THE ROYAL SOCIETY

Published online 2 June 2004

The aetiology of SARS: Koch's postulates fulfilled

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Proof that a newly identified coronavirus, severe acute respiratory syndrome coronavirus (SARS-CoV) is the primary cause of severe acute respiratory syndrome (SARS) came from a series of studies on experimentally infected cynomolgus macaques (*Macaca fascicularis*). SARS-CoV-infected macaques developed a disease comparable to SARS in humans; the virus was re-isolated from these animals and they developed SARS-CoV-specific antibodies. This completed the fulfilment of Koch's postulates, as modified by Rivers for viral diseases, for SARS-CoV as the aetiological agent of SARS. Besides the macaque model, a ferret and a cat model for SARS-CoV were also developed. These animal models allow comparative pathogenesis studies for SARS-CoV infections and testing of different intervention strategies. The first of these studies has shown that pegylated interferon-α, a drug approved for human use, limits SARS-CoV replication and lung damage in experimentally infected macaques.

Finally, we argue that, given the worldwide nature of the socio-economic changes that have predisposed for the emergence of SARS and avian influenza in Southeast Asia, such changes herald the beginning of a global trend for which we are ill prepared.

Keywords: SARS; SARS-CoV; Koch's postulates; aetiology

SARS emerged more than a year ago as a new human disease entity (Lee *et al.* 2003; WHO 2003), resulting in more than 800 deaths from more than 8000 documented probable cases of the disease. About six months after the disease was first recognized, the WHO declared this global outbreak to be under control, with only few sporadic cases having been diagnosed since. In response to the start of the outbreak, the WHO coordinated an international collaboration that included clinical, epidemiological and laboratory investigations to control the expanding epidemic. Identification of the causative agents was one of the first research priorities. Several viruses and bacteria were initially identified in SARS patients. The recently identified hMPV (van den Hoogen *et al.* 2001) and a newly discovered human coronavirus, SARS-CoV (Drosten *et al.* 2003) proved to be the most likely candidates on epidemiological grounds in a comparative study involving more than 300 patients fitting the WHO SARS case definition in 12 cohorts in Asia and Europe. hMPV infection was diagnosed in 12% and SARS-CoV infection in 75% of the patients. Other respiratory pathogens were identified only sporadically. SARS-CoV was therefore the most likely causal agent of SARS (Kuiken *et al.* 2003*a*). According to Koch's postulates, as modified by Rivers (1937) for virus diseases, six criteria should be met to establish a virus as the cause of a disease. The first three of these, namely isolation of the virus from diseased hosts, cultivation in host cells and proof of filterability, were met for both hMPV and SARS-CoV. The remaining three criteria were tested for in a series of experiments that we conducted in cynomolgus macaques (*Macaca fascicularis*): production of a comparable disease in the original host species or a related one, re-isolation of the virus and detection of a specific immunoresponse to the virus (Kuiken *et al.* 2003*a*). Four monkeys were infected with a SARS-CoV isolated in Vero cells from a patient who had died of SARS in Hong Kong. Three of these became lethargic from days 2–3 after infection onwards and two of them developed a temporary skin rash at day 4 after infection. Upon necropsy, carried out on day 6 after infection, three of the animals had multiple foci of pulmonary consolidation in both lungs.

Histologically, the main lesions in the consolidated lung tissue involved the alveoli and bronchioles, and consisted of areas with acute or more advanced phases of diffuse alveolar damage. Occasional multinucleated giant cells were found. In the macaque without clear macroscopic pulmonary lesions, minimum multifocal inflammatory lesions were observed histologically in the pulmonary tissue. By immunohistochemistry, the presence of SARS-CoV could be demonstrated in inflamed lung tissue of the three macaques showing pulmonary consolidation. The presence of SARS-CoV in the lungs was also confirmed by showing typical coronavirus-like particles in pneumocytes in affected pulmonary areas. From day 2 after infection onwards, SARS-CoV could be isolated or demonstrated by reverse transcription–polymerase chain reaction in sputum, nasal swabs and pharyngeal swabs of one or more animals. In a separate macaque infection experiment, it was shown that SARS-CoV-neutralizing antibodies were present from day 12 after infection onwards (Fouchier *et al.* 2003). The virus was also isolated from the faeces of one of the two monkeys involved in this experiment. No other relevant respiratory pathogens were identified in any of the macaques experimentally infected

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One contribution of 15 to a Discussion Meeting Issue 'Emerging infections: what have we learnt from SARS?'.

