Pflügers Arch - Eur J Physiol (2000) 440 [Suppl]: R94-R95

Changes in Th1/Th2 cytokine balance in Graves' desease

Tomaž Kocjan • Branka Wraber • Urška Repnik • Sergej Hojker

Correspondence to: T. Kocjan, Dept. of Endocrinology and Diabetes, Medical Centre Ljubljana, Zaloška 7, 1525 Ljubljana, Slovenia

B. Wraber, U. Repnik
Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia

S. Hojker
Dept. of Nuclear Medicine, Medical Centre Ljubljana, Zaloška 7, 1525 Ljubljana, Slovenia

Abstract Graves' disease (GD) is characterised by hyperthyroidism, caused by stimulatory thyrotropin receptor (TSHR) antibodies. Recent research shows that an important factor in the pathogenesis of autoimmune diseases is the change in the balance between Th1 cytokines, which promote cell mediated immunity, and Th2 cytokines, which promote humoral immunity. There are contradictory data about this balance shift in GD. Our objective was to determine the Th1/Th2 cytokine balance shift in patients with newly diagnosed GD, when compared to the same balance in healthy controls. We isolated mononuclear cells (MNC) from the peripheral blood of healthy donors and from patients with newly diagnosed GD before treatment. The MNC were activated with ionomycin in combination with phorbol 12-myristate 13-acetate (PMA). After 40-hour incubation, the concentrations of the cytokines produced (IFN-γ, IL-4, IL-10, IL-12) in the culture supernatants were measured by ELISA (Endogen, USA). The MNC cultures from patients with GD produced significantly less IL-12 and significantly more IL-10 and IL-4 than MNC cultures from healthy controls. All calculated ratios of Th1 against Th2 cytokines in MNC cultures from patients with GD were significantly lower than in MNC cultures from healthy controls. Our results show a systemic shift of cytokine production in patients with GD toward the Th2 cytokine response, thus confirming the key role of TSHR antibodies and humoral immunity in the pathogenesis of GD.

Key words Graves' disease · Th1/Th2 cytokine balance · Mononuclear cells · Ionomycin · Phorbol myristate acetate

Introduction

Graves' disease (GD) is an autoimmune condition of the thyroid, characterised by hyperthyroidism, which is caused by stimulatory thyrotropin receptor (TSHR) antibodies. Diffuse goiter, ophthalmopathy and praetibial myxoedema are other hallmarks of the disease, the latter two emphasizing the systemic nature of the autoimmune process. Genetic predisposition, in combination with some external factors (infections, stress, iodine) and internal conditions (female sex hormones) is probably required for the development of crucial abnormalities in CD4+ T helper (Th) lymphocytes' function, which lead to GD [5]. The Th lymphocytes are divided into at least two functionally antagonistic subsets, named Th1 and Th2 which are determined by the cytokine pattern produced by activated cells. The Th1/Th2 cytokines are also produced by other immune cells, such as CD8+ cells, B lymphocytes and macrophages. The IL-12 primes towards the Th1 response, where the cells secrete IFN-γ, IL-2 and LT and support cellular immunity. By contrast, IL-4 induces differentiation of Th2 cells, which secrete IL-5, IL-10 and IL-4 and support humoral immunity through B cell proliferation and production of antibodies. The Th1 cytokines inhibit the development of the Th2-mediated response, while Th2 cytokines cause reciprocal inhibition of Th1 responses. If the delicate balance between these two subsets is no longer sustained, this may lead to the development of different immune disorders, including autoimmune diseases [1]. In recent reports on Th1/Th2 balance in GD, the analysis has been mainly directed to the intrathyroidal cytokine pattern at the mRNA level in surgical specimens. The results proved contradictory, since Th1 [8] or Th2 prevalence [3] and an unrepresentative cytokine profile [6] were reported. There are fewer data about changes in the pattern of peripheral T lymphocytes in GD and about their behavior after unspecific stimulation "in vitro". It has already been postulated in diabetes mellitus type I [2] that after such stimulation peripheral T lymphocytes behave comparably to autoreactive T lymphocytes in diseased organs. Our objective was to determine the systemic Th1/Th2 cytokine balance shift in patients with newly diagnosed GD, when compared to the same balance in healthy controls.

Materials and methods

Peripheral venous blood samples were obtained from seventeen healthy volunteers and eighteen patients with newly diagnosed GD before treatment during the usual diagnostic procedure. Diagnosis was confirmed by clinical signs and symptoms, the characteristic ultrasound thyroid pattern, supressed TSH and elevated fT4, fT3, and TSHR antibodies values. The mononuclear cells (MNC) were isolated from the peripheral venous blood of both groups with centrifugation on Ficoll-Paque (Pharmacia, Sweden) density gradient. The MNC were suspended in RPMI 1640 medium containing antibiotics and fetal calf serum (Sigma, USA) and plated in cell culture plates (T grade, NUNC, Denmark). Polyclonal activators phorbol 12-myristate 13-acetate (PMA; conc. 3.33

ng/ml of MNC culture) and ionomycin (IONO; conc. 500 nM in MNC cultures) were then added. In the next step, the MNC cultures were incubated for 40 hours. After the incubation the concentrations of cytokines produced (IFN-γ, IL-4, IL-10, IL-12) were measured in the culture supernatants by ELISA kits (Endogen, USA). The T-test was used to compare the concentrations of cytokines produced and the ratios of Th1 against Th2 cytokines between the two groups studied.

