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Inhibition of TNF-α production in THP-1 macrophages by glatiramer acetate does not alter their susceptibility to infection by *Listeria monocytogenes* and does not impair the efficacy of ampicillin or moxifloxacin against intracellular bacteria

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Sir,

Listeriosis is one of the potential adverse effects of TNF-α-neutralizing treatments.[1] Glatiramer (copolymer 1; COPAXONE), a mixed, random polymer of Ala, Glu, Lys and Tyr used in the treatment of relapsing–remitting multiple sclerosis[2] blocks the secretion of TNF-α from IFN-γ- and endotoxin-stimulated THP-1 macrophages.[3] We have, therefore, examined the influence of glatiramer on the ability of IFN-γ to contain *Listeria* infection and on the activity of ampicillin and moxifloxacin to kill intraphagocytic bacteria in THP-1 macrophages. Glatiramer [CAS Registry no. 147 245-92-9; batch no. 242908102: average molecular weight, 7500 Da (limits, 4200–16 350); amino acid content (molecular fraction) L-Glu, 0.139; L-Ala, 0.432; L-Tyr, 0.091; L-Lys, 0.338; total amino acid residue content, 87.9%; bacterial endotoxin content, <0.25 endotoxin units/mg] was kindly received from Teva Pharmaceuticals Industries (Petah Tiqua, Israel). All experimental procedures and assay methods have been described in our previous publications.[4,5] We used a concentration of glatiramer of 20 mg/L, which was both non-toxic (based on lactate dehydrogenase release) and effective in blocking the production of TNF-α in THP-1 cells.[3]

Glatiramer (20 mg/L) did not influence the intrinsic antimicrobial activity of ampicillin or moxifloxacin towards *L. monocytogenes*, based on MIC determinations in broth [0.3 ± 0.1 and 0.5 ± 0.1 mg/L (arithmetic dilutions) for ampicillin and moxifloxacin, respectively]. Unstimulated cells produced only negligible amounts of TNF-α and glatiramer did not alter this behaviour. In contrast, the medium of cells exposed to IFN-γ (100 units/mL; 24 h) contained 38.3 ± 6.0 ng/L of TNF-α, and this concentration was decreased by ~2/3 in the presence of glatiramer. With infected cells, TNF-α production remained low in unstimulated cells and unaffected by the presence of glatiramer, whereas it amounted to 31.6 ± 1.2 ng/L (5 h post-phagocytosis) in IFN-γ-stimulated cells (24 h prior to infection). This production was again decreased by 2/3 if glatiramer was present. Glatiramer did not significantly modify the capacity of THP-1 macrophages to phagocytose *L. monocytogenes*. Figure 1 shows that glatiramer did not modify the growth of intracellular *L. monocytogenes* compared with untreated cells in the 24 h model (this model uses gentamicin at a concentration of 2 × its MIC to prevent the extracellular growth of *L. monocytogenes*). No change was seen either in the 5 h model (data not shown). As previously described,[6] IFN-γ impaired the intracellular growth of

