Cytokine Differences in Mature and Immature Human Macrophage Cell Lines

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1814

ABSTRACT

The goal of this investigation was to contrast the cytokine response profiles following LPS activation of mature and immature human macrophage phenotypes in order to assess the possibility that differences in macrophage maturity might have impact on the clinical outcomes following macrophage recruitment and activation in inflamed gingival tissues. Human macrophage cell lines previously characterized as promyelocytic (HL-60) and the more mature (THP-1) cells were cultured and used to assess the effects of LPS challenge. Following differentiation with PMA both cell lines were challenged with *E.coli* LPS and culture supernatants were collected and analyzed for TNF-alpha , GM-CSF , PGE2 and PGF2 alpha levels at various time points. HL-60 cells secreted TNF-alpha with a peak level achieved 6-8 hours following activation with LPS. HL-60 cells secreted very low levels of GM-CSF following 6-8 hours of incubation which increased only modestly before peaking at levels substantially less than that of TNF-alpha 24-48 hours after LPS addition. The mature macrophage cell line THP-1 peaked in production of TNF-alpha 6-8 hours following LPS challenge; however, THP-1 production of GM-CSF was substantial 6 hours following LPS-mediated activation and increased markedly for 24-48 hours to levels to exceeding that of TNF-alpha. PGE2 and PGF2 alpha were both significantly higher in the THP-1 cells than in the HL-60 cells. These findings demonstrate that the kinetics of the expression of the expression of these cytokines in macrophages differ markedly and that production of GM-CSF is substantially greater in the more mature THP-1 cell line. **Since TNF-alpha and GM-CSF mediate very different host responses and PGE2 is known to suppress TNF-alpha , these findings suggest that the maturity of macrophages infiltrating inflamed gingival tissues might have an important impact on the balance of the host response (inflammatory vs. protective) to LPS challenge.**

INTRODUCTION

Bacterial components such as LPS (lipopolysaccharide) are able to activate macrophages to synthesize and secrete many different inflammatory mediators that affect gum disease, such as TNF and PGE_2. Differing levels of maturity of macrophages within inflamed gingival tissues may influence the balance of the host response to LPS challenge.

OBJECTIVE

The goal of this investigation was to contrast the cytokine response profiles following LPS activation of mature and immature human macrophage phenotypes. The outcome would provide information as to whether differences in macrophage maturity might impact clinical outcomes following macrophage recruitment and activation in inflamed gingival tissues.

MATERIALS AND METHODS

THP-1 cells are a human, nonadherent, monocytic cell line purchased from the American Type Culture Collection (ATCC). HL-60 cells, a promyelocytic cell line (immature) were also obtained from ATCC; passages 20-40 were used in these experiments. Both cell lines were passaged three times per week in RPMI 1640 (GIBCO), supplemented with 10% heat-inactivated fetal bovine serum (Hyclone) and penicillin (100U/ml) and streptomycin (100 ug/ml).

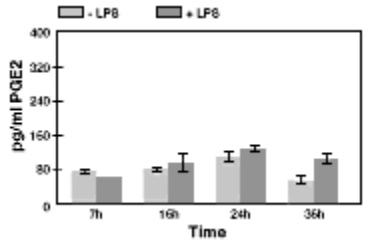
THP-1 cells (ATCC) were differentiated by exposure to 16nM Phorbol Myristate Acetate (PMA) for 18 hrs. Cells were pelleted by centrifugation, counted, and viability determined. Cells were seeded into 24-well plates at 5 $x10^5$ cells/well/ml.

HL-60 cells were pelleted, counted, and viability determined. These cells were seeded at 1.5 x 10^6 cells/well/2ml. directly into 24-well plates and differentiated by exposure to 32nM PMA for 24 hrs. The adherent cells were washed and checked for viability before beginning the time course.

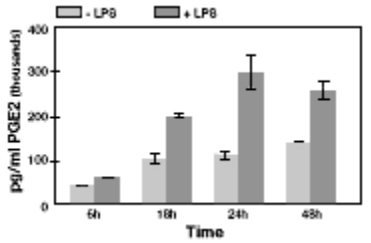
For both cell lines, each time point had unchallenged control wells (no LPS) and LPS- challenged wells at 1 ug/ml. Cell supernatants were removed at the various time points and frozen at -70° C. Analyses for TNF and GM-CSF were performed using R&D Systems ELISA kits. Eicosanoid assays were performed using Cayman EIA kits.

RESULTS

PGE2 Time course: HL-60 cells #2



Time Course: THP-1 cells #2



PGE2 levels are low in HL-60 and markedly higher in THP-1 cells.

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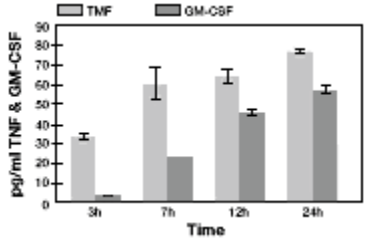
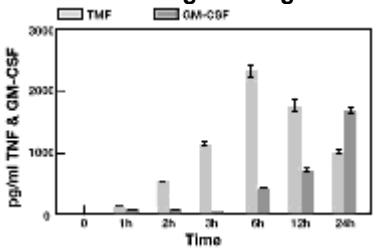
RESULTS (cont.)

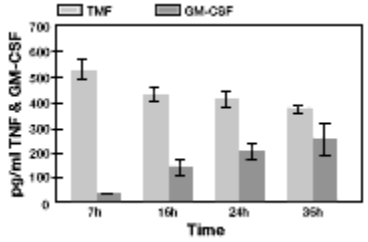
Time course: HL-60 cells #1
LPS challenge 1 ug/ml



Time course: THP-1 cells #1
LPS challenge at 1 ug/ml



Time course: HL-60 cells #2
LPS challenge 1 ug/ml



Time course: THP-1 cells #2
LPS challenge at 1 ug/ml

CONCLUSION

-TNF and GM-CSF have very different kinetics.

-TNF peaks early in both cell types but is directionally higher in THP-1 cells.

-GM-CSF production is delayed in both cell lines but is ~ 10x higher in THP-1 cells.

-PGE2 production parallels GM-CSF and is much higher in THP-1 cells.

-These findings suggest that maturity of macrophages impacts the balance of the cytokine responses: more mature cells secrete higher levels of GM-CSF and PGE2.

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