M2e-specific assays

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M2 of influenza A virus (IAV)

- Non-glycosylated transmembrane protein (97aa): 24 external, 19 transmembrane, 54 internal (Lamb et al.,PNAS 1981; Cell 1985).
- Forms homotetramers with pH-inducible proton transfer activity (Holsinger and Lamb, Virology 1991; Sugrue and Hay, Virology 1991; Steinhauer et al. PNAS 1991; Pinto et al. Cell 1992)
- Expressed at high density (~50% of HA) in plasma membrane of infected cells at time of virus maturation but low density (~2% of HA) in membrane of mature virus particles (Zebedee and Lamb,J Virol 1988).
- Antibodies specific for M2 ectodomain (M2e) restrict virus replication in vitro and in vivo (Zebedee and Lamb, PNAS 1989; Treanor et al. J Virol 1990).
- M2e remained relatively conserved in human isolates since 1918.

Measurement of M2e-specific Ab titers:

Functional assays: Reduction of virus yield or plaque size (not M2e-specific in presence of HA and NA-specific Abs).

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Ab binding assays (ELISA):

- **M2e peptide** (may not detect Abs specific for conformational determinants of mature tetrameric M2e)

- **M2-transfected cells**

  HeLa "Tet-on" cells (M2 under control of minimal CMV promoter and Tet control element; low constitutive expression can be upregulated by doxycycline): stable wt M2 (HeLa-M2) and control (HeLa-C10) transfectants.

ELISA using M2-transfected cells as immunosorbents.

ELISA performance:

HeLa-M2 and HeLa-C10 seeded into wells of flat bottom 96-well microtiter plates, 1/2 plate each.

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Grow for two days in presence of doxycycline to induce max. M2 expression.

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Fix cell monolayers with glutaraldehyde. Block and store at 4°C until use.

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Titer serum samples in parallel on HeLa-M2 and HeLa-C10.

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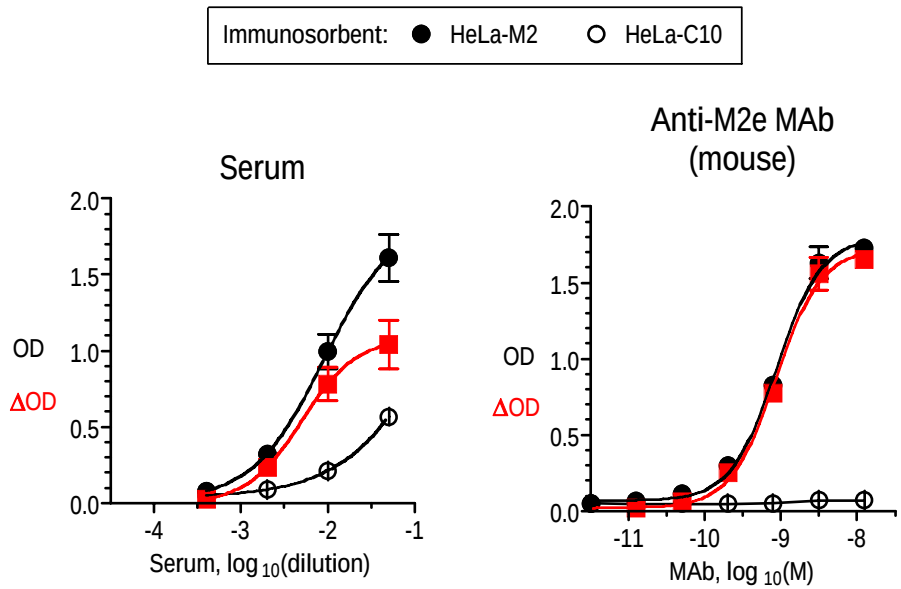
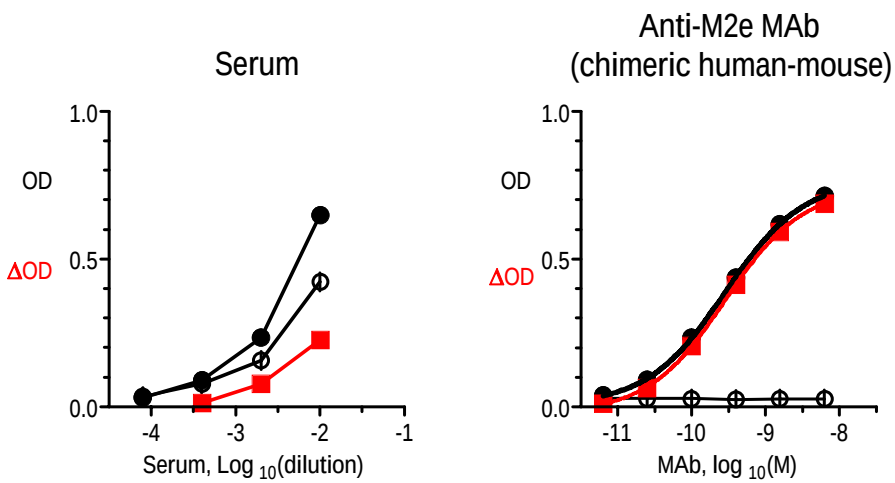
Use Δ OD (OD vs HeLa-M2 minus OD vs HeLa-C10) to quantify M2e-specific Ab concentration relative to a purified M2e-specific Ab standard.

