Enhanced Immune Response Induced by a Potential Influenza A Vaccine Based on Branched M2e Polypeptides Linked to Tuftsin

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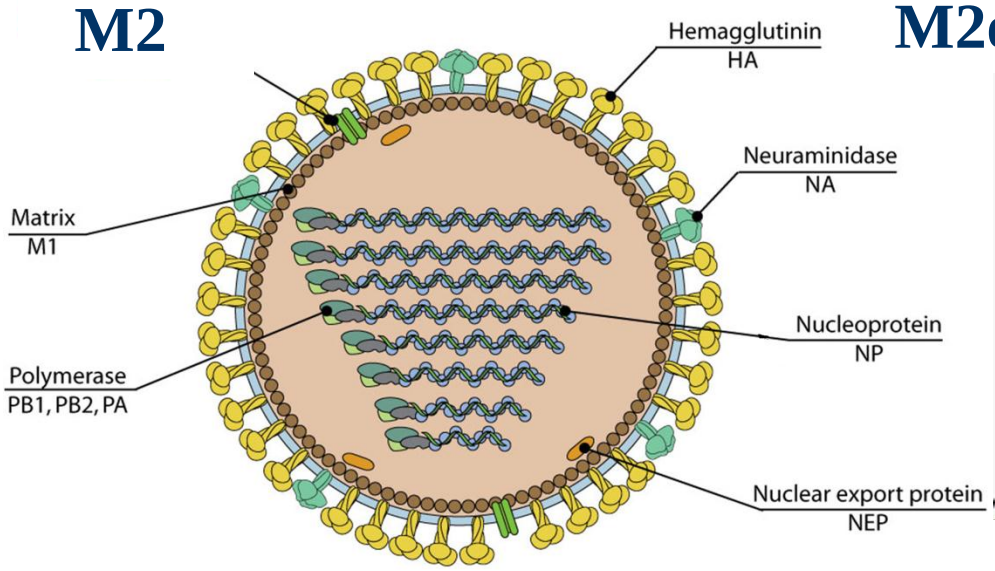
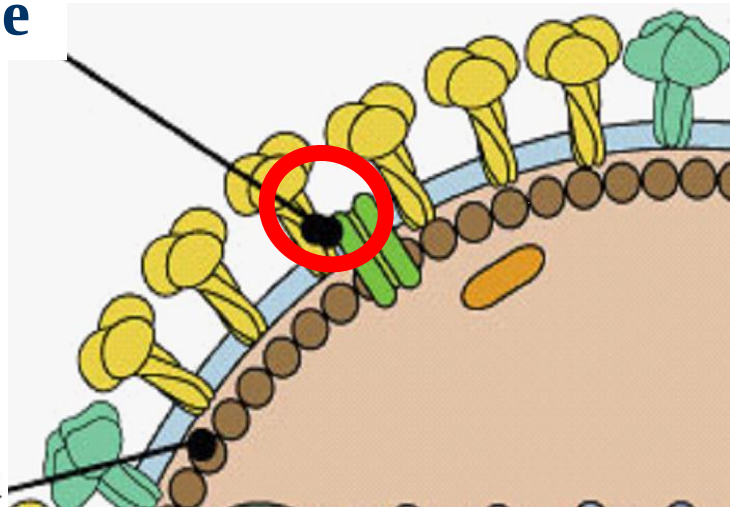
Contents

Background

Extracellular domain of matrix protein 2

- M2e is highly conserved in different subtypes of the influenza A virus .





- The problem is that the M2e has extremely low antigenicity and immunogenicity.

The research on influenza vaccine based on M2e

- **VLPs : M2e-HBc etc.**

- **Fusion constructs : KLH(keyhole limpet hemocyanin) ,OMPC (Neisseria meningitides outer membrane complex), PAMPs (pathogen associated molecular patterns) and BSA (Bovine Serum Albumin) etc.**

Multiple Antigen Peptide system (MAP)

- In 1988, James.P.Tam first reported the MAP.

Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 5409–5413, August 1988
Biochemistry

Synthetic peptide vaccine design: Synthesis and properties of a high-density multiple antigenic peptide system

(solid-phase peptide synthesis/peptide antigen/antipeptide antibody)

