HKU Discovers a Novel Therapeutic Strategy for Controlling Influenza Infections

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Influenza: a major threat to human health

- **Global impact of pandemics in the 20th century**
  - 1918 '*Spanish' influenza* (H1N1)
    - 40 million deaths.
  - 1957 '*Asian' influenza (H2N2)*
    - 1 million deaths
  - 1968 '*Hong Kong' influenza (H3N2)*
    - 1 million deaths

- Seasonal influenza takes a formidable toll in HK
- >1000 deaths annually
- Hospitalization
- Lost economic productivity

Pandemic Influenza in 2009

2009 H1N1	Mid-Level Range*	Estimated Range*
Cases		
0-17 years	~20 million	~14 million to ~28 million
18-64 years	~35 million	~25 million to ~52 million
65 years and older	~6 million	~4 million to ~9 million
Cases Total	~61 million	~43 million to ~89 million
Hospitalizations		
0-17 years	~87,000	~62,000 to ~128,000
18-64 years	~160,000	~114,000 to ~235,000
65 years and older	~27,000	~19,000 to ~40,000
Hospitalizations Total	~274,000	~195,000 to ~403,000
Deaths		
0-17 years	~1,280	~910 to ~1,880
18-64 years	~9,570	~6,800 to ~14,040
65 years and older	~1,620	~1,160 to ~2,380
Deaths Total	~12,470	~8,870 to ~18,300

*Data from US

Source: WHO

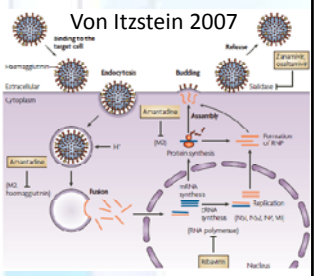
Current strategy for prevention of influenza infections

- Vaccine: effective prevention in healthy young adults.
- **Limitations of vaccine:**
  - Have to be updated every year with new strains that are expected to circulate in the coming year. When there is an unexpected change in the virus, vaccine efficacy may not be good enough.
  - Available too late in pandemics.
  - Limitations in the age groups who most need it
    - Efficacy in the elderly (above 65 year-old) is sub-optimal
    - Cannot be used in very young infants (below 6 months)

Current strategies for treatment of influenza infections

Anti-influenza drugs: Targeting specific viral proteins.

- Blocks virus entry into cell (M_2 ion channel functions):
  - Amantadine hydrochloride and rimantadine hydrochloride Associated with development of rapid resistance
- Blocks virus release from cells (by inhibiting viral neuraminidase:
  - Oseltamivir phosphate (e.g. Tamiflu)and Zanamivir



Problems with resistance:

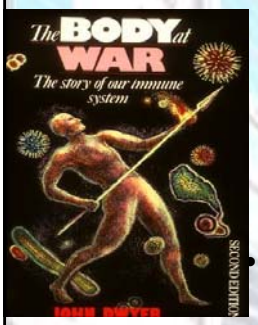
•Most H3N2 viruses resistant to amantadine and rimantadine.

•Most SEASONAL H1N1viruses resistant to oselatmivir

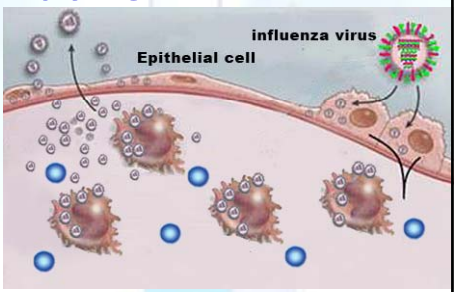
•Pandemic H1N1 resistant to amantadine / rimantadine but sensitive to oseltamivir & zanamivir. However, sporadic cases of oseltamivir resistance were already detected

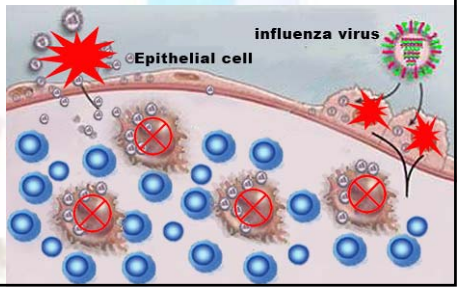
- **There is an acute need to develop alternative strategies for influenza therapy.**

Novel strategy: killing virus-infected cells



- Viruses have to replicate in living cells (of human or other animals).
- Killing virus-infected cells can clear virus.