with SARS-CoV. Collectively, these data completed the six criteria that needed to be fulfilled to identify SARS-CoV as the aetiological agent of SARS, which probably spilled over recently from an animal reservoir. Moreover, lesions in macaques infected experimentally with hMPV, isolated from a non-SARS individual, were limited to mild suppurative rhinitis and minimal erosions in conducting airways (Kuiken *et al.* 2003*a*, 2004). The disease was not exacerbated in two SARS-CoV-infected macaques that were subsequently inoculated with hMPV (Fouchier *et al.* 2003; Kuiken *et al.* 2003*a*). Besides cynomolgus macaques, we also succeeded in infecting ferrets and domestic cats experimentally with SARS-CoV (Martina *et al.* 2003). The virus was efficiently transmitted to non-infected cage mates. The cats did not develop clinical signs, whereas some of the ferrets became lethargic and one died. Infection of the respiratory tract was evident in all the infected animals and, histologically, SARS-CoV infection was associated with pulmonary lesions similar to those observed in the SARS-CoV infected macaques, except that they were milder, particularly in the cats. The availability of three animal models for SARS-CoV infection now allows us to carry out comparative pathogenesis studies as well as the testing of different intervention strategies like the use of antivirals, SARS-CoV-specific antibodies and candidate vaccines. Currently, we are using the ferret and the macaque models for such studies. Recently, we showed in the macaque model that prophylactic treatment of SARS-CoV-infected macaques with the antiviral agent pegylated interferon-α—a drug approved for human use—significantly reduces viral replication and excretion as well as pulmonary damage, compared with untreated macaques (Haagmans *et al.* 2004). Post-exposure treatment with this drug yielded intermediate results. These data warrant clinical studies with this drug, if and when SARS re-emerges. Prophylactic or early post-exposure treatment with pegylated interferon-α may help reduce the impact of SARS-CoV infections on healthcare workers and others possibly exposed to SARS-CoV, and may limit the spread of the virus in the human population.

Finally, one may wonder whether the recent emergence of SARS and the emergence of avian influenza A outbreaks, which also have resulted in animal to human transmissions with fatal consequences (de Jong *et al.* 1997; Claas & Osterhaus 1998; Webby & Webster 2003; Hien *et al.* 2004), in Southeast Asia and more particularly in Guandong Province of China, may be a result of key socio-economic changes in that region (T. Kuiken and A. D. M. E. Osterhaus, unpublished data). It may be argued that given the worldwide nature of these changes, the observed increase in the emergence of such virus infections in this area may herald the beginning of a global trend for which we are ill prepared (Kuiken *et al.* 2003*b*, 2004).

REFERENCES

Claas, E. C. & Osterhaus, A. D. M. E. 1998 New clues to the emergence of flu pandemics. *Nature Med.* **4**, 1122–1123.

de Jong, J. C., Claas, E. C., Osterhaus, A. D. M. E., Webster, R. G. & Lim, W. L. 1997 A pandemic warning? *Nature* **389**, 554.

Drosten, C. (and 25 others) 2003 Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *New Engl. J. Med.* **348**, 1967–1976.

Fouchier, R. A., Kuiken, T., Schutten, M., van Amerongen, G., van Doornum, G. J., van den Hoogen, B. G., Peiris, M., Lim, W., Stohr, K. & Osterhaus, A. D. M. E. 2003 Aetiology: Koch's postulates fulfilled for SARS virus. *Nature* **423**, 240.

Haagmans, B. L. (and 11 others) 2004 Pegylated interferon-alpha protects type 1 pneumocytes against SARS coronavirus infection in macaques. *Nature Med.* **10**, 290–293.

Hien, T. T. (and 29 others) 2004 Avian influenza A (H5N1) in 10 patients in Vietnam. *New Engl. J. Med.* **350**, 1179–1188.

Kuiken, T. (and 21 others) 2003 Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. *Lancet* **362**, 263–270.

Kuiken, T., Fouchier, R. A. M., Rimmelzwaan, G. F. & Osterhaus, A. D. M. E. 2003*b* Emerging viral infections in a rapidly changing world. *Curr. Opin. Biotechnol.* **14**, 641–646.

Kuiken, T., van den Hoogen, B. G., van Riel, D. A. J., Laman, J. D., van Amerongen, G., Sprong, L., Fouchier, R. A. M. & Osterhaus, A. D. M. E. 2004 Experimental human metapneumovirus infection of cynomolgus macaques (*Macaca fascicularis*) results in virus replication in ciliated epithelial cells and pneumocytes with associated lesions throughout the respiratory tract. *Am. J. Pathol.* (In the press.)

Lee, N. (and 13 others) 2003 A major outbreak of severe acute respiratory syndrome in Hong Kong. *New Engl. J. Med.* **348**, 1986–1994.

Martina, B. E., Haagmans, B. L., Kuiken, T., Fouchier, R. A., Rimmelzwaan, G. F., Van Amerongen, G., Peiris, J. S., Lim, W. & Osterhaus, A. D. M. E. 2003 Virology: SARS virus infection of cats and ferrets. *Nature* **425**, 915.

Rivers, T. M. 1937 Viruses and Koch's postulates. *J. Bacteriol.* **33**, 1–12.

van den Hoogen, B. G., de Jong, J. C., Groen, J., Kuiken, T., de Groot, R., Fouchier, R. A. & Osterhaus, A. D. M. E. 2001 A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nature Med.* **7**, 719–724.

Webby, R. J. & Webster, R. G. 2003 Are we ready for pandemic influenza? *Science* **302**, 1519–1522.

World Health Organization 2003 Severe acute respiratory syndrome (SARS). *Wkly Epidemiol. Rec.* **78**, 81–83.

GLOSSARY

hMPV: human metapneumovirus

SARS: severe acute respiratory syndrome

SARS-CoV: severe acute respiratory syndrome coronavirus

WHO: World Health Organization