

The effect of *Propionibacterium acnes* on maturation of dendritic cells derived from acne patients' peripheral blood mononuclear cells

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Abstract: *Propionibacterium acnes* (*P. acnes*) has been implicated in the pathogenesis of acne vulgaris which is the most common cutaneous disorder. It has a proinflammatory activity and takes part in immune reactions modulating the Th1/Th2 cellular response. The exposure of dendritic cells (DCs) to whole bacteria, their components, cytokines or other inflammatory stimuli and infectious agents induces differentiation from immature DCs into antigen-presenting mature DCs. The aim of the study was to evaluate the capability of *P. acnes* to induce the maturation of DCs. We stimulated monocyte derived dendritic cells (Mo-DCs) from acne patients with various concentrations of heat-killed *P. acnes* (10^6 - 10^8 bacteria/ml) cultured from acne lesions. The results showed an increase in CD80+/CD86+/DR+ and CD83+/CD1a+/DR+ cells percentage depending on the concentration of *P. acnes*. The expression of CD83 and CD80 (shown as the mean fluorescence intensity – MFI) increased with higher concentrations of *P. acnes*. There were also significant correlations between MFI of CD83, CD80, CD86 and concentration of *P. acnes*. The study showed that *P. acnes* in the concentration of 10^8 bacteria/ml is most effective in the induction of Mo-DCs maturation. Further studies concerning the influence on the function of T cells are needed.

Key words: antigen presenting cells (APCs), acne vulgaris, dendritic cells (DCs), *Propionibacterium acnes*

Introduction

Acne vulgaris is the most common cutaneous disorder affecting 70-87% teenagers with a predominance of men [1]. It is manifested by comedones, papules, pustules and cysts. There are many factors in the pathogenesis of acne. The mechanism triggering the development of the comedone and the stimuli causing the non-inflamed lesion to become inflamed are not well known. The microbiology of acne and its immunological implications are the main aim of the present research in the elucidation of the pathogenesis of the inflammatory acne lesions [2,3]. *Propionibacterium acnes* (*P. acnes*) has been implicated in the

pathogenesis of acne since its first isolation in 1896. It is now believed that *P. acnes* is a significant contributing factor to the inflammatory stages of the disease [4]. *P. acnes* is an anaerobic Gram-positive bacterium which has a proinflammatory activity and takes part in immune reactions modulating the Th1/Th2 cellular response. The main function of this process depends on dendritic cells (DCs). DCs play an important role in the innate and adaptive immune responses to microbial pathogens [5]. They are the most potent antigen presenting cells (APCs) which function as very efficient activators of naive and resting T cells and restimulators of memory T cells as well as B cells. DCs stimulated with *P. acnes* show increased expression of genes for adhesive molecules and cytokines, which is similar to the response of DCs activated with LPS – a prototype stimulus for DCs maturation [6,7]. Immature DCs are able to capture antigens by phagocytosis, macropinocytosis and endocytosis. Exposure of DCs

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to whole bacteria, their components, cytokines or other inflammatory stimuli and infectious agents induces differentiation of immature DCs into antigen-presenting mature DCs. DCs maturation is a process where major histocompatibility complex (MHC) class I or II and costimulatory molecules (e.g. CD80 and CD86) are upregulated and at the same time specific markers like CD83 and p55 are expressed [5].

The aim of the study was to evaluate the capability of *P. acnes* to induce the maturation of autologous DCs.

Patients and methods

Patients. Ten men, aged 15-23 years old, suffering from moderate to severe acne vulgaris were included in the study. Skin swab cultures from acne lesions were performed on G.C. Agar Base (Casman) (BIOCORDER Poland) in anaerobical conditions over 10 days using GENBags (bioMérieux, France). Next, *P. acnes* were identified with RAPID ID32A test (bioMérieux, France) and used for preparing bacterial suspensions. Suspensions in 0.9% NaCl corresponding to 3.0 McFarland standard (900×10^6 bacteria/ml) were treated with 0.5% Phenol analytical grade (SERVA Electrophoresis, Germany) and incubated at 80°C over 1.5 hours to kill the bacteria.

