Pre-existing strain specific neutralising antibodies abrogates the induction of interferon type I and cytotoxic T cell responses to subsequent homotypic influenza A virus challenge.

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ABSTRACT

Current inactivated influenza vaccines target the generation of influenza-specific antibodies to provide homotypic protection. However, little is known about the effects of annual vaccinations on the immune response during a subsequent influenza A virus infection. Here, we investigated the effect of pre-existing influenza-specific neutralising antibodies on innate and adaptive immunity during secondary infections. We report that the presence of pre-existing antibodies abrogates the induction of interferon type I (IFN-I) responses and cytotoxic T cell responses during subsequent influenza A virus infection. Wild-type mice were vaccinated intravenously with gamma-irradiated A/PR8 [H1N1] (γ-A/PR8) and challenged 3 weeks later with live A/PR8, and splenocytes were analysed 24 hours later for IFN-I mediated lymphocyte activation using fluorescent activated cell sorting. Our data clearly show absence of partial systemic lymphocyte activation and IFN-I responses in vaccinated mice. Furthermore, co-administration of A/PR8-specific sera and live A/PR8 virus abrogated the ability of live virus to induce partial lymphocyte activation, IFN-I responses as well as cytotoxic T cell responses. To test the clinical relevance of this observation, mice were mock or vaccinated with γ-A/PR8 and infected 3 weeks later with sub-lethal dose of A/PR8. These animals were then challenged 3 weeks later with lethal dose of A/PC [H3N2]. Our data clearly illustrate the effect of pre-existing antibodies on the ability of sub-lethal infection to generate cytotoxic T cell mediated heterosubtypic protection. I also investigated whether IFN-I responses are required for the generation of cytotoxic T cell responses in the presence of neutralising immune sera. My data show that addition of exogenous Poly I:C to A/PR8 virus pre-treated with A/PR8-specific sera did not rescue the induction of cytotoxic T cell responses. Thus, the presence of neutralising antibodies abrogates the induction of IFN-I and cytotoxic T cell responses during homotypic influenza A virus re-infection.

Declaration of Originality

I hereby declare that this thesis is my own work, and no material from this work has been submitted for the award of any other degree or diploma in the University of Adelaide or any other tertiary education. To the best of my knowledge and belief, this work contains no material previously published or written by others. Information derived from published and unpublished work of others has been acknowledged in the text and in the list of references.

I consent to this copy of my thesis being made available for circulation and photocopying for study and research purposes in accordance with the rules established by the University of Adelaide.

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Abbreviations

1x PBS	1x Phosphate buffer solution
^{60}Co	Cobalt-60 isotope
Ab (Abs)	Antibody/Antibodies
Ag (Ags)	Antigen/Antigens
Ag-Ab complex	Antigen-antibody immune complex
A/PC	A/Port Chambers/1/73 [H3N2]
A/PR8	A/Puerto Rico/8/43 [H1N1]
BCR	B cell receptor
CARD	Cytoplasmic DEx (D/H) box helicase cytoplasmic receptors
CFSE	Carboxyfluorescein succinimidyl ester
CpG	Unmethylated 2'-deoxyribo (Cytidine-phosphate-guanosine)
cRBC	Chicken red blood cells
DMEM	Dulbecco minimal essential medium
dpi	Days post-infection
dsRNA	Double stranded RNA
ECTV	Ectromelia virus
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescent activated cell sorting
FasL	Fas ligand
FcR	Fc receptor
FCS	Foetal calf serum
FPPS	Farnesyl diphosphate synthase enzyme
γ-A/PR8	Gamma-irradiated A/PR8
γ-SFV	Gamma-irradiated SFV
HA	Haemagglutinin protein
HAI	Haemagglutinin inhibition
HAU	Haemagglutinin unit
HRP	Horse radish peroxidase
IAV	Influenza A virus
IFN-I	Interferon type I
Ig	Immunoglobulin

IL-(1)	Interleukin-(1)
i.n.	Intranasal
i.p.	Intraperitoneal
IRF	Interferon regulatory factor
ISG	Type I IFN-I stimulated genes
i.v.	Intravenous
IVC	Individually ventilated cages
KGy	Kilo Gray
LAIV	Live attenuated influenza virus vaccine
LAS	Laboratory animal services
LPS	Lipopolysaccharide
M1	Matrix Protein 1
M2	Matrix Protein 2
mAbs	Monoclonal antibodies
MDA-5	Melanoma differentiation antigen 5
MDCK cells	Madin-Darby canine kidney cells
MHC-I	Major Histocompatibility molecule class I
MHC-II	Major Histocompatibility molecule class II
mRNA	Messenger RNA
MyD88	Myeloid Differentiation factor 88
NA	Neuraminidase protein
NF-κB	Nuclear factor kappaB
NK cells	Natural killer cells
NLR	Nod-like receptors
NP	Nucleoprotein
NPP	Nucleoprotein peptide
NS1	Non-structural Protein 1
NS2	Non-structural Protein 2
OAS	2' –5' oligoadenylate synthetase
PAMPs	Pattern-associated molecular patterns
PA1	Acidic Polymerase Protein 1
PA2	Acidic Polymerase Protein 2
PB1	Basic Polymerase Protein 1

PB2	Basic Polymerase Protein 2
pDC	Plasmacytoid dendritic cell
PKR	Protein kinase R
PRR	Pattern recognition receptor
Poly I:C	Polyinosinic: polycytidylic acid
RIG-1	Retinoic acid inducible gene-1
RNA	Ribonucleotide acid
ROS	Reactive oxygen species
RSV	Respiratory syncytial virus
RT	Room temperature
SA	Sialic Acid
SEM	Standard error of means
SFV	Semliki forest virus
ssRNA	Single stranded RNA
SU	Subunit vaccine
Tc	$CD8^+$ cytotoxic T cell
$TCID_{50}$	50% of Tissue culture infectious dose
TCR	T cell receptor
Th	$CD4^+$ helper T cell
TIR	Toll/interleukin 1 receptor (IL-1R) homology
TIV	Trivalent inactivated virus vaccine
TLR	Toll-like receptor
TMB	3, 3’, 5, 5’-Tetramethylbenzidine
Tmem	Memory T cell
TNF	Tumour necrosis factor
TNFL	Tumour necrosis factor ligand
TNFR	Tumour necrosis factor receptor
Viperin	Virus inhibitory protein, endoplasmic reticulum-associated, interferon-inducible
VNA	Virus neutralising assay
vRNP	Viral ribonucleoprotein complexes