Metabolomic analysis of the interleukin-10-deficient mouse model of Crohn's disease

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Abstract

Crohn's disease is an inflammatory disorder of the bowel, arising from the dysregulation of intestinal mucosal immunity. The interleukin-10-deficient ($IL10^{-/-}$) mouse develops intestinal inflammation with similar characteristics to Crohn's disease, due to the loss of immune tolerance towards intestinal microbiota. Metabolomic analysis is the study of small molecule metabolites, involving the measurement of large numbers of metabolites in biological samples. The aim of the research was to study the urinary metabolite profile of $IL10^{-/-}$ mice by gas chromatography-mass spectrometry metabolomic analysis. A metabolite profile of intestinal inflammation, consisting of 15 metabolites, was discovered by comparing the urinary metabolite profiles of $IL10^{-/-}$ and wildtype C57BL/6 mice. Xanthurenic acid and fucose were identified as the main urinary metabolites associated with the early stage of intestinal inflammation. Their levels were increased in $IL10^{-/-}$ mice relative to wildtype. Xanthurenic acid levels were attributed to increased tryptophan catabolism which produces kynurenine metabolites that may induce immune tolerance of T-cells towards intestinal microbiota. Plasma levels of kynurenine and 3-hydroxykynurenine were confirmed to be elevated in $IL10^{-/-}$ mice. The increased fucose levels may be due to abnormal fucosylation of plasma or intestinal mucosal proteins involved in leukocyte trafficking. Comparisons of the urinary metabolite profiles of $IL10^{-/-}$ and wildtype mice also revealed eleven metabolite differences that were unaffected by inflammation severity in $IL10^{-/-}$ mice. The main metabolites were glutaric acid, 2-hydroxyglutaric acid and 2-hydroxyadipic acid, which were decreased in $IL10^{-/-}$ mice. These eleven metabolite differences may be associated with residual genes from embryonic stem cells of the 129P2 mouse strain used to create the $IL10^{-/-}$ mouse, or novel functions of IL10 that are unrelated to inflammation. The metabolite profile of inflammation was not altered in $IL10^{-/-}$ mice fed with kiwifruit extracts, consistent with other measures of inflammation which showed that intestinal inflammation was not attenuated by the dietary intervention. The urinary levels of some kiwifruit metabolites differed between $IL10^{-/-}$ and wildtype mice, suggesting differences in absorption or intestinal microbial metabolism of these metabolites. Overall, the research demonstrates that metabolomic analysis of $IL10^{-/-}$ mice can identify potential biomarkers of intestinal inflammation and provide new insights into the metabolic effects of IL10-deficiency.

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Abbreviations

ACMSD	aminocarboxymuconate semialdehyde dehydrogenase
aq	aqueous extract
ATG16L1	autophagy-related 16-like 1
BSTFA	*N*,*O*-bis(trimethylsilyl)-trifluoroacetamide
bw	body weight
^{o}C	degree Celcius
CARD15	caspase recruitment domain family, member 15
CCR6	chemokine receptor 6
CEMS	capillary electrophoresis-mass spectrometry
cM	centi-Morgan
conv	conventional housing condition
CRP	C-reactive protein
CV	coefficient of variation
D2HGDH	D-2-hydroxyglutarate dehydrogenase
ddc	dopa decarboxylase
DSS	dextran sodium sulfate
DLG5	discs, large homolog 5 (Drosophila)
ELISA	enzyme-linked immunosorbent assay
ESR	erythrocyte sedimentation rate
FTIR	Fourier transform-infrared
g	gram
GABA	gamma-aminobutyric acid
GCMS	gas chromatography-mass spectrometry
GHB	gamma-hydroxybutyric acid / 4-hydroxybutyric acid
HEPA	high efficiency particulate air
HLA	human leukocyte antigen
IBD	inflammatory bowel disease
IDO	indoleamine-2,3-dioxygenase
IFNγ	interferon-gamma
IL1	interleukin-1
IL10	interleukin-10
IL10$^{-/-}$	interleukin-10-deficient
IL10R	interleukin-10 receptor
IL12	interleukin-12
IL17	interleukin-17
IL1α	interleukin-1-alpha
IL23	interleukin-23

IL23R	interleukin-23 receptor
IL6	interleukin-6
IRGM	immunity-related guanosine triphosphatase
kPa	kiloPascal
LCMS	liquid chromatography-mass spectrometry
MAP	*Mycobacterium avium* subspecies *paratuberculosis*
µg	microgram
MHC	major histocompatibility complex
min	minutes
µl	microlitre
ml	millilitre
µm	micron
mm	millimetre
MS	mass spectrometry
MSTFA	*N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide
MUC19	mucin 19
m/z	mass to charge ratio
NFKB	nuclear factor kappa-B
NMR	nuclear magnetic resonance
NOD	nucleotide binding oligomerisation domain protein
OCTN	organic cation transporter
org	organic solvent extract
PBS	phosphate buffered saline
PCA	principal components analysis
PPAR	peroxisome proliferator activated receptor
RAG	recombination activating genes
ret.	retention
R.I.	retention index
RT	retention time
s	seconds
SAA	serum amyloid-A
SAM	senescence accelerated mouse
SCID	severe combined immunodeficiency
SPF	specific pathogen-free
STAT	signal transducer and activator of transcription
Th1	T-helper 1
Th17	T-helper 17
Th2	T-helper 2
TIC	total ion chromatogram
TLR	toll-like receptor
TMS	trimethylsilyl derivative
TNBS	2,4,6-trinitrobenzene sulfonic acid
TNF	tumour necrosis factor-alpha
Tph1	tryptophan 5-monooxygenase 1

Treg	T-regulatory
WT	wildtype
w/w	weight/weight