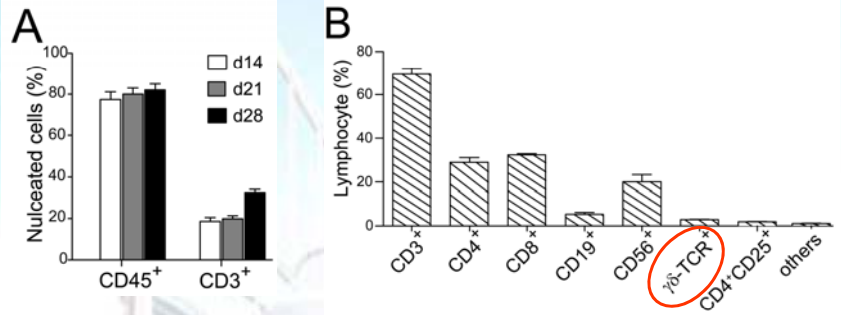
γδ-T cells

- One of the first lines of host immune defense.
- Constitute only a small proportion (2-10%) of T lymphocytes in blood.
- Previously we found γδ-T cells (gamma-delta T cell) could kill influenza virus-infected cells in vitro.
- It remains unknown whether these cells have same effects in vivo (in living animal).
- Not possible to use conventional mouse model for these studies because mice are not known to have these cells.

HKU developed humanized mice

- Studies in animals may not always reflect the situation in humans.
- It may not be possible to directly translate biomedical findings from animals to humans.
- HKU is one of the few laboratories worldwide to have successfully established the humanized mouse model.
- Humanized mice contain complete human immune system.
- Provides a realistic experimental model for studies on human immunology: reduces the gap between mice and humans.

Humanized mice with complete human immune system



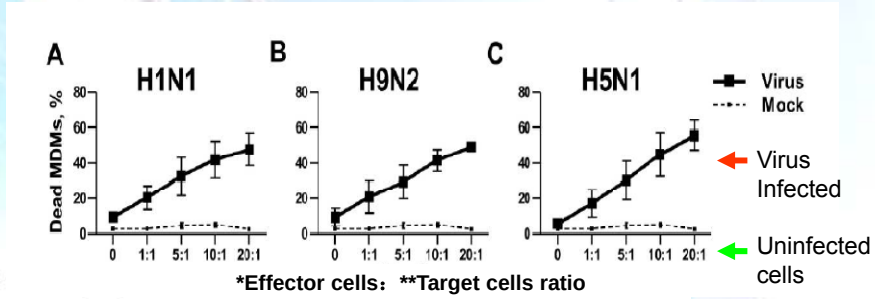
Once humanized mice were established,

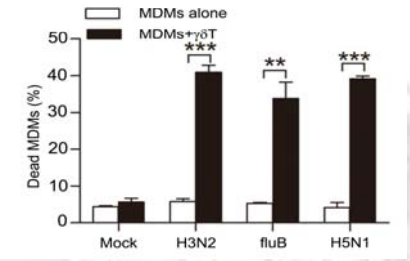
- About 80% and 30% of nucleated cells in the peripheral blood were human $CD45^+$ cells and $CD3^+$ T cells, respectively.
- The lymphocyte population comprised almost all the lymphocyte subsets: $CD3^+$ T, $CD4^+$ T, $CD8^+$ T, $CD19^+$ B, $CD56^+$ NK, $V\gamma9^+V\delta2^+$ T, $CD25^{high}CD4^+$ T cells.
- These T, B and $V\gamma9^+V\delta2^+$ T cells have normal function.

Pamidronate

- Pamidronate have been commonly used for over 20 years for the treatment of bone diseases such as osteoporosis and Paget's disease.
- The drug selectively activate and expand human $\gamma\delta$–T cells.

