



Data in Brief

Role(s) of IL-2 inducible T cell kinase and Bruton's tyrosine kinase in mast cell response to lipopolysaccharide

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ABSTRACT

Mast cells play critical roles during immune responses to the bacterial endotoxin lipopolysaccharide (LPS) that can lead to fatal septic hypothermia [1–3]. IL-2 inducible T cell kinase (ITK) and Bruton's tyrosine kinase (BTK) are non-receptor tyrosine kinases that act downstream of numerous receptors, and have been shown to modulate mast cell responses downstream of FcεRIα [4], however, their roles in regulating mast cell responses to endotoxic stimuli were unclear. We found that the absence of ITK and BTK alters the mast cell response to LPS, and leads to enhanced pro-inflammatory cytokine production by mast cells and more severe LPS-induced hypothermia in mice [5]. Here, we detail our investigation using microarray analysis to study the transcriptomic profiles of mast cell responses to LPS, and the roles of ITK and/or BTK expression in this process. Mouse whole genome array data of WT, *Itk*^{-/-}, *Btk*^{-/-}, and *Itk*^{-/-}*Btk*^{-/-} bone marrow-derived mast cells (BMMCs) stimulated by PBS (control) or LPS for 1 h were used in our latest research article [5] and is available in the Gene Expression Omnibus under accession number GSE64287.

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Specifications

Organism/cell line/tissue	<i>Mus musculus</i> /bone marrow-derived mast cells (BMMCs)
Sex	Female
Sequencer or array type	Agilent-014868 Whole Mouse Genome Microarray 4 × 44K G4122F (Probe Name version)
Data format	Raw and normalized Data
Experimental factors	WT, <i>Itk</i> ^{-/-} , <i>Btk</i> ^{-/-} , and <i>Itk</i> ^{-/-} <i>Btk</i> ^{-/-} BMMCs: LPS-treated vs PBS-treated
Experimental features	Transcriptomic analysis to investigate the role(s) of ITK and BTK in mast cell responses to endotoxin LPS. WT, <i>Itk</i> ^{-/-} , <i>Btk</i> ^{-/-} , and <i>Itk</i> ^{-/-} <i>Btk</i> ^{-/-} BMMCs were treated with 100 ng/ml LPS for 1 h and compared to controls (PBS-treated).
Consent	N/A
Sample source location	Ithaca, NY

1. Direct link to deposited data

Deposited data can be found here: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE64287>.

2. Experimental design, materials and methods

2.1. Generation of bone marrow-derived mast cells (BMMCs) and *in vitro* stimulation

WT, *Itk*^{-/-}, *Btk*^{-/-}, and *Itk*^{-/-}*Btk*^{-/-} mice were generated as previously described [4]. To generate BMMCs for *in vitro* stimulation, female mice were used at 6–8 weeks old. Bone marrow cells were harvested from the femurs and cultured in complete Dulbecco modified Eagle medium (DMEM, 4.5 g/L glucose, 10% low-endotoxin fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 1 mM non-essential amino acids, 100 U/ml penicillin/streptomycin) with 10 ng/mL recombinant murine interleukin-3 (rmIL-3, Cell Sciences, Canton, MA) and 50 ng/mL recombinant murine stem cell factor (rmSCF, Peprotech, Rocky Hill, NJ). After 5 weeks, cells were examined for purity of BMMCs based on their expression of mast cell lineage markers c-Kit and FcεRIα using flow cytometry: BMMCs were cultured in Fc Block (Clone 93; BioLegend, San Diego, CA) for 10 min, and anti-c-

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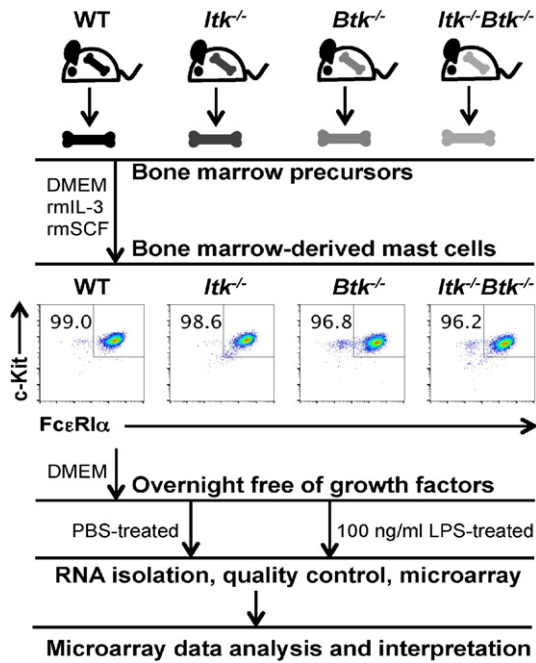


Fig. 1. Experimental scheme for transcriptomic analysis of mast cell response to LPS. Bone marrow-derived mast cells were generated using complete DMEM supplemented with mast cell inducing factors rmlL-3 and rmSCF. Highly pure populations (>96% c-Kit⁺ FcεRIα⁺) were factor starved for 12 h and stimulated with PBS or 100 ng/ml LPS for 1 h, followed by RNA isolation, quality control, and microarray.

