

UNDERSTANDING INFLUENZA VIRUS INFECTIVITY IN PIGS



Christopher W. Olsen, DVM PhD

Professor of Public Health and Associate Dean for Academic Affairs
School of Veterinary Medicine, Univ. of Wisconsin-Madison, USA

SWINE INFLUENZA SEROPOSITIVITY IN THE U.S., 1976-1998

1976-77:

H1: 47.0%

H3: 1.4%

1988-89:

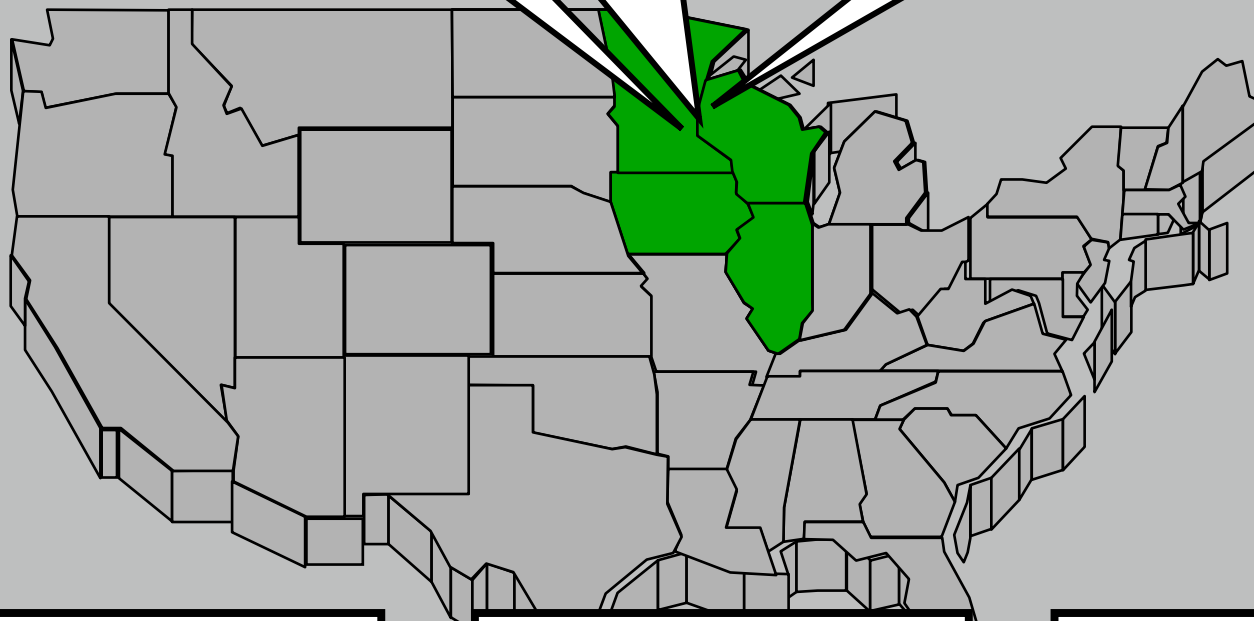
H1: 51.0%

H3: 1.1%

1997-98:

H1: 28.0%

H3: 8.0%

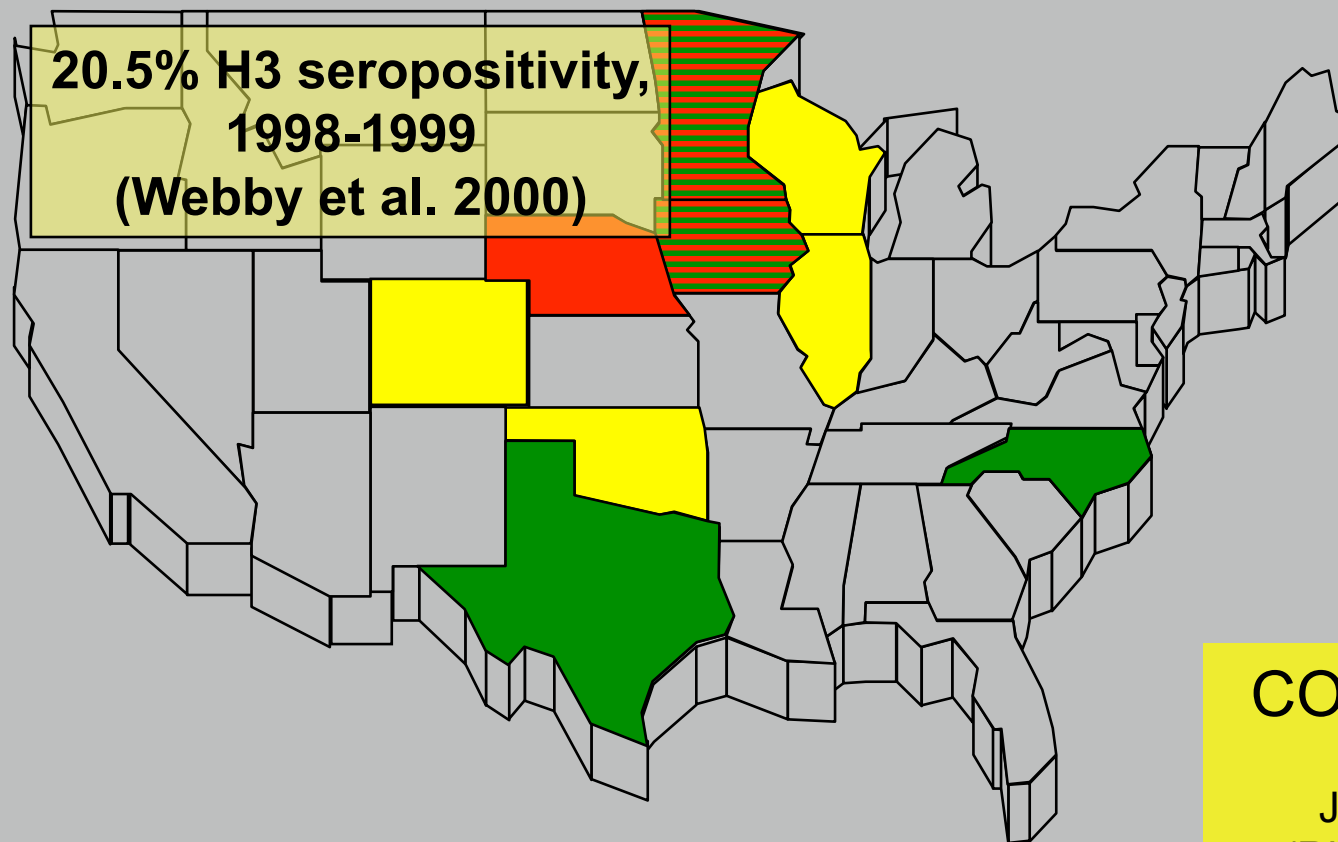


Hinshaw et al. 1978.
Virology 84:51-62

Chambers et al. 1991.
Arch. Virol. 116:261-265

Olsen et al. 2000.
Arch Virol. 145:1399-1419

EMERGENCE OF H3N2 VIRUSES AMONG PIGS IN THE UNITED STATES, 1998-2000



NB, IA, MN

3/98-3/99

(Karasin et al. 2000.
Virus Res. 68:71-85)

NC, TX, MN, IA

8/98-12/98

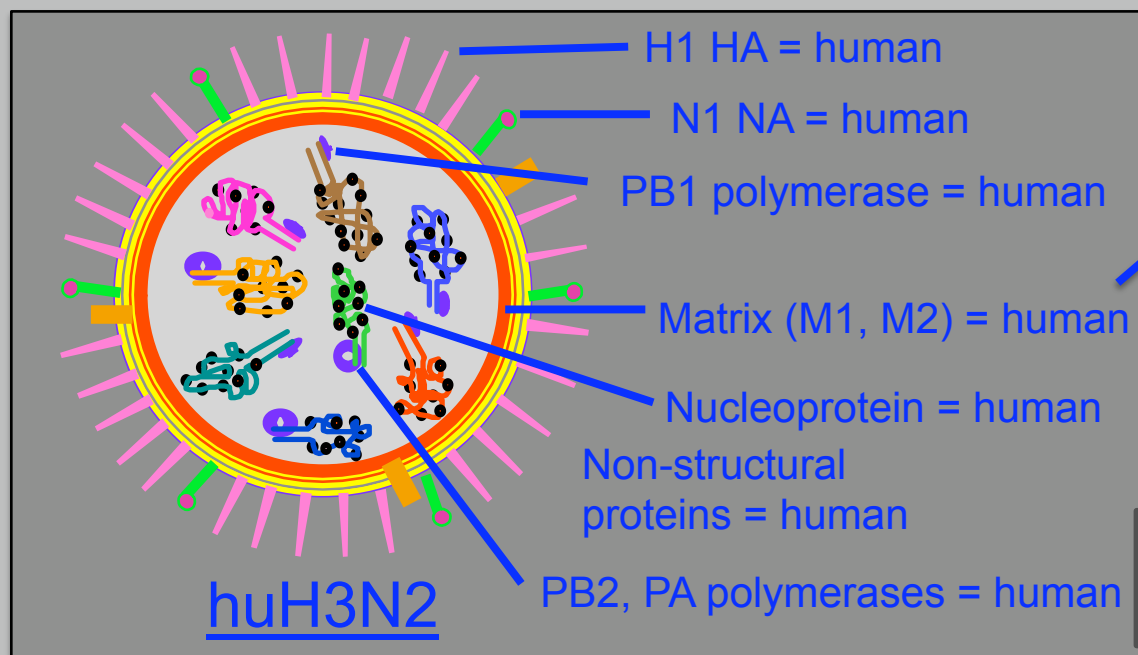
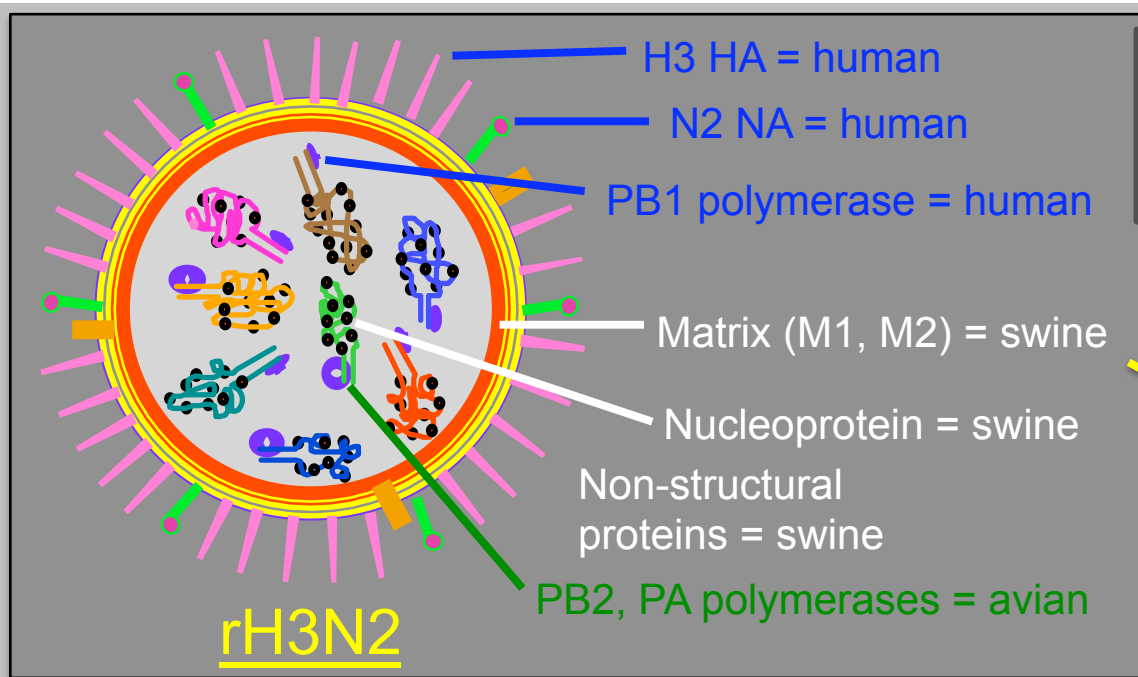
(Zhou et al. 1999.
J. Virol. 73:8851-8856)