Peripheral blood mononuclear cells. Isolation of peripheral blood mononuclear cells (PBMCs) and generation of monocyte-derived DCs (Mo-DCs) were performed according to previously used protocols [8]. PBMCs from the same individuals were isolated by density gradient centrifugation on Gradisol L (Aqua Medica, Poland). We used EasySep Human CD3 Positive Selection Cocktail and EasySep Magnetic Nanoparticles label CD3+ cells (StemCell Technologies, UK) for magnetic separation of PBMCs. CD3- cells ($10,000,000$ cells/well) were then cultured in RPMI 1640 (Biomed, Lublin, Poland) supplemented with 10% human autologous serum and antibiotics (Penicillin-Streptomycin; Sigma, Poland) in 6-well tissue culture plates at 37°C in 5% CO₂. After 1.5 hours nonadherent CD3- cells (e.g. CD19+) were removed and the culture plates were washed out by phosphate buffered saline (PBS) without Ca²⁺ and Mg²⁺ (Biochrom AG, Germany). CD3- adherent cells were then cultured in the medium described above with mGMP-rhuGM-CSF clinical grade (1000 IU/ml; GENTAUR, Belgium), rhIL-4 (500 IU/ml; Strathmann, Germany). Cytokines were added on the first, third and fifth day of the culture. On the sixth day of the culture various concentrations of heat-killed *P. acnes* (10^6 - 10^8 bacteria/ml) were added. After 48h adherent cells were detached with 0.02% trypsin-EDTA solution (Biochrome AG, Germany) and then washed out in PBS without Ca²⁺ and Mg²⁺. The cells were counted in the Neubauer chamber for the purpose of estimation of the efficiency of cultures and vitality of cells with trypan blue.

The control group for the study consisted of PBMC cultures without stimulation with *P. acnes*.

Flow cytometry. The maturation of DCs was determined in a flow cytometer (FACScalibur and CellQuest software). The following combinations of monoclonal antibodies (mAbs) were used: anti-CD45/CD14, anti-CD83/CD1a/HLA-DR, and anti-CD80/CD86/HLA-DR (Becton Dickinson Pharmingen, USA). Instrument settings were adjusted with CaliBRITE™³ (Becton Dickinson, USA). Samples were evaluated directly after the described protocol, without fixation.

Statistical analysis. The statistical analysis was performed using the Statistica 7.1 PL software and nonparametric Wilcoxon test. p values of 0.05 or less were considered statistically significant. Correlations were calculated using Spearman test.

Results

After the culture with different concentrations of *P. acnes* (P0 – culture without *P. acnes*, P6 – culture with *P. acnes* 10^6 bacteria/ml, P7 – culture with *P. acnes* 10^7 bacteria/ml, P8 – culture with *P. acnes* 10^8 bacteria/ml) we evaluated the percentage of cells of the following immunophenotypes:

- CD83+/CD1a+/HLA-DR+
- CD83+/CD1a-/HLA-DR+
- CD83-/CD1a+/HLA-DR+
- CD80+/CD86+/HLA-DR+

The percentage of cells with CD83+/CD1a+/HLA-DR+ increased depending on the *P. acnes* concentration. The significant differences were observed between P0 and P6 ($p < 0.05$), P0 and P8 ($p = 0.005$), P6 and P8 ($p = 0.005$), and P7 and P8 ($p = 0.005$) (Fig. 1A).

On the contrary, the percentage of CD83+/CD1a-/HLA-DR+ cells and CD83-/CD1a+/HLA-DR+ cells decreased with the increase of *P. acnes* concentration. For CD83+/CD1a-/HLA-DR+ cells these differences were statistically significant for P0 and P6 ($p < 0.04$), P0 and P8 ($p = 0.005$), P6 and P8 ($p = 0.005$), and P7 and P8 ($p = 0.005$) (Fig. 1B). Significant differences in the population of cells of the immunophenotype CD83-/CD1a+/HLA-DR+ were noticed between P0 and P7 ($p < 0.03$), P0 and P8 ($p = 0.005$), P6 and P8 ($p = 0.005$), and P7 and P8 ($p = 0.005$) (Fig. 1C).

The predominance of cells of CD80+/CD86+/HLA-DR+ immunophenotype was observed especially in the cultures with the highest concentration of *P. acnes*. Differences between P0 and P6 ($p < 0.02$), P0 and P7 ($p = 0.005$), P0 and P8 ($p = 0.005$), P6 and P8 ($p = 0.005$), and P7 and P8 ($p = 0.005$) were statistically significant (Fig. 1D).

We also evaluated the mean fluorescence intensity (MFI) of CD83, CD1a, CD80 and CD86 on DCs, representing the density of the receptors on the cell, derived from PBMCs incubated with different concentration of *P. acnes*. Statistically significant differences were observed for MFI of CD83 in the cultures P0 and P8 ($p < 0.02$), and P6 and P8 ($p < 0.03$) (Fig. 2A). A significant difference for MFI of CD1a was seen between P6 and P8 group ($p < 0.02$). Marked statistical differences were noticed for MFI of CD80 between P0 and P8 ($p = 0.005$), P6 and P8 ($p = 0.005$), and P7 and P8 ($p < 0.007$) (Fig. 2B). Differences for MFI of CD86 were not statistically significant.

