

High Prevalence of the CD14-159CC Genotype in Patients Infected with Severe Acute Respiratory Syndrome-Associated Coronavirus[∇]

Fang F. Yuan,^{1,3*} Ingrid Boehm,¹ Paul K. S. Chan,^{2,5} Katherine Marks,¹ Julian W. Tang,²
David S. C. Hui,^{4,5} Joseph J. Y. Sung,^{4,5} Wayne B. Dyer,^{1,3} Andrew F. Geczy,³
and John S. Sullivan^{1,3}

Australian Red Cross Blood Service-Endeavour, Sydney, NSW 2000, Australia¹; Department of Microbiology, The Chinese University of Hong Kong, Hong Kong²; Research Unit of Transfusion Medicine and Immunogenetics, Faculty of Medicine, University of Sydney, Sydney, Australia³; Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong⁴; Stanley Ho Centre for Emerging Infectious Diseases, School of Public Health, The Chinese University of Hong Kong, Hong Kong⁵

Received 28 February 2007/Returned for modification 15 May 2007/Accepted 27 September 2007

To investigate whether genetic factors of innate immunity might influence susceptibility and/or progression in individuals infected with SARS, we evaluated the CD14 gene polymorphism in 198 Hong Kong blood donors and 152 Hong Kong severe acute respiratory syndrome (SARS) patients who were previously genotyped for FcγRIIA polymorphisms. The prevalence of the CD14-159CC polymorphism was significantly higher in the patients with severe SARS than in those with mild SARS or controls (31% versus 15% [mild SARS] or 20% [controls]; mild SARS: $P = 0.029$; odds ratio, 2.74; 95% confidence interval, 1.15 to 6.57; controls, $P = 0.04$; odds ratio, 2.41; 95% confidence interval, 1.05 to 5.54), and both CD14-159CC and FcγRIIA-RR131 are risk genotypes for severe SARS-CoV infection.

The study of the relevance of polymorphisms of immunity-related genes in infectious diseases has been an important area of investigation, especially with regard to how these polymorphisms influence both susceptibility to the infection and the course of disease development. Previously, we investigated FcγRIIA and MBL polymorphisms in a group of 180 people from Hong Kong who were infected with severe acute respiratory syndrome-associated coronavirus (SARS-CoV) and revealed that in addition to age and comorbidity, FcγRIIA polymorphisms in individuals may influence outcome after infection with the SARS-CoV, the causative agent of SARS, as a significant association between FcγRIIA-R/R131 genotype and severe SARS was found (10). It is important to investigate whether other host genetic factors could influence susceptibility to SARS-CoV infection and its subsequent clinical course. The innate immune system plays a role in limiting an infectious challenge in the early stages after exposure, during the lag time required to initiate long-lasting adaptive immunity. The impairment of Toll-like receptors (TLRs) due to polymorphisms of TLR genes can alter immune response to a wide variety of microbial ligands, including viruses, and polymorphisms in TLR2 and TLR4 have been linked to infectious diseases in human. In particular, TLR-Arg677Trp was reported to be present in Korean patients with lepromatous leprosy exclusively (6), and this polymorphism was also found to be associated with tuberculosis in a Tunisian population (3). TLR2 and TLR4 SNPs also exhibit ethnic variation, with low and high

frequencies in some Asian populations compared with Caucasians and Africans (7). To examine the presence of polymorphisms in TLR2, TLR4, and their coreceptor CD14 in both healthy controls and SARS patients in Hong Kong, we further genotyped 152 patients who were diagnosed as having SARS both clinically and serologically in the Prince of Wales Hospital in Hong Kong and a group of 198 blood donors from Hong Kong (10) for polymorphisms in TLR2, TLR4, and CD14. The study was approved by the Institutional Human Ethics committees of the Prince of Wales Hospital and the Hong Kong Red Cross Blood Transfusion Service.

TLR2-Arg667Trp/Arg753Gln (2180C/T and 2408G/A), TLR4-Asp299Gly/Thr399Ile (12874A/G and 13174C/T), and CD14-159C/T were genotyped in individuals using PCR and restriction fragment length polymorphism analysis as described previously (2, 8, 9) with the following slight modifications: the PCR was performed by adding 1 μl DNA to a 13-μl solution containing 67 mM Tris base (pH 8.8), 16.6 mM ammonium sulfate, 2.5 mM magnesium chloride, 0.1% Tween-20, 200 μM of each deoxynucleoside triphosphate, 10 pmol of each oligonucleotide of a pair of appropriate primers, and 1 U Platinum *Taq* polymerase (Invitrogen); after PCR, 5 μl of the PCR product was used for overnight digestion at 37°C with the appropriate restriction enzymes (New England Biolabs): for TLR2 (Arg753Gln and Arg677Trp) polymorphisms, 2.5 U *AciI* was used, for TLR4 (Asp299Gly and Thr399Ile), 2 U *NcoI* or 2 U *HinfI* was used, and for CD14-159C/T, 5 U of *AvaII* was used. The digests were run on 2.5% high-resolution agarose gels. Data were analyzed for differences in distribution of CD14-159CC, -CT, and -TT genotypes among groups using the chi-squared test for linear trend or independence (two-by-three contingency tables and χ^2 analysis). Frequencies of combined genotypes (CD14-159 and FcγRIIA) were compared