Pamidronate-expanded γδ–T cells kill influenza-virus infected cells

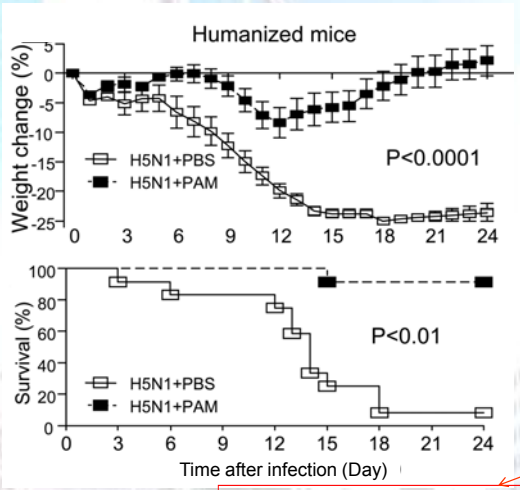




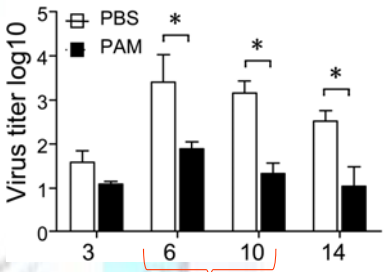
*Effector cells = γδ–T cells

**Target cells = virus-infected cells

Pamidronate controls influenza diseases and inhibits viral replication

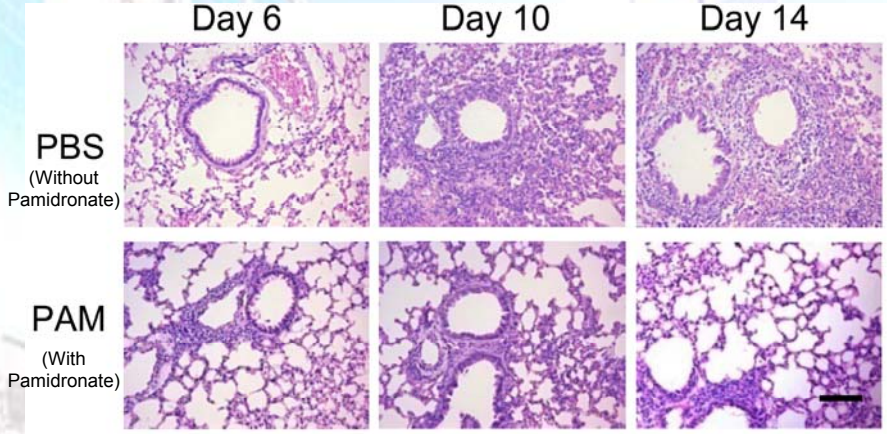


PAM=with Pamidronate
PBS=without Pamidronate (Control)

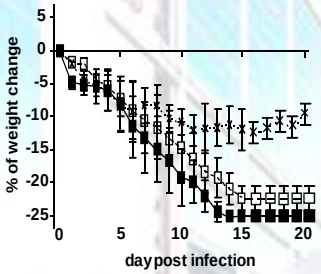


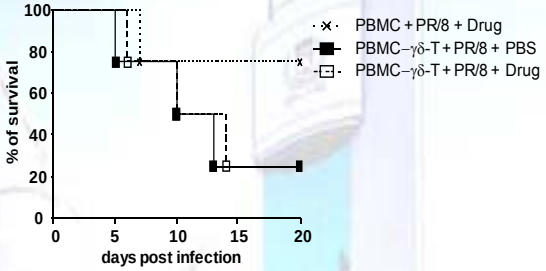
Virus titer decreased by **100 fold** after Pamidronate treatment