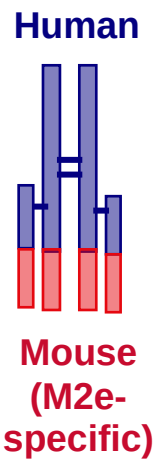
ELISA using M2-transfected cells as immunosorbents.

Assay of mouse sera:
Developed with rat-anti- mouse Cκ.



Assay of human sera:
Developed with mixture of goat-anti-human Cκ,Cλ.



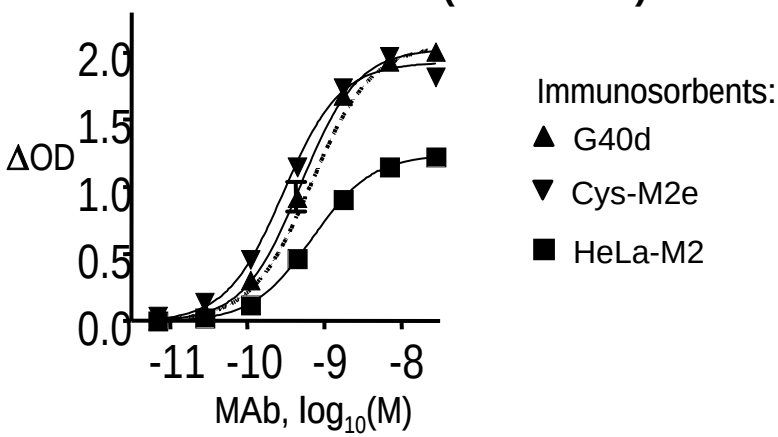


ELISA using M2e peptides as immunosorbents.

Specific immunosorbent:	Nonspecific immunosorbent:	ΔOD
G40d: M2e M2e S1 S1 M2e M2e A-K-G-K-G-K-G-C---C-G-K-G-K-G-K-A	BSA →	**Immunogen-specific Ab titer**
Cys-M2e: M2e M2e C-G-K-G-K-A	C-G-K-G-K-A →	**M2e peptide-specific Ab titer**

S1: SFERFEIFPKE **M2e**: SLLTEVETPIRNEWGSRSNDSSDP

Standard MAb (14C2-S1-4)



Three major M2e Ab specificities

Reaction in ELISA with:			Immunization:	
M2e peptide (2-24)	HeLa-M2	Ab specificity	M2e-MAP	Infection
+	-	M2e(pep)	~85%	
+	+	M2e(pep-nat)	~15%	~40%
-	+	M2e(nat)		~60%

M2e(pep-nat)- but not M2e(pep)-specific serum Abs correlate with protection after parenteral immunization of mice with M2e-MAP.

