<table>
<thead>
<tr>
<th><strong>Title</strong></th>
<th>Use of humanised mice to study antiviral activity of human γδ-T cells against influenza A viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Author(s)</strong></td>
<td>Tu, WW; Lau, YL; Peiris, JSM</td>
</tr>
<tr>
<td><strong>Citation</strong></td>
<td>Hong Kong Medical Journal, 2014, v. 20 n. 6, Suppl. 6, p. S4-S6</td>
</tr>
<tr>
<td><strong>Issued Date</strong></td>
<td>2014</td>
</tr>
<tr>
<td><strong>URL</strong></td>
<td><a href="http://hdl.handle.net/10722/210985">http://hdl.handle.net/10722/210985</a></td>
</tr>
<tr>
<td><strong>Rights</strong></td>
<td>Hong Kong Medical Journal. Copyright © Hong Kong Academy of Medicine Press.; This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.</td>
</tr>
</tbody>
</table>
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.

**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. 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 with influenza A viruses.
were infected intranasally with the human influenza A virus H1N1 (A/Hong Kong/54/98) [25 μL, 10^4TCID<sub>50</sub>], mouse-adapted influenza H1N1 (A/PR/8/34) [25 μL, 1×LD<sub>50</sub>], and avian influenza H5N1 (A/Hong Kong/483/97) or (A/Hong Kong/486/97) [25 μL, 1×LD<sub>50</sub>]. 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. 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<sup>−/−</sup>γc<sup>−/−</sup> and established humanised mice were infected intranasally with human influenza H1N1 virus, PR/8 virus, or avian influenza H5N1 (25 μL, 1×LD<sub>50</sub>) 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. We tried to establish the humanised mouse model with a human immune system. Using immunodeficient C57BL/6Rag2<sup>−/−</sup>γc<sup>−/−</sup> 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<sup>−</sup> 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<sup>−</sup> T cells in influenza A virus infection was determined in vivo. C57BL/10SgAiRag2<sup>−/−</sup>γc<sup>−/−</sup> mice were transplanted with huPBMCs or γδ<sup>−</sup> 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<sup>−</sup> T cell–depleted PBMCs, which indicated that unexpanded γδ<sup>−</sup> 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.

Acknowledgements
This study was supported in part by the Research Fund for the Control of Infectious Diseases, Food and Health Bureau, Hong Kong SAR Government (#07060482); University Grants Committee, (AoE/M-12/06); and Research Grants Council (General Research Fund, HKU 777108M).

References