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In vitro estimation of the infectious potential of the enterococcal strain by an analysis of monocytes' response to the formed biofilm*

Ocena potencjału infekcyjnego enterokoków na podstawie aktywacji wzorcowej linii monocytów przez komórki tworzonego biofilmu

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

The aim of our study was to assess the possibility of predicting *Enterococcus faecalis* pathogenicity based on morphological changes of monocytes interacting with bacteria and their adherence to biofilm.

Changes in the size and granularity of monocytes, as well as their adherence to biofilm were assessed using FACScan flow cytometer after 1h co-incubation of monocytes and enterococcal biofilm in 37°C. The obtained results were validated with respect to monocytes incubated without bacteria. The most prominent changes in size of monocytes were observed in the case of commensal bacteria. The interaction with bacteria isolated from the blood stream and urine caused comparable changes in the size of THP-1 cells and were smaller than the changes of cells incubated with commensal bacteria. Changes in THP-1 granularity were comparable regardless of the source of bacteria forming biofilm. The parameter which differed the most was connected to the adherence index relating to the monocytes remaining on the surface of biofilm after 1h of incubation.

Taking into consideration the obtained results, we conclude that changes in the morphology of monocytes can be treated as a tool assessing the potential pathogenicity of the bacteria. What is more, the adhesive properties towards bacterial biofilm alone might be considered as a tool allowing for the assessment of the pathogenicity of the isolated strain bypassing time-consuming techniques.

Keywords: ***Enterococcus faecalis*** **• adherence index • biofilm • pathogenicity • THP-1 monocytes**

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INTRODUCTION

Although they rarely cause infections, enterococci are currently the third leading cause of nosocomial infections including bacteremia in healthy individuals [6].

Enterococcal infections are believed to be mainly endogenous. The fact that PFGE (pulsed-field gel electrophoresis) comparison does not yield differences between intestinal and blood isolates of enterococci supports this opinion [16]. Shay et al. [18] proved that, amongst 11 paired stools and blood VRE isolates, 8 were identical. Similarly, Montecalvo et al. [14] found closely related stool and blood isolates in 3 patients during an outbreak in an oncology ward. On the other hand, since 2005, Willems et al. [23] and Leavis' team [10] proposed the identification of high-risk clonal complex of *Enterococcus faecium,* indicating the existence of at least two different genetic lineages of these bacteria.

In our previous study, significant differences in molecular properties between enterococcal isolates from different sites of infection have also been proved [3]. Additionally, the differences between commensal and RTx (renal transplantation) patient's enterococcal isolates include the susceptibility to phagocytosis [9].

The problem in the reaction of the host immune system toward enterococcal invasion has been until now examined only superficially [5]. The available data consider mostly the infections with bacteria in planctonic form and referencing these data to infections caused by bacterial biofilm may lead to many grievous simplifications. Due to the complexity of the biofilm matrix, interaction of biofilm with the host immune system may involve completely different mechanisms when compared to their planktonic counterpart [1].

The previous study had shown that *Enterococcus faecalis* recovered from the biofilm on dentin material is capable of stronger surface adherence and better survival inside monocytes *in vitro* than their planktonic counterparts [12]. Biofilm infected monocytes produce lower amounts of proinflammatory cytokines compared to monocytes infected with planktonic bacteria [5,12].

Monocytes combine 3 major functions: phagocytosis, presentation of antigens, and immunomodulation. Mononuclear phagocytes ingest material for 2 purposes: to eliminate waste and debris and to kill invading pathogens [4]. Monocytes are not the only cells with phagocytic activity in a living organism, but their longer persistence at a site of acute inflammation and the fact that they exhibit more diverse capacity to kill many microbes make them undisputedly one of the most essential elements of the innate immune system involved in fighting infection in the first line [4].

As the enterococcal infections turn out to be more and more problematic around the world [10], it becomes crucial to estimate a quick and easy test for assessing the virulence potential of strains isolated from various sites. Here, we hypothesize that the response of human monocytes to biofilm may be used as an estimator of virulence potential of the strains.

MATERIAL AND METHOD

Bacterial culture

Twenty enterococcal urine and blood isolates were collected from patients hospitalized at Medical University of Gdańsk and another twenty urine and faecal isolates were obtained from RTx patients who initially underwent induction with monoclonal (basiliximab) or polyclonal antibodies (ATG) and were prescribed subsequently TAC (tacrolimus) + MMF (mycophenolatemofetil)/MPS (mycophenolate sodium) + glucocorticosteroids or CsA (cyclosporine) + MMF/MPS + glucocorticosteroids or CsA + everolimus + glucocorticosteroids. As a reference group, 10 enterococcal strains of *E.faecalis* were isolated from healthy volunteers from Gdańsk region. The isolates were identified to species level by strep ID test (BioMerieux) and classified as different strains of *E.faecalis* by biochemical and resistance profiles. All bacterial strains were stored at (-70°C) in brain heart infusion (BHI) broth with 25% (vol/vol) glycerol.

