Different response to glucocorticoid therapy in autoimmune diseases of CNS

Različit odgovor na glukokortikoidnu terapiju primenjenu u autoimunskim oboljenjima centralnog nervnog sistema

Željka Stanojević, Marija Mostarica Stojković

Institut za medicinsku i kliničku biohemiju, Medicinski fakultet Univerziteta u Beogradu

Kontakt: zeljka.stanojevic@med.bg.ac.rs

Sažetak

Th17 ćelije i njihov glavni citokin interleukin 17 (IL-17), imaju ulogu u patogenezi autoimunskih oboljenja centralnog nervnog sistema kao što su multipla skleroza i eksperimentalni autoimunski encefalomijelitis (EAE). Efekat glukokortikoida (GK), lekova koji se primenjuju kao terapija inflamatornih i autoimunskih oboljenja, na ekspresiju i proizvodnju IL-17, još uvek nije detaljno ispitan. Nije precizno definisano ni mesto dejstva GK. U ovom radu su prikazani glavni rezultati doktorske disertacije posvećene ispitivanju dejstva GK na proizvodnju IL-17 na modelu EAE indukovano kod pacova, koji su povezani sa najnovijim saznanjima o efektima GK na Th17 ćelije kod ljudi i na animalnim modelima. Metilprednizolon (MP), sintetski glukokortikoid, inhibira *in vitro* proizvodnju IL-17 od strane ćelija limfnog čvora pacova stimulisanih mitogenom i antigenom na dozno zavisani način. Pod istim uslovima je značajno više izraženo inhibitorno delovanje MP na proizvodnju i ekspresiju gena za interferon gama (IFN- γ), citokina koji stvaraju Th1 ćelije. Isti obrazac delovanja MP na ekspresiju i proizvodnju IL-17 i IFN- γ zapažen je i na ćelijama izolovanim iz kičmene moždine pacova obolelih od EAE, čime je takođe pokazano da MP ostvaruje svoje efekte ne samo u perifernom limfnom tkivu, već i u ciljanom tkivu. Različita osetljivost Th1 i Th17 limfocita koji su glavni ćelijski izvori IFN- γ , odnosno IL-17 na delovanje GK zapažena je na drugim animalnim modelima i u bolestima kod ljudi. Razumevanje molekularskih mehanizama koji leže u osnovi relativne otpornosti Th17 ćelija na delovanje GK od ključnog je značaja za razvoj novih strategija u lečenju onih oblika autoimunskih i hroničnih bolesti koje su rezistentne na delovanje glukokortikoida.

Ključne reči: autoimunost, eksperimentalni autoimunski encefalomijelitis, metilprednizolon, IL-17, Th17

Abstract

Th17 cells and interleukin (IL-17), their signature cytokine, have the main role in the pathogenesis of autoimmune diseases of the central nervous system such as multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). The effect of glucocorticoids (GC) on expression and production of IL-17 has not been thoroughly tested yet. Also, the site of action of GC is not precisely defined. This paper presents the main results of the Doctoral thesis devoted to studies of GC on the production of IL-17 in the model of EAE, induced in susceptible laboratory animals. Methylprednisolone (MP), a synthetic glucocorticoid, inhibit *in vitro* production of IL-17 in mitogen-stimulated lymph node cells (LNC) as well as in myelin basic protein (MBP)-stimulated draining LNC in dose-dependent manner. However, under the same conditions inhibitory effect of the MP on production and expression of the genes for IFN- γ , a cytokine that TH1 cells generate, is significantly more pronounced. Interestingly, when we analyzed effects of MP applied *in vivo* in EAE, the same phenomenon was observed: the proportion of IFN- γ producing, but not all of IL-17 cells were reduced in cells isolated from MP treated rats in comparison to control rats which indicates that MP achieves its effects not only in the peripheral lymphoid tissues, but also in target tissue. Different sensitivities of Th1 and Th17 cells that are major cellular sources of IFN- γ or IL-17 in the effect of the GC has been observed in other animal models and in human disease. Understanding the molecular mechanisms underlying the relative resistance of Th17 cells on the operation of GC is very important for the development of new strategies in the treatment of those forms of autoimmune and chronic diseases that are resistant to the effect of glucocorticoids.

