphocyte development [16], and class switching [17]. In T-cells, NF-κB regulates the expression of cytokines, growth factors, and effector enzymes [12]. These NF-κB mediated effects are crucial for antibody response. T_3, the active thyroid hormone acts through two types of nuclear receptors (TRα and TRβ). TR receptors are found to be expressed in lymphocytes and the role of thyroid hormones on lymphocytes has been defined [18,19]. But it is not known if NF-κB plays any role in thyroid hormone mediated functions. No reports are available until date to show that hyperthyroid state activates NF-κB signaling pathway in lymphocytes.

In view of the above, this study was designed to explore: (a) if hyperthyroid state influences humoral immunity, (b) if NF-κB signaling pathway in lymphocytes is activated in hyperthyroid state, and (c) if there is a correlation between humoral immunity and activation of NF-κB signaling pathway in lymphocytes in hyperthyroid state.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 100–120 g were used for the experiment and given standard laboratory chow diet and water ad libitum. The animals were housed at 22 ± 2 °C with the 12/12 h light/dark cycle. All experimental protocols were approved by the institute ethical committee. Animals were randomly divided into two groups. An experimental model of hyperthyroid state was induced by treating the rats ($n = 6$) with L-thyroxin (25 μg/day/kg, PO, Knoll Pharmaceuticals, Jejuri, India) at 8 am daily for four weeks. Control rats ($n = 6$) were treated with vehicle. It was found that by third week of L-thyroxin treatment, the rats develop the features of overt hyperthyroid state (Table 1).

Table 1
Mean change in percentage in the parameters at 4th week or at the end of 4 weeks of thyroxin treatment in experimentally induced hyperthyroid rats with respect to controls

Parameters	Control rats (%)	Hyperthyroid rats
Body weight	100	Reduced by 24%
Food intake (4th week)	100	Increased by 26%
Heart rate	100	Increased by 68%
Serum cholesterol	100	Reduced by 37%
Serum thyroxin	100	Increased by 194%

2.2. Immunization of animals

At the end of third week of L-thyroxin treatment, animals were immunized with sheep red blood cells (SRBC). SRBC (5%) was prepared by dissolving 0.5 ml of packed cells in 10 ml of normal saline. The animals were immunized by injecting 1 ml of 5% RBC (1 ml contains 1×10^8 cells) through intraperitoneal route. L-Thyroxin treatment was continued for another one week after immunization.

2.3. Collection of blood

One week after immunization, the rats were anesthetized with intraperitoneal injection of thiopentone sodium (30 mg/kg). About 5–7 ml of blood was collected through cardiac puncture. Four milliliters of blood was heparinized for isolation of lymphocytes and serum was separated from the remaining blood sample.

2.4. Assay of humoral immune response

The anti-SRBC antibody titer from rat serum was assayed by direct hemagglutination technique as described by Banerjee and Hussain [20] and was expressed in terms of $-\log_2$ anti-SRBC titer.

2.5. Isolation of lymphocyte

Lymphocytes were isolated as described previously [21]. Four milliliters of heparinized blood was added slowly along the sides of the tube contains 4 ml of Ficoll hypaque (Histopaque 1077, Sigma, USA) and centrifuged at 400*g* for 20 min. The white interphase layer was separated and washed with PBS (pH 7.4) three times. Lymphocyte preparation was divided into two parts for preparation of nuclear fraction and whole cell lysate.

2.6. Preparation of lymphocyte nuclear proteins

Nuclear extracts were prepared as described before [22]. In brief, lymphocytes were lysed in 200 μl ice-cold buffer (10 mmol/L Hepes–KOH, pH 7.9, at 4 °C, 1.5 mmol/L $MgCl_2$, 10 mmol/L KCl, 0.5 mmol/L dithiothreitol (DTT), and 0.2 mmol/L PMSF) and centrifuged at 12,000*g* at 4 °C for 2 min. The supernatant was discarded and the nuclear pellet was resuspended in 100 μl ice-cold buffer (20 mmol/L Hepes–KOH, pH 7.9, at 4 °C, 25% glycerol, 1.5 mmol/L $MgCl_2$, 420 mmol/L NaCl, 0.2 mmol/L EDTA, 0.5 mmol/L DTT, and 0.2 mmol/L PMSF) incubated on ice for 20 min and then centrifuged for 2 min as above. The supernatant containing nuclear protein was estimated by Bradford method [23].

2.7. Lymphocyte whole cell lysate preparation

Lymphocyte whole cell lysate was prepared in lysis buffer (1% Triton X-100, 10 mmol/L sodium pyrophosphate,