LETTER

Influence of HLA gene polymorphisms on susceptibility and outcome post infection with the SARS-CoV virus

Dear Editor,

Severe Acute Respiratory Syndrome (SARS) has been described as the first pandemic of the 21st century and between November 2002 and July 2003, over 8000 people were infected with the SARS-associated coronavirus (SARS-CoV). Polymorphisms in the human leukocyte antigen (HLA) system have been shown to influence susceptibility to SARS-CoV but here we were unable to demonstrate any association between HLA and susceptibility or outcome post infection with the SARS-CoV.

Between November 2002 and February 2003 approximately 300 cases of atypical pneumonia were diagnosed in Guangdong province in Southern China (Peris J S M et al., 2004). In March 2003 the World Health Organisation (WHO) issued a global health alert followed by a travel advisory and by the time the epidemic had run its course (by July 2003), there was a total of 8096 cases from 26 countries, with a case fatality rate of almost 10% (http://www.whoint/csr/sars/country/table2004_04_21/en/). In a remarkable global collaborative effort, a novel Coronavirus (SARS-CoV) was identified and the entire genome was sequenced by the middle of April 2003 (Peris J S M et al., 2004).

A number of host factors have been proposed to influence susceptibility and outcome post infection with the SARS-CoV and these include the ACE2 receptor (Li W et al., 2003) and L-SIGN (Jeffers S A et al., 2004). Both *in vivo* (Reghunathan R et al., 2005) and *in vitro* (Ng M H L et al, 2004) studies have clearly highlighted the importance of the innate inflammatory immune response rather than the specific immune response as the more important determinants for infection with the SARS-CoV. A number of studies have demonstrated a possible association between HLA gene polymorphisms and susceptibility to SARS-CoV, with HLA-B*4601 (Lin M et al., 2003), HLA-B*0703 (Ng L F P et al., 2005), HLA-DRB4*01010101 (Ng M H L et al., 2010), HLA-DR B1*1202 (Keicho N et al., 2009) and HLA-Cw*0801 (Chen Y M et al., 2006) found to be significantly associated with genetic susceptibility to infection with the SARS-CoV.

A possible protective effect was also seen for certain other HLA alleles including HLA-Cw*1502 (Wang S F et al., 2011) and HLA-DRB1*0301 (Ng M H L et al., 2004, Wang S F et al., 2011). In associated coverage in the scientific literature (Chiu Y T, 2003), it was suggested that the screening and isolation of genetically at risk health care workers might prevent the spread of the virus in any future SARS outbreak.

Here we have investigated a possible association between HLA gene polymorphisms and susceptibility and outcome post infection with the SARS-CoV in a large cohort of Hong Kong Chinese patients who were infected with SARS-CoV. This cohort (n=176 patients) is almost twice the size of the groups studied previously and gives a better opportunity to look for a possible influence of HLA on susceptibility and outcome post infection with the SARS-CoV.

This study was conducted on samples collected at the teaching hospital of the Chinese University of Hong Kong, Prince of Wales Hospital, where a major outbreak of SARS had occurred (Lee N et al., 2003). All included patients were unrelated and fulfilled the WHO's surveillance definition of SARS including high fever > 38 °C, cough or breathing difficulty, a contact history within 10 days prior to the onset of symptoms and radiological evidence of pneumonia (http://www.who.int/csr/sars). The majority of study patients had serological evidence of SARS-CoV infection as determined by an in-house indirect immunofluoresence assay (Chan P K et al., 2004a) and of the few patients that died before a convalescent serum sample could be collected, SARS-CoV infection was confirmed by RT-PCR and/or virus isolation (Chan P K et al., 2004b). HLA- A, B (Kennedy L J et al., 1997) and HLA-DR (Kimura A et al., 1992) typing was carried out using PCR and sequence specific oligonucleotide probes. The HLA gene frequencies for all the SARS patients (all of Chinese ethnic origin) were compared with a published control population of unrelated Hong Kong ethnic Chinese bone marrow donors (Chang Y W et al., 1997) and are shown in Table 1). For the Hong Kong Chinese bone marrow donors, HLA-A and -B typing was by serology and HLA-DRB1 from sequence based typing (Chang Y W et al., 1997). There were no significant differences in HLA gene frequencies between the SARS infected and control groups. Further stratification on the basis of disease severity did not demonstrate any

Table 1. HLA gene frequencies (GF) in SARS patients and healthy controls

		GF Mild Infection N=128 for HLA-A and HLA-AB N=128 for HLA-DRB1	GF Dead /ICU N=48 HLA-A and HLA-AB N=48 for HLA-DRB1	GF Normal N=18774 HLA-A and HLA-AB N=250 for HLA-DRB1
HLA-A	01	0.016	0.010	0.008
	02	0.333	0.339	0.316
	03	0.012	0.010	0.011
	11	0.304	0.339	0.338
	24	0.161	0.134	0.145
	26	0.012	0.010	0.020
	29	0.004	0	0.012
	30	0.012	0.043	0.016
	31	0.020	0.010	0.015
	33	0.112	0.076	0.099
	34	0.004	0	0.001
HLA-B	07	0.008	0	0.015
	13	0.091	0.149	0.092
	15	0.163	0.137	0.167
	18	0.004	0.011	0.004
	27	0.024	0.032	0.021
	35	0.024	0.011	0.032
	37	0.012	0.011	0.005
	38	0.048	0.043	0.051
	39	0.016	0.022	0.014
	60	0.163	0.149	0.149
	61	0.028	0.032	0.027
	44	0.020	0.011	0.014
	46	0.177	0.162	0.147
	48	0.012	0.032	0.017
	50	0.008	0	0.003
	51	0.053	0.022	0.047
	52	0.004	0.011	0.006
	54	0.032	0.066	0.021
	55	0.016	0	0.021
	56	0.004	0.022	0.009
	58	0.078	0.055	0.067
	67	0.008	0.011	0.002
	81	0.004	0	0
HLA-DRB1	03	0.056	0.022	0.088
	04	0.129	0.113	0.100
	07	0.044	0.055	0.053
	08	0.060	0.055	0.045
	09	0.161	0.188	0.159
	10	0.016	0.032	0.022
	11	0.052	0.089	0.047
	12	0.176	0.201	0.176
	13	0.032	0.011	0.026
	14	0.069	0.066	0.075
	15	0.157	0.089	0.126
	16	0.056	0.077	0.062

