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The role of influenza virus gene constellation and viral morphology on cytokine induction, pathogenesis, and viral virulence

Key Messages

1. H5N1 viruses that cause severe disease in humans are potent inducers of proinflammatory cytokines in contrast to seasonal influenza viruses, and this may play a role in the mechanism of H5N1 pathogenesis.
2. H5N1 viruses are predominantly spherical in morphology. Virus morphology does not influence the ability to induce proinflammatory cytokines.
3. The NS1 viral protein may play a role in the potency of proinflammatory induction.
4. The H5N1 haemagglutinin and neuraminidase do not appear to transfer the high cytokine phenotype.
5. The ability to induce cytokines is a polygenic trait, involving a combination of different viral genes.

Introduction

Since the first emergence of highly pathogenic H5N1 avian influenza in 1997 (H5N1/97), the virus has continued to genetically re-assort and evolve to become increasingly pathogenic with an expanding range of hosts.¹ Between late 2003 and mid 2006, the newly emerged Z genotype H5N1 avian influenza has spread across 10 Asian countries and beyond Southeast Asia, causing high morbidity and mortality in aquatic birds, poultry and humans.² The pathogenesis of these highly virulent H5N1 viruses in humans is still largely unknown. Virus-induced cytokine dysregulation may play a crucial role in H5N1 pathogenesis and the induction of cytokines is replication dependent.³ The replication efficiency and the morphology of influenza A viruses may be linked.⁴

In addition, investigation of the viral determinants responsible for such cytokine imbalance is critical to the understanding of the possible mechanisms underlying the disease. This may help define potential therapeutic interventions in human disease associated with H5N1 to enable better influenza pandemic preparedness. Therefore the aims of this study were: (1) to investigate the role of virus morphology on the ability of influenza A H5N1 to induce tumour necrosis factor (TNF) and other proinflammatory cytokines from macrophages, and (2) to elucidate the viral gene component(s) involved in induction of cytokines by the use of influenza reverse genetics and naturally available reassortant viruses.

Methods

This study was conducted from February 2005 to January 2007. We used a panel of naturally occurring and recombinant viruses (generated by reverse genetics), which hyper-induce cytokine release from macrophages in either a virus-replication-dependent or independent manner. We proposed to correlate these phenotypes with virus replication competence, virus morphology (filamentous vs spherical particles) and virus genotype. In the second phase of the study, we used reverse genetics to engineer recombinant viruses, which contain human or avian influenza M or N genes that are known to alter virus morphology.³

Results

H5N1/97 had been demonstrated to cause hyper-induction of proinflammatory cytokines, most notably TNF- α , in primary human macrophages in vitro. We further explored the TNF- α induction potential of the recent Z genotype H5N1 viruses in a human macrophage model and the viral determinants involved in high TNF- α induction. By measuring mRNA levels using quantitative polymerase chain reaction and protein levels using enzyme-linked immunosorbent assay, the Z genotype viruses were found to be potent inducers of TNF- α ; some strains induced up to 4 folds greater TNF- α than did H5N1/97 (Fig 1). In light of these findings, the viral determinants responsible for the TNF- α hyperinduction were further investigated.

We hypothesised that TNF- α hyperinduction in primary human macrophages

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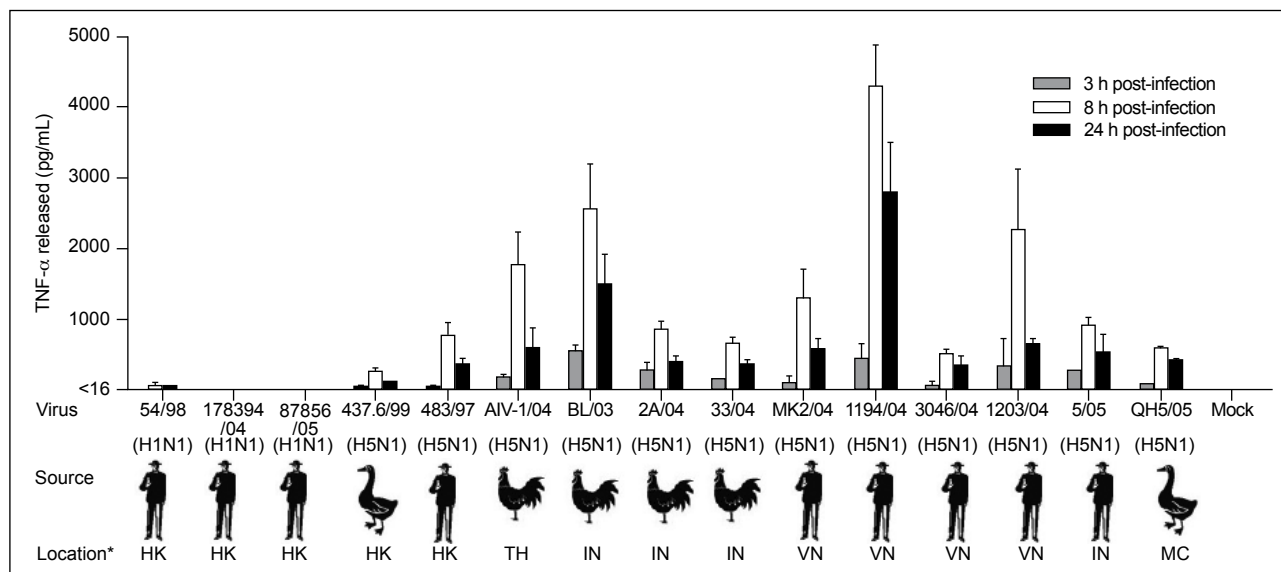