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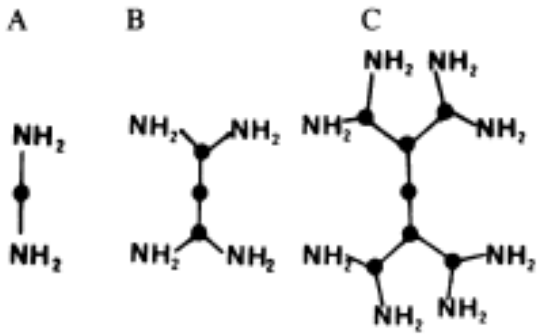
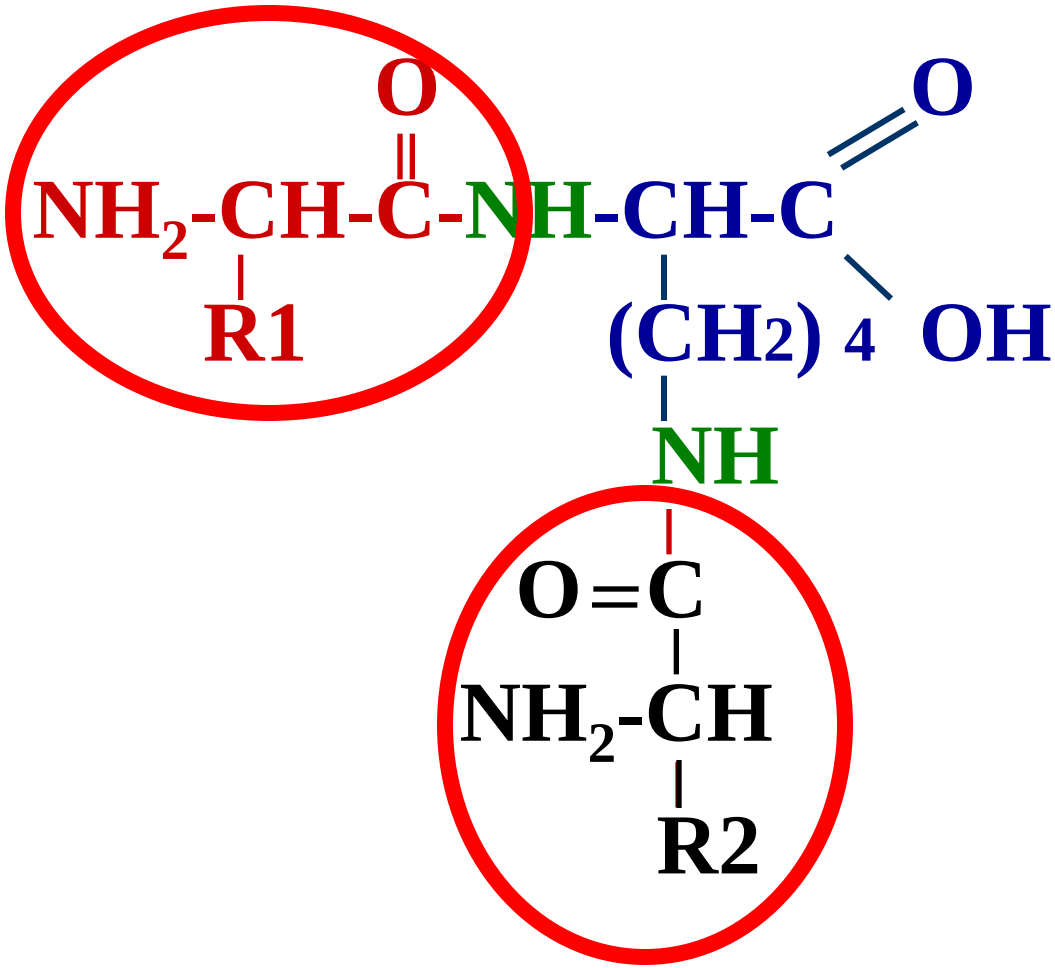
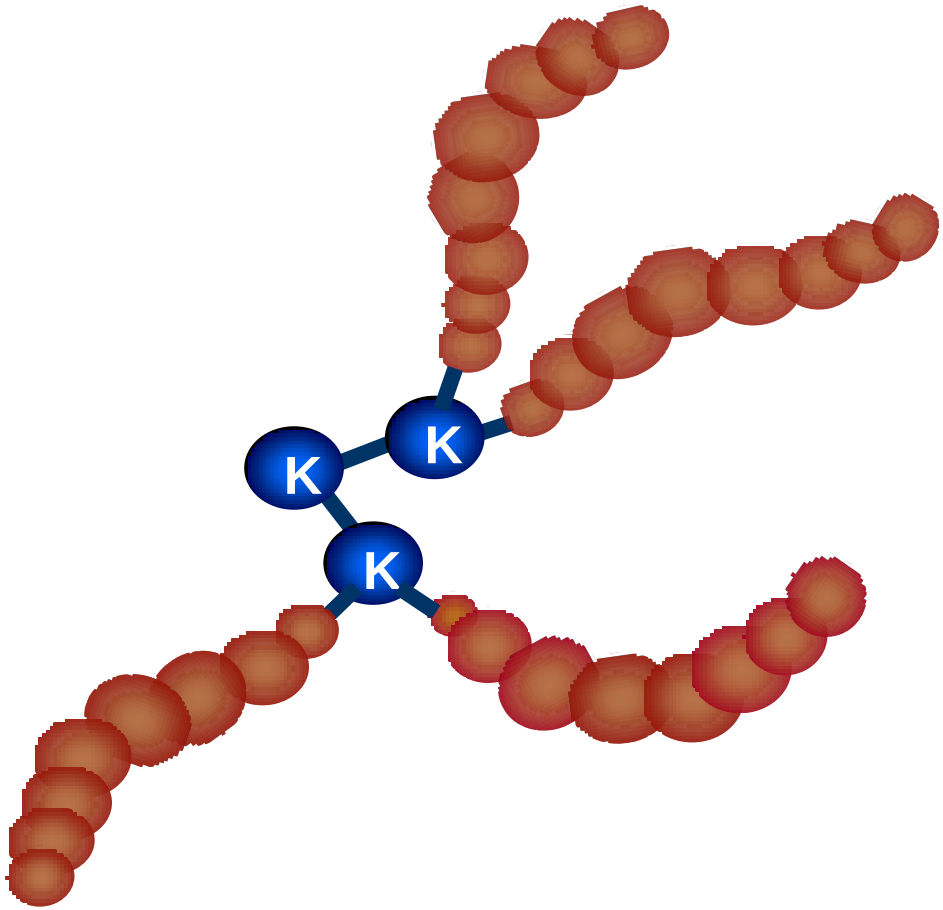
Communicated by Bruce Merrifield, March 31, 1988

ABSTRACT A convenient and versatile approach to the direct synthesis of a peptide-antigen matrix by the solid-phase method is described. The approach is called the multiple antigen peptide system (MAP) and it utilizes a simple scaffolding of a low number of sequential levels (n) of a trifunctional amino acid as the core matrix and 2^n peptide antigens to form a macromolecule with a high density of peptide antigens of final M_r 10,000. The MAP model chosen for study was an octabranching MAP consisting of a core matrix made up of three levels of lysine and eight amino terminals for anchoring peptide