Pamidronate prevents inflammation in infected lung

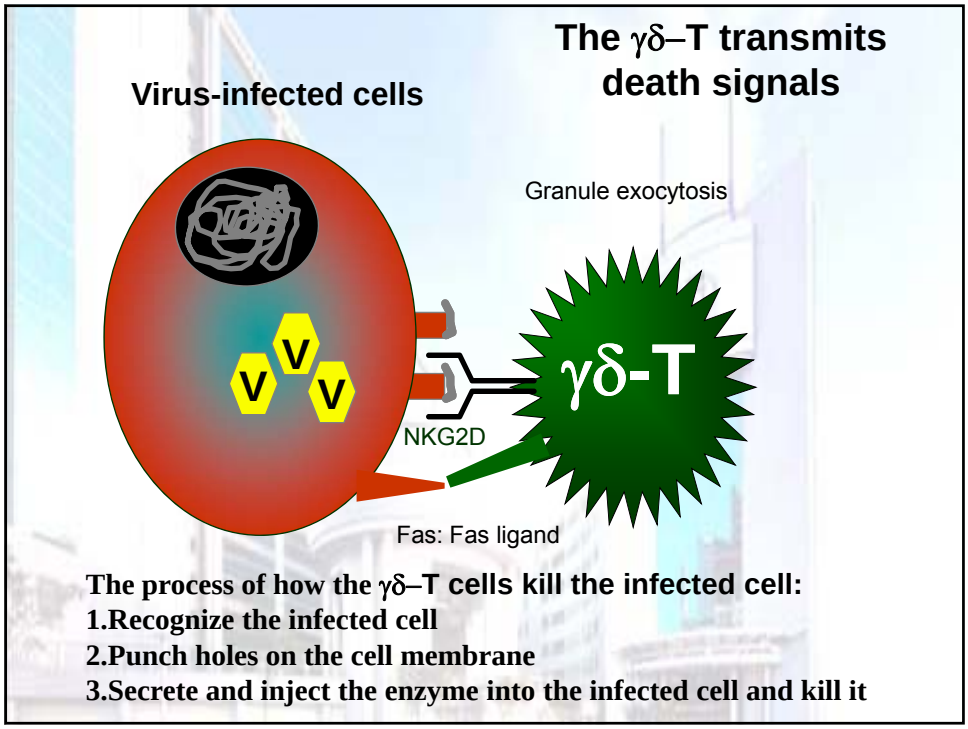


γδ-T cells mediate the control of influenza diseases by Pamidronate





- Treatment with pamidronate significantly decreased weight loss and mortality in humanized mice with γδ-T cells .
- Pamidronate had no such effects in H1N1 virus-infected humanized mice without γδ-T cells .

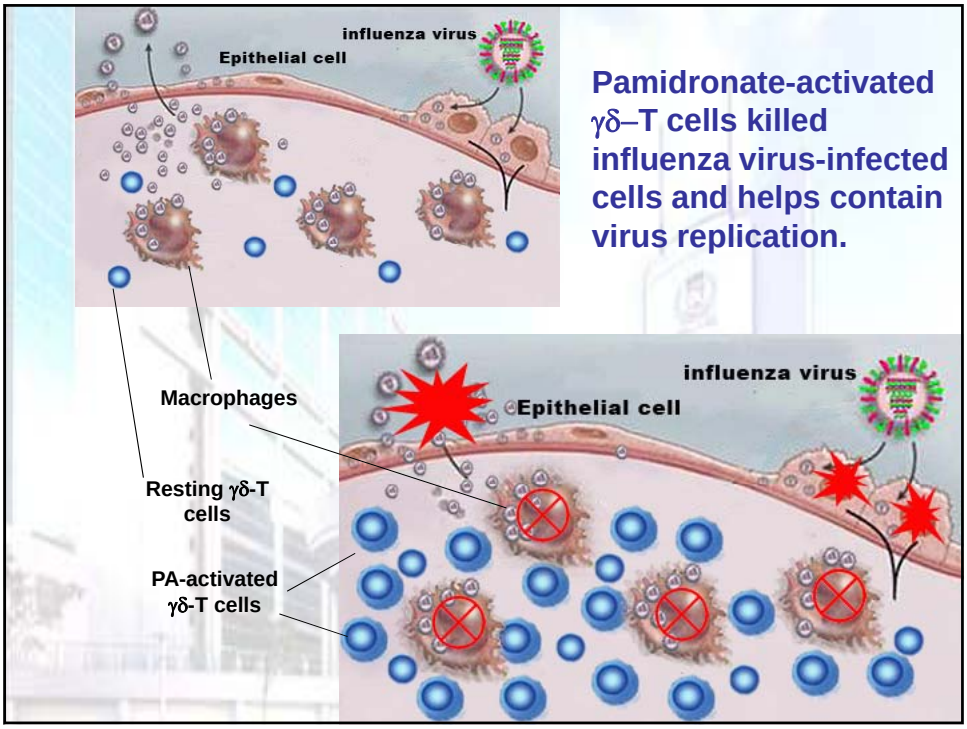


The process of how the γδ–T cells kill the infected cell:

1.Recognize the infected cell

2.Punch holes on the cell membrane

3.Secrete and inject the enzyme into the infected cell and kill it



Significance

- Our study, for the first time, demonstrated that pamidronate can control all the strains of influenza diseases caused by seasonal, pandemic or avian H5N1 influenza viruses through boosting γδ–T cell immunity in humanized mouse model.
- Influenza viruses are unlikely to be able to acquire resistance to the action of γδ T cells which are part of the natural immune system of the body.
- "New application of an old drug" potentially offers a ready and cost-effective solution to viral infections.
- Humanized mouse model provides a powerful and low-cost platform for preclinical study of drugs such as pamidronate.
- Humanized mice may provide an ideal model in therapeutic studies of human pathogens related to infectious diseases, for vaccine testing and stem cell studies.
- This research is at pre-clinical stage and will be expanded to human clinical trials.

Acknowledgments

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