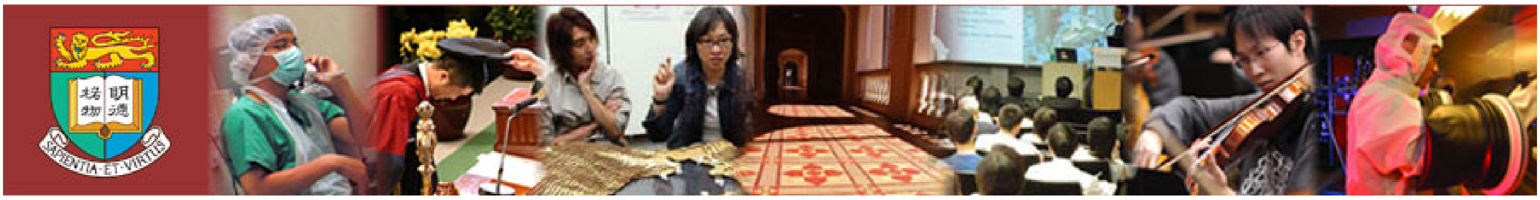

Use of humanised mice to study antiviral activity of human γδ-T cells against influenza A viruses

WW Tu *, YL Lau, JSM Peiris

KEY MESSAGES

1. Phosphoantigens could protect against influenza A (H1N1) virus infection in humanised mice with a human immune system.
2. This protection was strain-dependent for avian influenza A (H5N1) virus infection.
3. This suggests a novel therapeutic approach against influenza by using phosphoantigens to inhibit influenza A virus infection.
4. The humanised mouse model provides a low-cost platform for further studies of vaccines, stem cell biology, and therapeutics for human pathogens.

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[1] WW Tu *, [1] YL Lau, [2] JSM Peiris

The University of Hong Kong:
[1] *Department of Paediatrics and Adolescent Medicine*
[2] *Department of Microbiology*

* Principal applicant and corresponding author: wwtu@hku.hk

Introduction

Influenza A virus can cause substantial morbidity and mortality. Potential emergence of a new pandemic strain (eg avian influenza A virus [H5N1]) through natural reassortment is a concern. Both innate and adaptive immune systems play critical roles in protecting against influenza A viruses, and direct manipulation of the host immune system can help protect against influenza A virus infections. Vaccines targeting the adaptive immune system may be less effective against a new pandemic strain. The currently available neuraminidase inhibitors may become ineffective with the emergence of resistant virus strains. Therefore, activating early innate responses enables protection against influenza A virus infection early after virus exposure.

As the first line of the host defence, the antiviral activities of γδ–cells against other viruses have been demonstrated in different models.[1-3] Human Vδ2–T cells, representing most peripheral blood and lymphoid organ γδ–T cells, can be selectively activated and expanded by a phosphoantigen such as isopentenyl pyrophosphate, which also triggers γδ–T cells to produce interferon-γ and other cytokines and chemokines as antiviral activities.[2,3] In human in vitro systems, phosphatidic acid–activated Vδ2–T cells can inhibit virus replication and kill virus-infected cells caused by hepatitis C virus and severe acute respiratory syndrome coronavirus.[4,5] Whether phosphoantigen-activated Vδ2–T cells have antiviral activities against human and avian influenza A viruses in vivo remains unknown.

Unlike other cells in humans, Vδ2–T cells are the dominant γδ–T cells in the circulation, whereas murine γδ–T cells do not express the homologue of the Vγδ–T-cell receptor, and no functional equivalent for these cells has been identified in mice so far. Studies on non-human primates are constrained by high costs, limited availability, paucity of genetic models for human diseases, and lack of genetically inbred strains suitable for cell transplantation. Creation of humanised mice that carry partial or complete human immune systems may help to overcome these obstacles. In this study, conducted from August 2007 to July 2009, a humanised mouse model was established to investigate the antiviral activities of human T cells against human and avian influenza A viruses in vivo.

Methods

Generation of humanised mice

C57BL/10SgAiRag2$^{-/-}$γc$^{-/-}$ mice (Taconic, Hudson, NY, USA) were kept in individual ventilated cages in the Laboratory Animal Unit of the University of Hong Kong. To establish humanised mice models, human peripheral blood mononuclear cells (huPBMCs) were obtained from healthy donors after approval by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. Humanised mice were then established as previously described with some modifications.[6] All manipulations were in compliance with the guidelines for the use of experimental animals.

Infection of humanised mice with human and avian influenza A virus

Under anaesthesia, 10-week-old humanised mice were infected intranasally with the human influenza A virus H1N1 (A/Hong Kong/54/98) [25 μL, $10^{8.4}$TCID$_{50}$], mouse-adapted influenza H1N1 (A/PR/8/34) [25 μL, 1×LD$_{50}$], and avian influenza H5N1 (A/Hong Kong/483/97) or (A/Hong Kong/486/97) [25 μL, 1×LD$_{50}$]. The weight and survival of the infected mice were checked daily post-infection.