Procedure:

M2e-MAP + Adj. sc/im/ip, 2-3x

↓

Determine Ab titer in blood 3 wk after last immunization

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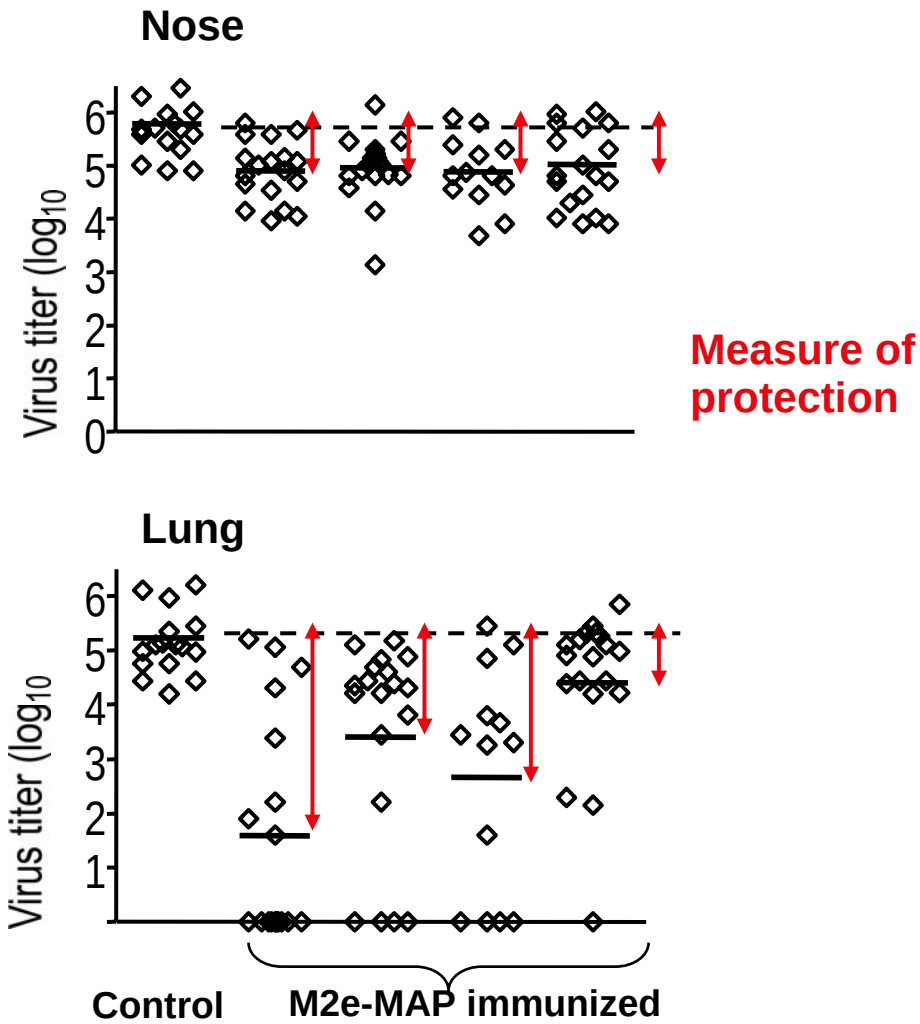
Nasally confined IAV challenge

↓

Determine virus titer in nose, trachea and lung 5 days later.



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Correlation between serum Ab titer and protection

Specificity of serum Abs	Spearman correlation coefficient r		
	Nose	Trachea	Lung
M2e(pep),total	0.07	-0.03	0.18
M2e(pep-nat),total	0.96	0.82	0.8

M2e(pep-nat)-specific serum Abs engendered by parenteral immunization of mice with M2e-MAP correlate with protection.

