A role for microRNA-155 in the control of infection

By
Victoria Francis John

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Trinity Hall
University of Cambridge

Abstract

MicroRNAs (miRNAs) are small (~22 nucleotide) non-coding regulatory RNA molecules which influence the expression of genes within eukaryotic cells. miRNAs function through targeted binding to the 3' un-translated region (UTR) of messenger RNAs (mRNAs) in a sequence specific manner. Recent studies have implicated microRNA-155 (miR-155) as an important player in the development and function of a number of immune cells including B and T cells, macrophages and dendritic cells. The aim of this study was to investigate the role of miR-155 in controlling either a mucosal *Citrobacter rodentium* or a systemic *Salmonella enterica* serovar Typhimurium infection in the context of the overall immune response. Here we present evidence that miR-155-deficient mice are less competent in their ability to eradicate a mucosal *C. rodentium* infection compared with wild type control C57BL/6 mice. We show that miR-155-deficient mice have a higher *C. rodentium* burden in gastrointestinal tissue and also exhibit spread into systemic tissues. Additionally, we demonstrate that germinal centre formation and humoral immune responses are impaired in the absence of miR-155. In view of the fact that this phenotype is largely reproduced in μMT (B cell-deficient) mice reconstituted with miR-155-deficient B cells we conclude that miR-155 is required to control *C. rodentium* infection. Further, miR-155-deficient mice were able to clear a primary infection with an attenuated strain of *S.* Typhimurium but were defective in their immune response to *Salmonella*.

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Declaration

I hereby declare that this dissertation is the result of my own work and contains no material written by any other person. It includes nothing that is the outcome of work done in collaboration except where specifically indicated here or in the Materials and Methods section. I was fully involved in all aspects of the design and the experimental work presented.

Dr S Clare (Wellcome Trust Sanger Institute, Hinxton, Cambridge), assisted the author in performing tissue collection from experimental animals and performed immunisations and animal procedures during the studies.

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Dr Elena Vigorito (Babraham Institute, Cambridge) created all chimeric mice used throughout this study.

None of the material presented herein has been submitted previously for the purpose of obtaining another degree. I confirm that this thesis does not exceed 300 single-sided pages of double spaced text, or 60,000 words.

Victoria F. John

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Abbreviations

ADCC	Antibody-dependent cell-mediated cytotoxicity
A/E lesion	Attaching and effacing lesion
AICDA	Activation-induced cytidine deaminase
ALV	Avian leukosis virus
AP	Activator protein
APCs	Antigen presenting cells
BALT	Bronchus-associated lymphoid tissue
BCR	B-cell receptor
Bfp	Bundle forming pili
BIC	B-cell integration cluster
BL	Burkitt lymphoma
BSA	Bovine serum albumin
CFU	Colony forming units
CGG	Chicken gamma globulin
CSR	Class switch recombination
CTLs	Cytotoxic T-lymphocytes
CXCR	Chemokine, CXC Motif, Receptor
DC	Dendritic cell
D-PBS	Dulbecco’s phosphate buffered saline

DNP	Dinitrophenylated
EHEC	Enterohaemorrhagic *Escherichia Coli*
ELISA	Enzyme-linked immunosorbant assay
EPEC	Enteropathogenic *Escherichia Coli*
ESP	Expressed surface protein
EST	Expressed sequence tag
FAE	Follicle associated epithelium
FasL	Fas Ligand
FDC	Follicular dendritic cell
GA	Glutaraldehyde
GALT	Gastric-associated lymphoid tissue
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GC	Germinal centre
gDNA	Genomic DNA
GO	Gene ontology
HEV	High endothelial venule
HL	Hodgkin lymphoma
HRP	Horse radish peroxidase
HUL	Haemolytic uraemic syndrome
IBD	Inflammatory bowel disease

ICAM	Intracellular adhesion molecule
IFN	Interferon
Ig	Immunoglobulin
IHC	Immunohistochemistry
IL	Interleukin
ILF	Isolated lymphoid follicle
INOS	Inducible nitric oxide synthase
ITAM	Immunoreceptor tyrosine activation motif
i.v.	Intravenous
KEGG	Kyoto Encyclopedia of Genes and Genomes
KDa	Kilo Dalton
KLH	Keyhole limpet hemocyanin
LB	Luria Bertani
LEE	Locus of enterocyte effacement
LPS	Lipopolysaccharide
LT	Lymphotoxin
MALT	Mucosal-associated lymphoid tissue
M cell	Microfold cell
MHC	Major histocompatability complex
miRNA	microRNA

mLN	Mesenteric lymph node
MMP	Matrix metalloproteinase
MMR	Mismatch repair
MPEC	Murine-pathogenic *Escherichia Coli*
mRNA	messenger RNA
Myd88	Myeloid differentiation factor 88
Mø	Macrophage
μMT mice	B cell-deficient mice
NALT	Nasal-associated lymphoid tissue
NEO	Neomycin
NF	Nuclear factor
NFAT	Nuclear factor of activated cells
NHL	Non-Hodgkin lymphoma
NK	Natural Killer cell
NP	3-hydroxy-4-nitro-phenylacetyl
OPD	O-Phenylenediamine dihyrochloride
ORF	Open reading frame
PAMPs	Pathogen associated molecular patterns
PBS	Phosphate buffered saline
PFA	Paraformaldehyde

pi	Post-inoculation
pIgR	Polymeric immunoglobulin receptor
PNA	Peanut agglutinin
PO	Propylene oxide
Pol	Polymerase
PP	Peyer's patch
RAG	Recombination activating gene
RISC	RNA-induced silencing complex
PKC	Protein kinase C
rpm	Revolutions per minute
PRRs	Pattern recognition receptors
RSS	Recombination signal sequences
RT	Room temperature
RNIs	Reactive nitrogen intermediates
RT-PCR	Reverse transcription-polymerase chain reaction
SCs	Stromal cells
SEM	Standard error mean
SHM	Somatic hypermutation
STAT	Signalling transducer and activator of transcription
TBMs	Tingible body macrophages

TCR	T-cell receptor
TdT	Terminal deoxynucleotidyl transferase
TetC	Tetanus toxin fragment C
T_H	T helper
TIR	Translocated intimin receptor
TLR	Toll-like receptor
TMCH	Transmissible murine colonic hyperplasia
TNF	Tumour necrosis factor
Tween	Polyoxyethylene-sorbitan monolaurate
T3SS	Type 3 Secretion System
UTR	Untranslated region