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Short communication

Effect of tigecycline on the production of selected cytokines and counts of murine CD4$^+$ and CD8$^+$ T cells - an *in vitro* study

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Abstract

Due to the unrecognized effect of tigecycline (TIG) on CD4$^+$ and CD8$^+$ T cells, the present study has been undertaken in order to determine whether the drug can affect these cells in respect of their counts, and the production of IFN-γ, IL-17 (pro-inflammatory and immune-protective cytokines), IL-4 (anti-inflammatory and immune-protective cytokine), IL-10 and TGF-β (anti--inflammatory and immune-suppressive cytokines). Murine lymphocytes were treated with TIG for 48 and 96 h at concentrations reflecting its plasma levels obtained in vivo at therapeutic doses, and at 10-fold lower concentrations. It was found that TIG neither affected substantially the percentage and absolute counts of entire CD4$^+$ and CD8$^+$ T cell populations nor influenced the Foxp3$^+$CD25$^+$CD4$^+$ regulatory/suppressive T cell subset. Furthermore, the percentages of IL-4-, IL-10-, IL-17- and TGF-β-producing CD4$^+$ T cells were not altered following the exposure to TIG. Similarly, TIG did not influence IFN-γ production by CD8$^+$ T cells. Thus, with respect to the parameters evaluated, TIG does not seem to exert immune-suppressive and anti--inflammatory effects.

Key words: tigecycline, CD4+ T cells, CD8+ T cells, cytokines, mouse

Introduction

Tigecycline (TIG), the first glycylcycline antibiotic, is structurally derived from minocycline. TIG has antimicrobial activity against most Gram-positive and Gram-negative aerobic and anaerobic bacteria, and is used for the treatment of complicated skin infections, community-aquired pneumonia and complicated intra-abdominal infections (Traunmüller et al. 2009). Several reports indicate that tetracyclines have additional effects that are separate from their antimicrobial function, such as anti-inflammatory and immune--suppressive activity (Sun et al. 2015). However, the effect of TIG on the immune system is almost unknown. According to the available literature, all the knowledge on this question is limited to the information that TIG did not affect IL-1β, IL-6, IL-8 and TNF-α levels (Traunmüller et al. 2009, Sun et al. 2015, von Seth et al. 2015). Therefore, we decided to investigate whether TIG exhibits anti-inflammatory and/or immune-suppressive properties similar to those represented by other tetracyclines. To achieve this aim, the effects of TIG

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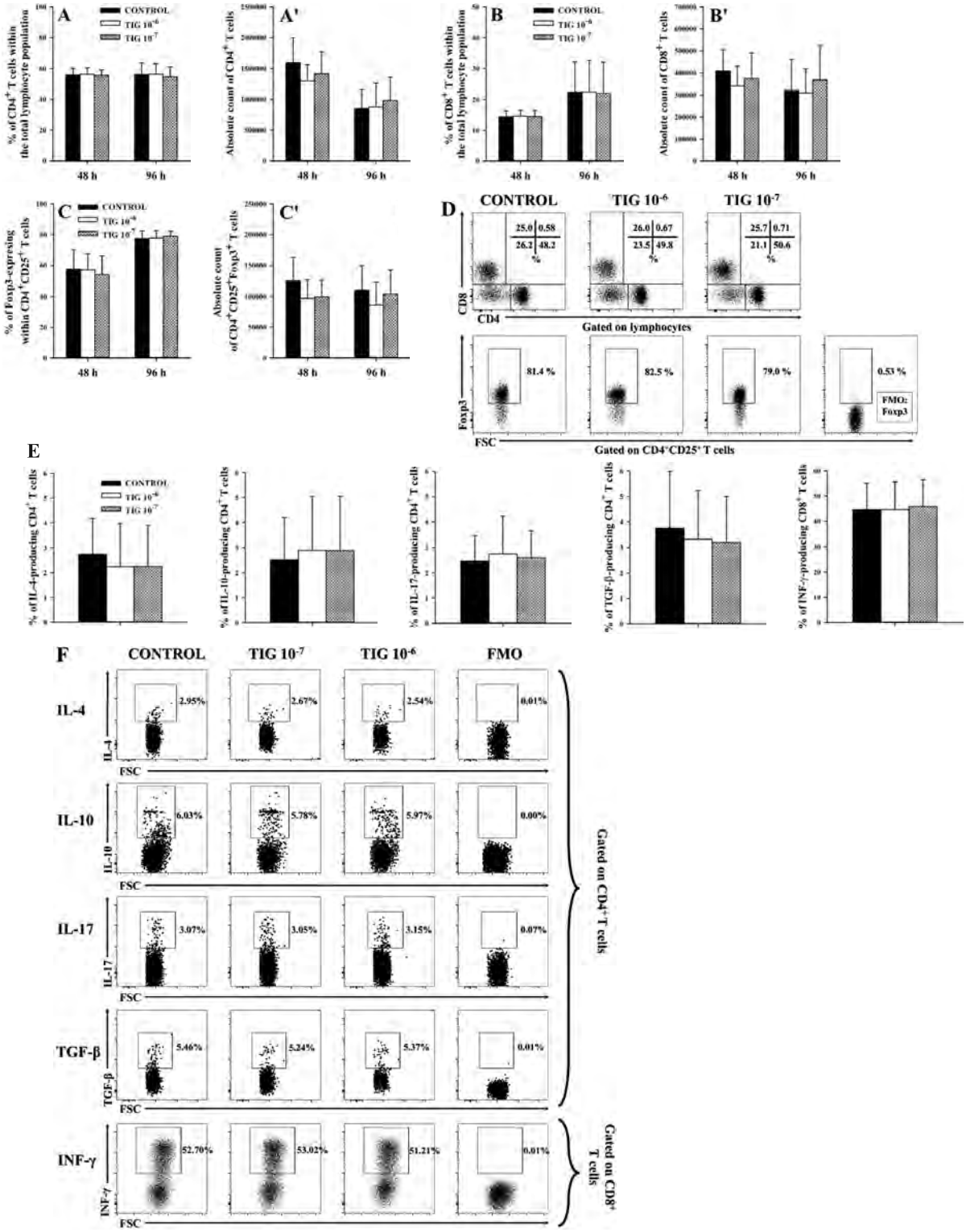

