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BSI2014 - Poster Acceptance Notification A-686-0002-00386

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Dear Khalid Alshaghdali,

Thank you for submitting your abstract for the British Society for Immunology Congress 2014, taking place from Monday 1 December to Thursday 4 December 2014 at The Grand Hotel and Brighton Centre in Brighton, UK.

We are pleased to inform you that your abstract A-686-0002-00386 entitled "***Macrophage subsets exhibit selective endotoxin tolerance induced by Escherichia coli LPS***" has been accepted for **POSTER** display at the Congress.

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To secure your abstract at the Congress and for it to be published in the journal *Immunology*, the presenting author must register and pay to attend the Congress.

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Poster guidelines

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The British Society For Immunology Annual Congress
1-4 December 2014 Brighton, UK

386
Macrophage subsets exhibit selective endotoxin tolerance induced by Escherichia coli LPS

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Macrophages (MΦs) control gut mucosal responses; facilitating tolerance to commensal bacteria and food components, while keeping the ability to trigger immune defences to pathogens. MΦ reactions are

Macrophage subsets exhibit selective endotoxin tolerance induced by Escherichia coli LPS

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Introduction

Immunity can be achieved by both immune activation or deactivation mechanisms. Intestinal tolerance is an important mucosal mechanism, whereby host cells and commensal organisms are tolerised whilst maintaining responsiveness to harmful pathogens. This state of tolerance is broken in the case of mucosal inflammatory pathologies, causing a destruction of gut mucosal tissue (1). Mucosal macrophages (MΦs) have a dual functionality that determines tolerance to commensal organisms or immune response entro pathogens such as Escherichia coli. Endotoxin tolerance (ET) is a circumstance where cells go through a hypo responsive state, unable to respond to further endotoxin-LPS challenge (1). Previously, tolerisation studies showed differential suppression between M1 (pro-inflammatory) and M2 (regulatory) MΦs in response to LPS of an oral pathogen, *Porphyromonas gingivalis* (2). *Escherichia coli* is an intracellular gut mucosal pathogen; *E. coli*-LPS is already understood to be able to induce ET in macrophages (2).

Objectives

The aim of this study was to investigate the susceptibility of functionally distinct MΦ subsets to *E. coli* K12 LPS induced suppression.

Macrophage (MΦ) culture

THP-1 cells were maintained in R10 medium (RPMI-1640) supplemented with 10% v/v foetal calf serum (FCS). MΦ subsets M1 like and M2 like cells were generated by differentiation of THP-1 in the presence of 25ng/ml phorbol-12-myristate acetate (PMA) for 3 days for M1 like MΦ or 10nM 1,25-$(OH)_2$ Vitamin D_3 for 7 days for M2 like MΦ(2).

Activation and Tolerisation of macrophage subsets

THP-1 derived M1-like MΦs and M2-like MΦs were pre-treated with 100 ng/ml K12 LPS for 24 hours. Then, Pre-stimulus culture medium was removed carefully and MΦs were washed in fresh R10 before re-stimulation by 100 ng/ml K12 LPS for a further 18 hours at 37° C /5% CO_2. The supernatants were harvested and stored at -20° C until required for cytokine assay by sandwich ELISA whereas the cell lysates were used for detection of gene expression by Real Time polymerase chain reaction (RT-PCR)

ELISA **RT-PCR**

Statisti

Data were analysed using Minitab version 16. Significant differences among treatments were evaluated by balanced analysis of variance, one or two-way analysis of variance (ANOVA) when applicable.