Fig 1. Tumour necrosis factor (TNF)- α secreted by primary human macrophages infected with H1N1 and H5N1 viruses

Primary human macrophages were infected at multiplicity of infection of 2 and the amount of TNF- α released into the supernatant was quantified by enzyme-linked immunosorbent assay at 3 h (■), 8 h (□) and 24 h (■) post-infection. Error bars indicate the standard deviation calculated from the results of 3 independent experiments using macrophages from different donors. H5N1 genotype Z viruses were found to hyper-induce TNF- α to a level similar or higher as compared to A/HK/483/97 (H5N1)

* HK denotes Hong Kong, TH Thailand, IN Indonesia, VN Vietnam, and MC mainland China

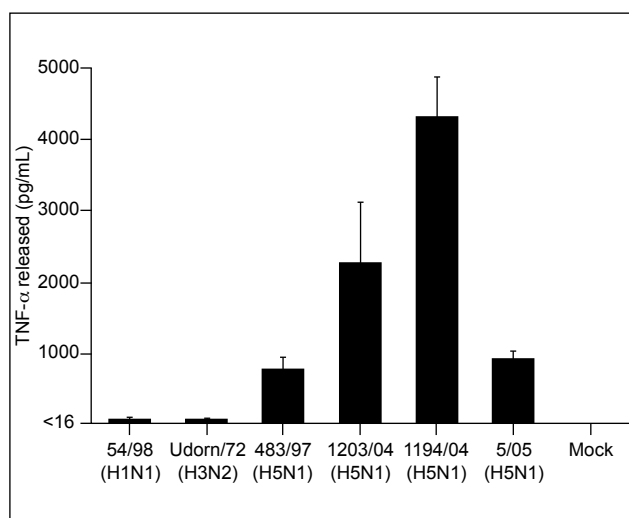


Fig 2. Comparison of tumour necrosis factor (TNF)- α secreted by Udorn/72 (H3N2) and H5N1-infected human macrophages

Human macrophages were infected at a multiplicity of infection of 2 and the amount of TNF- α released was quantified by enzyme-linked immunosorbent assay at 8 h post-infection. Macrophages infected with filamentous virus Udorn/72 induced significantly lower levels of the cytokine than those infected with H5N1 (Student's *t* test, $P < 0.05$)

in vitro may be related to filamentous viral morphology, or to specific viral genetic determinants. It was demonstrated that TNF- α hyperinduction was not associated with the morphology of virus particles, but was closely linked to the presence of certain H5N1 viral gene components (Fig 2).

By employing reverse genetics, recombinant viruses containing genes from H5N1 (A/Vietnam/1203/2004)

and genes from a low TNF- α -inducing virus, H1N1 (A/WSN/33), were constructed to further explore the genetic contribution of the eight viral segments of Z genotype H5N1 viruses towards TNF- α hyperinduction (Fig 3). Although recombinants containing the H5 NS, M or NP gene alone in an H1N1 genetic background did not confer the high TNF- α -inducing phenotype, such induction was likely a polygenic trait involving specific combinations of H5 genes. One of these combinations entailed the H5 replication complex (NP/PA/PB1/PB2), while another involved H5 surface proteins in addition to H5 matrix proteins (HA/NA/M). Both of these combinations led to significantly increased TNF- α production in primary human macrophages in vitro.

Discussion

A severe inflammatory reaction was triggered when cells were infected with the avian influenza H5N1 virus. The extreme immune response could then aggravate the resulting inflammatory reaction and lung damage. This may be a key contributor to what makes the H5N1 viruses more pathogenic than ordinary influenza viruses. These studies also provide a framework for understanding the disease severity that may ensue if the H5N1 virus becomes pandemic. Should this occur by reassortment (as happened in 1957 and 1968), cytokine induction would be less extensive and the disease may become attenuated. However, if the virus directly adapts to human-to-human transmission, as happened in 1918, the severity of human disease may remain very severe.