Fig. 1. The effect of tigecycline (TIG) on the relative and absolute counts of $CD4^+$ (A, A'), $CD8^+$ (B, B') $Foxp3^+CD25^+CD4^+$ (C, C') and T cells and on the percentage of $IL\text{-}4^+CD4^+$, $IL\text{-}10^+CD4^+$, $IL\text{-}17^+CD4^+$, $TGF\text{-}\beta^+CD4^+$ and $IFN\text{-}\gamma^+CD8^+$ T cells (E). The relative count is expressed as a percentage of: CD4- and CD8-expressing T cells within the total lymphocyte population, Foxp3-expressing cells within the $CD25^+CD4^+$ T cell subset, IL-4-, IL-10-, IL-17- and TGF-β-producing cells within the $CD4^+$ T cell subset and IFN-γ-secreting cells among $CD8^+$ T cells. The absolute count represents the number of $CD4^+$, $Foxp3^+CD25^+CD4^+$ and $CD8^+$ T cells per sample. Representative cytograms showing percentages of $CD4^+$, $Foxp3^+$, $CD8^+$, $IL\text{-}4^+$, $IL\text{-}10^+$, $IL\text{-}17^+$, $TGF\text{-}\beta^+$ and $IFN\text{-}\gamma^+$ cells within the respective cell subsets. Fluorescence minus one controls were used to confirm the gating strategy used to identify Foxp3-expressing and IL-4-, IL-10-, IL-17-, IFN-γ- and TGF-β-producing cells. The results are the mean (± S.D.) of two independent experiments with eight wells per experiment (n = 16).

on $CD4^+$ and $CD8^+$ T cells were evaluated with respect to: (a) the percentage and absolute counts of entire $CD4^+$ and $CD8^+$ T cell populations, (b) the percentage and absolute counts of the $Foxp3^+CD25^+CD4^+$ regulatory/suppressive T cell subset (Treg cells), (c) the percentages of IL-17-, IL-4-, IL-10- and TGF-β-producing $CD4^+$ T cells, and (d) the percentage of IFN-γ^+CD8^+ T cells. In order to evaluate the effect of TIG on cytokine production, the lung lymphocytes were used because severe respiratory bacterial infections are important indications for the use of TIG.