CO, OK, WI, IL, NC

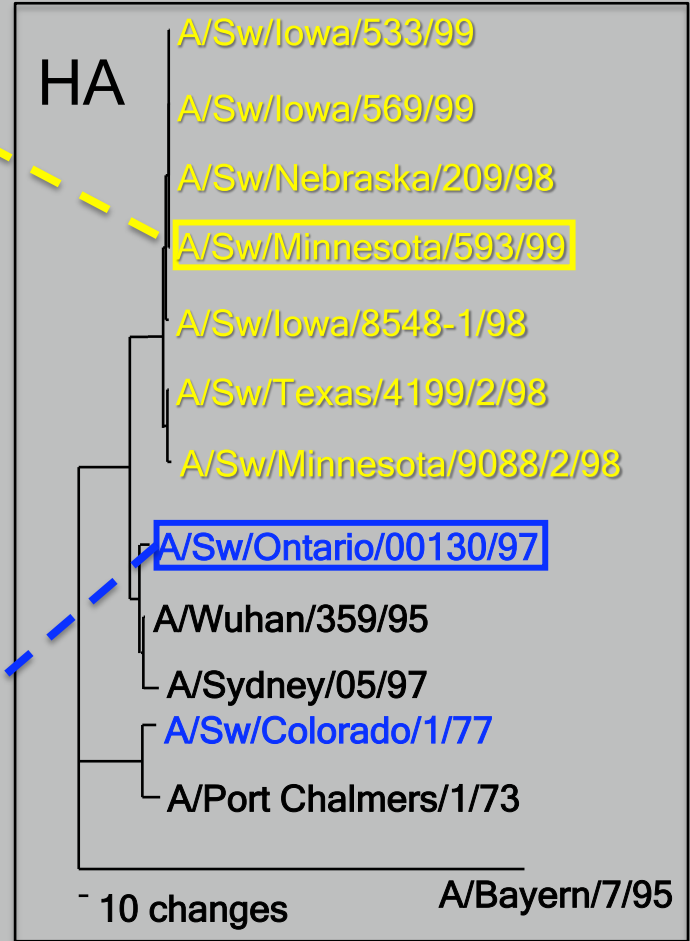
(Webby et al. 2000.

J. Virol. 74:8243-8251;

(Richt et al. 2003. J. Clin.
Microbiol. 41:3198-3205)



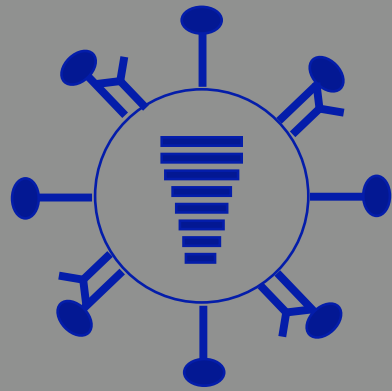
Spread throughout North America subsequent to initial appearance



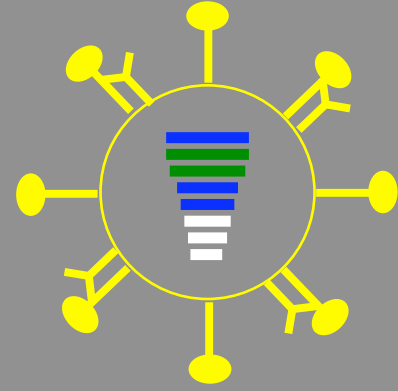
Not reisolated subsequent to initial appearance

(Karasin et al. 2000. Virus Res. 68:71-85)

INITIAL *IN VIVO* EXPERIMENTS



A/Sw/Ontario/00130/97



A/Sw/Minnesota/593/99



Infectivity

Nasal shedding

Lung Pathology

Serology

(Landolt et al. 2003. J. Clin. Microbiol. 41:1936-41)

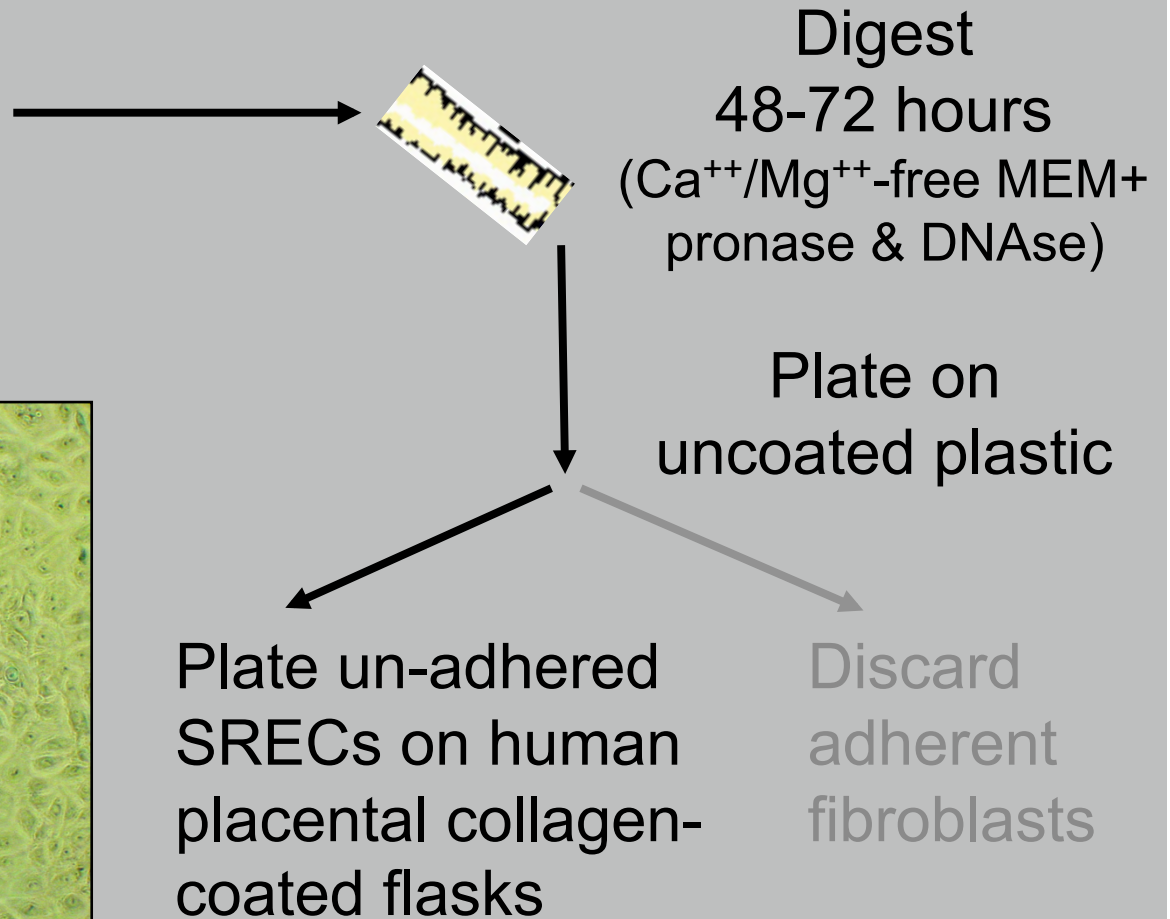
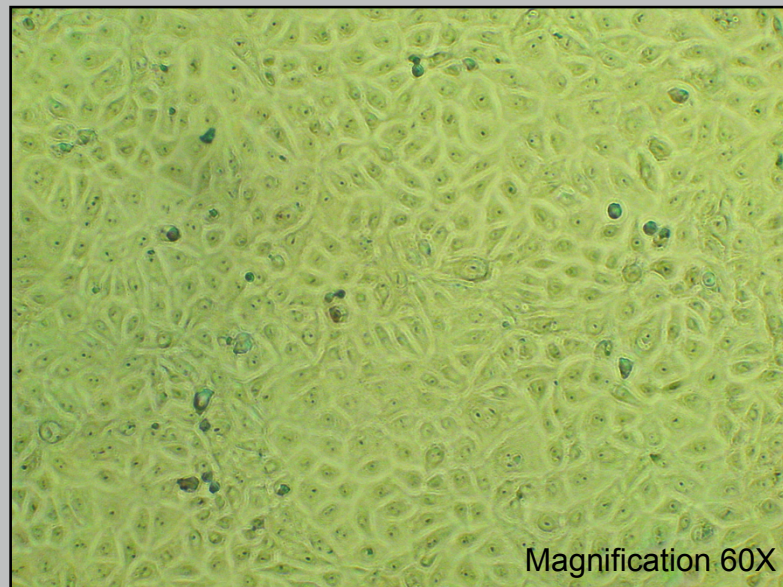
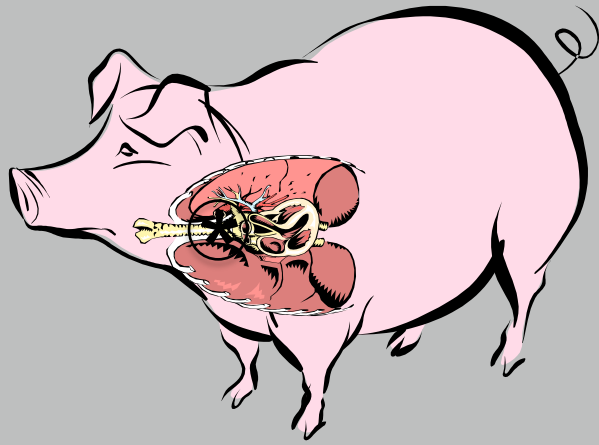
INITIAL *IN VIVO* EXPERIMENTS

	<u>A/Sw/Ontario/00130/97</u>	<u>A/Sw/Minnesota/593/99</u>
Infectivity	Limited infectivity	Infectious even at low doses
Shedding	Delayed shedding kinetics	Shedding kinetics similar to classical swine viruses
Lung pathology	Mild lesions	Moderate to severe lesions
Serology	Absent/Low HI titers	High HI titers