Lysine （ K ）


K
K
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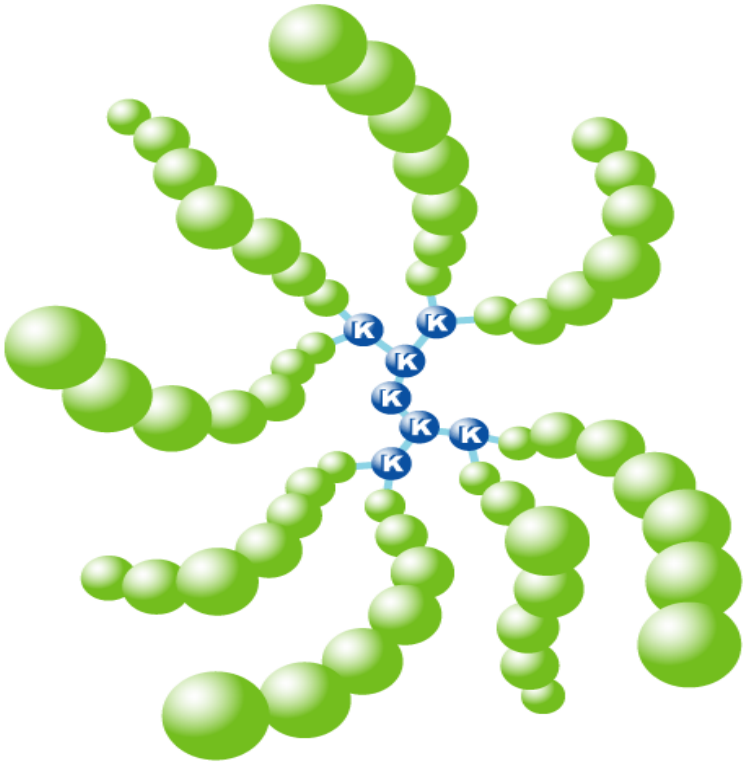
K
K
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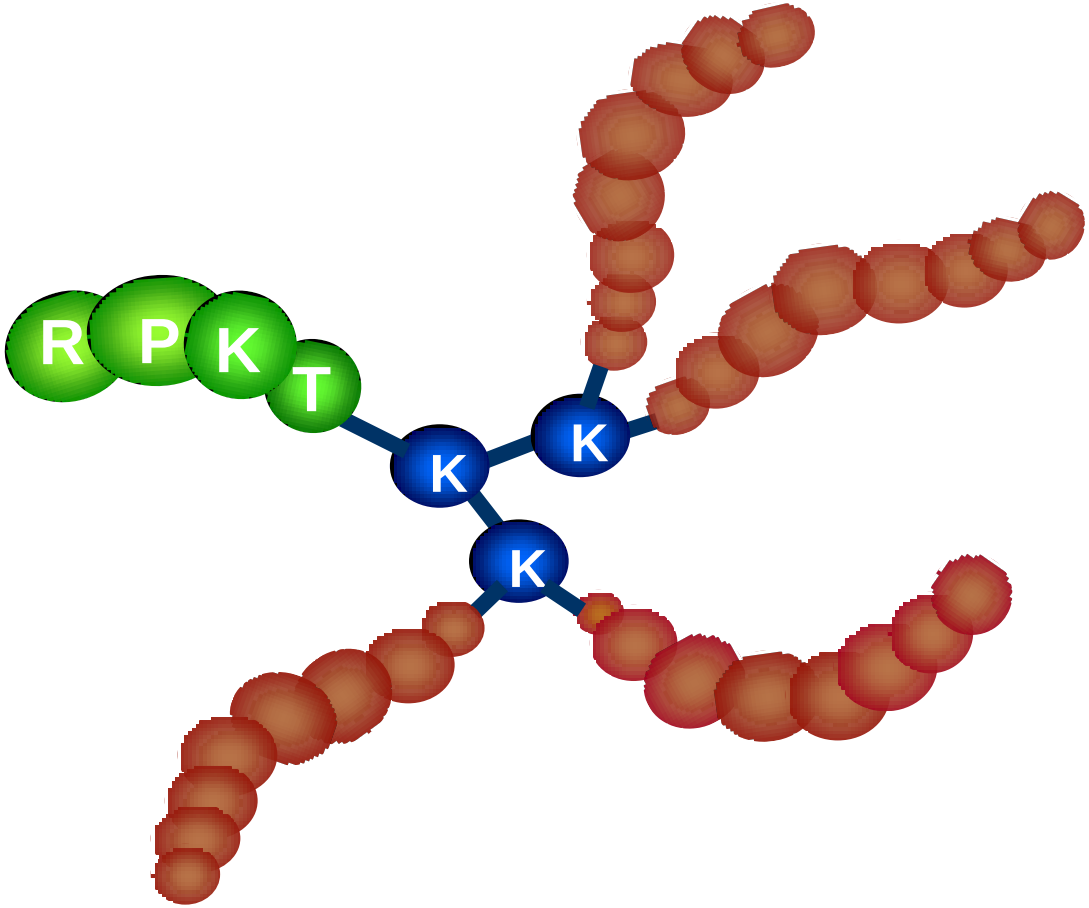
K

Tuftsin

Tuftsin is a fraction (Thr-Lys-Pro-Arg) of the IgG Fc fragment.

- **Tuftsin could be recognized by specific receptors on phagocytic cells, and is capable of targeting proteins and peptides to these sites.**
- **It also could modulate the antigen-presenting capacity of macrophages.**