Procedure:

M2e-MAP + Adj. sc/im/ip, 2-3x

↓

Determine Ab titer in blood 3-4 wk after last immunization

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Nasally confined IAV challenge

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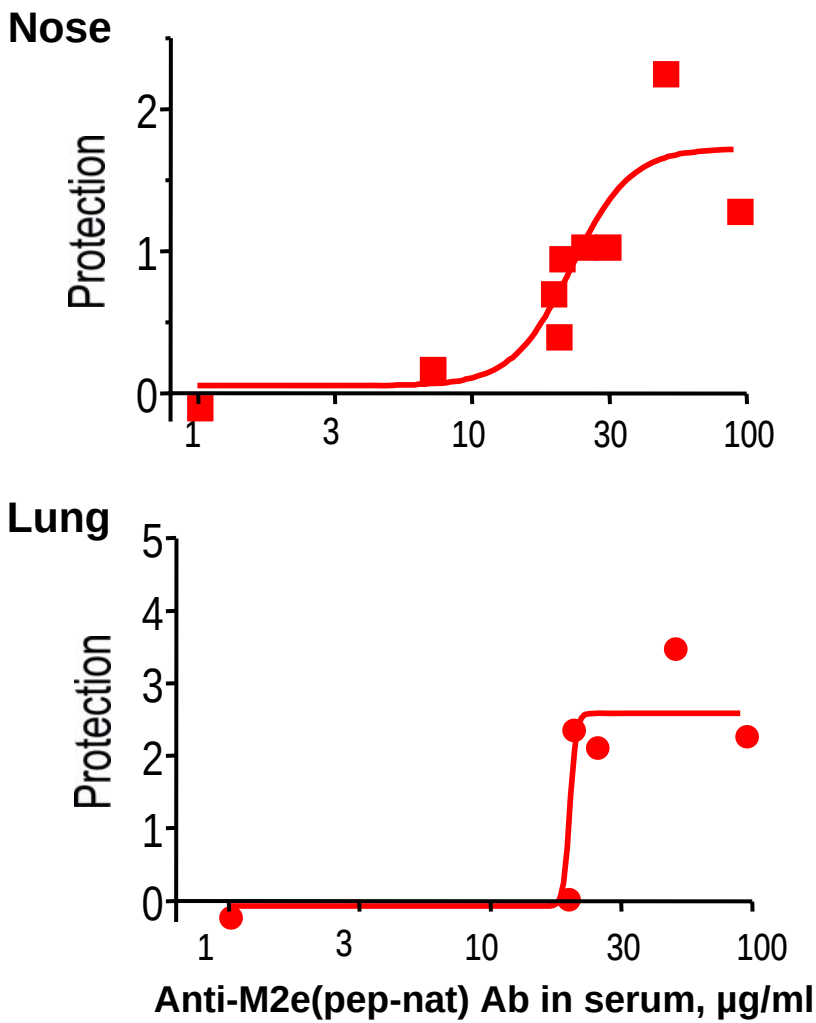
Determine virus titer in nose, trachea and lung 5 days later.



M2e(pep-nat)-specific serum Abs resulting from intranasal immunization of mice with M2e-MAP fail to correlate with protection.

Procedure:

M2e-MAP + Adj. sc/im/ip, 2-3x

↓

Determine Ab titer in blood 3-4 wk after last immunization

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Nasally confined IAV challenge

