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Published in:
British Journal of Pharmacology

Document Version:
Early version, also known as pre-print

Queen's University Belfast - Research Portal:
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Download date:08. Jun. 2022
DATA SUPPLEMENT

INDUCTION OF THE INFLAMMATORY REGULATOR A20 BY GIBBERELLIC ACID IN AIRWAY EPITHELIAL CELLS

J A Reihill¹*, B Malcomson¹*, A Bertelsen¹, S Cheung¹, A Czerwiec¹, R Barsden¹, J S Elborn¹, H Dürkop², B Hirsch³, M Ennis¹, C Kelly⁴* and B C Schock¹*$. 

¹Centre for Infection and Immunity, Queen’s University of Belfast, Belfast, BT9 7AE, UK
²Institute für Pathodiagnostik, 12099 Berlin, Germany
³Charité-University, Institute of Pathology, 12200 Berlin, Germany
⁴Northern Ireland Centre for Stratified Medicine, University of Ulster, Londonderry, BT47 6SB, UK

*Both junior and senior authors contributed equally to this work

$Corresponding author contact details:
Address: Centre for Infection and Immunity, Queen’s University of Belfast, Health Sciences Building, 97 Lisburn Road, Belfast, UK, BT9 7AE.
Phone: +44 (0) 28 9097 5876
Fax: +44 (0) 28 90 972671
Email: b.schock@qub.ac.uk

Key Words: A20 protein; NF-kappaB; Gibberellic Acid, Airway epithelial cells; Inflammation.
EXPERIMENTAL PROCEDURES

Quantitative real time qPCR

Primers were designed using gene accession numbers and Primer3 open-source PCR primer design software and obtained from Invitrogen Ltd. (Paisley, UK). Primer sequences are given in Table S1.

Table S1: PCR primer sequences.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession Number</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>Beta-actin</td>
<td>NM_001101.3</td>
<td>5’ ctcttccagcctctctctct 3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3’ agcactgtgtggcgtacacg 5’</td>
</tr>
<tr>
<td>A20</td>
<td>NM_006290</td>
<td>5’ gagagcacaatggctgaacatg 3’</td>
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<tr>
<td></td>
<td></td>
<td>3’ tccagtgtgtctgttcggtcagc 5’</td>
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<tr>
<td>p65</td>
<td>NM_021975</td>
<td>5’ cctggagcaggctatcaagtc 3’</td>
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<tr>
<td></td>
<td></td>
<td>3’ cactgtcacctgagcaggcta 5’</td>
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<tr>
<td>TLR4</td>
<td>NM_003266.3</td>
<td>5’ tggacaattggctagaggg 3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3’ gatccagccactgctgtct 5’</td>
</tr>
</tbody>
</table>
SUPPLEMENTARY RESULTS

Cell Proliferation

Figure S1: GA3 does not induce cellular proliferation. 16HBE14o- cells were incubated with GA3 (0-300 μM) for 72h and cell proliferation was determined in using the CellTiter 96® AQeuous One Solution Cell Proliferation Assay (Promega, UK) according to the manufacturer’s recommendations. No change in proliferation was observed.

Purity of GA3 preparation - Expression of TLR4 mRNA

Figure S2: GA3 preparation does not induce TLR4 mRNA expression. To determine if the GA3 preparation used in this study may contain traces of endotoxin, expression of TLR4 and β-actin (housekeeping gene) were assessed by qPCR. LPS alone [10 μg/ml] significantly increased TLR4 mRNA expression in a time dependent manner (p<0.01 LPS 1h vs. LPS 24h, Kruskal Wallis with Dunn’s post hoc test, n=5), but exposure of cells to GA3 [30 μM] did not induce TLR4.