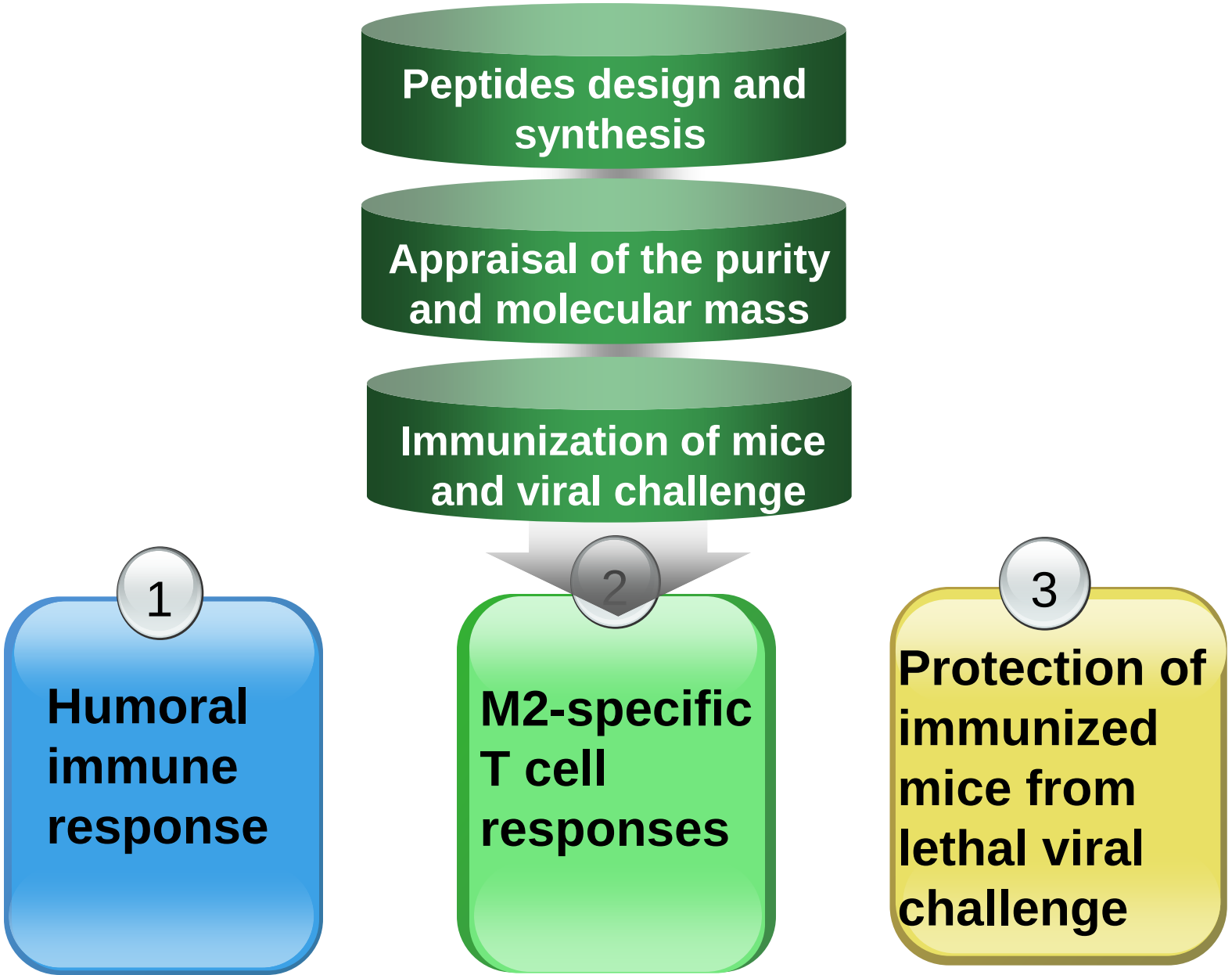
a novel influenza A vaccine based on M2e