Kit (Clone 2B8; eBioscience, San Diego, CA) and anti-FcεRIα (Clone MAR-1; eBioscience) for 30 min, followed by analysis on LSRII (BD Bioscience, San Jose, CA). BMMCs with more than 96% purity (c-Kit⁺ FcεRIα⁺) were used for stimulation (Fig. 1). Density of BMMCs was adjusted to 2 million/ml for stimulation. To reduce background signals caused by growth factors rmlL-3 and rmSCF, BMMCs were factor starved overnight in complete DMEM prior to stimulation by PBS or 100 ng/ml LPS for 1 h.

2.2. RNA isolation and microarray

Cells were subjected to total RNA extraction using RNeasy Plus Mini Kit with removal of genomic DNA following the manufacturer's instruction (Qiagen, Valencia, CA). RNA was quantified using a NanoDrop-1000 spectrophotometer (Wilmington, DE) and quality was monitored using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA samples with RNA integrity number (RIN) between 9.8 and 10 were used for microarray. Cyanine-3 (Cy3) labeled complementary RNA (cRNA) was prepared from 200 ng RNA using the One-Color Low RNA Input QuickAmp Labeling Kit (Agilent). Dye incorporation and cRNA yield were checked with the NanoDrop ND-1000 Spectrophotometer. 1650 ng per sample of Cy3-labelled cRNA (specific activity >10.0 pmol Cy3/μg cRNA) was fragmented at 60 °C for 30 min in a reaction volume of 55 μl containing 11 μl 25× Agilent fragmentation buffer and 2.2 μl 10× Agilent blocking agent following the manufacturer's instructions. On completion of the fragmentation reaction, 55 μl of 2× Agilent hybridization buffer was added to the fragmentation mixture and hybridized 100 μl to Agilent Whole Mouse Genome Microarray Kit, 4 × 44K (G4122F) for 17 h at 65 °C in a rotating Agilent hybridization oven. After hybridization, microarrays were washed 1 min at room temperature with GE Wash Buffer 1 (Agilent) and 1 min with 37 °C GE Wash buffer 2 (Agilent), then dried immediately by brief centrifugation. Slides were scanned immediately after washing on the

Agilent DNA Microarray Scanner (G2505B) using one color scan setting for 4 × 44K array slides (scan area 61 × 21.6 mm, scan resolution 5 μm, dye channel is set to Red & Green and both the Red and Green photomultipliers (PMT) is set to 100%).

2.3. Microarray data analysis

The scanned microarray images were analyzed with Feature Extraction Software 9.1 (Agilent) using parameters (protocol GE1-105_DEC8 and Grid: 012391_D_20060331) to obtain background subtracted and spatially detrended Processed Signal Intensities (PSI). Features flagged in Feature Extraction as Feature Non-uniform outliers were excluded. Data were further analyzed in GeneSpring (Agilent). GAPDH-normalized probe values were converted into gene expression values with Quantile normalization for further analysis. Average values of the triplicates in WT PBS control group were set as basal level 1.

3. Discussion

Upon obtaining the microarray data, we analyzed, technically, whether the microarray data were generated with good quality to reflect the biological features of the cells by checking the correlation coefficients computed on raw, non-normalized data in linear scale of all pairs within the dataset. The Pearson Similarity Metric calculates the mean of all elements in vector *a*, then subtracts that value from each element in *a* and calls the resulting vector *A*; it does the same for *b* for vector *B*; and eventually, correlation coefficient is computed as $\mathbf{A} \cdot \mathbf{B} / (|\mathbf{A}| |\mathbf{B}|)$. We found that the raw data of all samples were highly correlated (≥ 0.92), indicating comparable data quality in individual array data (Fig. 2).