Key words: autoimmunity, experimental autoimmune encephalomyelitis, methylprednisolone, IL-17, Th17

Introduction

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the CNS with a putative autoimmune pathogenesis (1). Experimental autoimmune encephalomyelitis (EAE), an organ-specific autoimmune inflammatory disease, induced in susceptible laboratory animals, shows significant similarities to MS in both clinical and pathological aspects and therefore is widely used as its experimental model (2). Both diseases are assumed to be mediated by myelin-specific CD4⁺ T lymphocytes, more specifically by Th1 and Th17 cells and their signature cytokines, IFN- γ and IL-17, respectively (3,4). Th17 cells, at least in mice, develop from naive CD4⁺ T cells under the influence of TGF- β and IL-6 (5,6), require IL-23 for survival and expansion (7) and secrete a profile of inflammatory cytokines including IL-17 and IL-17F, IL-6, GM-CSF, TNF- α , IL-21 and IL-22 (8). The proinflammatory functions of these cytokines suggest their important role in pathogenesis of both MS and EAE because the main feature of their pathophysiology is neuroinflammatory reaction (9).

Glucocorticoids and central nervous system inflammation

Glucocorticoids (GC) are used to treat a wide range of inflammatory, allergic and autoimmune diseases and they have been shown effective in the treatment of acute relapses in MS (10), as well as of EAE (11). Multiple mechanisms are proposed to explain glucocorticoid therapeutic efficacy in autoimmune damage to the CNS (12), since GC down-regulate both innate and adaptive immune responses. Namely, GC have been shown to inhibit lymphocyte proliferation and lymphocyte expression and production of various proinflammatory cytokines and mediators (e.g. IL-1 β , IL-6, TNF- α), while enhancing the expression of anti-inflammatory cytokines (e.g. IL-10, TGF- β), T-cell apoptosis and redistribution, a shift in the population of Th cells from Th1 to Th2 and the proportion of regulatory cells (12).

Since ample evidence indicates that GCs differentially regulate the production of Th1 and Th2 cytokines (12) it is important to know how GCs affect the production of IL-17, a cytokine accused to be critically involved in the pathogenesis of autoimmune and chronic inflammatory diseases frequently treated with GCs. Therefore, the aim of my thesis (13) was to analyze effects of methylprednisolone (MP), a synthetic glucocorticoid drug on IL-17 expression and production, and compare these effects with corresponding effects on IFN- γ . Obtained results partially contributed better understanding of glucocorticoids on Th-17 effects in health and disease.

First, the effect of MP was studied in an in vitro system consisting of rat lymph node cells (LNC) stimulated with polyclonal T cell mitogen, Concanavalin A (Con A) and determined by measuring the expression of IL-17 and IFN- γ mRNAs in cultivated cells by PCR and concentration of the cytokines in culture supernatants by ELISA. MP decreased expression and production of both cytokines, but interestingly, its effect was less pronounced on IL-17 than on IFN- γ (14). The observed inhibition was, at least partially, conducted through inhibition of ROR γ T transcription factor. However, the influence of MP is less effective if the drug is applied to purified T cells than to mixed population of LNC. It suggests that action of MP on IL-17 generation in rat LNC includes both direct influence on T cells and indirect influence on other LNC populations contributing to T cells IL-17 production (14). Indeed, MP was shown to affect activity of accessory LNC cells, including dendritic cells and macrophages which produce numerous cytokines that are important for the stimulation of IL-17 in T cells, such as IL-1, TNF- α , IL-6, IL-18 (12).

In line with these data, our results showed that MP inhibited IL-6 production in LNC population devoid of T lymphocytes. It is thus expected that MP affects IL-17 production in LNC more potently than in purified T cells (14). However, the same phenomenon was not observed with IFN- γ , as MP had almost equal inhibitory effect on T cells as on LNC. It seems that the direct effect of MP on T cells is crucial for IFN- γ inhibition, although it was convincingly demonstrated that MP inhibit the production of IL-12, necessary for Th1 differentiation (15). The potency of the direct effect of MP on IFN- γ production in T cells is supported by recent findings that GCs directly interfere with T-bet, the essential transcription factor of IFN- γ -producing cells (16).