(M2e) 4 -Tuftsin

Methods and Results

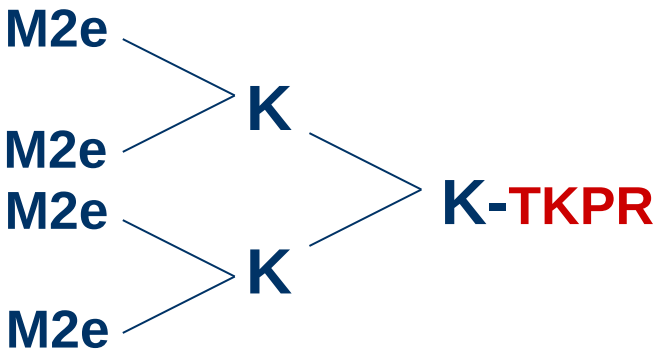
Research framework



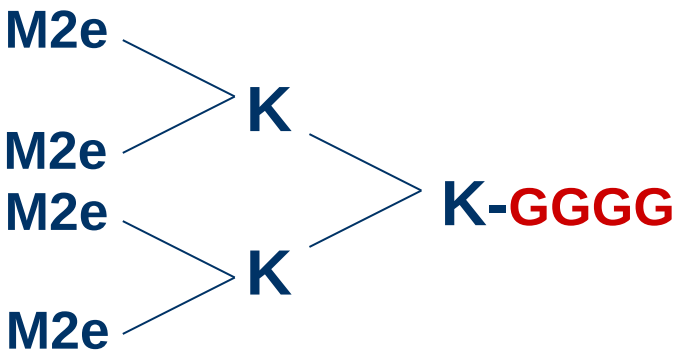
Peptides design and synthesis

M2e SLLTEVETPIRNEWGCRCNDSSD

(M2e) 4 -Tuftsin

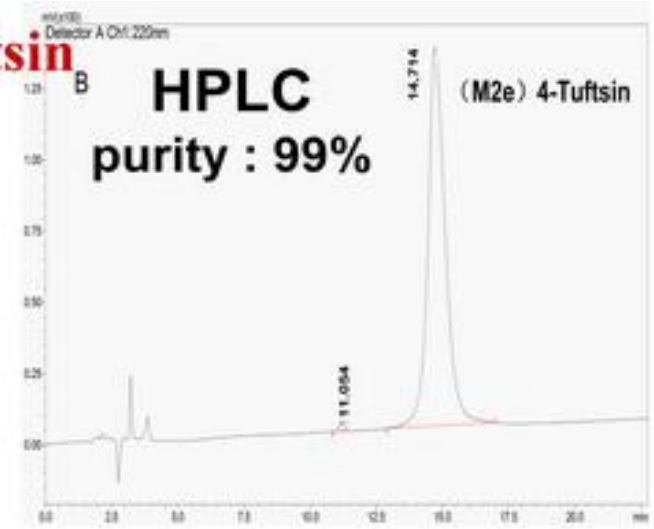


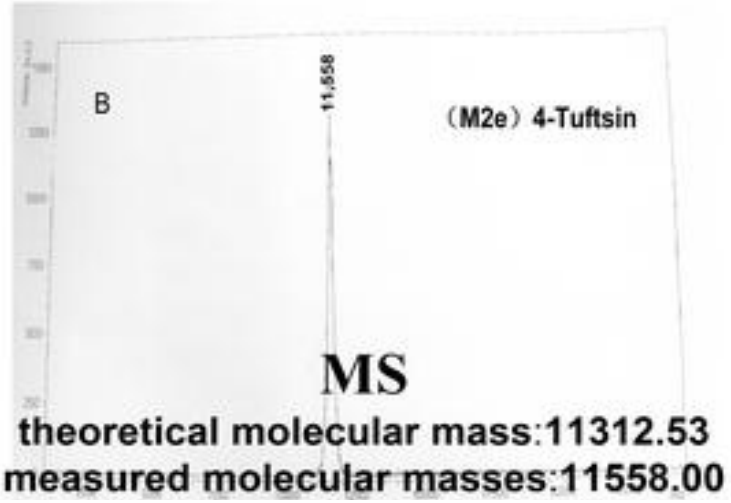
(M2e) 4 -GGGG



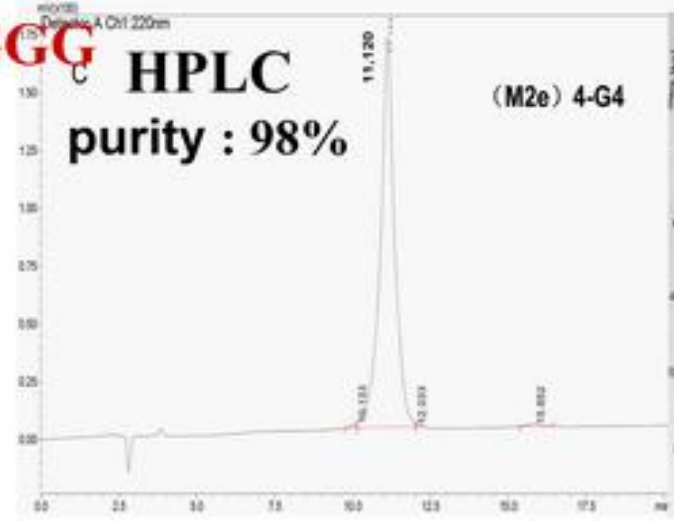
Appraisal of the purity and molecular mass

