Methicillin-resistant *Staphylococcus aureus* ST80 induce lower cytokine production by monocytes as compared to other clonal (ST) types

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Introduction and purpose

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains an important cause of nosocomial and community-associated infections due to its ability to produce toxins. The aim of the present study was to investigate the association of monocytes immune response in terms of cytokines produced after inoculation with different MRSA clones.

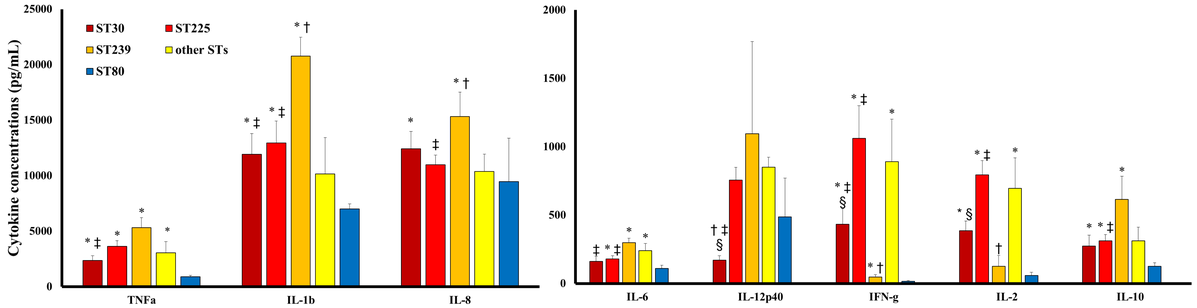
Methods

Thirty-one clinical MRSA strains recovered from patients with variable staphylococcal infections at the University General Hospital of Patras during a four-year period were selected on the basis of clonal types, *agr* groups and toxin genes carriage. Isolates were identified as *S. aureus* by Gram stain, catalase, coagulase production and PCR for *nuc* gene. The presence of *mecA*, *lukS/lukF-PV* (PVL) and *tst* (TSST-1) genes, as well as, the determination of *agr* groups (accessory gene regulator) was performed by PCR. Clonality was investigated by means of multi-locus sequence typing (MLST). Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats of healthy volunteers by density centrifugation on Ficoll density gradient. Cells at a concentration of $1x10^6$ /mL per well, were then seeded in 24-well flat bottom tissue culture plates and cultured at 37°C in a humidified, 5% CO_2 atmosphere. Peripheral blood mononuclear cells (10^6 cells/mL) were stimulated with live bacterial cells for 45 min at a ratio of 1:10. Cells were incubated for 10 hours and supernatants were collected. The levels of TNFa, IL-1b, IL-8, IL-6, IL-12p40, IL-10, IFN-gamma and IL-2, were measured by Human Cytokine Multiplex Immunoassay kit. SPSS ver. 23.0 was used for statistical analysis.

Results

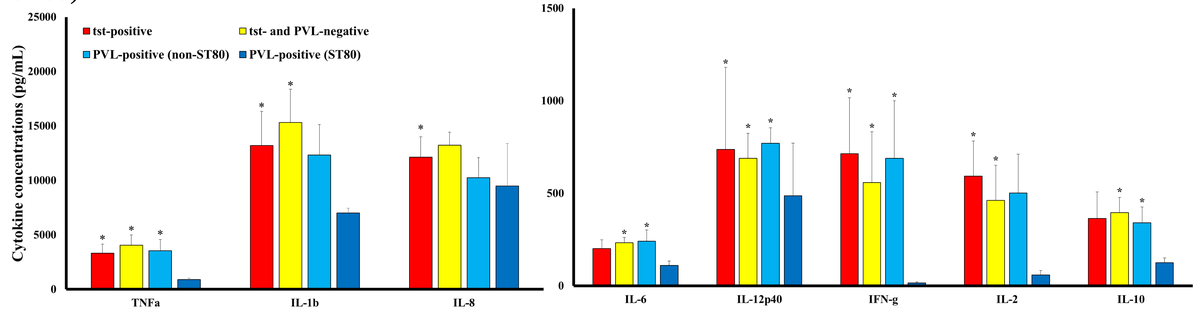
Toxin genes presence was verified in 25 strains (13 *tst* and 12 *lukS/lukF-PV*-positive). Clones identified by MLST were ST80 (seven strains), ST225 (seven), ST30 (five), ST239 (five), while the remaining seven isolates were grouped together as “other” (two ST5, two ST377, one ST22, one ST217 and one ST770). Strains belonging to ST80 induced statistically lower levels of TNFa, IL-1b, IL-8, IL-6, IL-10, IFN-gamma and IL-2 production in supernatants of PBMCs (Figure 1). As toxins are concerned, PVL-positive strains classified into ST80 clone induced statistically lower concentrations of TNFa, IL-6, IL-12p40, IL-10, IFN-gamma and IL-2 production as compared to PVL-positive strains belonging to other clones, *tst*-positive strains and toxin-negative ones. Strains of *agr*3 group belonging to ST80 induced statistically lower concentrations of all tested cytokines with the exception of IL8 as compared to *agr*3 strains not-belonging to ST80, *agr*2 or *agr*1 types.

Figure 1. Differences of cytokine concentrations in supernatant upon incubation of PBMCs with *S. aureus* between different STs



* P<0.05 in comparison with ST80; † P<0.05 in comparison with other STs; ‡ P<0.05 in comparison with ST239; § P<0.05 in comparison with ST225

Figure 2. Differences of cytokine concentrations in supernatant upon incubation of PBMCs with *S. aureus* between *tst*-positive, *tst*- and PVL-negative and PVL-positive (belonging to ST80 and to STs other than ST80)



* P<0.05 in comparison with PVL-positive belonging to ST80

Conclusions

MRSA strains of ST80 clone induce lower cytokines production by monocytes as compared to all other clones. This difference cannot be attributed to the presence of *lukS/lukF-PV* genes or *agr*3 type that are usually present in ST80. This low induction of immune response by ST80 MRSA can partially explain its silent and successful dissemination in the community and the hospital.