Biofilms of these strains were obtained by culturing at 37°C on flat-bottom wells (TPP, Switzerland) for 72h in BHI medium. After another 28h, medium was replaced with the fresh 2ml of BHI.

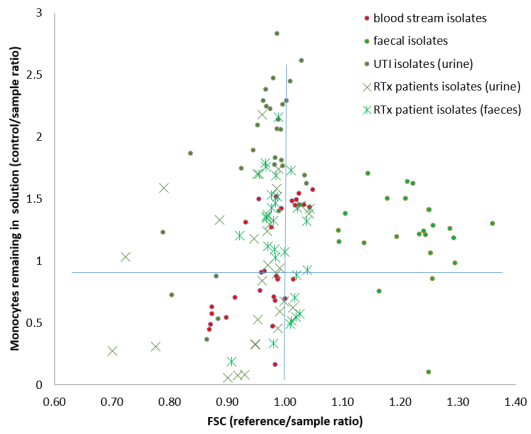

Fig. 1. Distribution of monocytes properties in relation to enterococcal biofilm of different origin

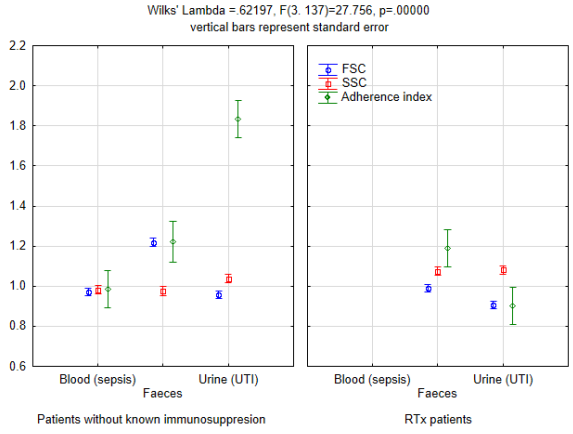

Fig. 2. Monocytes' characteristic after incubation with enterococcal biofilm of different origin (ANOVA comparison)

Phagocytosis assay

For assay of phagocytosis, THP-1 cell line (ATCC) was used. The cells were cultured in RPMI-1640 medium supplemented with 2mM L-glutamine, 100U/ml penicillin, 100µg/ml streptomycin and 10% (vol/vol) heat-inactivated foetal bovine serum (FBS) (all from Sigma-Aldrich). Wells with biofilm were washed with 0.9% NaCl; suspension of monocytes was then added and incubated at 37°C for 60 min. Sterile wells were used as a reference for adhesion and the activation of monocytes. The number of monocytes and their morphology (described by FSC – forward scatter parameter featuring the size of the cell, and SSC – side scatter parameter referring to the observed cell's granulation) was estimated by using a FACScan flow cytometer (Becton-Dickinson, Franklin Lakes, NJ, USA) and standardised with results obtained for reference wells. The differences were tested by an analysis of variance (ANOVA) by StatSoft software (Statistica 10, USA).

RESULTS

During the experiments 23.3% to 46.2% monocytes bound to the bacterial biofilm. The affinity of monocytes differed significantly depending on the origin of the strains. The adherence index for commensal strains was higher (1.24) than in the control plate that was not covered with bacterial biofilm. This ratio was only 0.87 for blood stream isolates. The adherence was the lowest for isolates from urinary tract infections, with median remains ratio of 1.88.

Interaction with biofilm results also in changes of monocytes' size (FSC). After monocytes were incubated with commensal strains, mean FSC value for monocytes was 1.24 compared to the control value (1.00), and 0.89 and 0.98 for blood stream and urine isolates, respectively (Fig.1.). The SSC values showed the lowest diversity and varied from 0.95 to 1.03.

DISCUSSION

The resistance of enterococci to phagocytosis was described many years ago and the role of some of the virulence traits in this phenomenon was proved [21]. For example, it has been demonstrated that *E.faecalis* lacking aggregation substance (AS) bound only minimally to PMNs in the absence of opsonins, while *E.faecalis* INY1801 expressing AS bound to PMNs in large numbers, with the majority of the bacteria appearing to be internalized [15]. It is assumed that biofilm cells resist phagocytosis by immune cells [5] and the formation of biofilm protects bacteria from being eradicated. Although what is worth mentioning is that there is some evidence suggesting that biofilm can be destroyed by PMNs [7].