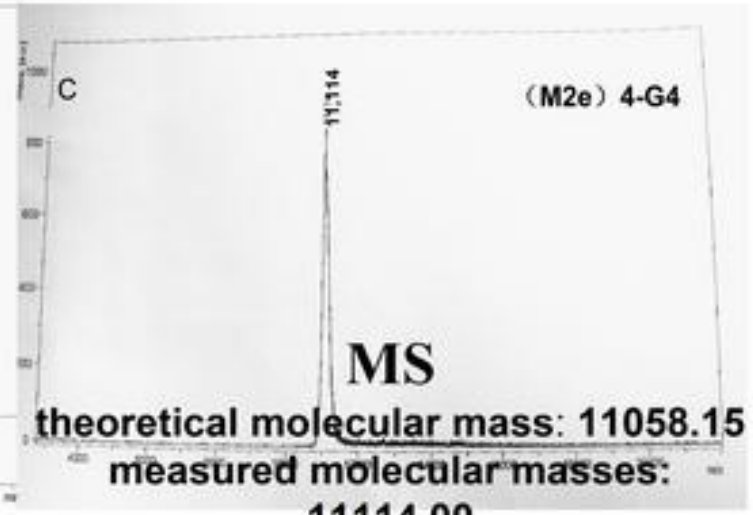
(M2e) 4 -Tuftsin



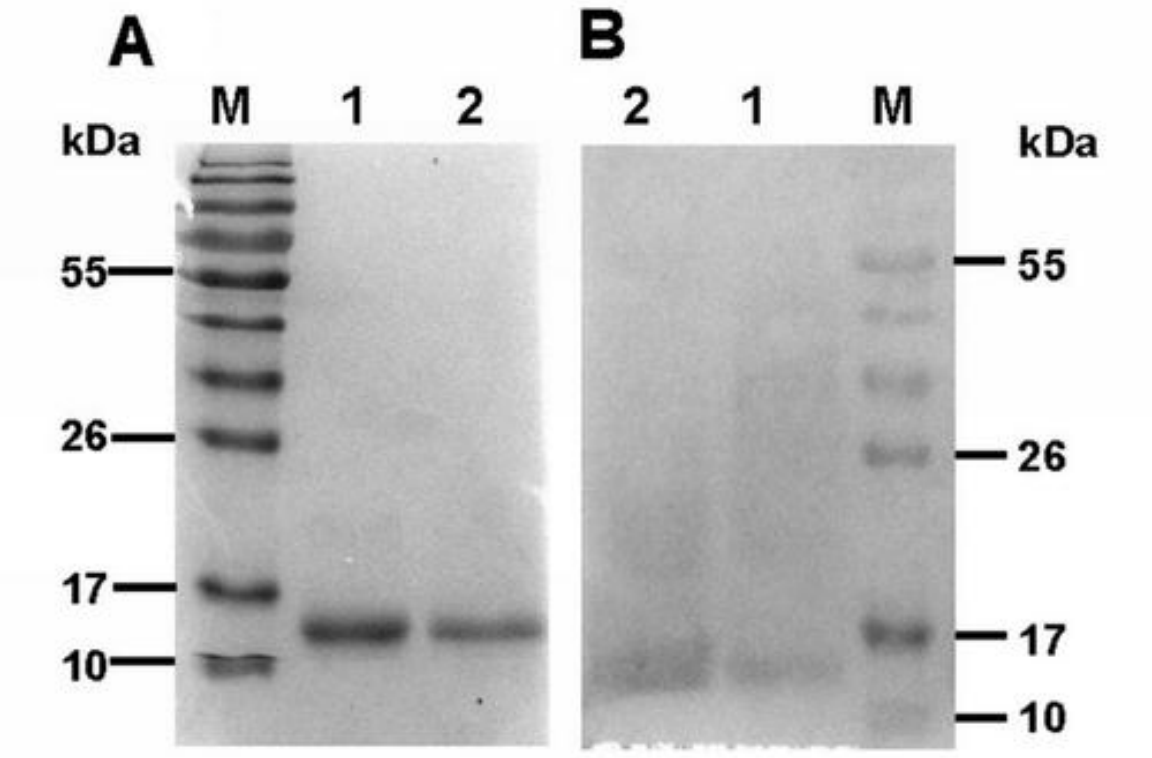


(M2e) 4 -GGGG





SDS-PAGE and Western blot



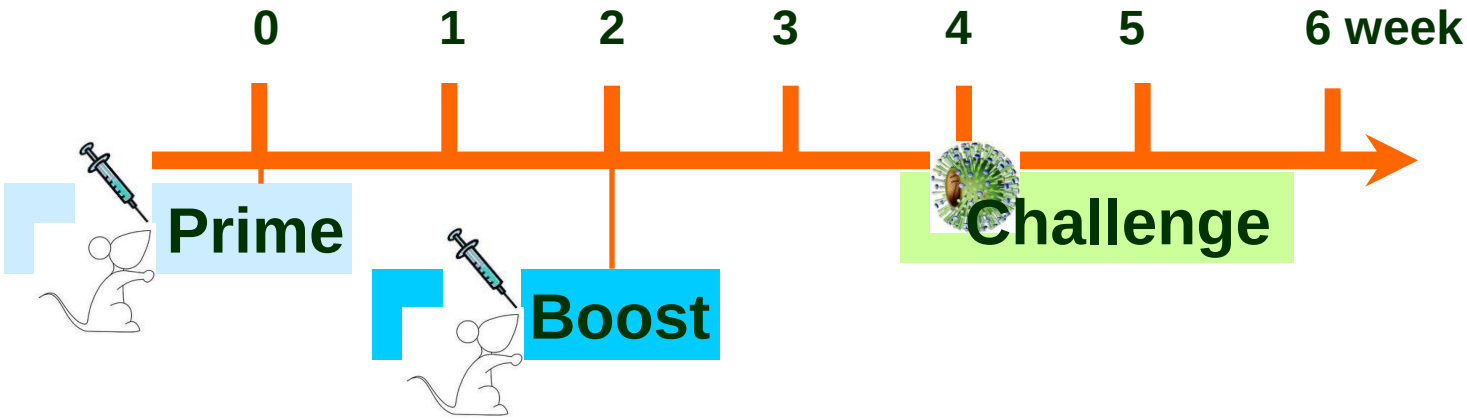
(A) SDS-PAGE bands of (M2e)4-tuftsin (Lane 1) and (M2e)4-G4 (Lane 2) indicated molecular masses corresponding to their molecular mass.

(B) The synthetic peptides were tested for possession antigenicity of M2 by Western blot using mAb against M2.

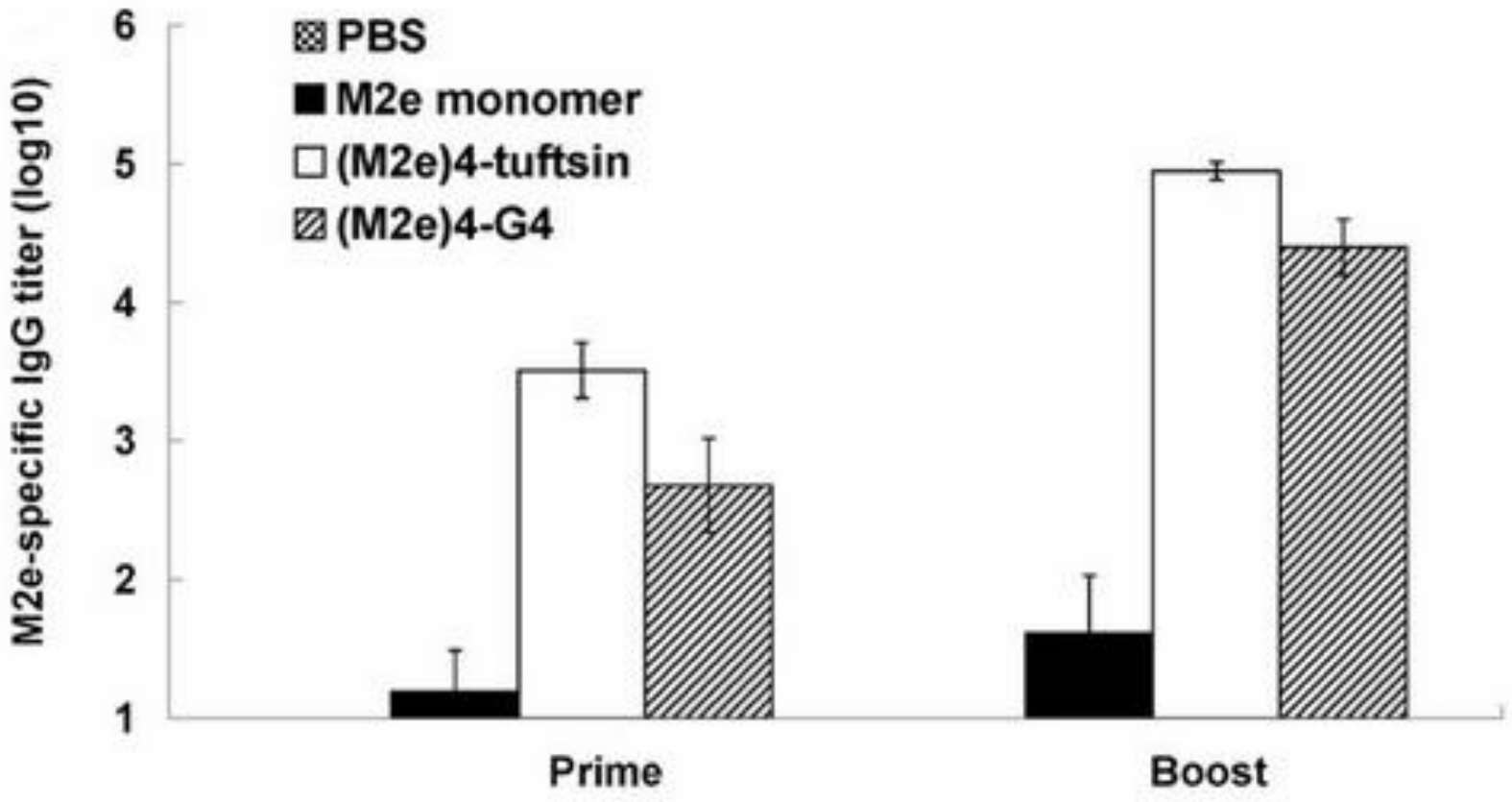
Immunization and viral challenge

The mice were immunized intramuscularly with 10μg of M2e monomer, (M2e)4-tuftsin or (M2e)4-G4 plus aluminum adjuvant respectively. PBS plus aluminum adjuvant was injected for control group. Booster immunization was given 2 weeks later.

