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Short communication

Inhibitory effect of antidepressant drugs on contact hypersensitivity reaction is connected with their suppressive effect on NKT and CD8$^+$ T cells but not on TCR delta T cells

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ABSTRACT

Background: Contact hypersensitivity (CHS) reaction induced by a topical application of hapten is a cell-mediated antigen-specific type of skin inflammation mediated by interaction of several subtypes of T cell subpopulations. Recently, it has been shown that antidepressant drugs inhibit CHS reaction, although the mechanism of this effect remains unknown. The aim of the present study was to investigate the effect of 2-week desipramine or fluoxetine administration on the CHS reaction induced by picryl chloride (PCL) application in B10.PL mice and in knock-out mice established on B10.PL background: TCR$\delta^{-/-}$ mice lacking TCR$\gamma\delta$ T lymphocytes; $\beta_2m^{-/-}$ mice lacking CD8$^+$ T lymphocytes and CD1d$^{-/-}$ mice lacking CD1d dependent natural killer T (NKT) lymphocytes.
Methods: B10.PL, TCR$\delta^{-/-}$, $\beta_2m^{-/-}$ and CD1d$^{-/-}$ mice were divided into six groups: 1) vehicle-treated negative control group; 2) desipramine-treated negative control group; 3) fluoxetine-treated negative control group; 4) vehicle and PCL-treated group (positive control group); 5) desipramine and PCL-treated group; and 6) fluoxetine and PCL-treated group. CHS to PCL was tested by evaluation of ear swelling. Metabolic activity of spleen and lymph node cells were estimated by MTT test.
Results: The antidepressants significantly suppressed the CHS reaction in B10.PL mice: desipramine by 55% and fluoxetine by 42% compared to the positive control. This effect was even stronger in TCR$\delta^{-/-}$ mice, in which fluoxetine reduced the ear swelling by 73% in comparison with the vehicle-treated positive control group. On the other hand, desipramine and fluoxetine did not inhibit CHS reaction in $\beta_2m^{-/-}$ and CD1d$^{-/-}$ mice. Moreover, PCL increased metabolic and/or proliferative activity of splenocytes in all four strains of mice whereas the antidepressants decreased this activity of splenocytes in B10.PL, TCR$\delta^{-/-}$ and CD1d$^{-/-}$ mice.
Conclusion: The results of the present study show that lack of CD8$^+$ T cells or NKT cells abolishes the immunosuppressive effect of antidepressant drugs on PCL-induced CHS reaction in mice. These results suggest that antidepressant drug-induced inhibition of CHS reaction is connected with their inhibitory effect on ability of CD8$^+$ T cells and NKT cells to induce and/or escalate CHS reaction. TCR$\gamma\delta$ cells seem not to be involved in antidepressant-induced suppression of CHS.

1. Introduction

Contact hypersensitivity (CHS) reaction is a classic example of in vivo T cell immunity in which sensitization of the skin with a reactive hapten leads to induction of T effector cells that are then recruited locally to mediate antigen-specific inflammation after subsequent skin challenge [1]. Moreover, it is known that the components of innate immunity are involved in initiation of CHS reaction, and some studies have shown that complement and mast cells play an important role in recruitment of the CHS-effector T cells to the skin at the early CHS "initiating" stage, as quickly as 2 h, in previously sensitized mice [2].

Some of us have previously reported that innate-like B-1 B lymphocytes played a role in this innate immunity mediated recruitment of CHS-effector T cells [3]. The B-1 B lymphocytes were activated within 1 h post immunization and produced IgM antibodies. Upon local skin challenge with hapten, IgM antibodies form immune complexes with the antigen, which activate complement cascade, to generate C5a that

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stimulates mast cells to secrete tumor necrosis factor (TNF)-α and serotonin involved in T cell recruitment [4]. Campos et al. [5] also reported that innate-like invariant $V\alpha14^+$ $J\alpha18^+$ NKT (iNKT) cells were required to activate the B-1 B lymphocytes along with the antigen by producing IL-4 within minutes after immunization.

Our recently published papers showed that antidepressant drugs suppressed the T helper (Th)1-mediated CHS reaction induced by PCL in CBA/J mice and $CD8^+$ T cytotoxic (Tc)1 cell mediated CHS reaction induced by 2,4-dinitrofluorobenzene (DNFB) in Balb/c mice [6,7]. In the above-mentioned CHS reactions, Th1 or $CD8^+$ Tc1 cells, respectively, played a dominant role although several other subtypes of cells, like natural NKT cells and TCRγδ T cells were involved in induction and modulation of these reactions.

The mechanism of the suppressive effect of antidepressant drugs on CHS reaction is unknown. To determine the mechanism of antidepressant-induced immunosuppression, we examined the effect of antidepressant drugs on CHS reaction in B10.PL mice and three strains of knock-out mice created on B10.PL background: $TCR\delta^{-/-}$(mice lacking TCRγδ T lymphocytes), $\beta_2m^{-/-}$ (mice lacking CD8 T lymphocytes), and $CD1d^{-/-}$ (mice lacking CD1d dependent NKT lymphocytes). Our studies were designed to answer the question whether the suppressive action of antidepressant drugs on CHS reaction can be associated with the effect of these drugs on specific subpopulations of lymphocytes.

2. Materials and methods

2.1. Mice

Eight weeks old male $TCR\delta^{-/-}$, $\beta_2m^{-/-}$ and $CD1d^{-/-}$ developed on the B10.PL ($H\text{-}2^u$) background and B10.PL control mice were used in experiments. The weight of the animals was between 20 and 25 g. Animals originated from the breeding unit of the Department of Medical Biology, Jagiellonian University, College of Medicine. All mice were kept under pathogen-free conditions using filter-topped microisolator cages and sterile equipment and fed autoclaved food and water. Experiments were carried out according to the guidelines of the Animal Use and Care Committee of the Jagiellonian University (dated 16.03.2004).