HLA-DRB1 gene frequencies (GF) were calculated by direct counting of observed alleles. For HLA-A and -B phenotype frequencies were first determined by direct counting and the method of Ceppellini (Ceppellini R et al., 1955) was used to calculate GF. Differences in allele number observed between cases and controls were evaluated using Fisher's exact test. P values were corrected for the number of observations made. No significant differences were observed.

significant differences between the severe SARS group (n=48, intensive care unit (ICU) and/ or death) and the mild SARS group (n=128, no ICU stay and mild symptoms) again compared with the control bone marrow donor cohort.

Based on a larger cohort of patients than reported in previous studies, we were unable to demonstrate any significant association between HLA gene polymorphisms and susceptibility or outcome post infection with the SARS-CoV. Similar findings were reported for a SARS cohort from Guangdong in Southern China (Xiong P et al., 2008). While this does not preclude such an association, the present data are fully consistent with the current view that susceptibility and outcome post infection with the SARS-CoV are more likely to be influenced by the innate inflammatory response and factors such as age and associated underlying illness (Peiris J S M et al., 2004) than the specific arm of the immune response. Issues of small sample numbers have the ability to bias interpretations and this has been noted by others (http://

www.the-scientist.com/?articles.view/articleNo/16130/ title/Gene-Association-Studies-Typically-Wrong/) and it would be worthwhile to combine the various studies and perform a meta-analysis. While on-going studies to further understand the molecular details of the inflammatory and immune response to infection with the SARS-CoV are essential, it is important to remember and reinforce the public health message that the most effective tool in the fight against SARS is the same as that used against an age old infection like cholera, namely rapid tracing, identification and isolation of infected individuals and their contacts in order to limit the spread of the infectious agent.

FOOTNOOTES

Fang Fang Yuan[1], Zlatibor Velickovic[1,2], Lesley J Ashton[3], Wayne B Dyer[1,4], Andrew F Geczy[1], Heather Dunckley[1], Garry W Lynch[1,5], John S Sullivan[1✉]

1. Central Clinical School, Sydney Medical School, University of Sydney, NSW 2006, Australia;
2. Department of Cell & Molecular Therapies, Royal Prince Alfred Hospital, NSW 2050, Australia;
3. Faculty of Medicine, University of New South Wales, High St, Kensington, NSW 2052, Australia;
4. Australian Red Cross Blood Service, 17 O'Riordan St, Alexandria, NSW 2015, Australia;
5. Veterinary Science, University of Sydney, NSW 2006, Australia
✉Correspondence:
Phone: +61-2-94284829, Email: john.sullivan@sydney.edu.au

REFERENCES

Ceppellini R, Siniscalco C A, Smith C A. 1955. Ann Hum Genet, 20: 97-115.

Chan P K, To W K, Ng K C, et al. 2004a. Emerg Infect Dis, 10: 825-831.

Chan P K, Ng K C, Chan R C, et al. 2004b. Emerg Infect Dis, 10: 530-532.

Chang Y W, Hawkins B R. 1997. Hum Immunol, 56: 125-135.

Chen Y M, Liang S Y, Shih Y P, et al. 2006. J Clin Microbiol, 44: 359-365.

Chiu Y T. 2003. Nat Med, 9: 1335.

Jeffers S A, Tusell S M, Gillim-Ross L, et al. 2004. Proc Natl Acad Sci USA, 101: 15748-15753.

Keicho N, Itoyama S, Kashiwase K, et al. 2009. Hum Immunol, 70: 527-531.

Charron D. 1997. HLA: Genetic diversity of HLA: Function and medical implications. EDK Press, Paris. pp216-225.

Tsuji K, Aizawa M, Sasazuki T. 1992. HLA 1991. Oxford University Press, Oxford. pp397-419.

Lee N, Hui D, Wu A, et al. 2003. N Engl J Med, 348: 1986-1994.

Li W. Moore M J, Vasilieva N, et al. 2003. Nature, 426: 450-454.

Lin M, Tseng H K, Trejaut J A, et al. 2003. BMC Medical Genetics, 4: 9-15.

Ng L F P, Hibberd M L, Ooi E E, et al. 2004. BMC Infect Dis, 4: 34.

Ng M H L, Cheng S H, Lau K M, et al. 2010. Hong Kong Med J, 16 (Suppl 4): S29-33.

Ng M H L, Lau K M, Li L, et al. 2004. J Infect Dis, 190: 515-518.

Peiris J S M, Guan Y and Yuen K Y. 2004. Nat Med, 10: S88-S97.

Reghunathan R, Jayapal M, Hsu L Y, et al. 2005. BMC Immunol, 6:2.

Wang S F, Chen K H, Chen M, et al. 2011. Viral Immunol, 24: 421-426.

Xiong P, Zeng X, Song M S, et al. 2008. Int J Immunogenetics, 35: 69-74.