Results

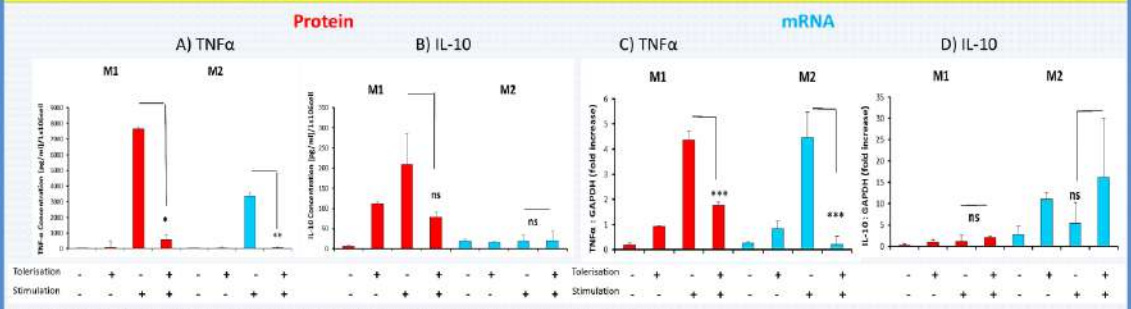

Figure: K12 LPS differentially suppresses M1 & M2 MΦ cytokines production and gene expression.

M1 (Red) and M2 (Blue) MΦ subsets were pre-stimulated with 100 ng/ml K12 LPS for 24 hours (determined as optimal time period for expression of all cytokines TNFα, IL-1β, IL-6 and IL-10, data not shown) prior to stimulation with 100 ng/ml K12 LPS incubated for a further 18 hours { (-)= no LPS whereas (+)= LPS added for either or both pre-stimulated and stimulated cells}. At the end of the treatment time, supernatants were harvested to detect the production Levels of TNFα (A) and IL-10 (B) by ELISA kits according to the manufacturer's instructions. The cell lysates were used for detection of gene expression of TNFα (C) and IL-10 (D) by (RT-PCR) . Cytokine production is expressed as the mean secretion ± SD in pg/ml and gene expression (mRNA level) is expressed as fold change using GAPDH as reference gene. Data showed represents triplicate samples for n = 3 replicate experiments. Significant effects on suppression compared to the untolerised LPS control for the specified MΦ subset are indicated as *p< 0.05, **p< 0.01 , *** P<0.005, and ns, not significant.

	4 hours pre-treatment				24 hours pre-treatment			
M1	TNFα (68%) ⇓	IL-1β ↔	IL-6 ↔	IL-10 (45%) ⇓	TNFα (92%) ⇓	IL-1β (80%) ⇓	IL-6 (92%) ⇓	IL-10 (66%) ⇓
M2	TNFα (95%) ⇓	IL-1β (60%) ⇓	IL-6 ↔	IL-10 ↔	TNFα (97%) ⇓	IL-1β (55%) ⇓	IL-6 ↔	IL-10 ↔

Table: K12 LPS differentially suppresses M1 & M2 MΦ cytokines.

Data in the table summarise the level of change on cytokine production in percentage by tolerised MΦ subsets M1 and M2 comparing to untolerised LPS controls. M1 and M2 MΦ were pre-treated with K12 LPS as mentioned above for 4 hours and 24 hours before challenge with the same stimulus.

Conclusion

- **K12 LPS tolerised M1 and M2 Macrophage subsets exhibit down-regulation of pro-inflammatory cytokines TNFα and IL-1β whereas IL-6 is only suppressed in M1 MΦs subset.**
- **K12 LPS tolerised M1 and M2 Macrophage subsets showed a clear suppression of TNFα mRNA expression persisting to 24 hours.**
- **Anti-inflammatory cytokine, IL-10, showed no significant change in cytokine production and mRNA expression by both M1 like MQs and M2 like MΦs.**

References

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3. Foey, A. D. 2011. Butyrate regulation of distinct macrophage subsets: opposing effects on M1 and M2 macrophages. Int. J. Probiotics & Prebiotics, 6. 147-158.

CERTIFICATE OF ATTENDANCE

This is to certify that

khalid Alshaghdali

attended the

British Society for Immunology Congress 2014

held at the Brighton Centre and Grand Hotel
Brighton, UK

from 1-4 December 2014

The BSI 2014 Congress has received CPD accreditation as follows:
Royal College of Pathologists to a maximum of 23 credits
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