We observed the correlation between MFI of CD83, CD80, CD86 and concentration of *P. acnes* (respectively, Spearman's rank correlation coefficient $R = 0.3389$, 0.5481 , 0.4563 ; $p < 0.05$).

Discussion

Propionibacterium acnes causes a range of infections and is associated with many inflammatory conditions.

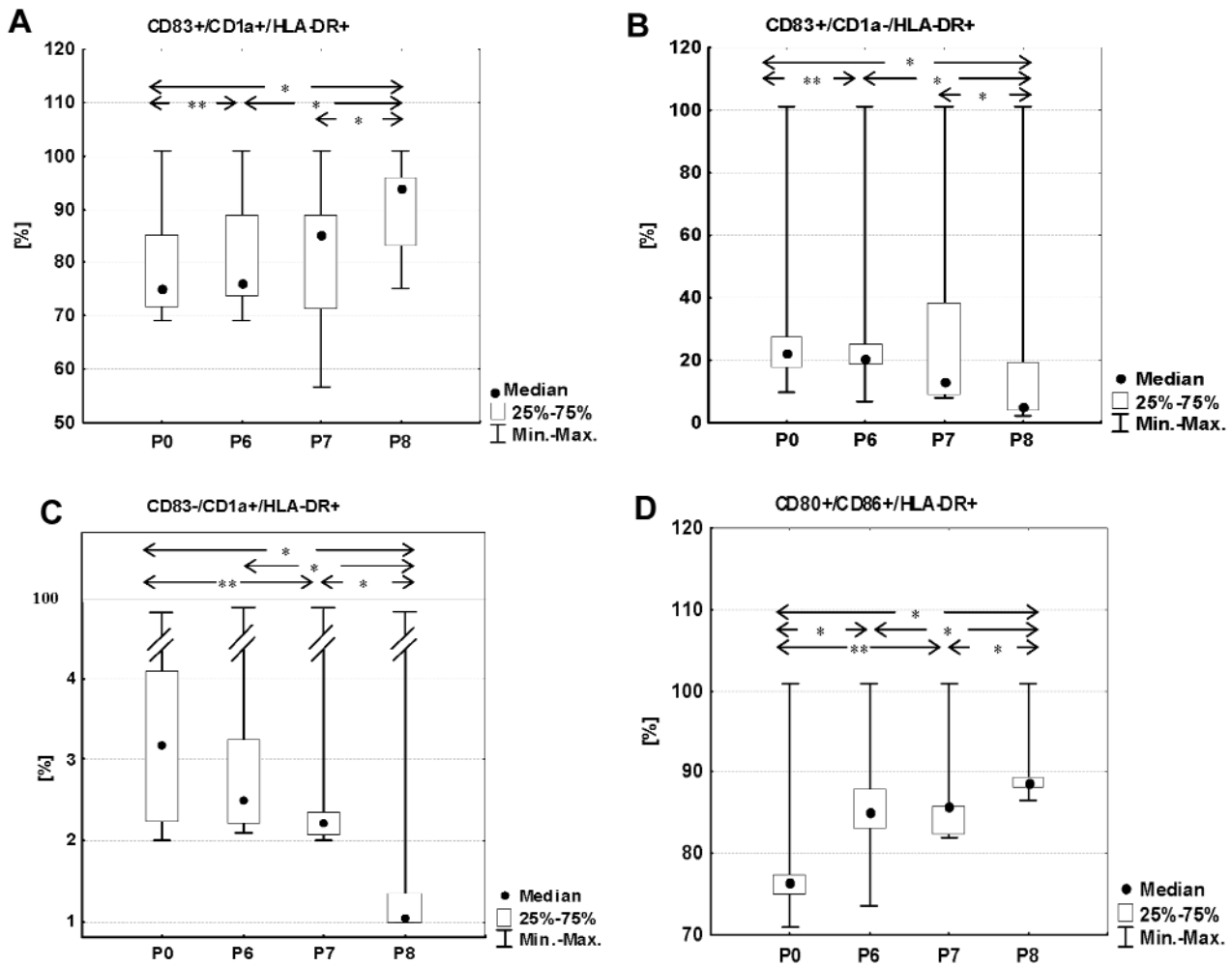


Fig. 1. Evaluation of cells of selected immunophenotypes after the culture with different concentrations of *P. acnes* (P0 – culture without *P. acnes*, P6 – culture with *P. acnes* 10⁶ bacteria/ml, P7 – culture with *P. acnes* 10⁷ bacteria/ml, P8 – culture with *P. acnes* 10⁸ bacteria/ml). **A.** Percentage of cells of CD83+/CD1a+/HLA-DR+ immunophenotype. Significant differences between P0/P6, P0/P8, P6/P8 and P7/P8. *(p=0.005), **(p<0.05). **B.** Percentage of cells of CD83+/CD1a-/HLA-DR+ immunophenotype. Significant differences between P0/P6, P0/P8, P6/P8 and P7/P8. *(p=0.005), **(p<0.04). **C.** Percentage of cells of CD83-/CD1a+/HLA-DR+ immunophenotype. Significant differences between P0/P7, P0/P8, P6/P8 and P7/P8. *(p=0.005), **(p<0.03). **D.** Percentage of cells of CD80+/CD86+/HLA-DR+ immunophenotype. Significant differences between P0/P6, P0/P7, P0/P8, P6/P8 and P7/P8. *(p=0.005), **(p<0.02).