Virus phenotype reversed by exchanging the HA + NA genes

(Landolt et al. 2003. J. Clin. Microbiol. 41:1936-41; 2006. J. Clin. Microbiol. 44:297-301)

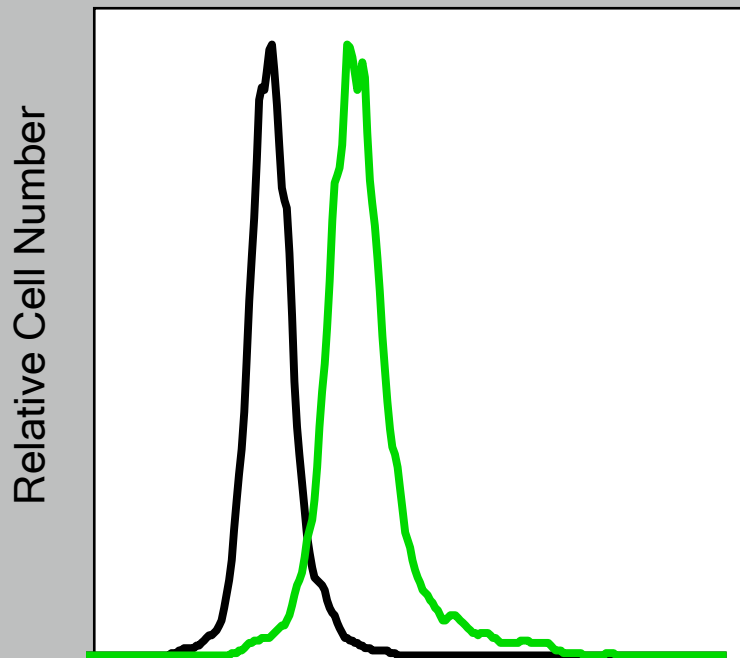
ISOLATION OF SWINE RESPIRATORY EPITHELIAL CELLS (SRECS)



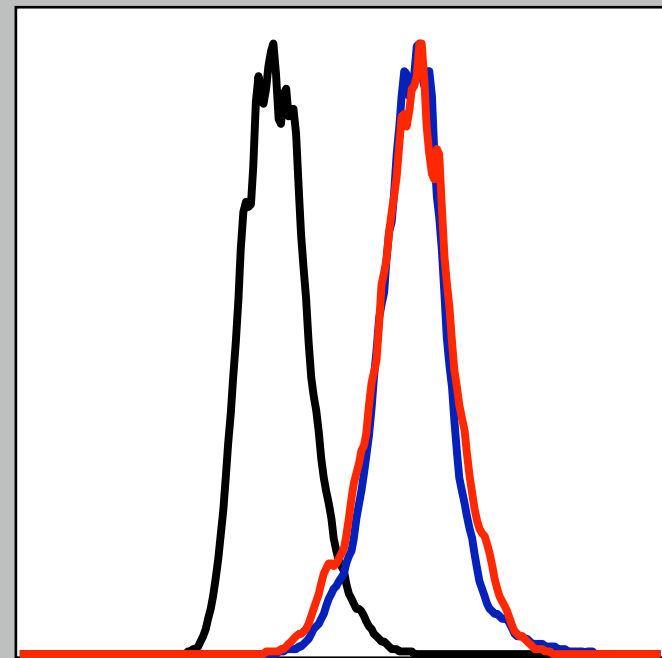
(Busch et al. 2008. Virus Research 133:269-279)

SREC ARE A MONOMORPHIC POPULATION OF CYTOKERATIN⁺ AND SA⁺ CELLS

Cytokeratin positive



2,6 and 2,3 SA positive



■ Anti-Cytokeratin
■ Isotype Control

■ SNA (2,6)
■ MAA (2,3)
■ Mock

(Busch et al. 2008. Virus Research 133:269-279)

INFECTION PROTOCOL

Inoculate cells with 3 TCID₅₀ / cell



Incubate 1 hr at 37°



Wash cells 2x



Incubate cells 11 hrs 37°



Formalin fix cells



Immunocytochemistry
or FLOW staining (α NP)

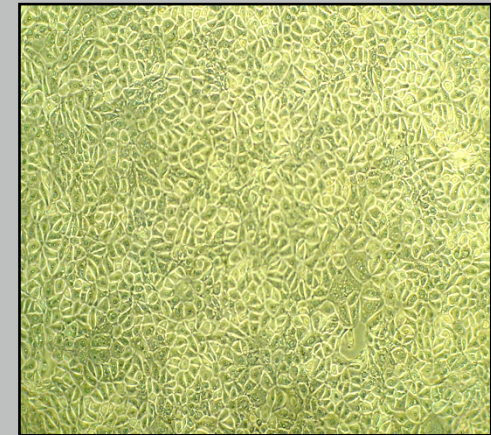
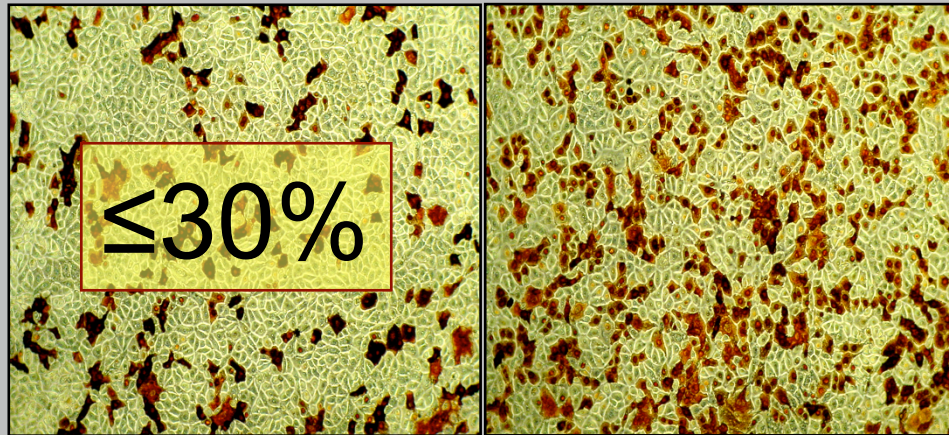
Single round of
infection –
no trypsin

INFECTIVITY IN SRECS

A/Sw/ONT/00130/97

A/Sw/CO/1/77

Mock

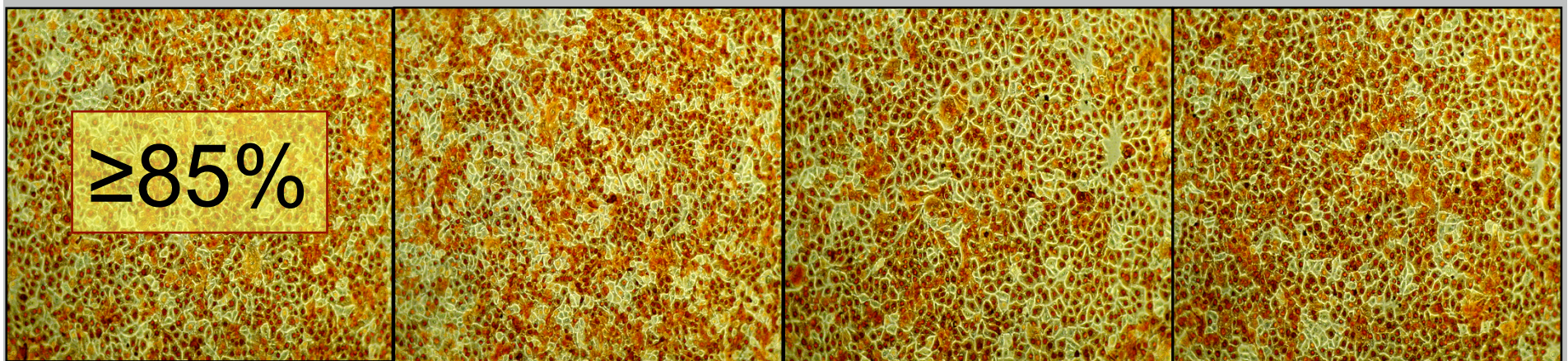


A/Sw/MN/593/99

A/Sw/IA/569/99

A/Sw/IA/533/99

A/Sw/NE/209/98



Magnification 30X

(Busch et al. 2008. Virus Research 133:269-279)