Acknowledgements

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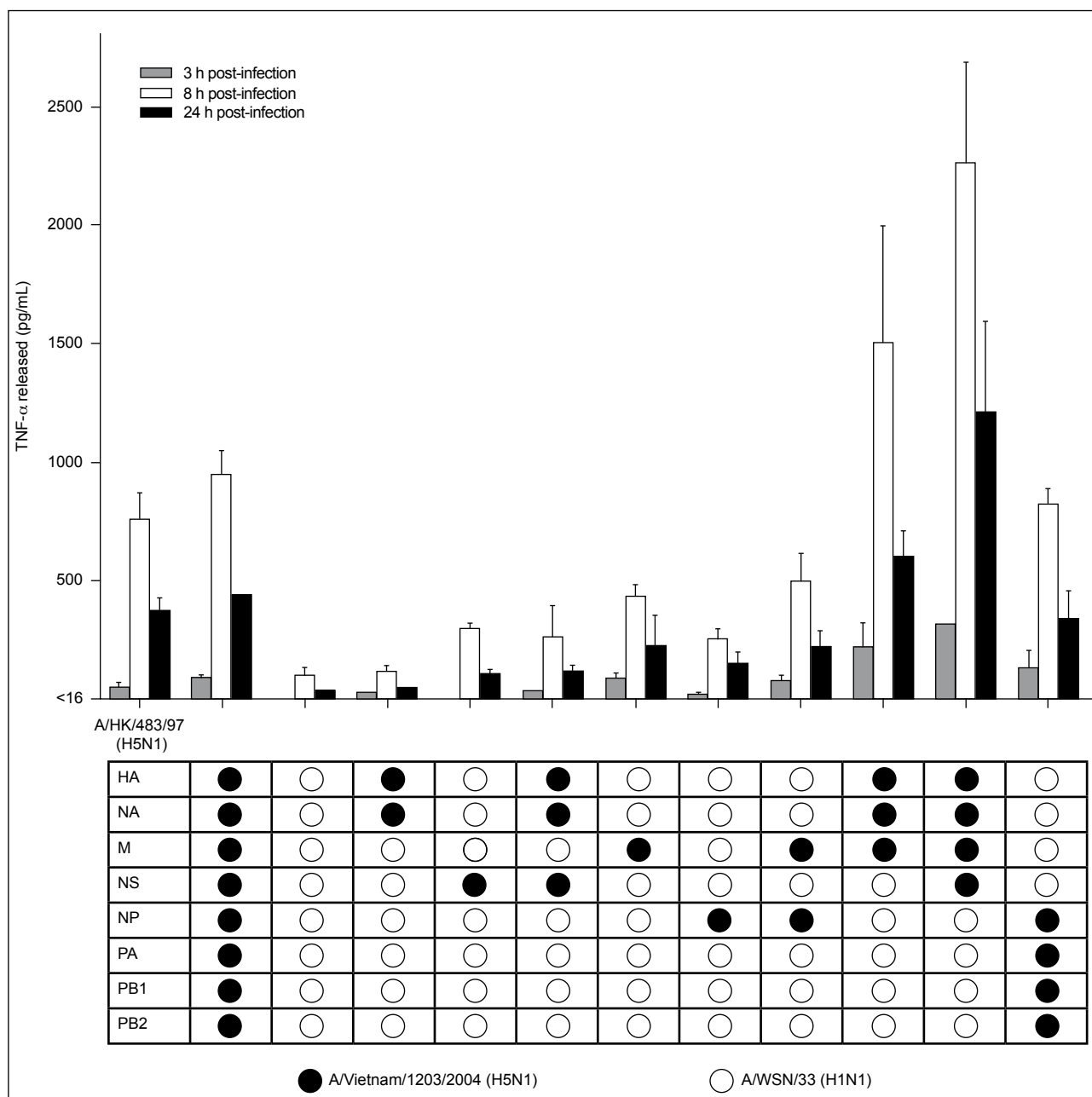


Fig 3. The contribution of 1203/04 gene segments to tumour necrosis factor (TNF)- α induction in human macrophages
 Recombinant viruses with varying combinations of 1203/04 and WSN/33 genes were tested (see text for details)

Control of Infectious Diseases (RFCID: 01030172), Food and Health Bureau, Hong Kong SAR Government. Results of this study have been used for writing a paper currently under review by the Journal of Infectious Diseases: Mok CK, Wong CH, Cheung CY, et al. The viral genetic determinants of H5N1 influenza viruses that contribute to cytokine dysregulation.

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