Apart from its involvement in the pathogenesis of acne vulgaris [3] *P. acnes* is associated with synovitis, acne, pustulosis, hyperostosis and osteitis (SAPHO) [9], and infections of the central nervous system, endocardium, joints, eyes, bone marrow and articular discs after surgical procedures [10]. Roszkowski *et al.* studied the modulation of the immune system by propionibacteria and showed that *P. acnes* is one of the most potent adjuvants [11]. It was shown that pretreatment with heat-killed cells of *P. acnes* provides protection against infection and anti-tumor activity in animal models [12]. In some Polish research centres autovaccines prepared from the patient cultures of *P. acnes* and/or *Staphylococcus (epidermidis, aureus)* are used. However, there is not much evidence of the efficacy of this treat-

ment in acne vulgaris [13-15]. It should be considered if vaccines with DCs loaded with *P. acnes* antigen were more effective in the acne therapy.

In this study we would like to evaluate the capability of *P. acnes* to induce the maturation of DCs. We would like to choose the concentration of *P. acnes* which induces the strongest modulation of Mo-DCs. The stimulation of Mo-DCs with *P. acnes* resulted in their maturation to APCs, as evidenced by enhanced expression of cell surface molecules CD83, CD80, CD86 and HLA-DR. The results showed an increase of CD80+/CD86+/DR+ cells percentage depending on the concentration of *P. acnes*. Subpopulations of CD83+/CD1a+/DR+, CD83+/CD1a-/DR+ and CD83-/CD1a+/HLA-DR+ cells were observed, however the