***Which viral gene(s) are
responsible for the
difference in infectivity?***

REASSORTANT VIRUSES

 MN

12 plasmid rg system
Neumann et al. 1999.
PNAS 96:9345-9350

 ONT

rgMN

PB1
PB2
PA
HA
NP
NA
M
NS

rgONT

PB1
PB2
PA
HA
NP
NA
M
NS

rgMN+
ONT HA/NA

PB1
PB2
PA
HA
NP
NA
M
NS

rgONT+
MN HA/NA

PB1
PB2
PA
HA
NP
NA
M
NS

rgMN+
ONT HA

PB1
PB2
PA
HA
NP
NA
M
NS

rgONT+
MN HA

PB1
PB2
PA
HA
NP
NA
M
NS

rgMN+
ONT NA

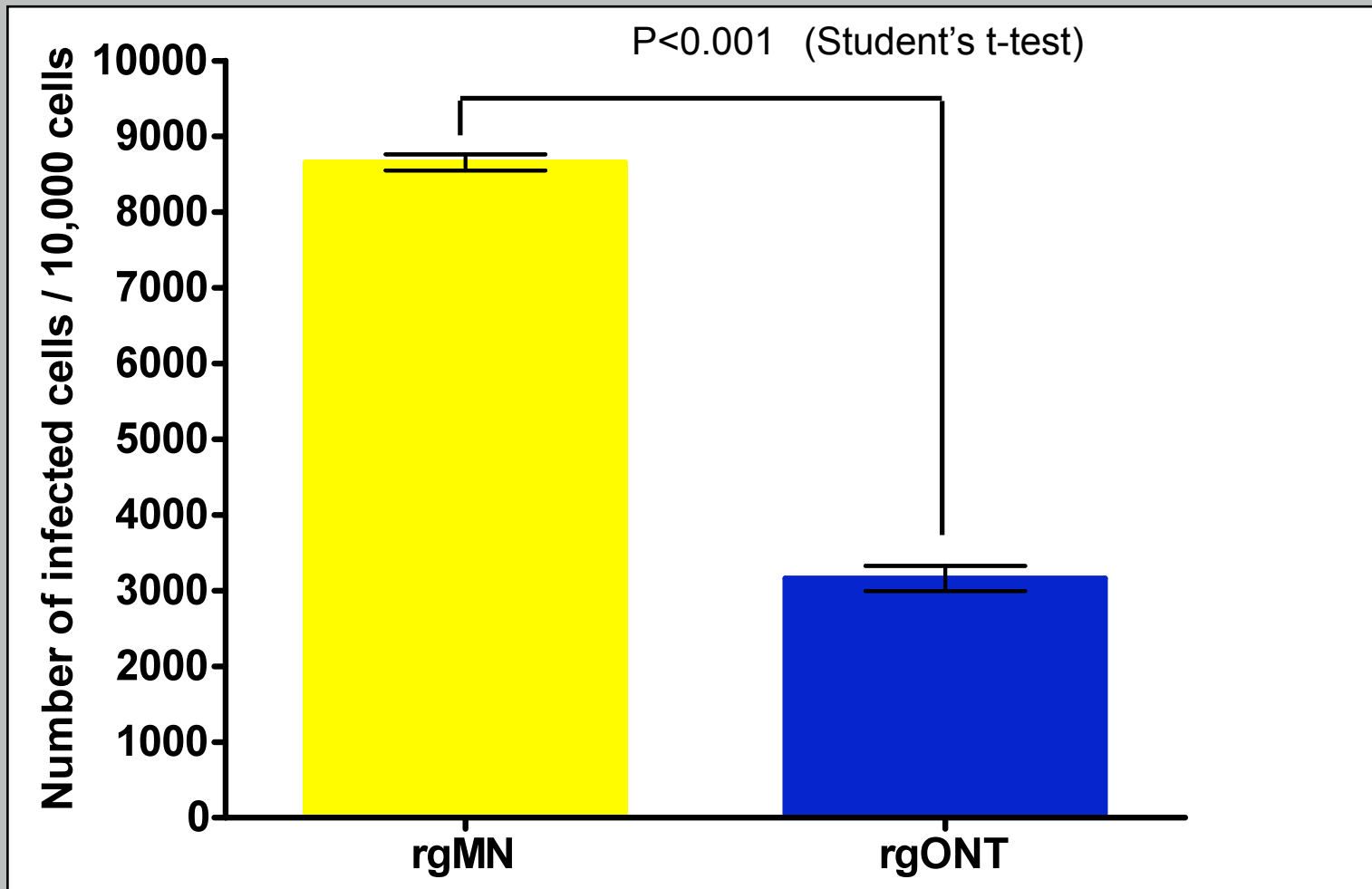
PB1
PB2
PA
HA
NP
NA
M
NS

rgONT+
MN NA

PB1
PB2
PA
HA
NP
NA
M
NS

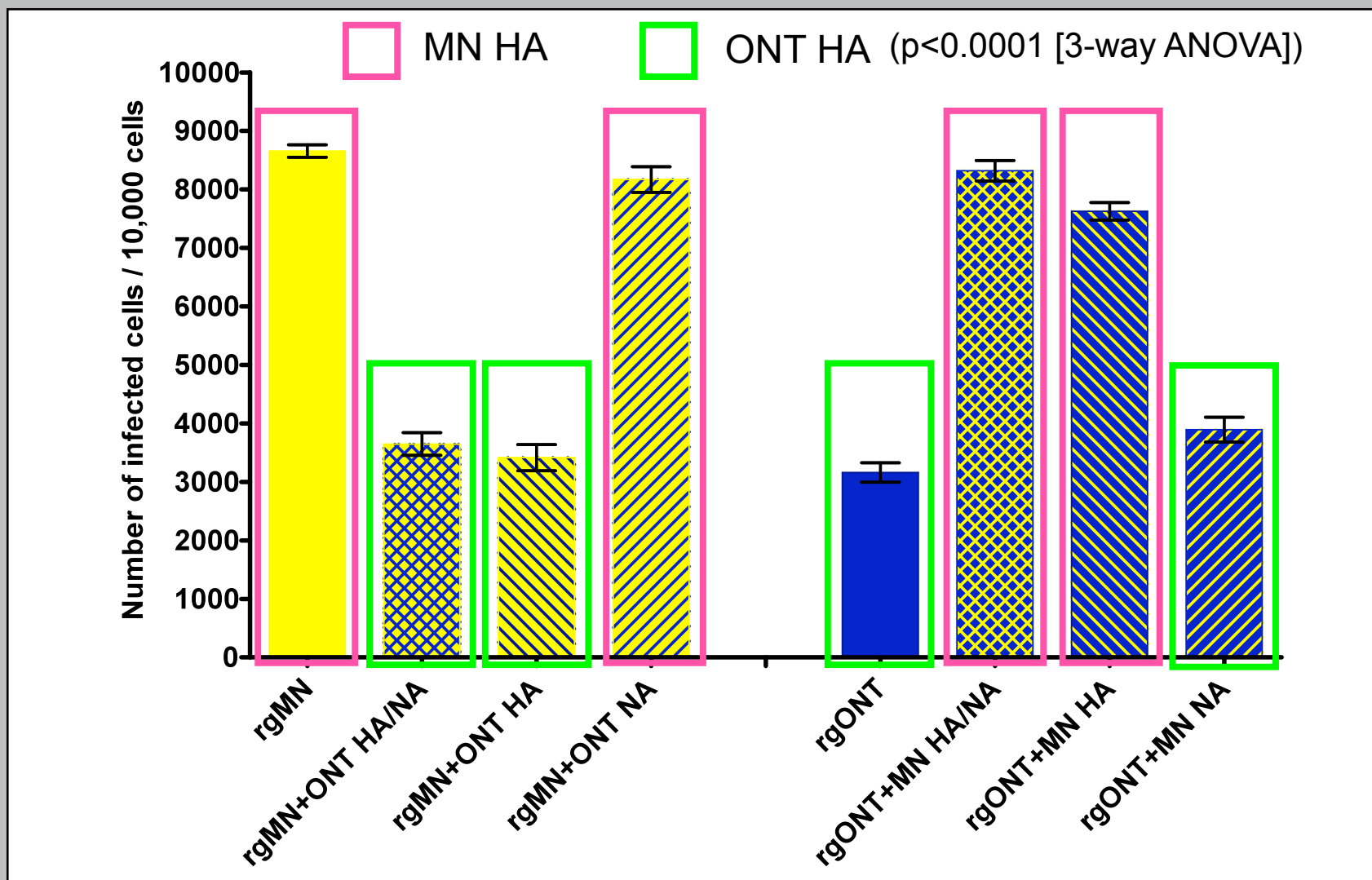
(Busch et al. 2008. Virus Research 133:269-279)

COMPARISON OF INFECTIVITY OF MN VS. ONT IN SRECS (FLOW CYTOMETRY)



(Busch et al. 2008. Virus Research 133:269-279)

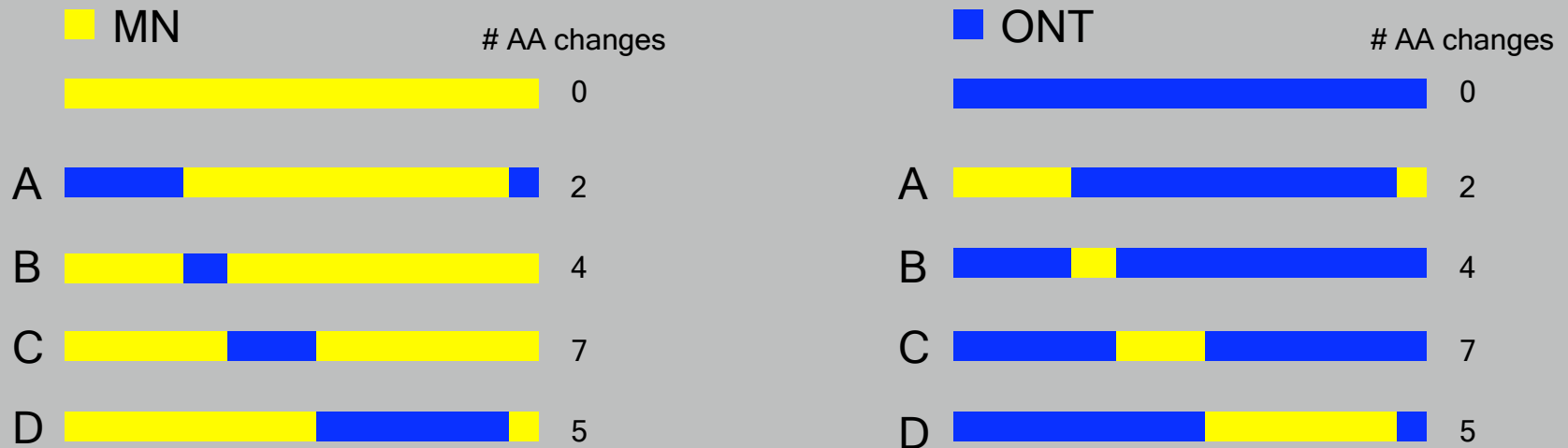
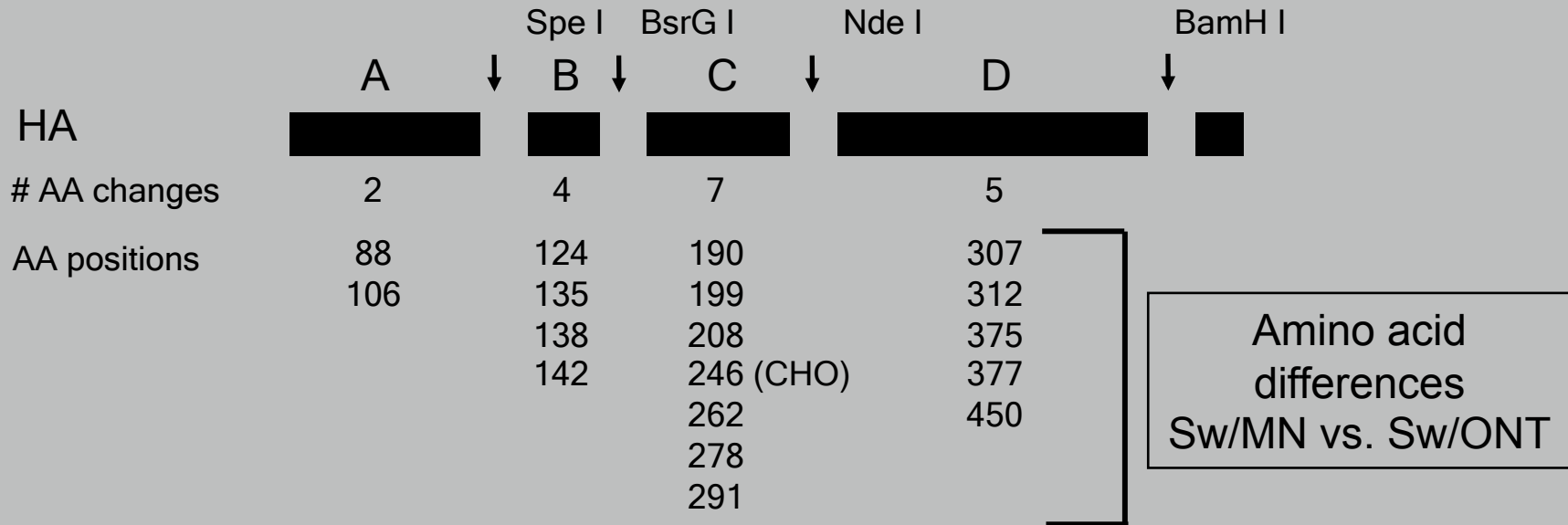
INFECTIVITY OF REASSORTANT VIRUSES



(Busch et al. 2008. Virus Research 133:269-279)

