Mapping the stimulus-specific signaling pathways involved in THP-1 cells exposed to porphyromonas gingivalis LPS vs Fimbria vs live *P.g.*

Julian A. Saba[1,2]; Donna L. Potts[1]; Qingde Zhou[2]; Mark E. McComb[1]; Catherine E. Costello[1]; Salomon Amar[2]

[1]Boston University School of Medicine, Boston, MA;
[2]Boston University School of Dental Medicine, Boston, MA

Introduction

Periodontitis is an inflammatory disease with host-parasite interactions which contribute to connective tissue destruction and alveolar bone resorption. *Porphyromonas gingivalis* (*P.g.*) a black-pigmented Gram-negative anaerobic bacterium, is a major pathogen in the development and progression of periodontitis. Cell wall and appendage structures, such as lipopolysaccharide (LPS) and fimbriae (Fim A), play important roles in the induction of innate immune responses, including cytokine production by localized and circulating monocyte/macrophage. Based on preliminary data, live P.g. stimulates unique pro-inflammatory signal transduction pathways in human peripheral blood monocytes (PBM) and THP-1 as opposed to purified bacterial components. In these experiments we test at the level of protein expression that unique signaling pathways are differentially induced by live P.g., LPS and Fim A.

Materials and Methods

Monocyte-like THP-1 cells were exposed to live *P.g.*, *P.g.* LPS and *P.g.* Fim A. 2-dimensional gel electrophoresis (2-DGE) was performed on the cell lysate proteins of PBM and THP-1. Spots corresponding to differentially expressed proteins were excised and subjected to tryptic in-gel digestion. MALDI mass spectra were acquired and protein identification was performed using online protein database searches.

Results

We had previously shown, using a proteomic approach, that host immune cells sense live *P.g.* and its components differently. Our results reveal unique protein expressions in response to live *P.g.* as compared to its bacterial components. We are currently investigating, at the levels of protein expression, to determine how unique signaling pathways are differentially induced by live *P.g.*

Analysis of the 2-DGE profiles has led to the identification of several differentially expressed protein spots. Treatment with *P.g.* LPS and *P.g.* fimbriae resulted in differential expression of eleven and eight protein spots, respectively, relative to unstimulated controls (Figures 1b,1c). Spots of interest were excised, in-gel digested with trypsin, and analyzed via MALDI-TOF MS for protein identification (Figure 2). Our experiments demonstrate that the proteomics approach holds promise for revealing unique signaling pathways that are differentially induced by LPS and fimbriae. The data generated in these experiments using 2-DGE provided qualitative comparison of the proteins expressed in response to exposure to LPS and Fim A, as well as the identification of several proteins. LPS stimulation of THP-1 cells up-regulated the expression of the following proteins compared to control: deoxyribonuclease, actin, carbonic anhydrase 2, alpha enolase, GRAP 2 human, PDI, grp78 and HSP70. In contrast, fimbriae treatment did not result in statistically significant changes to protein levels relative to control. The changes seen after fimbriae exposure were small and down-regulatory relative to controls. This suggests that comparing LPS to fimbriae is no different from comparing LPS to control. Thus, it is not Fim A that is involved in the critical role of up-regulating the innate immune response but LPS.

Conclusions

Our laboratory had previously shown that host immune cells sense live *P.g.* and its components differently. We have now carried out preliminary studies using proteomic approaches to address

this situation. Our 2-DGE profiles show several unique protein expressions in response to live *P.g.* as compared to its bacterial components (Figure 1d). So far we have identified 14 protein spots.

This research is supported by the NIH Grants R01 DE15989 (SM), P41 RR10888 and S10 RR15943 (CEC) and NHLBI contract N01 HV28178 (CEC).

