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Clinical Immunology 116 (2005) 182 – 191

Clinical IMMUNOLOGY

www.elsevier.com/locate/yclim

Identification of prolactin as a novel immunomodulator on the expression of co-stimulatory molecules and cytokine secretions on T and B human lymphocytes

Karina Chavez-Rueda[a], Jesús Hérnández[b], Edgar Zenteno[b], Alfredo Leaños-Miranda[a], Ma Victoria Legorreta-Haquet[a], Francisco Blanco-Favela[a,*]

[a]*Laboratorio de Auto-inmunidad, Unidad de Investigación Medica en Inmunología, Hospital de Pediatría, Centro Medico Nacional "Siglo XXI", IMSS. Av. Cuauhtémoc 330 Col. Doctores PO Box: 73-032, CP: 03020 IMSS. México, D.F., México*

[b]*Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), México D.F., México*

Received 8 October 2004; accepted with revision 21 March 2005
Available online 28 April 2005

Abstract

We investigate the immunomodulator role of prolactin (PRL) on $CD4^+$ and B cell activation from healthy subjects in comparison with hyperprolactinemic patients. Peripheral blood mononuclear cells, $CD4^+$ or B cells, purified, were cultured under different conditions, as follows: with mitogen, without stimulus, with different concentrations of human PRL, with unspecific mitogen plus PRL, or with antibodies against PRL. The results revealed that PRL is produced by lymphocytes, the expression of CD69 and CD154 molecules, and interleukin secretions depend partially on the autocrine PRL, this is supported by the findings that secretions of IL-2, IFNγ, and co-stimulatory molecule expression were markedly reduced when autocrine PRL was blocked with anti-PRL antibodies. Furthermore, PRL activity was only observed during the first 2 h after activation. In contrast, B cell culture did not show any alteration by adding or blocking PRL in the expression of CD40 and CD86 in both groups: healthy subject and hyperprolactinemic patients.

Keywords: Prolactin; CD69; CD154; CD40; CD86; Hyperprolactinemia; Immunomodulatory; Cell activation; IL-2; IFNγ

Introduction

Communication between cells is mediated by endocrine, nervous, and immune systems, which constitute an interlocking network. Several evidences have shown that the neuro-endocrine peptide hormone, prolactin (PRL), participates in the immune response. PRL is mainly synthesized by the anterior pituitary. However, the decidua, breast, and the lymphocytes [1–4] are also capable of synthesizing this hormone. Structural analysis of the PRL receptor has demonstrated that it is related to the cytokine/hematopoietin family, like the growth hormone (GH), erythropoietin, granulocyte-macrophage colony stimulating factor (GM-CSF), and some interleukins (IL), such as IL-2 to IL-7, IL-9, IL-13, and IL-15 [5]. The receptor for PRL is also present in T- and B-lymphocytes and macrophages [6–9]. Excess prolactin (hyperprolactinemia) can be caused by diverse conditions, which can be divided into three groups: physiological (pregnancy), drugs (metoclopramide), and disease (prolactinomas and chronic renal failure). Hyperprolactinemia in women can cause irregular menses, galactorrhea, hypogonadism, and infertility. In contrast, clinical manifestations in men are widely variable and include decreased libido, impotence, and infertility [10]. Recently, it has been described that some hyperprolactinemic patients develop autoimmune rheumatic diseases [11] and some women develop natural autoantibodies during

* Corresponding author.
E-mail address: fblanco1@terra.com.mx (F. Blanco-Favela).

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doi:10.1016/j.clim.2005.03.013

pregnancy probably due to the hyperprolactinemic state [12], suggesting a strong PRL participation in immune system activation.

Other evidences about PRL's role in the immune system are: in vitro, PRL acts as a co-mitogen for T, B, and NK cells in both human [13–16] and mice [17,18], independently from stimulation with antigen and/or mitogen. In NZB/W mice, which display a disease similar to systemic lupus erythematosus (SLE), implants of syngenic pituitary glands induce a hyperprolactinemic state, resulting in accelerated autoimmunity and early mortality [19,20] that can be improved with bromocriptine treatment [20]. In humans, clinical trials have shown that a subset of SLE patients course with hyperprolactinemia during disease activity [21,22]. In contrast, hypophysectomized rats decreased responses to both red blood cells and *Escherichia coli* lipopolysaccharides, which can be restored by the administration of exogenous PRL, but not with other pituitary hormones [23], suggesting that PRL's low levels are associated with immunodeficiency.