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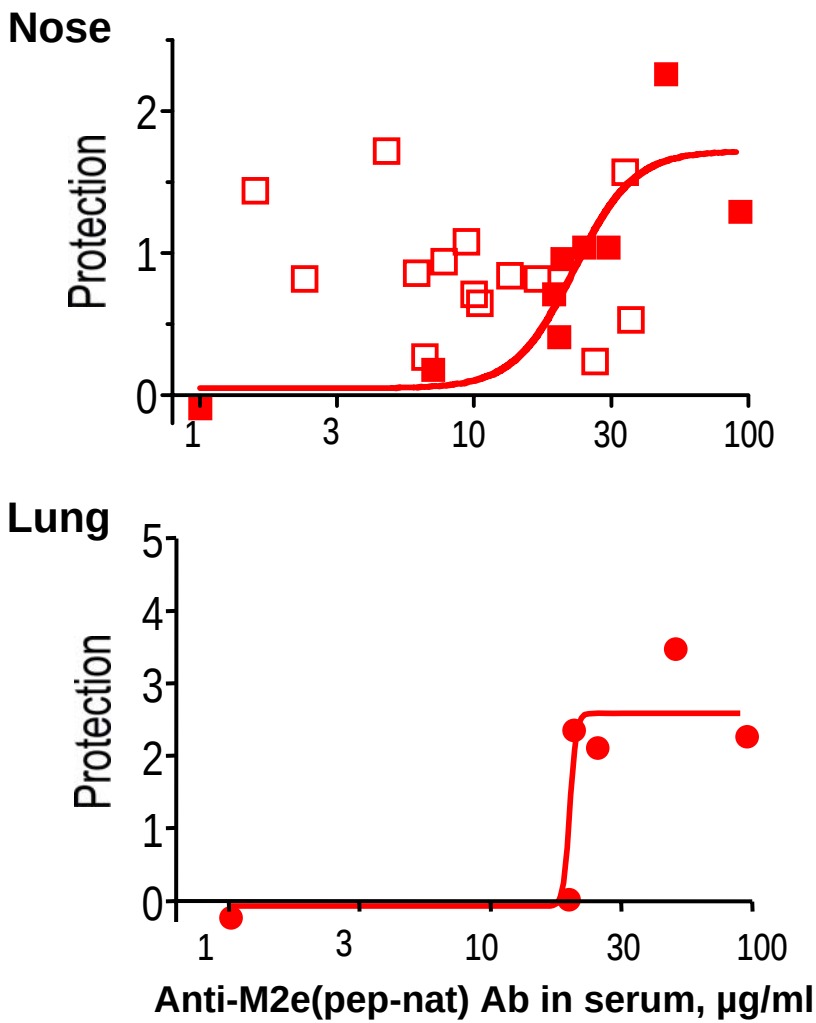
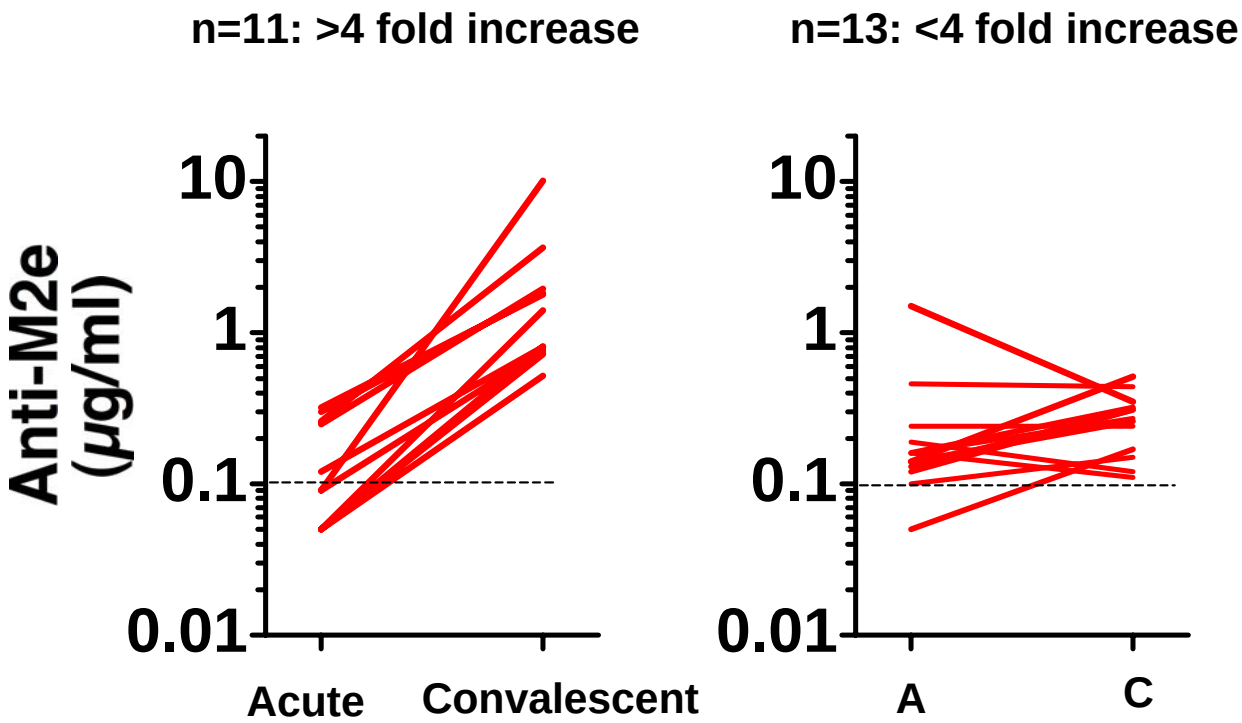
Determine virus titer in nose, trachea and lung 5 days later.