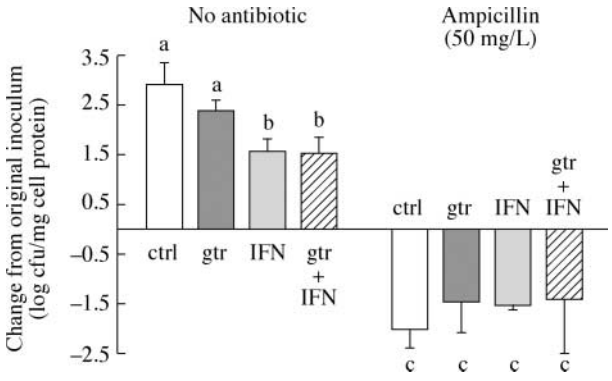

Figure 1. Influence of glatiramer on the course of *L. monocytogenes* intracellular infection in J774 macrophages. Cells were exposed to *L. monocytogenes* for 1 h (phagocytosis) and then returned to fresh medium for 24 h before cell collection and enumeration of cell-associated bacteria. The growth of extracellular bacteria, which would otherwise occur after 5–6 h through the release of dying cells, was prevented by addition of gentamicin [2 mg/L (2×MIC); this concentration does not prevent the intracellular growth of *L. monocytogenes*, data not shown]. The four left blocks refer to cells unexposed to ampicillin and treated as follows: ctrl (control), no treatment; gtr (glatiramer), cells incubated with glatiramer (20 mg/L) for 24 h, and then maintained in the presence of the same concentration of glatiramer during the phagocytosis of *L. monocytogenes* as well as during the post-phagocytosis period (fresh solutions of glatiramer were used each time); IFN (IFN-γ), cells exposed to IFN-γ (100 units/mL) for 24 h before phagocytosis (IFN-γ was not present during phagocytosis or during the post-phagocytosis period); gtr + IFN, cells treated as for the IFN group but with glatiramer present throughout the experiment as in the gtr group. The four right blocks refer to experiments with the same design but for which ampicillin (50 mg/L) was added during the 24 h post-phagocytosis period. The ordinate shows the change in bacterial counts from original inoculum (post-phagocytosis). All data points are the mean of three determinations ± S.D. Blocks with the same letters denote groups that are not significantly different from one another by one-way ANOVA (P>0.05). This experiment was performed twice with essentially similar results.

L. monocytogenes by ~50%, and glatiramer did not modify this effect. When infected cells were exposed to ampicillin for 24 h after phagocytosis, the bacterial load was reduced by ~1.7 log compared with the original, post-phagocytosis inoculum. Glatiramer, IFN-γ, or the combination of glatiramer and IFN-γ did not significantly modify this effect of ampicillin. In the next series of experiments, we examined the activity of moxifloxacin (4 mg/L) using the 5 h model. We observed a decrease in the post-phagocytosis inoculum of 1.34 ± 0.03, 1.26 ± 0.16, 1.31 ± 0.07 and 1.32 ± 0.07 $\log_{10}$ units for cells treated with moxifloxacin alone, glatiramer and moxifloxacin, IFN-γ and moxifloxacin, and the combination of glatiramer, IFN-γ and moxifloxacin, respectively. In parallel experiments, we examined the influence of glatiramer on the accumulation of moxifloxacin and no effect was seen [apparent cellular to extracellular drug concentration ratios at 2 h of 9.6 ± 2.0 in controls versus 9.4 ± 1.1 in cells exposed to glatiramer (20 mg/L) during the uptake period; similar values were found for cells pre-exposed to glatiramer (20 mg/L) for 24 h]. Glatiramer did not influence the accumulation of three other quinolones (ciprofloxacin, levofloxacin and garenoxacin).

Our data, therefore, show that the production of TNF-α is not critical in IFN-γ-stimulated THP-1 cells for anti-*Listeria* activity. The model used has been validated to analyse the behaviour of intracellular *L. monocytogenes* with respect to the action of cytokines[5,6] and to the influence of antibiotics. THP-1 cells display functional receptors for TNF-α[7] and their presence in the cell line used here has been confirmed (J. Zanon, unpublished data). TNF-α may be more a potentializer of IFN-γ[8] than a true effector for the control of *L. monocytogenes* growth in THP-1 cells. Because intracellular multiplication of *L. monocytogenes* is an important determinant in the persistence and the spread of the infection, our results suggest that glatiramer (i) may actually not increase this risk, and (ii) may not adversely affect ampicillin or quinolone-based antibiotic treatments should the necessity arise. This will need to be confirmed by *in vivo* studies.

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Serotonin syndrome due to co-administration of linezolid and venlafaxine

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Sir,

Linezolid is an oxazolidinone antibiotic with non-selective, reversible monoamine oxidase inhibitor (MAOI) action. It has been reported to interact with selective serotonin reuptake inhibitors (SSRIs) and other sympathomimetic drugs resulting in serotonin syndrome. We report the first published case of serotonin syndrome due to interaction of linezolid and venlafaxine.

An 85-year-old man was referred for management of a chronically infected total hip joint prosthesis. He had a past history of Parkinson's disease, ischaemic heart disease, atrial fibrillation, diabetes, previous stroke and a permanent pacemaker. The hip prosthesis was removed and surgical specimens isolated *Pseudomonas aeruginosa*, methicillin-susceptible *Staphylococcus aureus* and methicillin-resistant *Staphylococcus epidermidis*. Intravenous antibiotics were given for 6 weeks. Following closure of the wound, oral therapy was commenced with ciprofloxacin 750 mg twice daily, rifampicin 300 mg twice daily