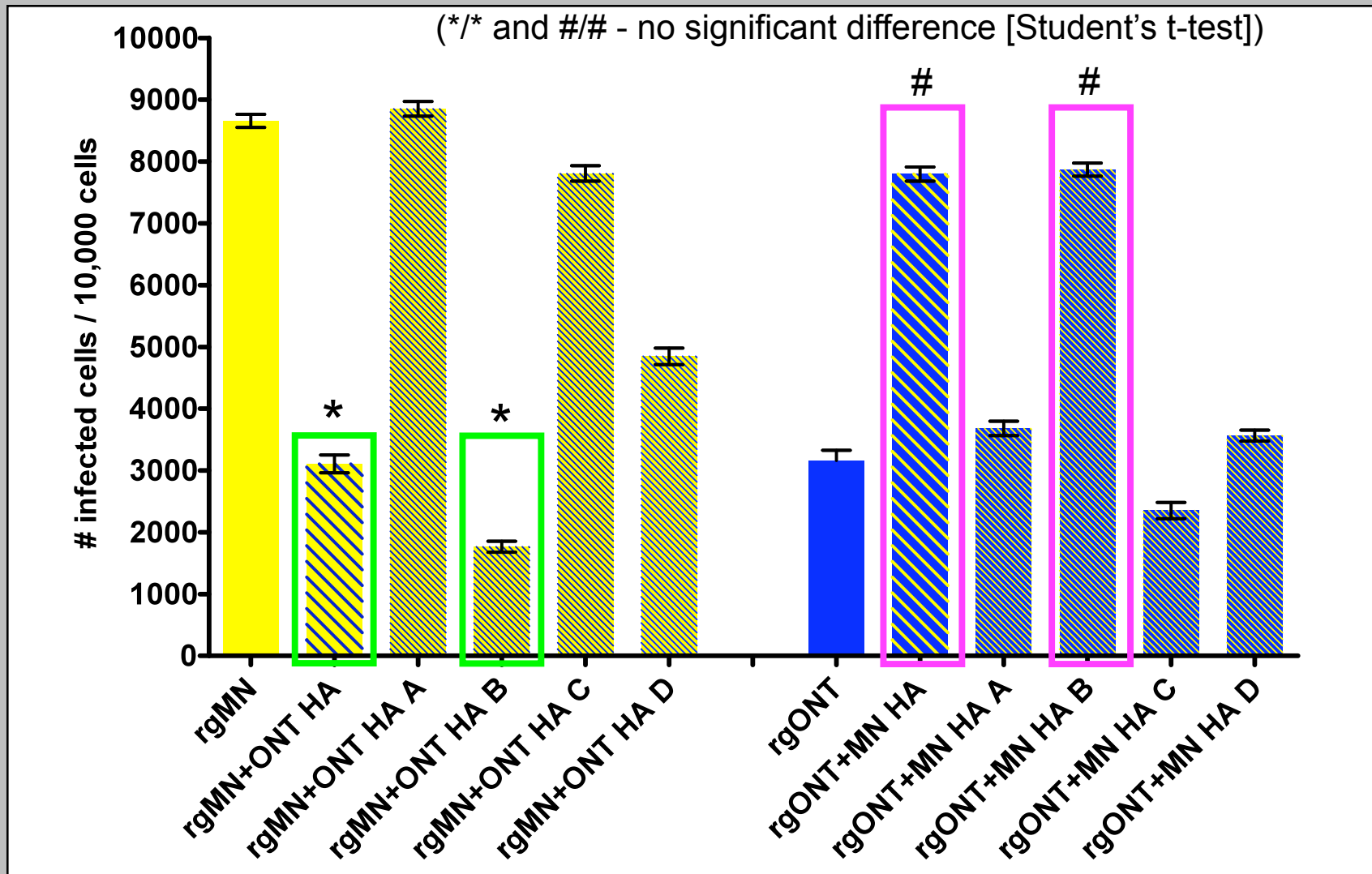
***Which amino acids
are responsible for
the differences in infectivity?***

GENERATION OF HA CHIMERIC VIRUSES



(Busch et al. 2008. Virus Research 133:269-279)

INFECTIVITY OF HA CHIMERAS



(Busch et al. 2008. Virus Research 133:269-279)