Virus titre determination and immunohistochemistry assays

The lungs of infected humanised mice were harvested at the indicated time and homogenised with phosphate buffered saline (PBS) 2 mL, and the virus titre was determined as described previously.[7] Lung immunohistochemistry staining was performed as described previously.[8]

Treatment of virus-infected humanised mice

Humanised mice were separated into mock, PBS-treated, and drug-treated groups, matched according to sex, age, and the source of huPBMCs. Four weeks after transplantation of huPBMCs, Rag2$^{-/-}$γc$^{-/-}$ and established humanised mice were infected intranasally with human influenza H1N1 virus, PR/8 virus, or avian influenza H5N1 (25 μL, 1×LD$_{50}$) under anaesthesia. For human H1N1 and PR/8 virus infections, phosphoantigen or an equivalent volume of PBS was injected intraperitoneally on days 3, 5, 7, and 9 after virus infection, whereas for avian influenza H5N1 infection, injections were on days 1, 3, 5, 7, and 9 after virus infection. Survival was monitored and the infected mice were weighed daily; mice with >25% weight loss were sacrificed and counted as dying.

Results

Phosphoantigen-activated cells could efficiently kill human and avian influenza H1N1, H9N2, and H5N1 virus-infected cells and inhibited virus replication in vitro.[9] We tried to establish the humanised mouse model with a human immune system. Using immunodeficient C57BL/6Rag2$^{-/-}$γc$^{-/-}$ mice that lack functional T, B, and natural killer (NK) cells, after 4 weeks of transplantation of huPBMCs, around 80% of nucleated cells in peripheral blood were human lymphoid cells expressing human CD45 and >20% expressed human CD3. Within lymphocytes, cell subsets were composed of human T cells (69.4%), B cells (5.5%), NK cells (20.0%), and T cells (2.8%). Human CD3^{+} T cells were found in spleen, liver, and intestine, but not in lung or kidney of humanised mice at 4 weeks post-transplantation. In addition, human immunoglobulin G could be detected in the humanised mice. The humanised mice could survive for >1 year after human immune system reconstitution.

Using the humanised mouse model, the role of γδ–T cells in influenza A virus infection was determined in vivo. C57BL/10SgAiRag2$^{-/-}$γc$^{-/-}$ mice were transplanted with huPBMCs or γδ–T cell–depleted PBMCs. Four weeks later, humanised mice were infected intranasally with human H1N1 influenza A virus. The weight of humanised mice were monitored. Although the weight of humanised mice decreased significantly after infection, there was no significant difference in the weight of humanised mice transplanted with huPBMCs or γδ–T cell–depleted PBMCs, which indicated that unexpanded γδ–T cells had little effect on influenza A virus infection.

The number of Vδ2-T cells could be increased 8-fold after 2 days of phosphoantigen treatment. This suggested that phosphoantigen could expand human Vδ2-T cells in vivo.

To determine whether phosphoantigen could be used for treatment of human influenza A virus infection in a humanised mouse model, the effect of phosphoantigen treatment on human influenza H1N1 virus infection in vivo was examined. During 20 days of observation, humanised mice could be effectively infected by human influenza H1N1 virus as indicated by the significant weight loss. However, for humanised mice treated with phosphoantigen, the weight did not decrease at all during this period. The phosphoantigen treatment significantly inhibited virus replication in the lung compared with the control group. Similarly, phosphoantigen treatment significantly decreased weight loss and mortality, and reduced virus titres in the lung infected with mouse-adapted influenza H1N1 PR/8 virus.

Similar to observations in humanised mice infected with influenza H1N1 virus, treatment with phosphoantigen significantly decreased weight loss and mortality, and reduced virus titres in the lung infected with highly pathogenic avian influenza H5N1 virus (A/Hong Kong/486/97). However, phosphoantigen had no protective role against another highly pathogenic avian influenza H5N1 virus infection (A/Hong Kong/483/97); all the humanised mice died 9 days after avian influenza H5N1 virus infection.

Discussion

Using a humanised mouse model, phosphoantigen was demonstrated to control human influenza A virus infection in vivo. For avian influenza A virus infection, this protection was strain-dependent. Phosphoantigen could control avian influenza A H5N1/486 but not H5N1/483 virus infection in vivo. The control of influenza A virus infection may be mediated by the selective activation and expansion of human Vδ2-T cells in the humanised mouse model. This variance between the different strains of H5N1 virus may be explained by the fact that H5N1/483 virus can invade mouse brain and cause death, but the human Vδ2-T cells cannot cross the brain-blood barrier. Our study suggests a novel therapeutic approach against influenza by using phosphoantigens to activate and expand γδ–T cells against influenza infection.

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