For many years, one explanation of a biofilm's resistance to the immune system was that immune cells cannot penetrate into the biofilm. However, Leid *et al.* [11] documented such a penetration in an *in vitro* study of freshly isolated human leukocytes into *Staphylococcus aureus* biofilms, under static and flowing fluid conditions that mimic physiological shear. Leukocytes also penetrated 7-day-old *S.aureus* biofilms under laminar-shear conditions. Leukocytes produced a Th1-type response to both the 7- day-old shear and 2-day-old static *S.aureus* biofilms. Such leukocytes were able to penetrate biofilms but were unable to phagocytose bacteria, suggesting that other mechanisms may inhibit normal leukocytes function.

Furthermore, studies of Kasturee Daw and others [5], who compared the uptake of *E.faecalis* biofilm cells to their planktonic counterparts, revealed that biofilm cells may be taken up efficiently by immune cells, such as macrophages and dendritic cells and in fact at significantly higher numbers depending on the strain background.

One may guess that the host's defense should discriminate between pathogens and commensals to prevent the

unnecessary activation of the immune response [7]. In our previous study we have observed a significant difference in biofilm composition and metabolic activity of biofilm formed by commensal and virulent strains [13]. It has been demonstrated [17] that immune response can be estimated by determining, whether monocytes differentiate after the activation by bacteria. Ciabattini and others [2] showed that human monocytes react to bacterial cells by increased the expression of mRNA for tool-like receptors, up-regulation of surface markers, such as CD54 – an adhesion molecule involved in leukocyte trafficking toward inflammatory stimuli, and production of proinflammatory cytokines but reduction of phagocytic activity. It has also been hypothesized that common survival of enterococcal cells within macrophages let them spread and create distant sites of infection [21], the same applies to other phagocytes [22], which may explain why the strains with the strongest attraction to monocytes were the ones isolated from blood in our experiment. Macrophages function is a dynamic process, which may be dependent on the changing local microenvironment [19,20]. In the setting of inflammation, macrophages can also be activated by microbial organisms. The classic forms of the activation results in the generation of large amounts of reactive oxygen species and inflammatory cytokines, which all serve to augment killing of microbes, phagocytosis of intracellular parasites and local cell-mediated immune response. One should note that all markers of monocytes/macrophages activation in response to bacteria invasion reported above were based primarily on monocytes' interaction with freely suspended cells or their components, which may not reflect true monocytes response to biofilm-bound bacteria.

Our current study demonstrated higher adherence of monocytes to biofilm formed by bloodstream isolates than to biofilm formed by urine isolates. What is significant, similar results were observed in our previous investigation of macrophages phagocytosis of enterococcal isolates of different origin [8]. Additionally, we have observed interesting differences on monocytes reaction to biofilm formed by isolates from RTx patients when compared with isolates of the same origin from patients without immunosuppression. The changes observed were similar to those of the blood stream isolates and can explain the higher risk of infection in RTx patients. The differences in reactions toward bacteria of different origin were also observed during our study with neutrophils [9]. Despite the fact that mononuclear phagocytes share many properties with neutrophils, they also have distinctive morphologic and functional properties, depending on their state of differentiation. Monocytes have a preserved capacity to augment the,production of granule proteins through new protein synthesis, in contrast to mature neutrophils. At a site of acute inflammation, monocytes accumulate more slowly, but remain longer. Their metabolic burst is not so intensive, but their capacity to kill many microbes is more diverse compared with that of neutrophils [4].

REFERENCES

[1] Bryers J.D.: Medical biofilms. Biotechnol. Bioeng., 2008; 100: 1-18

[2] Ciabattini A., Cuppone A.M., Pulimeno R., Iannelli F., Pozzi G., Medaglini D.: Stimulation of human monocytes with the gram-positive vaccine vector *Streptococcus gordonii*. Clin. Vaccine Immunol., 2006; 13: 1037-1043

[3] Daca A., Jarzembowski T., Witkowski J.M., Bryl E., Rutkowski B., Dębska-Ślizień A.: Prophages in enterococcal isolates from renal transplant recipients. Renal failure etiologies promote selection of strains. Biomed Res. Int., 2014; 2014: 514689

[4] Dale D.C., Boxer L., Liles W.C.: The phagocytes: neutrophils and monocytes. Blood, 2008; 112: 935-945