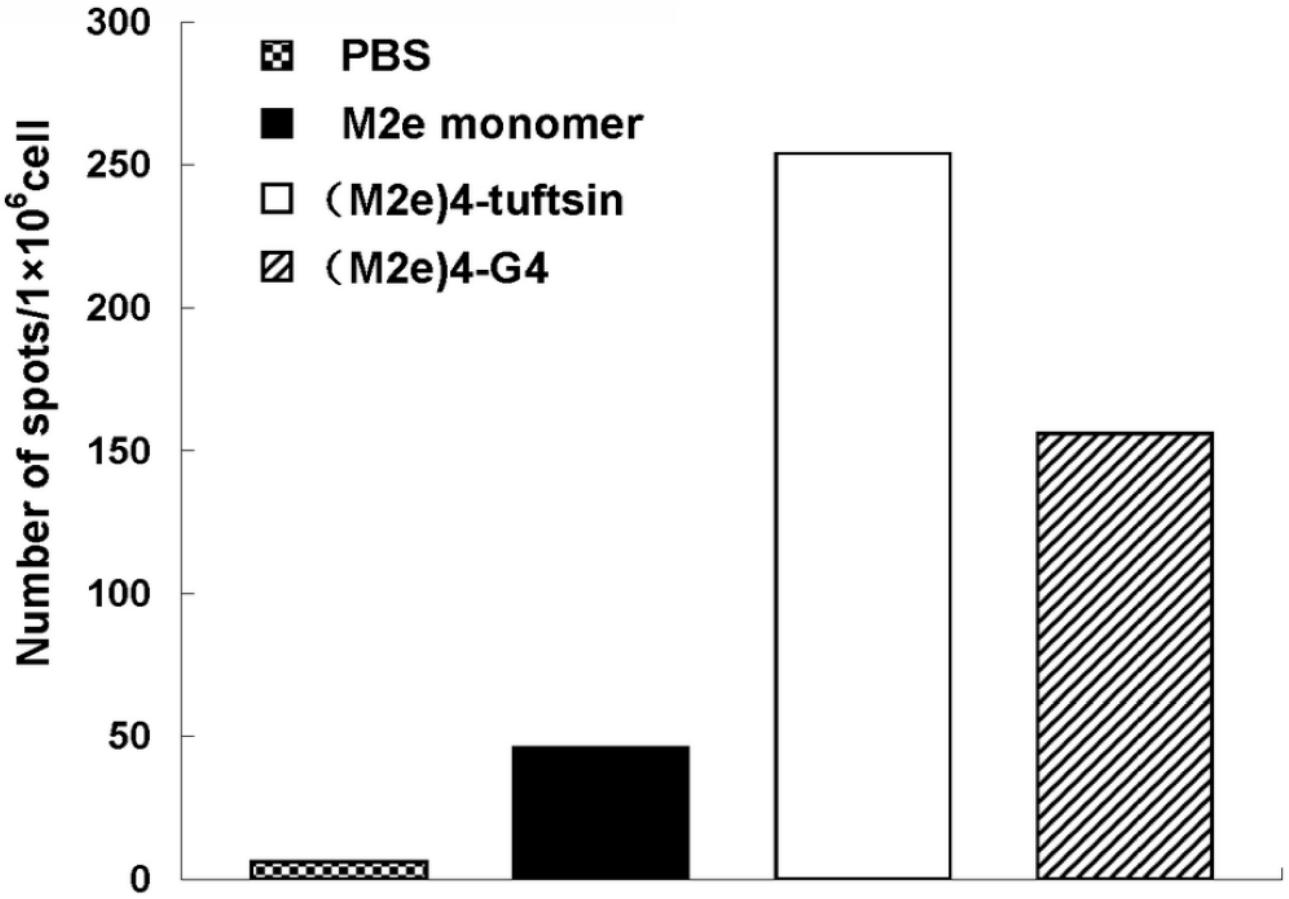
Two weeks after final immunization, anesthetized mice were challenged intranasally with 10 LD50 of influenza virus PR8 strain .



