Phenotypic and Functional Characterisation of Innate and Adaptive Immune Responses after Mucosal Vaccination.

By

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Abstract

The successful development of mucosal vaccines is still impeded by our lack of understanding of how the mucosal immune system regulates antigen-specific responses. As most pathogens interact with or invade their host through a mucosal surface we may learn more about mucosal immunity by investigating the interaction of pathogens and their products with host factors and cells at mucosal surfaces. Here I examine the properties of a live *Salmonella*-based vaccine and a mucosal adjuvant based on a bacterial protein. Initially I examined the immunogenicity of the *M. tuberculosis* fusion antigen Ag85B-ESAT6 using a number of different mucosal vaccination strategies. These strategies included (i) intranasal immunisation with Ag85B-ESAT6 protein with and without Heat Labile toxin as an adjuvant (ii) oral immunisation with *Salmonella enterica* Typhimurium expressing Ag85B-ESAT6 from *in vivo* inducible or constitutive promoters (with and without intranasal boosts). Mice immunised with the various vaccine candidates were found to have significant anti-Ag85B-ESAT6 serum and mucosal antibody titres as well as strong T_H1 type cytokine responses, with IFN-γ levels particularly high. Intranasal boosting served to further enhance these immune responses. Following vaccination with the constitutive *Salmonella* vector, mice challenged with *M. tuberculosis* were found to have significantly reduced CFU in the liver when compared to non-vaccinated animals. Mice primed with *Salmonella* and then boosted intranasally with Ag85B-ESAT6/LTK63 led to a significant increase in protection, equivalent to that observed in mice vaccinated with BCG.

The nasal route for vaccination offers some important opportunities for the prophylaxis of many diseases, however the description of immune responses involved early after intranasal administration of antigen have not been clearly established. In a separate study, flow cytometry and confocal microscopy were used to examine the frequencies and localisation of innate immune cells, their activation status, as well as the expression of cell adhesion molecules following intranasal immunisation. I found striking differences between the cell surface phenotype of leukocytes and their pattern of distribution in the tissues examined at all time points tested after immunisation. Following on from these results one particular cell type was examined in more depth to determine its role in adaptive immune responses.

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Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text.

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All other immunisations, tissue collections, tissue processing (including cutting of frozen sections and immunostaining) were performed by the author.

Dr Jes Dietrich (Statens Serum Instiut, Denmark) performed the aerosol *M. tuberculosis* challenge experiments following vaccination with the recombinant constitutive *Salmonella* vaccine strain in their category 3 animal facilities.

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Abbreviations

Ag85B	Antigen 85B (30kDa mycolyl transferase)
AIDS	Acquired immunodeficiency syndrome
APC	Antigen presenting cells
bp	Base pair
BCG	Bacille Calmette-Guérin
BSA	Bovine serum albumin
BALT	Bronchial-associated lymphoid tissue
CAMs	Cell adhesion molecules
CBA	Cytometric Bead Analysis
CD	Cluster of Differentiation
CFUs	Colony forming units
CLN	Cervical lymph nodes
CMI	Cell mediated immunity
CMIS	Common mucosal immune system
conA	Concavalin A
CT-B	Cholera toxin B-subunit
CTL	Cytotoxic T lymphocyte
DC	Dendritic cells
ELISA	Enzyme-linked immunosorbant assay
ESAT-6	Early secreted antigenic target 6kDa
FACS	Fluorescence-activated cell sorting
FDC	Follicular dendritic cells
GALT	Gut-associated lymphoid tissue
GC	Germinal Centres
GM-CSF	Granulocyte-macrophage colony stimulating factor
HEV	High Endothelial Venules
HIV	Human immunodeficiency virus
HRP	Horseradish peroxidase
ICAM-1	Intracellular adhesion molecule-1
IFN	Interferon
Ig	Immunoglobulin

i.n.	Intranasal
i.p.	Intraperitoneal
i.v.	Intravenous
IL	Interleukin
kDa	Kilo Dalton
KO	Knock-out
LB	Luria Bertani
LNs	Lymph nodes
LT	Heat-labile toxin
LT-B	LT B-subunit
LTK63	LT with mutation in A subunit (ser63→lys)
mAb	Monoclonal antibody
MAdCAM-1	Mucosal addressin cell adhesion molecule-1
MCP-1	Macrophage chemoattractant protein-1
MHC	Major histocompatibility Complex
NALT	Nasal-associated lymphoid tissue
NKC	Natural Killer cells
NKT	Natural Killer T cells
NRAMP1	Natural resistance-associated macrophage protein-1
OD	Optical density
OPD	O-Phenylenediamine dihyrochloride
p	Plasmid
PBS	Phosphate Buffered Saline
PBST	PBS + 0.01% Tween 20
PCR	Polymerase chain reaction
PP	Peyer's patches
PPD	Purified protein derivative
PNA	Peanut agglutinin
PNAd	Peripheral Node Addressin
rBCG	Recombinant BCG
RD	Region of difference
rpm	Revolutions per minute
RT	Room temperature
s.c.	Subcutaneous

TBS	Tris buffered saline
TCR	T cell receptor
TetC	Tetanus toxin fragment C
T_H	T-helper
TNF	Tumour necrosis factor
TLR	Toll-like receptor
Tween	Polyoxyethylene-sorbitan monolaurate
URT	Upper respiratory tract
VCAM-1	Vascular cell adhesion molecule-1
WHO	World Health Organisation