2.2. Drugs and chemicals

Desipramine hydrochloride and fluoxetine (Sigma, USA) were used. Drugs were dissolved in sterile water and administered intraperitoneally (ip) in a dose of 10 mg/kg.

2,4,6-Trinitro-1-chlorobenzene (TNCB, known also as picryl chloride, PCL) from Chemica Alta (Edmonton, Canada) was used as a contact allergen.

2.3. Mouse CHS test

B10.PL control mice, $TCR\delta^{-/-}$, $\beta_2m^{-/-}$ and $CD1d^{-/-}$ mice were actively sensitized by topical application of 0.15 ml of 5% PCL in an acetone–ethanol mixture (1:3) to the shaved abdomen. Mice were shaved and painted with the acetone–ethanol mixture alone as a sham immunization. Four days later, mice were challenged to both sides of the ear with 10 μl of 0.4% PCL in an olive oil–acetone mixture (1:1). The subsequent increase in ear thickness was measured 24 h later with an engineer's micrometer and expressed in units of 10^{-2} mm ± SD. Background increase in ear thickness of the non-immunized littermates that were similarly challenged, was subtracted from each experimental group, to yield the net ear swelling expressed in units of 10^{-2} mm ± SD. Each experimental and control group consisted of seven animals.

The animals were divided into six groups: 1) vehicle-treated negative control group; 2) desipramine-treated negative control group; 3) fluoxetine-treated negative control group; 4) vehicle-treated PCL group (positive control group); 5) desipramine-treated PCL group; and 6) fluoxetine-treated PCL group.

Fourteen-day intraperitoneal treatment with the antidepressant drugs desipramine or fluoxetine began 9 days before sensitization. Then, contact sensitization (PCL challenge) was induced and treatment with antidepressant drugs was continued for another 5 days. Four days later (24 h before ending of the experiment), PCL challenge were re-exposed (elicitation phase). Final ear measurement was carried out 1 h after the last drug injection.

2.4. Preparation of cell suspensions

For in vitro studies, the animals were sacrificed 1 h after the last injection of an antidepressant drug and immediately their spleens, thymuses, axillary and inguinal lymph nodes were gently removed and weighed. The relative weights of these organs was expressed as the weight of thymus, spleen or lymph nodes (in mg) divided by body weight (g).

The spleens and lymph nodes were crushed in individual glass homogenizers, dipped in ice. The obtained cells were suspended in RPMI-1640 medium (Sigma, USA), and were centrifuged at 500 × *g* for 5 min. Cell pellets were resuspended in the RPMI-1640 medium, supplemented with antibiotics and a 10% fetal bovine serum (Sigma, USA).

MTT 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma Chem., St Louis, MO, USA) was dissolved in PBS at 5 mg/ml. A stock MTT solution (10 μl per 100 μl of cell culture; a suspension of 2×10^6 splenocytes per ml and 2×10^5 lymph node cells per ml) was added to all wells, and the plates were incubated at 37 °C for 1.5 h. The lysis buffer (HCl, 2-propanol, POCH, Gliwice, Poland), 100 μl, was added to each well and the plates were evaluated 30 min later with a Uniscan II reader (Labsystem, Finland) at a wavelength of 570 mm. Splenocytes in this assay were tested unstimulated after 24 h of culture, lymph nodes cells were tested unstimulated and concanavalin A (Con A, 0.6 μg/ml and 2.5 μg/ml) or lipopolysaccharide (LPS, 5 μg/ml) stimulated, after 48 h of culture.

The proliferative response of the spleen cells to mitogen was described earlier by Kubera et al. [13]. Briefly, 2×10^5 cells per well were stimulated with concanavalin A (Con A, 0.6 μg/ml and 2.5 μg/ml) and were incubated in 96-well plates at 37 °C at a final volume of 0.2 ml for 72 h. Cell proliferation was determined by adding 0.5 μCi of [^{3}H]-thymidine per well (ICN, USA, SpA 6.7 Ci/mmol) 16 h before the end of incubation. We report here on the 2.5 μg/ml of Con A concentration only, because there were not differences between strains at the 0.6 μg/ml Con A stimulation level.

3. Statistical analysis

The results were statistically assessed by analysis of variance (ANOVA). Multiple post-hoc differences were checked by means of Duncan significant difference test ($p = 0.05$).

4. Results

4.1. Antidepressant drugs inhibit contact hypersensitivity reaction in B10.PL and $TCR\delta^{-/-}$ mice but not in $\beta_2m^{-/-}$ and $CD1d^{-/-}$ mice

Treatment of B10.PL mice with the antidepressant drugs desipramine and fluoxetine, reduced the ear swelling by 55% ($p < 0.05$) and 42% ($p < 0.05$), respectively, in comparison with the vehicle-treated positive control group (Table 1). Desipramine and fluoxetine had no effect on the ear thickness in negative control group (data not shown).

Contact hypersensitivity reaction in vehicle-treated $TCR\delta^{-/-}$ mice was lower by 40% ($p < 0.05$) in comparison to vehicle treated B10.PL mice. Treatment of $TCR\delta^{-/-}$ mice with the antidepressant drugs desipramine and fluoxetine, reduced the ear swelling by 56% ($p < 0.05$) and 73% ($p < 0.05$), respectively, in comparison with the vehicle-treated