Materials and Methods

The mice were housed and treated as described previously (Maślanka et al. 2016). Law in Poland (Act of 15 January 2015 on the Protection of Animals Used for Scientific or Educational Purposes) does not require a permit from an ethics commission to conduct experiments in which samples for research are obtained *post mortem* from animals not submitted to any procedure while alive. Mice were euthanized by asphyxiation with CO_2. Whole sets of head and neck lymph nodes (HNLNs) were removed and subjected to homogenization in a Dounce tissue grinder. Lung lymphocytes were isolated by enzymatic digest and enriched by density gradient centrifugation as described previously (Maślanka et al. 2016). Lymphocytes were exposed to TIG (Sigma-Aldrich, Schnelldorf, Germany) at concentrations reflecting its plasma levels obtained *in vivo* at a typical dose (10^{-6}) and at a ten-fold lower concentration. HNLN lymphocytes were used to evaluate the effect of TIG on counts of $CD4^+$ and $CD8^+$ T cells. In turn, lymphocytes isolated from the lungs were used to determine the effect of TIG on cytokine production. HNLN and lung lymphocytes were adjusted to 4×10^6 and 1×10^6 cells/mL, respectively, in a complete medium (Maślanka et al. 2016), and seeded in 24-well plates in 1 mL aliquots. The counts of $CD4^+$, $Foxp3^+CD25^+CD4^+$ and $CD8^+$ T cells were determined after 48 and 96 h of culture. In order to examine the intracellular production of cytokines, lung lymphocytes were pre-incubated for 10 h without (control) or with the drug followed by 6 h stimulation with 50 ng/ml of phorbol-12-myristate-13-acetate and 1 μg/mL of ionomycin (both from Sigma-Aldrich) in the presence of 1 μL/mL of protein transport inhibitor (BD Biosciences, San Jose, USA) during the last 4 h to raise intracellular cytokine stores. Each experiment included 8 wells of cells (obtained from the individual mice) for each condition tested. All experiments were repeated twice (overall $n = 16$). Cells were stained for surface antigens with fluorochrome-conjugated monoclonal antibodies (mAbs): FITC rat anti-mouse CD4 (clone H129.19), APC-Cy7 rat anti-mouse CD8a (clone 53-6.7), PE-Cy7 rat anti-mouse CD25 (clone PC61; all from BD Biosciences). As regards intracellular staining for Foxp3, cells were washed, fixed, and permeabilized using a mouse Foxp3 buffer set (BD Biosciences) according to the manufacturer's protocol. Subsequently, cells were labeled with PE-conjugated rat anti-mouse Foxp3 mAb (clone MF23; BD Biosciences). As regards intracellular staining for cytokine-producing cells, the samples were washed, fixed, and permeabilized using Cytofix/Cytoperm solution and Perm/Wash buffer (both from BD Biosciences) according to the manufacturer's protocol. Subsequently, cells were stained with PE-CF594 rat anti-mouse IL-4 (clone 11B11), APC rat anti-mouse IL-10 (clone JES5-16E3), PerCP-Cy5.5 anti-mouse IL-17 (clone TC11-18H10), PE rat anti-mouse TGF-β (clone TW7-16B4) and APC rat anti-mouse IFN-γ (clone XMG1.2; all from BD Biosciences). Flow cytometry analysis was performed using a FACSCanto II cytometer (BD Biosciences). The data were acquired by FACS Diva version 6.1.3 software (BD Biosciences) and analyzed by FlowJo software (Tree Star Inc., Stanford, USA). Statistical analysis was performed using one-way analysis of variance followed by the Bonferroni's *post hoc* test. Differences were deemed significant when the p values were < 0.05.

Results and Discussion

The exposure to TIG in either of the concentrations used neither affected substantially the percentage and absolute counts of entire $CD4^+$ (Figs. 1A and A') and $CD8^+$ (Figs. 1B and B') T cell populations nor influenced the Treg cell subset (Figs. 1C and C'). Similarly, the percentages of IL-4-, IL-10-, IL-17- and TGF-β-producing $CD4^+$ T cells and IFN-γ-producing $CD8^+$ T cells were not altered following the exposure to both concentrations of TIG (Fig. 1E). These results indicate that TIG does not inhibit production of key pro-inflammatory and immune-protective cytokines such as IL-17 and IFN-γ by $CD4^+$ and $CD8^+$ T cells, respectively. Furthermore, they show that TIG does not increase the production of important anti-inflammatory/immune-suppressive (i.e. IL-10 and TGF-β) and anti-inflammatory and immune-protective (i.e. IL-4) cytokines. These results are compatible with results of other investigators, who did not find any influence of TIG on such pro-inflammatory cytokines as IL-1β, IL-6 and TNF-α (Traunmüller et al. 2009, von Seth et al. 2015). In turn, it is well known that tetracycline and doxycycline can reduce the production of pro-inflammatory cytokines such as IL-6, IL-8 and TNF-α (Tai et al. 2013, Sun et al.

2015, von Seth et al. 2015). Moreover, minocycline, which is a structural analog of TIG, also suppressed the production of TNF-α and IL-8 (Tai et al. 2013, Sun et al. 2015). The cited and present results strongly suggest that TIG, in contrast to other tetracyclines, does not exert an anti-inflammatory effect via inhibition or induction of the production of crucial pro- or anti-inflammatory cytokines, respectively. On the one hand, the lack of an anti-inflammatory effect could be considered as a disadvantage of TIG, because such an effect can provide therapeutic benefits in some cases (von Seth et al. 2015). On the other hand, the lack of an effect of TIG on pro-inflammatory cytokines in certain infections could be desirable because some of these cytokines have important immune-protective properties, i.e. they play important roles in host defense against extracellular bacterial and fungal infections. Moreover, TIG did not affect counts of $CD4^+$ and $CD8^+$ T cells, hence most clearly this agent does not exert destructive effects on lymphocytes involved in the production of cellular immunity. Furthermore, we did not find any influences of TIG on the expression of Foxp3 or the abundance of Treg cells. In conclusion, with respect to the evaluated parameters, TIG does not exert any immune-suppressive and anti-inflammatory effects, and seems to be a safe drug for administration in bacterial infections, especially in patients with suppressed immunity.

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