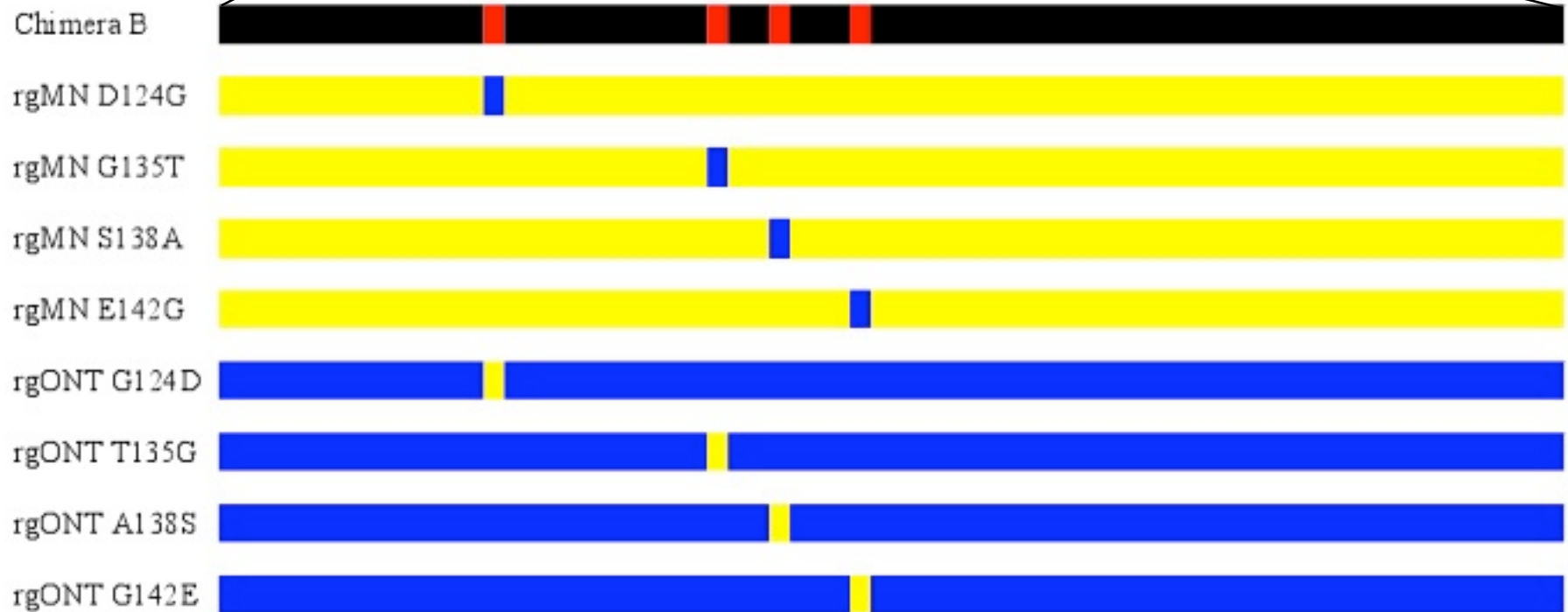
HA B POINT MUTATIONS

Spe I BsrG I



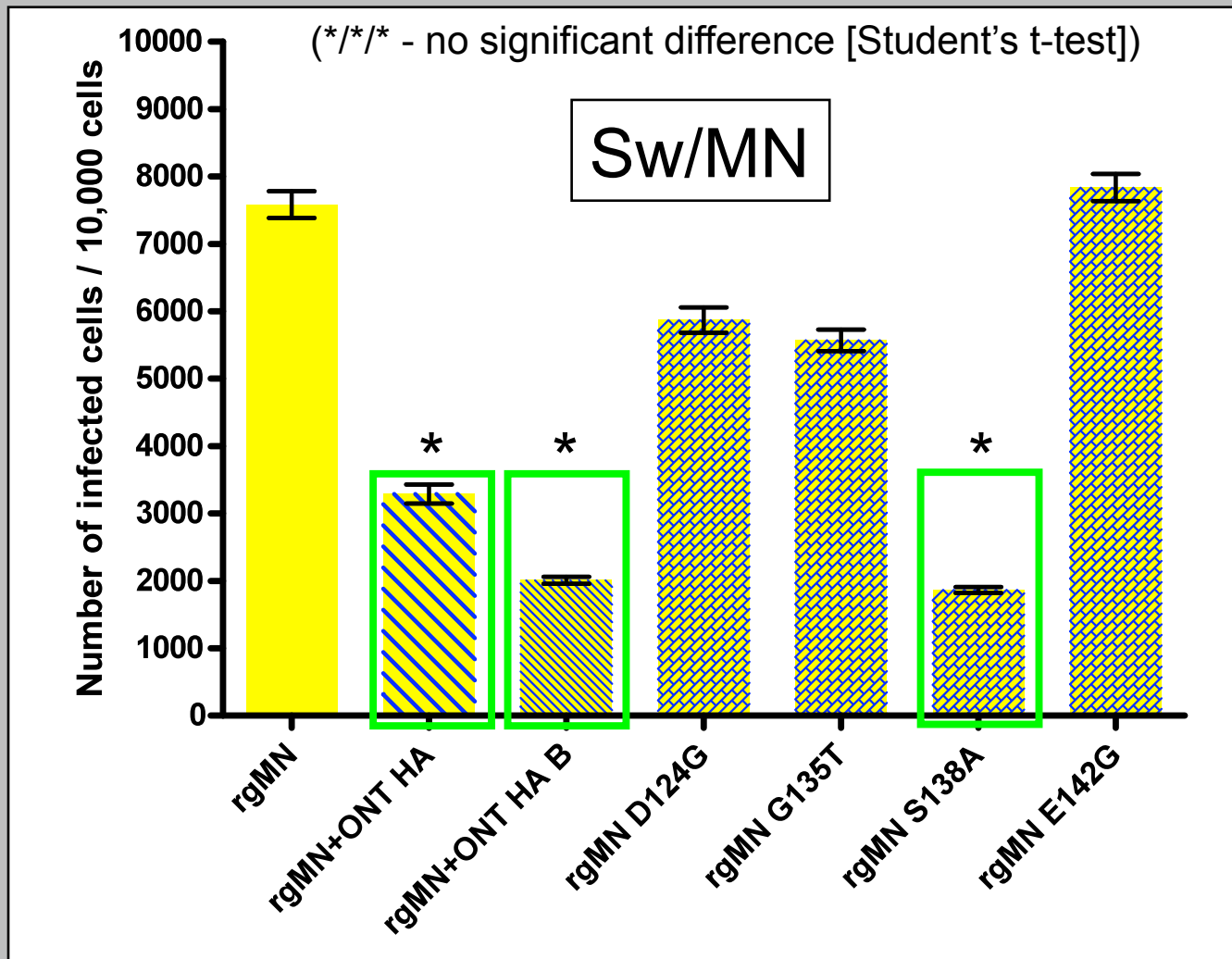
124

135 138 142



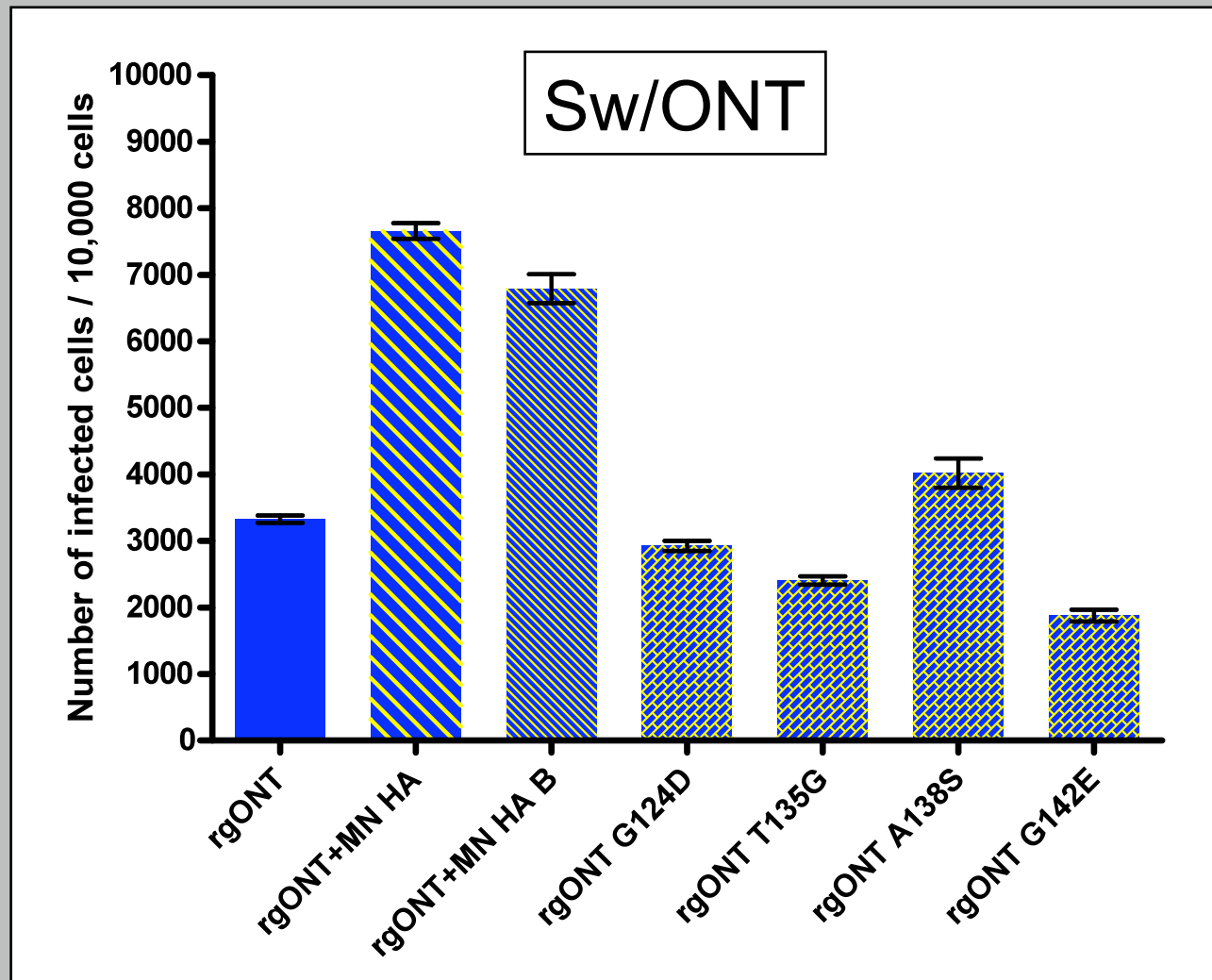
(Busch et al. 2008. Virus Research 133:269-279)

INFECTIVITY OF rgMN HA POINT MUTANTS



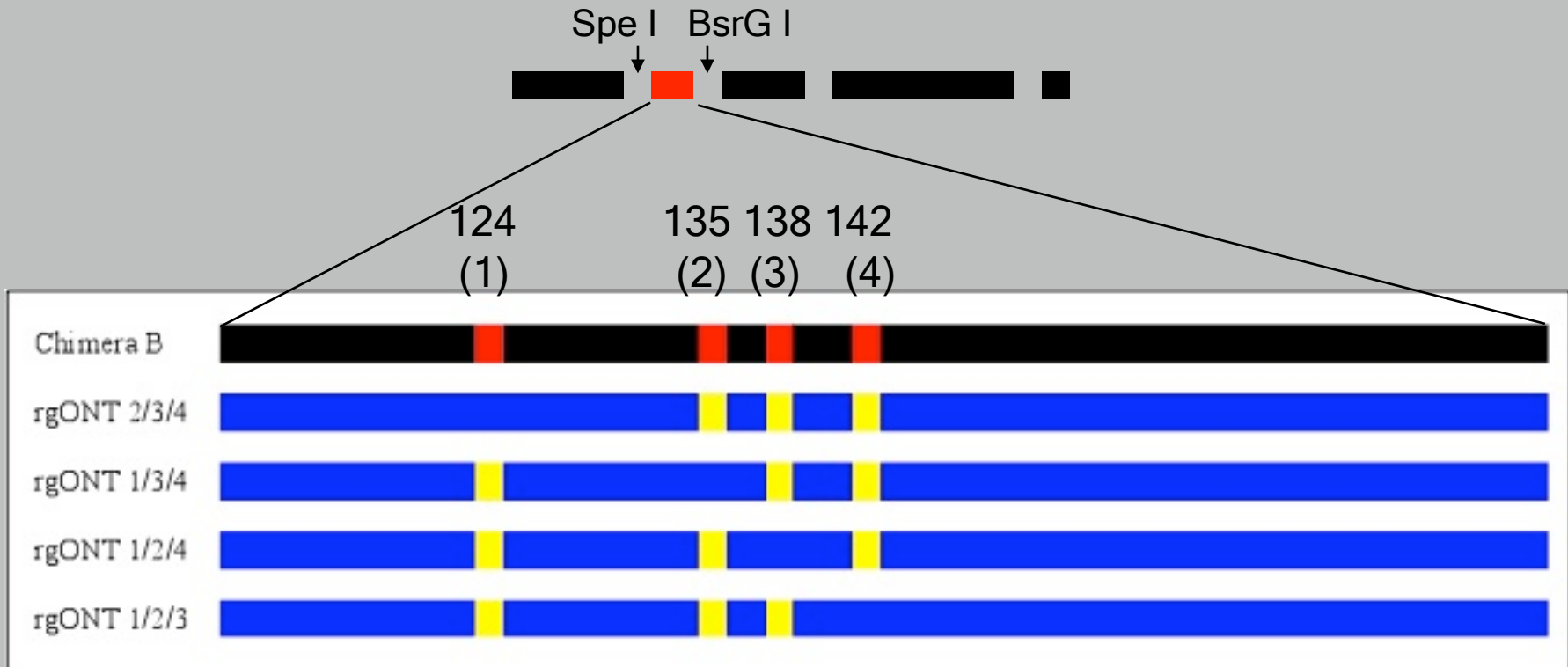
(Busch et al. 2008. Virus Research 133:269-279)

INFECTIVITY OF rgONT HA POINT MUTANTS

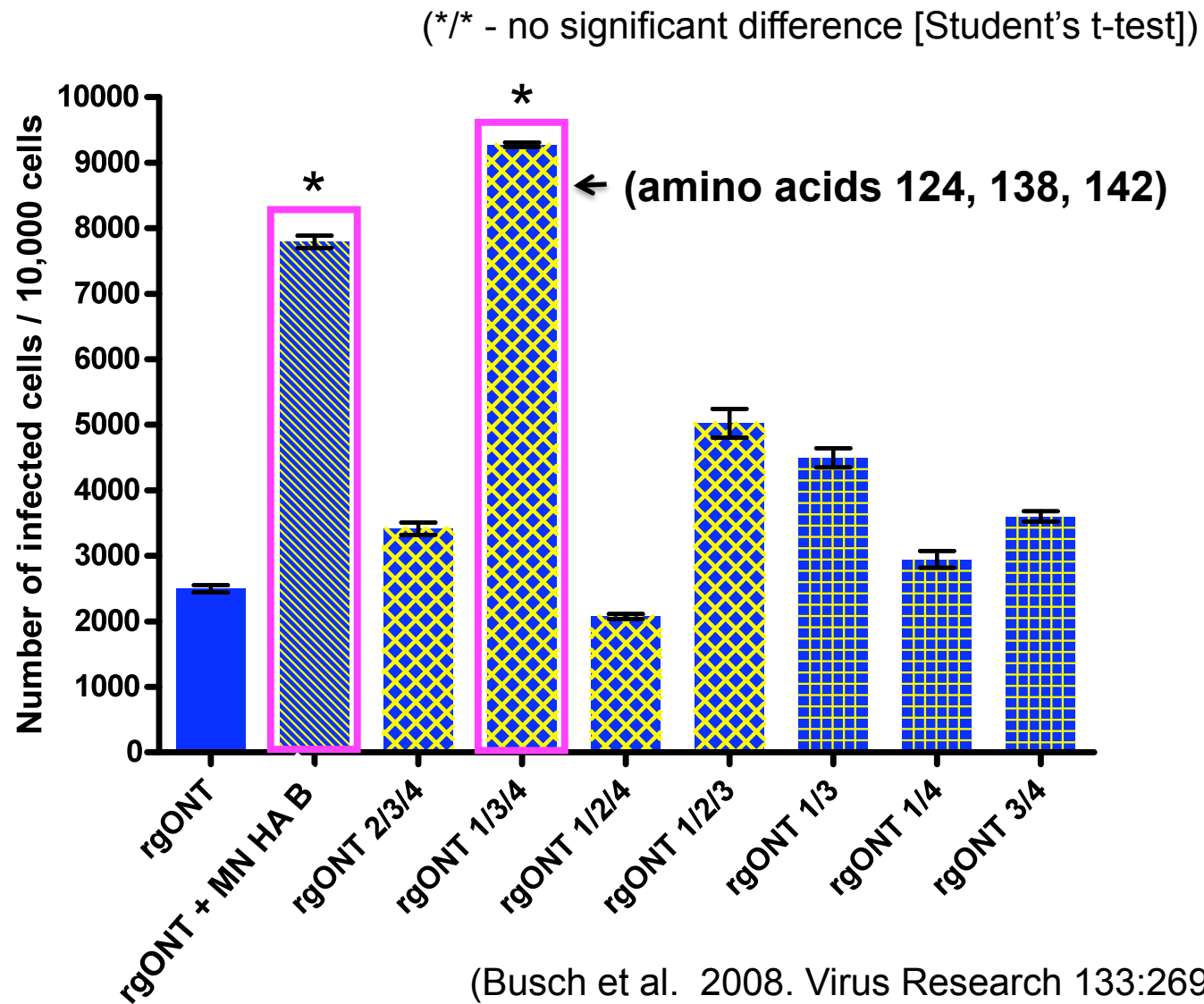


(Busch et al. 2008. Virus Research 133:269-279)

rgONT TRIPLE POINT MUTATIONS



MINIMAL AMINO ACID CHANGES NEEDED TO INCREASE rgONT INFECTIVITY



ISOLATION AND CHARACTERIZATION OF H4N6 AVIAN INFLUENZA VIRUSES FROM PIGS WITH PNEUMONIA IN CANADA

Karasin et al. 2000. J. Virol. 74:9322-9327.