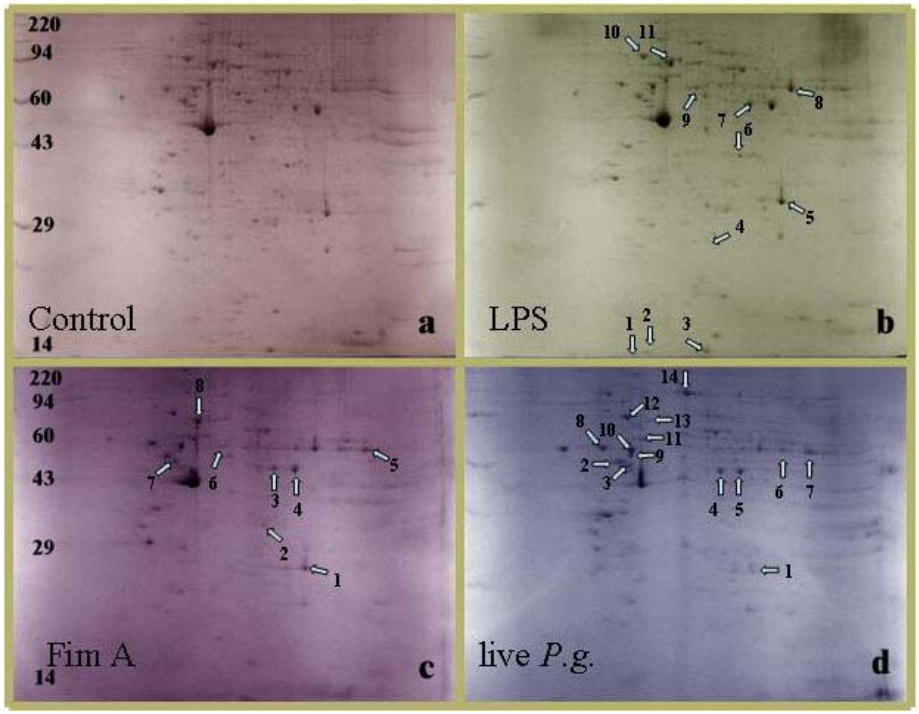

Figure 1. 2-DGE using isoelectric focusing with pH range 3–10 in the horizontal dimension and SDS-PAGE (10%) in the vertical dimension for 400 µg of proteins from differentiated THP-1 cells exposed to (b) LPS, (c) Fim A and (d) live *P.g.* (10 µg/ml, 24 hours) and stained with Coomassie blue. Labeled gel spots indicate differentially induced proteins (compared to (a) control) that were cut out for in-gel trypsin digestion and subsequently were analyzed by MS.

2-D Gel Spot No.[a]	Protein Name	Relative Volume[b] (C:LPS)	*MJpI* 2-DGE[c]	*MJpI* Database[d]	Masses Matched[e] #(%)	Accession Number Swiss-prot
1	Deoxyribonuclease	1:2	1.5/5.2	29.1/5.1	5(27)	P24855
2	Deoxyribonuclease	1:2	1.6/5.2	29.1/5.1	5(24)	P24855
3	Deoxyribonuclease	1:2	1.4/6.0	29.1/5.1	5(33)	P24855
4	Actin	1:3.3	20.0/6.5	40.6/5.6	12(26)	P60709
5	Caborric anhydrase 2	1:5.6	29.0/8.0	29.2/6.9	8(33)	P00918
6	Annexin A1	1:3.3	38.2/6.4	38.8/6.6	20 (76)	P04083
7	Alpha enolase	1:6.1	48.0/7.2	47.4/7.0	11(18)	P06733
8	GRAP 2 human	1:14.4	49.0/8.2	37.9/6.4	19 (60)	O75791
9	Protein disulfide isomerase	1:3.2	58.0/6.5	57.1/6.0	20(63)	P30101
10	Glucose-regulated protein, 78 kDa	1:6.7	73.0/5.3	72.4/5.1	25(39)	P11021
11	Heat shock 70 kDa protein 8 isoform 2	1:5.9	70.0/5.6	71.1/5.4	11(24)	P11142

a) The proteins correspond to spots whose volume changed ± two fold after *P.g.* LPS stimulation. Spots (numbered in Figure 2) were cut from 2-DGE, digested with trypsin and subjected to MALDI-TOFMS for analysis.
b) Relative volumes of matched spots were determined by PD Quest 7.0 (BioRad).
c) Apparent mass and *p*I determined from 2-DGE.
d) Mass and *p*I obtained from Swiss-Prot database.
e) The peptide masses were submitted to the Swiss-Prot database and to the NCBI database. # indicates the number of peptides matched and (%) reports the total sequence coverage.

Table 1. Identification of proteins from LPS-treated THP-1 cells compared to untreated control cells.

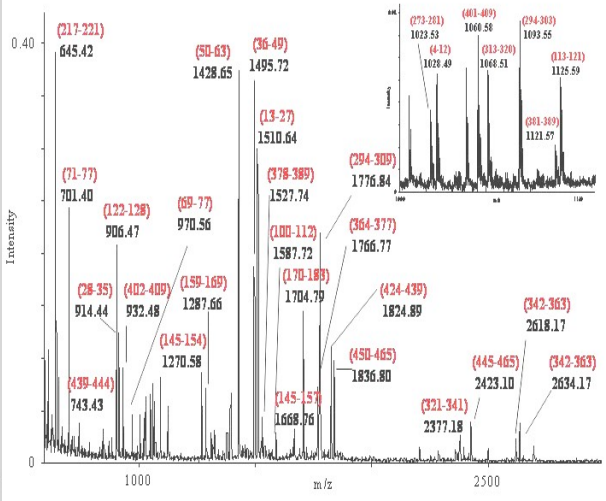

Figure 2. MALDI-TOF mass spectrum of tryptic in-gel digested protein 10 (Figure 1d) from LPS-treated THP-1 cells.