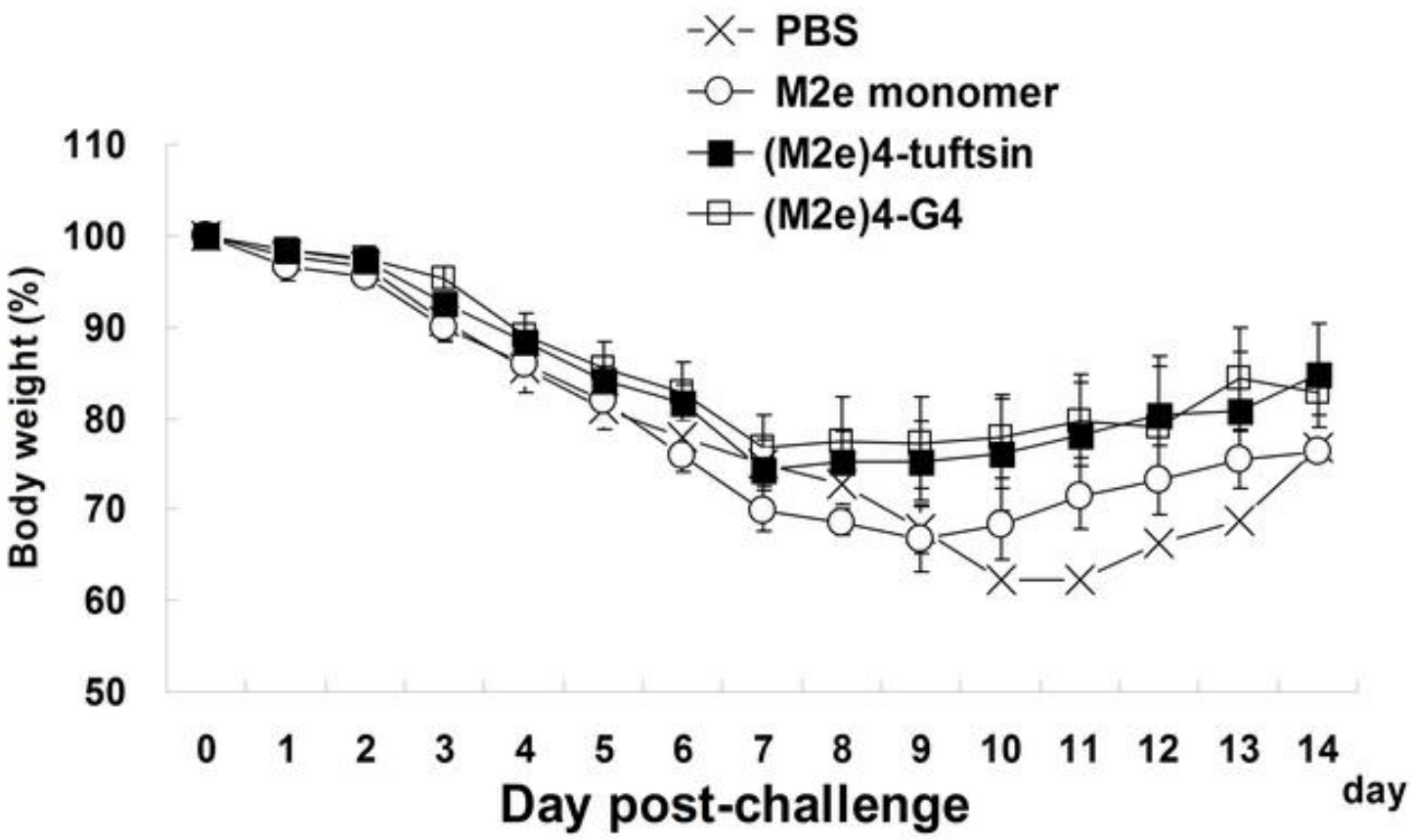
Humoral immune response



M2-specific T cell response



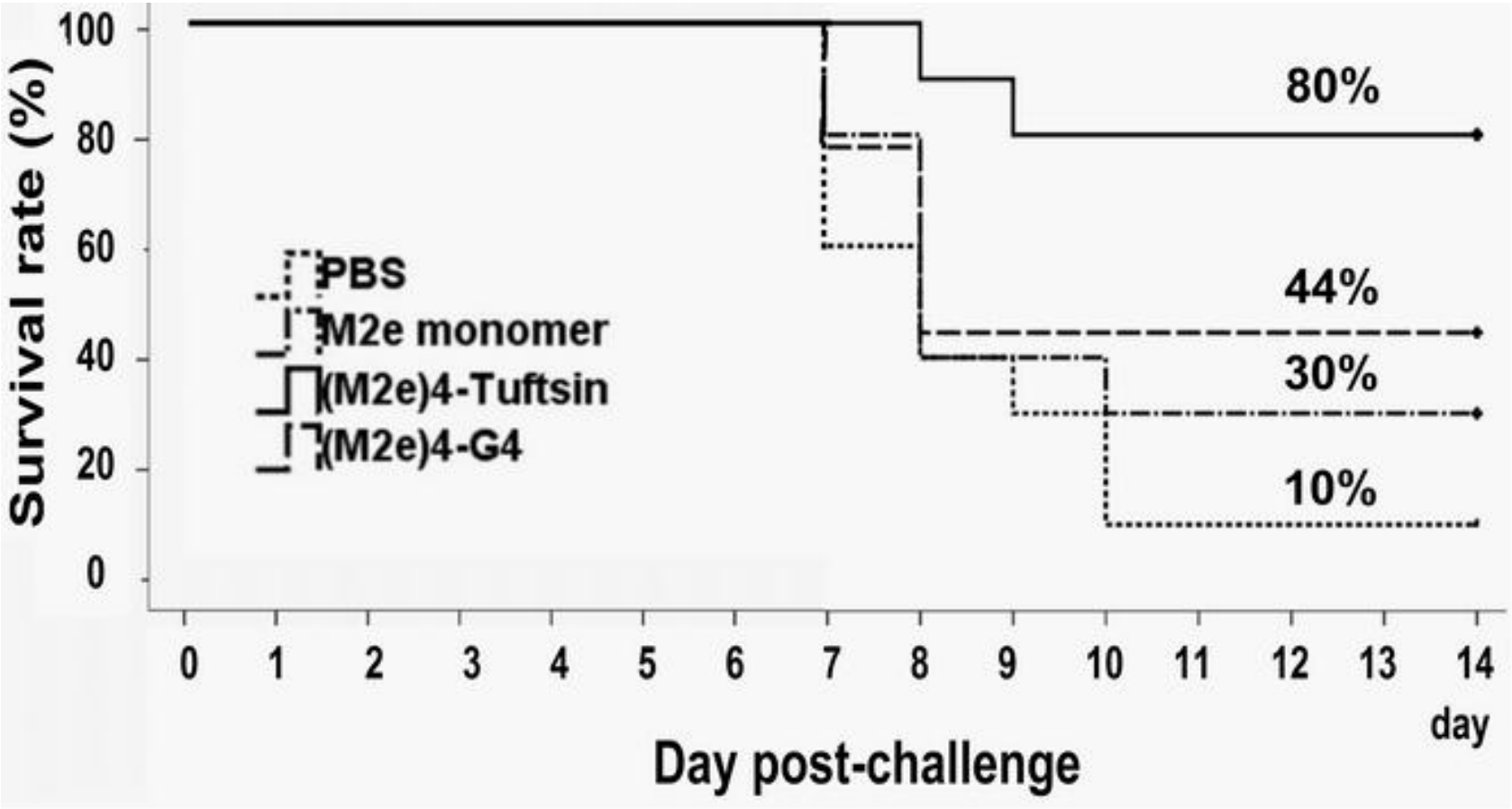
Percentage change of mouse body weight



Changes in relative weight were calculated as follows:

(group mean weight on the day specified / group mean weight on day 0) ×100%

Kaplan-Meier Survival analysis



Conclusion

- The (M2e)4-Tuftsin induce highest level of M2 specific antibody.
- The (M2e)4-Tuftsin was the most effective in stimulating T cell response.
- The (M2e)4-Tuftsin could protect mice against influenza A virus PR8 strain (H1N1).

Further research

- The immune responses of mice to intranasal immunization of (M2e)4-tuftsin have been studied (data not shown).
- The protective effects of branched NP epitopes or branched NP epitopes mixed with M2e is being studied.
- The protective effects of (M2e)4-Tuftsin against other subtypes of influenza A viruses will be studied.


Thank You!