- Approximately 5% of 2,600 pigs affected
 - coughing, weight loss, labored breathing, 12 deaths
- Virus isolation in MDCK cells from lung tissue
- All 8 gene segments of avian-lineage
- Farm used nearby lake water for barn cleaning
- Amino acids at HA 226/228 (H3 numbering) differed from avian consensus (Q226L, G228S)



HA AMINO ACIDS 226 AND 228

- Receptor preference for H3 (H2, H9) influenza viruses
 - Connor et al. 1994. Virology 205:17-23
 - Rogers et al. 1983. Nature 304:76-78
 - Matrosovich et al. 2000. J. Virol. 74: 8502-8512
 - Matrosovich et al. 2001. Virology 281:156-162
- H3 and H4 are phylogenetically closely related
 - Nobusawa et al. 1991. Virology 182:475-485

***Do residues 226 and 228
control binding preference,
and/or infectivity,
of H4 Sw/ONT/99?***

HA AMINO ACIDS 226 AND 228

Virus Name	Residue 226	Residue 228
rgSw/ONT/99	L (2,6*)	S (2,6)
L226Q	Q (2,3)	S
S228G	L	G (2,3)
L226Q/S228G	Q (2,3)	G (2,3)

(* 2,3/2,6 predicted binding based on: Conner et al. 1994. Virology 205:17-23; Matrosovich et al. 1997. Virology 233:224-234; Rogers et al. 1983. Nature 304:76-78)

(Bateman et al. 2008. J. Virol., in press)

226 AND 228 ROLE IN SIALIC ACID BINDING

Virus	2,3 SL		2,3 SLN		2,6 SLN	
	app K_d	R^2	app K_d	R^2	app K_d	R^2
rgSw/ONT/99	288	0.88	371	0.90	4	0.99
L226Q	311	0.91	314	0.96	90	0.89
S228G	443	0.88	389	0.90	3	1.00
L226Q/S228G	31	0.87	25	0.83	156	0.89

(Lower apparent K_d = higher affinity binding)

226 AND 228 ROLE IN SREC INFECTIVITY

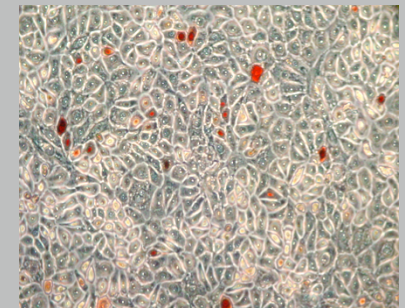
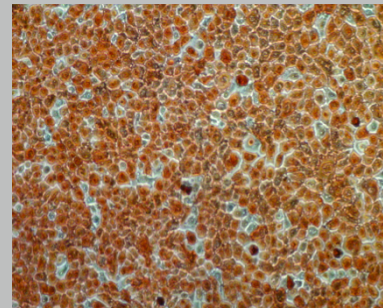
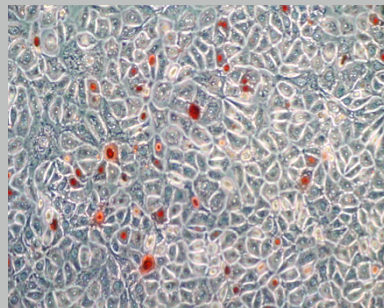
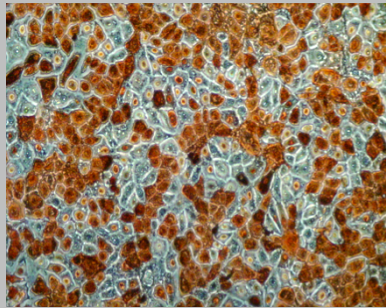
rgSw/ONT/99

L226Q

S228G

L226Q/S228G

SREC



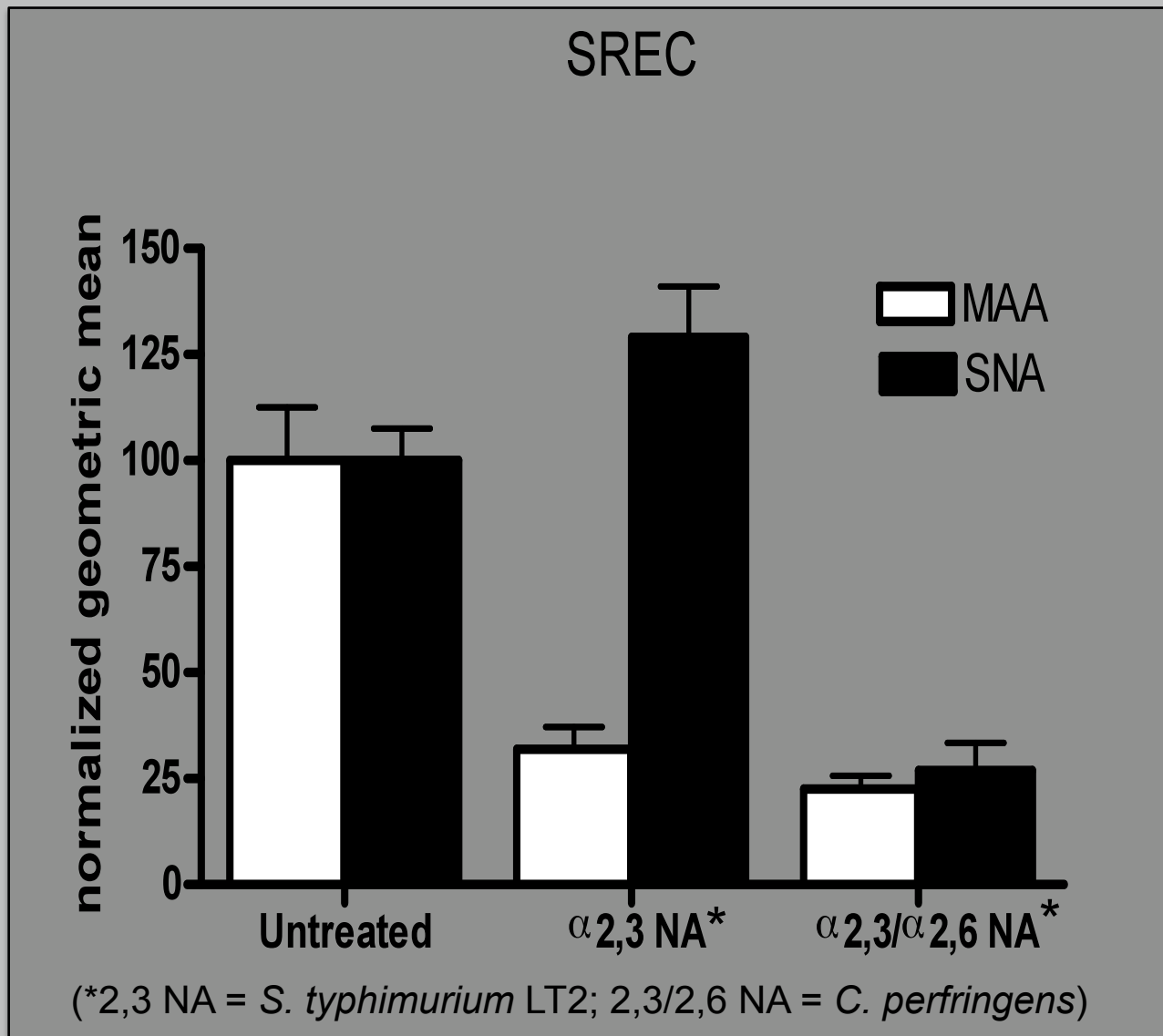
(Bateman et al. 2008. J. Virol., in press)

SIALIC ACID UTILIZATION FOR INFECTION OF SREC

Virus	Affinity for 3'SL	Affinity for 6'SLN	SREC Infectivity
Parent	Low	High	High
L226Q	Low	Low	Low
S228G	Low	High	High
L226Q/S228G	High	Low	Low

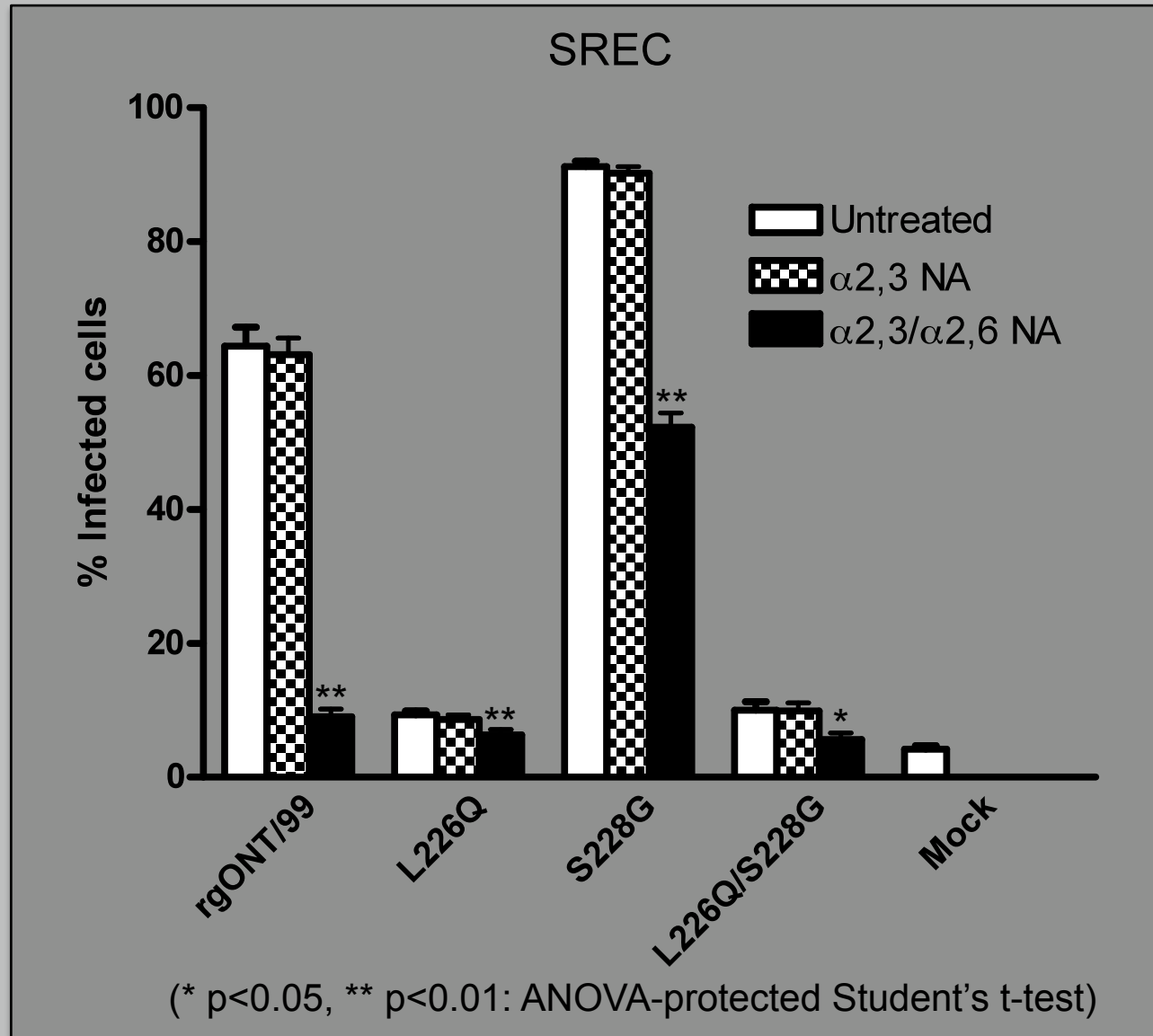
(Bateman et al. 2008. J. Virol., in press)