* Corresponding author. Mailing address: Cell Biology Laboratory, ARCBS-Endeavour, 153 Clarence Street, Sydney, NSW 2000, Australia. Phone: 61 2 9229 4523. Fax: 61 2 9229 4521. E-mail: fyuan@arcbs.redcross.org.au.

[∇] Published ahead of print on 3 October 2007.

TABLE 1. Comparison CD14 polymorphisms and "risk" genotypes (CD14-159CC, FcγRIIA-R/R131) between Hong Kong patients with SARS and control group of Hong Kong blood donors

Factor	No. (%) of:		
	SARS patients (n = 152) ^a		Blood donors (n = 198)
	Mild	Severe (ICU) ^c	
Presence of CD14-159 polymorphism ^b			
CC	19 (15)	8 (31)	39 (20)
CT	67 (53)	14 (54)	95 (48)
TT	40 (32)	4 (15)	64 (32)
Presence of risk genotypes	30 (24)	12 (46)	53 (27)
Absence of risk genotypes	96 (76)	14 (54)	145 (73)

^a Mild SARS refers to patients who recovered without the need for intensive care or ventilation.

^b The difference in linear tread distribution of CD14-159 genotypes (CC, CT, and TT) between the ICU and mild SARS groups was significant (two-by-three contingency table; $P = 0.027$).

^c In comparison with the mild SARS group and controls, the incidence of patients carrying risk genotypes in the severe SARS (ICU) group is significantly higher (two-by-two contingency table; for mild SARS, two-sided $P = 0.029$; odds ratio, 2.74 [1.15 to 6.57]; for controls, $P = 0.04$; odds ratio, 2.41 [1.05 to 5.54] for controls.

among groups using Fisher's exact test (two-by-two contingency tables, two sided). Differences were considered significant when P was <0.05 . TLR4-Asp299Gly and TLR4-Thr399Ile polymorphisms, which are common in Caucasians (1), were found in one Hong Kong blood donor only, and no individuals carried the TLR2-Arg677Trp or the TLR2-Arg753Gln polymorphism. Difference in the linear tread distribution of CD14-159 genotypes (CC, CT, and TT) between the severe-SARS (intensive care unit [ICU]) and mild-SARS subgroups was significant (31%, 54%, and 15% versus 15%, 53%, and 32%, respectively; $P = 0.027$) (Table 1). Taken together with our previous investigation of FcγRIIA polymorphisms in those patients, these results revealed that CD14-159CC and FcγRIIA-R/R131 are risk genotypes for severe SARS-CoV infection, as nearly half of the ICU patients carried the risk genotype(s), compared to less than a quarter of the mild SARS group or controls (46% versus 28% [mild SARS] or 27% [controls]; mild SARS: $P = 0.029$; odds ratio, 2.74 [1.15 to 6.57]; controls: $P = 0.04$; OR, 2.41 [1.05 to 5.54]) (Table 1). The results of this study demonstrate a possible link between CD14-159CC and severity of SARS-CoV infection, apart from FcγRIIA-R/R131. CD14 plays a unique role in innate immunity as a coreceptor for both TLR2- and TLR4-mediated microbial ligand signaling to initiate innate immune activity. It has been shown that CD14 could induce or elevate

TLR2/TLR4-dependent cytokine response to lipopolysaccharide and cytomegalovirus (4, 7). The possible functional role of the CD14-159 polymorphism is to alter the inflammatory response in individuals, since CD14-159 influences serum CD14 (sCD14) levels, with elevated levels of sCD14 being found in CD14-159TT carriers compared to -159CC carriers (2, 5), and sCD14 binds to circulating bacterial endotoxin and reduces its biological activity. The finding that severe SARS (ICU) patients exhibit higher a frequency of CD14-159CC and low CD14-159TT genotypes may suggest that those patients could have reduced antiviral activity and thus experience enhanced viral toxicity, resulting in a more severe form of SARS-CoV infection, but the exact mechanism of CD14 in response to viruses is not clear. The major finding of our study is a significant association between risk genotypes (CD14-159CC and FcγRIIA-R/R) and the severity of SARS-CoV infection. Although polymorphisms of TLR4-299/399 and TLR2-677/753 are rare or nonexistent in Hong Kong populations, the question of what exact role TLRs play in the clinical and pathological features of SARS remains.

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