[5] Daw K., Baghdayan A.S., Awasthi S., Shankar N.: Biofilm and planktonic *Enterococcus* faecalis elicit different responses from host phagocytes *in vitro*. FEMS Immunol. Med. Microbiol., 2012; 65: 270-282

[6] Fisher K., Phillips C.: The ecology, epidemiology and virulence of *Enterococcus*. Microbiology, 2009; 155: 1749-1757

[7] Hirschfeld J.: Dynamic interactions of neutrophils and biofilms. J. Oral Microbiol., 2014; 6: 26102

[8] Jarzembowski T., Daca A., Witkowski J.M., Bryl E., Rutkowski B.: The high PMNs phagocytosis resistance of enterococcal isolates from RTx patients. Biomed. Res. Int., 2015; 2015: 432579

[9] Jarzembowski T., Daca A., Witkowski J.M., Bryl E., Rutkowski B., Świeć K.: The high phagocytic activity of macrophages against blood stream isolates of Enterococci. Austin Therapeut., 2016, 3: 1025

[10] Leavis H.L., Bonten M.J., Willems R.J.: Identification of high-risk enterococcal clonal complexes: global dispersion and antibiotic resistance. Curr. Opin. Microbiol., 2006; 9: 454-460

[11] Leid J.G., Shirtliff M.E., Costerton J.W., Stoodley P.: Human leukocytes adhere to, penetrate, and respond to *Staphylococcus aureus* biofilms. Infect. Immun., 2002; 70: 6339-6345

[12] Mathew S., Yaw-Chyn L., Kishen A.: Immunogenic potential of *Enterococcus faecalis* biofilm under simulated growth conditions. J. Endod., 2010; 36: 832-836

[13] Meissner W., Jarzembowski T.A., Rzyska H., Botelho C., Pałubicka A.: Low metabolic activity of biofilm formed by *Enterococcus faecalis* isolated from healthy humans and wild mallards (*Anas platyrhynchos*). Ann. Microbiol., 2013; 63: 1477-1482

[14] Montecalvo M.A., Horowitz H., Gedris C., Carbonaro C., Tenover F.C., Issah A., Cook P., Wormser G.P.: Outbreak of vancomycin-, ampicillin-, and aminoglycoside-resistant *Enterococcus faecium* bacteremia in an adult oncology unit. Antimicrob. Agents Chemother., 1994; 38: 1363-1367

[15] Murapa P., Gandhapudi S., Skaggs H.S., Sarge K.D., Woodward J.G.: Physiological fever temperature induces a protective stress response in T lymphocytes mediated by heat shock factor-1 (HSF1). J. Immunol., 2007; 179: 8305-8312

[16] Olivier C.N., Blake R.K., Steed L.L., Salgado C.D.: Risk of vancomycin-resistant *Enterococcus* (VRE) bloodstream infection among patients colonized with VRE. Infect. Control Hosp. Epidemiol., 2008; 29: 404-409

[17] Palucka K.A., Taquet N., Sanchez-Chapuis F., Gluckman J.C.: Dendritic cells as the terminal stage of monocyte differentiation. J. Immunol., 1998; 160: 4587-4595

[18] Shay D.K., Maloney S.A., Montecalvo M., Banerjee S., Wormser G.P., Arduino M.J., Bland L.A., Jarvis W.R.: Epidemiology and mortality risk of vancomycin-resistant enterococcal bloodstream infections. J. Infect. Dis., 1995; 172: 993-1000

[19] Stout R.D., Jiang C., Matta B., Tietzel I., Watkins S.K., Suttles J.: Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. J. Immunol., 2005; 175: 342-349

[20] Stout R.D., Suttles J.: Functional plasticity of macrophages: reversible adaptation to changing microenvironments. J. Leukoc. Biol., 2004; 76: 509-513

[21] Süssmuth S.D., Muscholl-Silberhorn A., Wirth R., Susa M., Marre R., Rozdzinski E.: Aggregation substance promotes adherence, phagocytosis, and intracellular survival of *Enterococcus faecalis* within human macrophages and suppresses respiratory burst. Infect. Immun., 2000; 68: 4900-4906

[22] Wells C.L., VandeWesterlo E.M., Jechorek R.P., Erlandsen S.L.: Effect of hypoxia on enterocyte endocytosis of enteric bacteria. Crit. Care Med., 1996; 24: 985-991

[23] Willems R.J., Top J., van Santen M., Robinson D.A., Coque T.M., Baquero F., Grundmann H., Bonten M.J.: Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex. Emerg. Infect. Dis., 2005; 11: 821-828

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