This microarray dataset was then subjected to analysis to determine whether ITK and/or BTK play a prominent role in regulating mast cell transcriptional responses to LPS. Statistical analysis of fold change of gene expression comparing LPS-treated to PBS-treated groups showed consistent results to those observed by RT-PCR, flow cytometry, and ELISAs for cytokine detection, that TNF-α is significantly up-regulated in *Itk*^{-/-}*Btk*^{-/-} mast cells. In addition, a significantly larger number of genes responded to LPS treatment in the absence of both ITK and BTK, among which there were genes involved in the NF-κB signaling transduction. These data suggested a synergistic role of ITK and BTK negatively regulating NF-κB signal transduction during the mast cell pro-inflammatory response to endotoxin LPS [5].

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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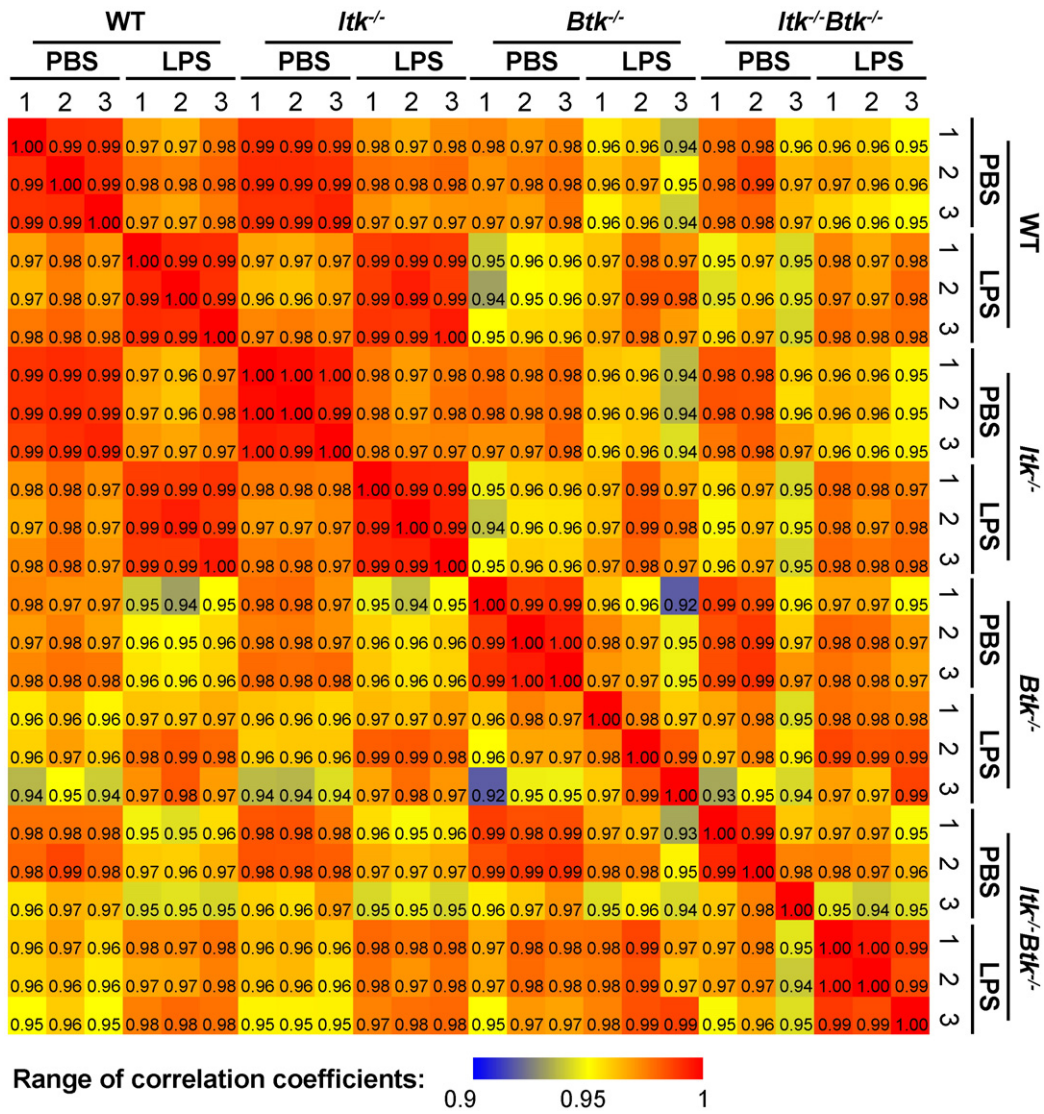


Fig. 2. Similarity metrics of raw microarray data. Pearson Correlation Coefficients of all pairs in the dataset were computed and plotted. This metrics indicates similar data quality in all samples.

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