Results

The MNC cultures from patients with GD produced significantly less IL-12 and significantly more IL-10 and IL-4 than normal cells. The difference in production of IFN-γ was not statistically significant (Figure 1).

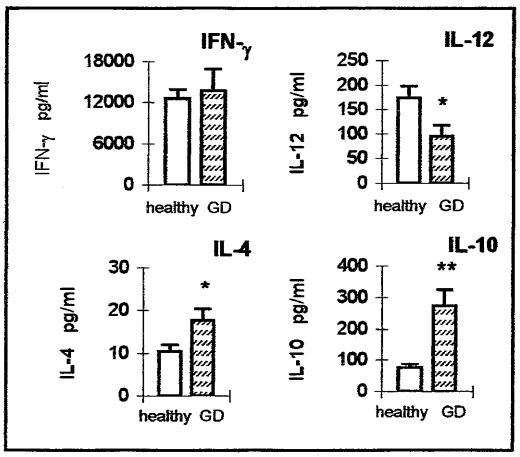

Fig. 1 Comparison of cytokine concentration in supernatants of MNC cultures between healthy controls (n=17) and patients with Graves' disease (GD) (n=18). The mononuclear cells (MNC) were stimulated with IONO&PMA for 40 hours. The cytokines were measured by ELISA (Endogen, USA). The results are expressed as mean + SE in pg/ml.
* = $p \leq 0.05$; ** = $p \leq 0.01$ with regard to healthy controls.

All calculated ratios of Th1 against Th2 cytokines in MNC cultures from patients with GD were significantly lower than in MNC cultures from healthy controls (Figure 2).

Discussion

Disturbances in systemic immunoregulation which is probably mediated through the cytokine network imposing a delicate Th1/Th2 balance are considered as one of the primary causes of autoimmune diseases. The aim of this study was to display differences in the pattern of the circulating immune cells and the cytokines they produce between patients with newly diagnosed GD and the healthy controls. Our results show a systemic shift of cytokine production by peripheral MNC in patients with GD toward the Th2 immune response, when compared to healthy controls. This study is consistent with previous works [4], while at the same time introducing IL-12, bearing in mind the data which point to the critical role of IL-12-induced Th1 cells in the development of organ-specific autoimmune diseases [7].

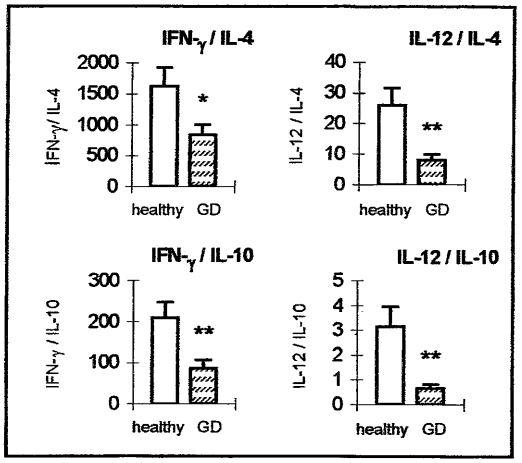

Fig. 2 Comparison of ratios of counterregulatory cytokines in supernatants of MNC cultures between healthy controls (n=17) and patients with Graves' disease (GD) (n=18). The mononuclear cells (MNC) were stimulated with IONO&PMA for 40 hours. The cytokines were measured by ELISA (Endogen, USA). The results are expressed as mean + SE.
* = $p \leq 0.05$; ** = $p \leq 0.01$ with regard to healthy controls.

In conclusion, the results of our study confirm the key role of TSHR antibodies and humoral immunity in the pathogenesis of GD, which is not generally reflected in most of the other organ specific autoimmune diseases [1], where the opposite usually occurs. Current therapeutic options for patients with GD differ little from those that were available in the 1950s; nevertheless recent advances in our understanding of the immune system regulation hold promise for the development of new, highly specific interventions.

References

1. D'Elios M, Del Prete G (1998) Th1/Th2 balance in human diseases. Transplant Proc 30: 2373-2377.
2. Foulis AK, McGill M, Farquharson MA (1991) Insulitis in type I DM in man: macrophages, lymphocytes and IFN-gama containing cells. J Pathol 164: 97-103.
3. Heuer M, Aust G, Ode-Hakim S, Scherbaum WA (1996) Different cytokine mRNA profiles in Graves' disease, Hashimoto's thyroiditis and nonautoimmune thyroid disorders determined by quantitative RT-PCR. Thyroid 6(2): 97-106.
4. Kallmann BA, Huether M, Tubes M, Feldkamp J, Bertrams J, Gries FA, Lampeter EF, Kolb H (1997) Systemic bias of cytokine production toward cell-mediated immune regulation in IDDM and toward humoral immunity in Graves' disease. Diabetes 46: 237-243.
5. McIver B, Morris JC (1998) The pathogenesis of Graves' disease. Endocrinol Metab Clin North Am 27(1): 73-89.
6. Paschke R, Schuppert F, Taton M, Velu T (1994) Intrathyroidal cytokine gene expression profiles in autoimmune thyroiditis. J Endocrinol 141(2): 309-315.
7. Trembleau S, Germann T, Gately MK, Adorini L (1995) The role of IL-12 in the induction of organ-specific autoimmune diseases. Immunol Today 16(8): 383-386.
8. Watson PF, Pickerill AP, Davies R, Weetman AP (1994) Analysis of cytokine gene expression in Graves' disease and multinodular goiter. J Clin Endocrinol Metab 79: 355-360.