Similar results were obtained when the MP effect on lymphocytes from immunized rats was analyzed. MP decreased IL-17 and IFN- γ expression and production in cells from lymph nodes draining the site of immunization with encephalitogen, as well as in cells isolated from the spinal cords of rats which had developed EAE (14). Results convincingly demonstrate that MP inhibits IFN- γ production more potently than IL-17 production, irrespectively of experimental setting used. Additionally, these results present evidence that small production of IFN- γ remained after MP action is still adequate to inhibit IL-17 production since the addition of anti-IFN- γ -neutralizing antibody eliminated such an inhibitory effect (14). Therefore, it seems that although MP inhibits IFN- γ , they still cooperate to limit IL-17 generation. The lack of deepening IL-17 inhibition by the addition of exogenous IFN- γ is unexpected but it might be explained by saturating effect of IFN- γ that remained upon MP treatment. We can

conclude that the expression and production of IL-17 is less sensitive to the influence of MP than the IFN- γ production.

Besides various mechanisms that underlie the different response to glucocorticoid therapy, among populations of autoreactive T cells, (12) there is a diverse response to glucocorticoid therapy depending on the environment, purposely the site of action. Recent evidence from EAE induced in mice suggests that the major targets of GC action are peripheral rather than CNS-residing T lymphocytes (17). On the contrary, plentiful data convincingly demonstrate that GC directly influences cells within the target tissue (18). In our experiments, the number of cells isolated from the spinal cord of MP-treated animals immunized with encephalitogen was significantly lower than the number of cells isolated from control diseased rats and their IFN- γ and IL-17 expression and production were inhibited (19). This could be a consequence of the inhibition of encephalitogenic cell infiltration into the CNS that has been previously reported following treatment of EAE with glucocorticoids (20). The limitation of the infiltration might be explained by increased apoptosis and weaker activation of encephalitogenic cells in lymphoid organs, by decrease in expression of adhesion molecules on leukocytes and endothelial cells of the blood brain barrier (BBB), as well as by increased integrity of BBB (2). Fewer infiltrating cells within the CNS of MP treated rats might also be a consequence of increased rate of apoptosis of these cells in the CNS, as previously suggested (20). Surprisingly, we could not detect increased cell death rate under the influence of MP in EAE rats (19), which is in accordance with the recent investigation performed in mice (17). Most recent data demonstrate that both murine and human Th17 cells are resistant to glucocorticoid-induced apoptosis and the underlying mechanism is ascribed to high levels of BCL-2 (21).

When we analyzed effects of MP applied *in vivo* in EAE, the same phenomenon was observed: the proportion of IFN- γ producing, but not of IL-17 producing cells was reduced in cells isolated from MP treated rats in comparison to control rats (19). The proportion of cells isolated from the spinal cord of diseased rats expressed both cytokines, in accordance with recently described findings in DA rat EAE (22). It is tempting to speculate that the influence of MP on Th1 might be direct, while the drug might affect Th17 cells and their cytokines through its effect on CNS resident cells, which have previously been shown to modulate IL-17 production.

Conclusion

Our results demonstrating low sensitivity of Th 17 cells, both *in vivo* and *in vitro*, to the inhibitory ac-

tion of MP (13,14,19) have been later confirmed by findings that T lymphocytes derived from MS patients with an enhanced Th17-like phenotype are less sensitive to hydrocortisone inhibition (23) and severe forms of allergic asthma associated with Th17 response are poorly controlled by corticosteroids (24). Moreover, it has been recently shown that not only Th17 cells are refractory to the inhibitory actions of steroids but also that these cells are actively and specifically selected for within mixed T cell cultures upon exposure to steroids (25). These data extend current notions of steroid resistance in Th17 cells by identifying a distinctive subset of Th17 cells that may underlie steroid hyporesponsiveness (25). It is also interesting to speculate, that steroid treatment of autoimmune patients may directly enrich for precisely the proinflammatory T cell subsets linked with inflammation and drug resistance. The most recent data demonstrate that although Th17 cells are refractory to glucocorticoid suppression at a genome-wide level, there is a reciprocal sensitivity of this subset of cells to calcineurin inhibition with cyclosporin A, explaining the clinical efficacy of the latter in the treatment of diseases with steroid resistant form (26). However larger studies will be required to understand the mechanisms underlying steroid resistance of Th17 cells and how these mechanisms might be used for the selection of optimal therapy of autoimmune and other chronic inflammatory diseases.

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