SA REMOVAL VIA NA TREATMENT



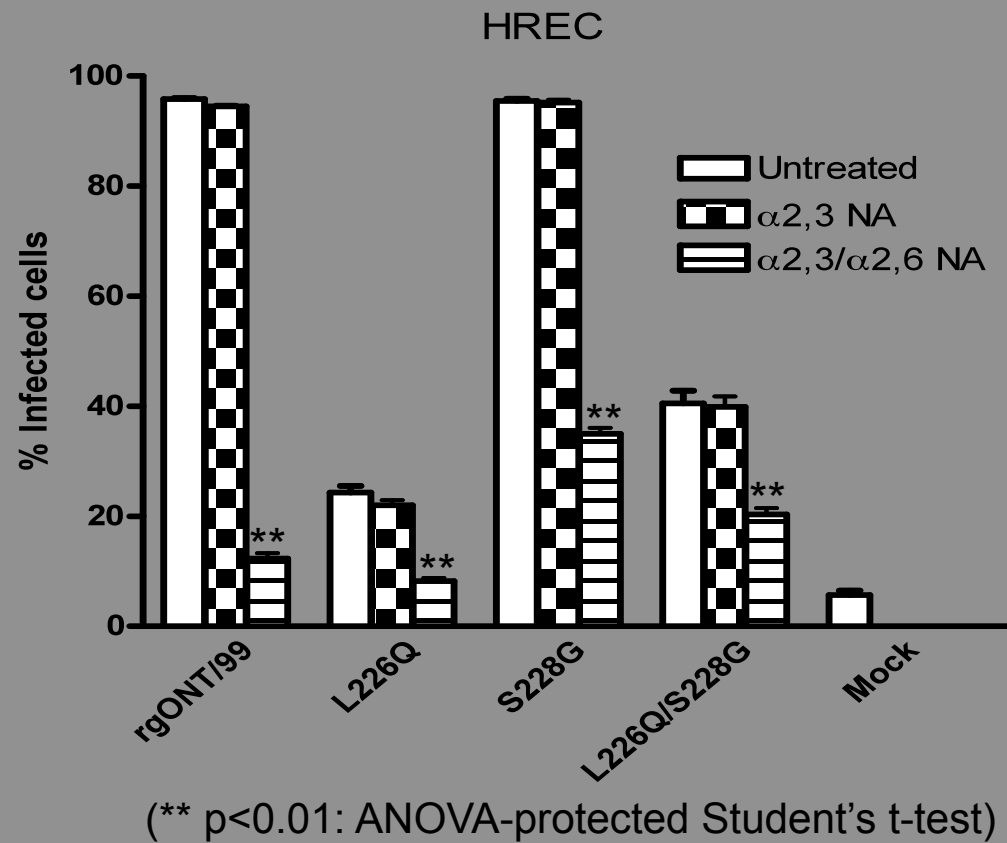
(Bateman et al. 2008. J. Virol., in press)

INFECTIVITY AFTER NA TREATMENT



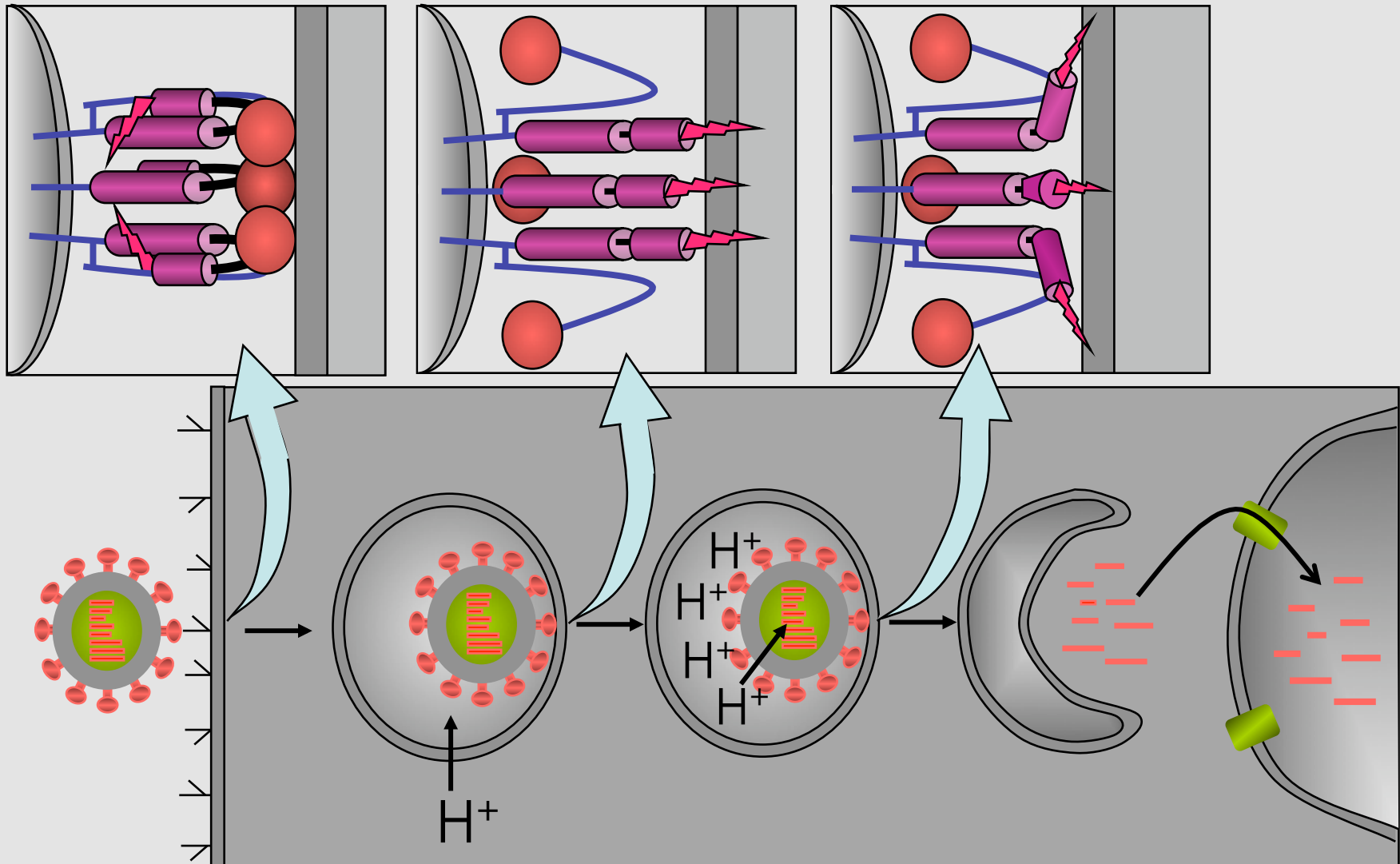
(Bateman et al. 2008. J. Virol., in press)

226 AND 228 ROLE IN HREC INFECTIVITY



(Bateman et al. 2008. J. Virol., in press)

INFLUENZA VIRUS ENTRY



(G. Landolt)

CONCLUSIONS (H3N2)

- Early triple reassortant viruses exhibit higher infectivity in SRECs than previous H3N2 swine isolates
 - Important in population-level emergence?
- Infectivity phenotype is controlled by HA
 - Amino acid 138 is a major determinant of infectivity
 - S138A decreases infectivity of Sw/MN to the level of Sw/ONT
 - A138S + G124D & G142E increases infectivity of Sw/ONT to the level of Sw/MN
- Mechanism?
- Relationship between *in vitro* infectivity & *in vivo* infectivity and transmissibility at aa level?
 - On-going experiments

CONCLUSIONS (H4N6)

- Residues 226 and 228 control sialic acid binding
- Both 226Q and 228G are necessary for high affinity binding to SA α 2,3Gal
- Amino acid 226 alone can determine affinity for SA α 2,6Gal, *AND* is also the major determinant of SREC infectivity

(Replication of H3 and H9 viruses in cultures of human airway epithelium

–Matrosovich et al. 2007. Virology 361:384-390

–Wan & Perez. 2007. J. Virol. 81:5181-5191)

- Regardless of 2,3/2,6 receptor binding preference, the H4N6 viruses predominantly utilize SA α 2,6Gal to enter SRECs (and HRECs)

(Bateman et al. 2008. J. Virol., in press)



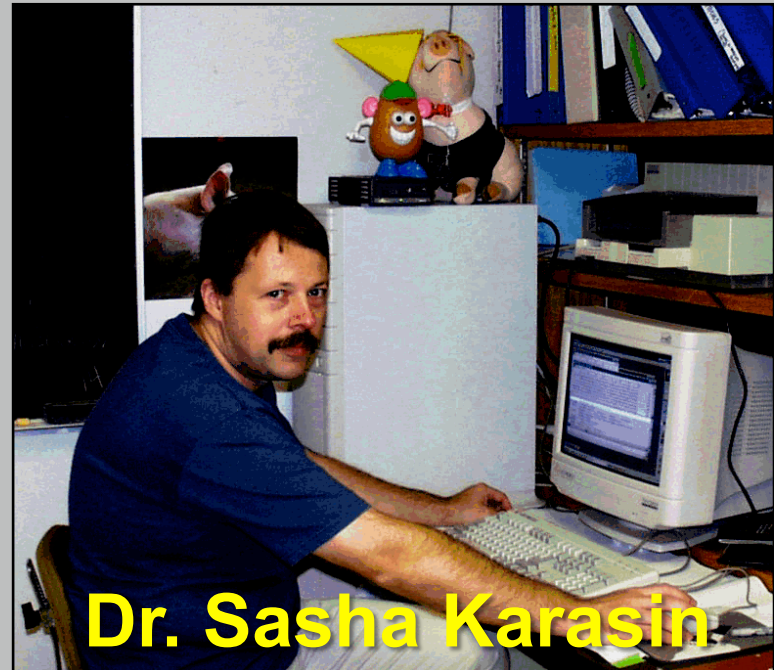
Dr. Gabriele Landolt



Dr. Marc Busch



Mr. Allen Batemann



Dr. Sasha Karasin

- 
- UW School of Veterinary Medicine
 - M. Suresh
 - UW School of Medicine and Public Health
 - Rebecca Brockman-Schneider
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