The adaptive immune response is a complex process, in which activation of the immune cells is fundamental. The process is initiated by antigen presentation of antigenic peptides bound to class I or II MHC molecules by professional antigen-presenting cells (APC) [24]. Then the antigen is recognized by the T cell receptor (TCR) who dictates antigen specificity and plays a central role in initiating T cell activation [24]. However, this interaction, by itself, is not sufficient to fully activate naive T cells. Thus, for virgin T cell activation, subsequent non-antigen-specific co-stimulatory signals are necessary to trigger cytokine gene expression. The best-known co-stimulatory signal for T cells is provided by the interaction of CD28 on the T cell with the members of the B7 family (CD80 and CD86) on the APC. On the other hand, B cells and other antigen-presenting cells are also targets of co-stimulatory signals, mainly received through the CD40 receptor after engagement by its ligand CD154 (CD40L) on activated T cells. This signal promotes growth, differentiation, survival, and isotype switching on B cells. A third type of signal with a crucial role in T and B cell activation is mediated through binding of soluble cytokines to their respective receptors [25,26]. To further define the participation of prolactin in the mechanism of immune cell response activation, cells from two different sources were studied: (a) from hyperprolactinemic patients with high serum levels of prolactin at the moment the samples were taken and (b) from healthy humans with normal serum levels of PRL. Cells were in vitro cultivated under different stimuli to recreate different levels of PRL at the moment that cell activation is achieved. The markers for $CD4^+$ T cell activation were the expression of co-stimulatory molecules, such as CD69, CD154, and cytokine secretions; CD86, CD40, and immunoglobulin production were used as markers of B cell activation.

Materials and methods

Patients

The criteria of five idiopathic hyperprolactinemia patients were high PRL serum level (>20 ng/ml) plus menstrual disorder and galactorrhea without any evidence of conditions otherwise associated with elevated PRL, such as pregnancy, PRL-secreting pituitary adenoma (prolactinoma), intracranial tumors compressing the pituitary stalk or hypothalamus, drugs, hypothyroidism, chest wall diseases, or hepatorenal disorders. The criteria of ten healthy subjects were to be disease-free, women without menstrual disorders, and normal PRL serum levels (<20 ng/ml). Venous blood samples were drawn between 8:00 AM and 10:00 AM.

Cells

Human peripheral blood mononuclear cells (PBMNCs) were isolated by density gradient centrifugation (Lymphoprep, Nycomed Pharma AS, Oslo, Norway). PBMNCs were recovered from the interface, washed in PBS, and resuspended in serum-free culture medium (AIM-V medium, Life Technologies, Grand Island, NY), which is a synthetic medium without prolactin. Cell viability was determined by trypan blue exclusion, it was always above 95%.

The used human PRL (hPRL) and polyclonal antibodies against prolactin were kindly donated by Dr. A.F. Parlow from the National Hormone and Pituitary Program Harbor-UCLA Medical Center (batch # AFP3855A). The non-related antibody was obtained through serum precipitation from normal rabbits.

T and B cell isolation

Isolation of human $CD4^+$ T cells from PBMC was achieved by depletion of non-CD4+ cells. PBMCs were reacted with a cocktail of hapten-conjugated monoclonal antibodies (mAb) against CD8, CD11b, CD16, CD19, CD36, and CD56. B cells (95% CD19+) were negatively selected after reacting PBMC with hapten-labeled mAb to non-B cells, including anti-CD2, CD4, CD11b, CD16, CD36, and anti-IgE. These cells were exposed to magnetic beads coupled to an anti-hapten monoclonal antibody (Miltenyi Biotec, Auburn, CA). The magnetically labeled cells were depleted on a MACS column (Miltenyi Biotec, Auburn, CA) with the magnetic MidiMACS field (Miltenyi Biotec, Auburn, CA).

Cell proliferation assay

PBMNCs were plated at 2×10^6 cells/ml, 0.1 ml/well, in 96-well plates and cultured in synthetic serum-free medium with 0, 0.5, 1, and 2 μg/ml of concanavalin A (conA, Sigma, St Louis, MO). For the rest of the experiment, we decided to