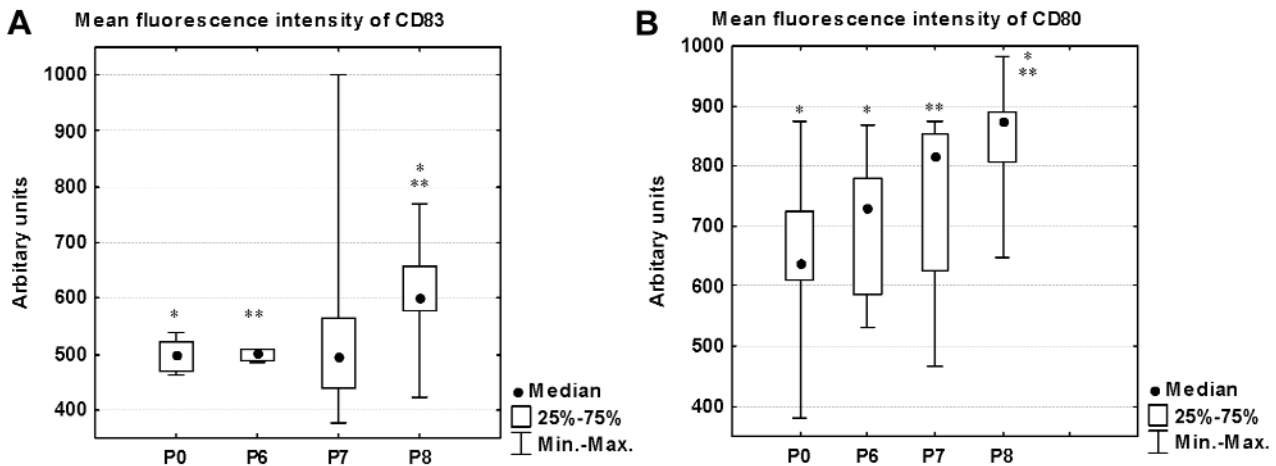


Fig. 2. Mean fluorescence intensity (MFI) of CD83 and CD80 on DCs derived from PBMCs incubated with different concentration of *P. acnes* (P0 – culture without *P. acnes*, P6 – culture with *P. acnes* 10^6 bacteria/ml, P7 – culture with *P. acnes* 10^7 bacteria/ml, P8 – culture with *P. acnes* 10^8 bacteria/ml). **A.** Mean fluorescence intensity of CD83 on DCs. Significant differences between P0/P8 and P6/P8. *($p < 0.02$), **($p < 0.03$). **B.** Mean fluorescence intensity of CD80 on DCs. Significant differences between P0/P8, P6/P8 and P7/P8. *($p = 0.005$), **($p < 0.007$).

population of CD83+/CD1a+/DR+ increased, and CD83+/CD1a-/DR+ and CD83-/CD1a+/HLA-DR+ decreased dose-dependently. The expression of CD83 and CD80 (shown as MFI) increased with higher concentrations of *P. acnes*. There were also correlations between MFI of CD83, CD80, CD86 and concentrations of *P. acnes*.

There are no similar studies comparing the influence of different concentrations of *P. acnes* on the DC maturation. Kopitar *et al.* stimulated Mo-DCs with different oral bacterial antigens (prepared from *Streptococcus mitis*, *Propionibacterium acnes* and *Bacteroides* spp.) and observed an enhanced expression of CD83, CD80 and HLA-DR compared with monocytes at the beginning of the experiment [16]. Cervi *et al.* showed up-regulation of the surface expression of CD80 and MHC II in mice injected with *P. acnes* [17]. A similar experiment was conducted by Squaiella *et al.* They investigated the role of *P. acnes* and its soluble polysaccharide extract on murine bone marrow DCs. The increase of CD80 and CD86 expression was observed [18].

Similarly, enteric pathogens, such as *Campylobacter jejuni* [5], *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) [19], *Helicobacter pylori* [20] and *Escherichia coli* [21] have been shown to up-regulate the expression of cell surface costimulatory molecules CD40, CD83, CD80 and CD86, and MHC II in the immature DCs stimulated with the aforementioned bacteria.

Veckman *et al.* characterized the effect of *Lactobacillus rhamnosus* (*L. rhamnosus*) and *Streptococcus pyogenes* (*S. pyogenes*) on the expression of CD80, CD83 and CD86 after 24h-culture with different doses

of bacteria. They observed that *S. pyogenes* stimulated the expression of CD83 and CD86 better than *L. rhamnosus*. The best bacteria:DC ratio after the experiments with different bacteria concentrations was estimated for 5:1 [22]. Veckman *et al.* evaluated also plasmacytoid DCs (PDCs) and myeloid DCs (MDCs) stimulated with *Streptococcus pyogenes* at 5:1 bacteria: DC ratios for 24h or 48h and with influenza A virus. The expression of CD86 in PDCs after 24h stimulation was slightly increased, however after 48h it was higher in microbe-stimulated PDCs. Stimulation of MDCs with *S. pyogenes* resulted in significantly increased expression of CD40 and CD86 at 24h and 48h [23].

The strong up-regulation of CD83 expression was observed also in DCs infected with *Listeria monocytogene*, *Treponema pallidum* and *Mycobacterium tuberculosis* [24].

Lactobacillus casei similarly to *L. rhamnosus* induced a moderate maturation of DCs in the presence of epithelium – a low increase in CD86 and CD54 expression [25]. *Lactobacillus plantarum* induced a lower expression of CD80 and CD83 compared with Gram-negative pathogenic bacteria [26].

On the contrary, *Mycobacterium leprae* [27], *Coxiella burnetti* [28] and pneumococcal polysaccharides [29] do not induce DC maturation.

On the basis of the presented experiments we would like to use *P. acnes* in the concentration of 10^8 bacteria/ml for further studies concerning the influence on functions of T cells. Some studies were conducted to evaluate the immune response after the stimulation with DCs copulsed with *P. acnes* in animal models [17,30], however there is still not enough knowledge on the influence of DCs primed with

P. acnes on T cell functions [16,31]. This knowledge is important for better understanding of the immunopathology of acne vulgaris. It also offers new possibilities for modulating the immune response with vaccination of DCs loaded with *P. acnes* antigen in acne vulgaris patients.

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