M2e-specific Ab titers in human sera

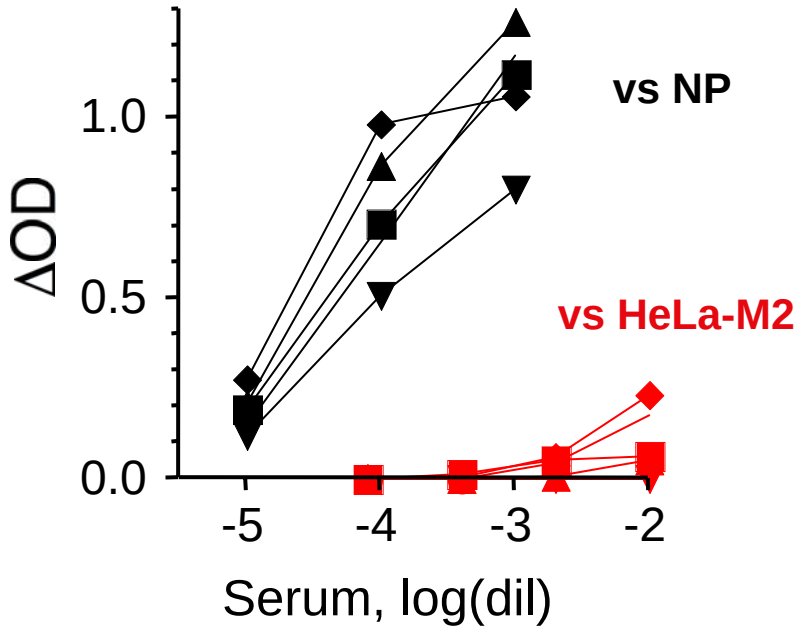
Sera from 24 human subjects, obtained during acute and convalescent phase of natural IAV infection.



- **In humans, IAV infection engenders an M2e(nat) or (pep-nat)-specific Ab response that is irregular and of short duration.**

M2e(nat)-specific Ab titers in human sera

Titration of sera from healthy human subjects



- **Normal human sera contain 100-1000 fold lower concentrations of M2e(nat)- or (pep/nat)-specific than NP-specific Abs.**

Summary

- **ELISA against M2e-peptide and HeLa-M2 revealed three major specificities: M2e(pep), M2e(nat) and M2e(pep-nat).**
- **Only the M2e(nat)- and M2e(pep-nat)-specific Abs (detected by HeLa-M2 ELISA) appear to be protective.**
- **In mouse and human, IAV infection engenders a poor M2e(nat)-specific Ab response. Therefore, in spite of previous IAV infections, adult human sera contain low M2e(nat)-specific Ab titers. This provides a strong rationale for the development of a vaccine capable of inducing a protective M2e(nat)-specific immune response.**

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