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Association of cytokine and chemokine gene polymorphisms with severe acute respiratory syndrome

Key Messages

1. The *IFN-γ* +874A allele and *RANTES* -28 G allele are risk factors for SARS susceptibility.
2. The *RANTES* -28 G allele plays a role in the pathogenesis of SARS.
3. The polymorphisms of *IL-10, TNF-α, IL-12, IP-10, Mig* and *MCP-1* are not associated with SARS susceptibility.

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Introduction

Severe acute respiratory syndrome (SARS) is an infectious disease caused by the SARS coronavirus,[1] but the pathogenesis is still far from clear.[2] We have demonstrated that genetic haplotypes associated with low-serum mannose-binding lectin (MBL) are associated with SARS,[3] and our findings have been replicated recently.[4] Hong Kong Chinese who are homozygotes for *CLEC4M* tandem repeats have recently been reported to be less susceptible to SARS. Other susceptibility genes, such as OAS-1 and MxA have also been identified.

Cytokines are known to take an important role in antiviral action. Interferon (IFN)-γ from T and natural killer (NK) cells is important for driving the T helper cell type 1 (Th1) responses. It also activates monocytes and macrophages, which in turn take part in antiviral responses by producing free radicals and pro-inflammatory cytokines like tumour necrosis factor (TNF)-α. On the other hand, interleukin (IL)-10 counteracts the inflammatory response by inhibiting TNF-α production and neutrophil activation. Interleukin-12 is important for the development of the T helper type 1 (Th1) response in the initial phase of bacterial, parasitic, and viral infections. In many viral infections, IL-12 promotes viral clearance and host recovery from infection.

Chemokines play an important role in cell trafficking during immune responses. Acute respiratory viruses commonly induce inflammatory chemokines in local tissue. In a previous study we confirmed that the SARS coronavirus induces upregulation of a number of inflammatory chemokines, ie Regulated upon Activation Normal T cell-Expressed and Secreted (RANTES), interferon-gamma inducible protein 10 (IP-10) and Monocyte Chemoattractant Protein-1 (MCP-1). The upregulation of these chemokines, including the monokine induced by interferon gene (Mig) may recruit inflammatory cells and leukocytes into the tissue.

In this study, we hypothesised that polymorphisms of the cytokine genes, *IFN-γ, IL-10, TNF-α*, *IL-12,* and the chemokine genes, *RANTES, IP-10, Mig* and *MCP-1*, might be associated with SARS. These genes were chosen for their key roles in antiviral action and inflammation regulation and their polymorphisms based on their potential regulation of gene expression.

Methods

This study was conducted from September 2005 to August 2006.

Patient populations

The study was approved by the Clinical Research Ethics Committee of the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. This study included 495 Hong Kong Chinese patients with SARS (211 males and 284 females, mean±SD age was 40.74±15.73 years) and 578 ethnically matched healthy controls from the Red Cross (343 males and 235 females, mean±SD age was 30.05±9.49 years). At least 95% of the patients were documented as having SARS-CoV antibody seroconversion and/or detectable SARS-CoV RNA in respiratory secretions by RT-PCR. The death group consisted of 57 patients who died from SARS and their mean±SD age was

Table 1. Genotype frequencies and allele frequencies of cytokines in SARS patients and controls

Genotype	SNP	No. (%)		OR (95% CI)*	P value*
		SARS (n=476)	Control (n=449)		
IFN-γ +874	A/A	332 (69.8)	203 (45.2)	5.19 (2.78 - 9.68)	<0.001
	A/T	127 (26.7)	189 (42.1)	2.57 (1.35 - 4.88)	
	T/T	17 (3.6)	57 (12.7)	Reference	
IL-10 -1082	A/A	439 (92.2)	411 (91.5)		NS†
	A/G	35 (7.4)	38 (8.5)		
	G/G	2 (0.4)	0 (0)		
IL-10 -592	A/A	244 (51.3)	209 (46.6)		NS
	A/C	188 (39.5)	214 (47.7)		
	C/C	44 (9.2)	26 (5.8)		
TNF-α -308	GG	403 (84.7)	377 (83.9)		NS
	GA	70 (14.7)	70 (15.6)		
	AA	3 (0.6)	2 (0.5)		
		SARS (n=470)	Control (n=558)		
IL-12B promoter 318/322	318/318	122 (26.0)	145 (26.4)		NS
	318/322	252 (53.6)	238 (43.4)		
	322/322	96 (20.4)	166 (30.2)		
IL-12B intron 2 218/221	218/218	153 (32.6)	160 (29.3)		NS
	218/221	234 (49.8)	251 (45.9)		
	221/221	83 (17.7)	135 (24.7)		
IL-12B intron 4 268/272	268/268	70 (14.9)	133 (23.8)		NS
	268/272	222 (47.2)	237 (42.5)		
	272/272	147 (31.3)	150 (26.9)		
	Others	31 (6.6)	38 (6.81)		
IL-12B 3'UTR	A/A	157 (33.4)	155 (27.9)		NS
	A/C	235 (50.0)	294 (52.9)		
	C/C	78 (16.6)	107 (19.2)		
Allele					
IFN-γ +874	A	791 (83.1)	595 (66.3)	2.23 (1.75 - 2.83)	<0.001
	T	161 (16.9)	303 (33.7)		
IL-10 -1082	A	913 (95.9)	860 (95.8)		NS
	G	39 (4.1)	38 (4.2)		
IL-10 -592	A	676 (71.0)	632 (70.4)		NS
	C	276 (29.0)	266 (29.6)		
TNF-α -308	G	876 (92.0)	824 (91.8)		NS
	A	76 (8.0)	74 (8.2)		
IL-12B promoter 318/322	318	496 (52.8)	528 (48.1)		NS
	322	444 (47.2)	570 (51.9)		
IL-12B intron 2 218/221	218	540 (57.5)	571 (52.3)		NS
	221	400 (42.6)	521 (47.7)		
IL-12B intron 4 268/272	268	376 (40.0)	517 (46.4)		NS
	272	533 (56.7)	560 (50.3)		
	Others	31 (3.3)	37 (3.30)		
IL-12B 3'UTR	A	549 (58.4)	604 (54.3)		NS
	C	391 (41.6)	508 (45.7)		

* P value and OR (95% CI) were calculated with the use of logistic regression models, adjusted for sex and age. After correction by Bonferroni method, the significant P value should be less than 0.003

† NS denotes not significant

56.2±15.3 years, with 33 males and 24 females.

Genotyping

IFN-γ +874A/T, *IL-10* -1082G/A, *IL-10* -592A/C, *IL-12B* promoter 318/322, *IL-12B* intron 2 218/221, *IL-12B* intron 4 268/272, *IL-12B* 3'UTR A/C and *TNF-α* -308 G/A were genotyped using the TaqMan system (Applied Biosystems, CA, USA). *RANTES* -28C/G and *MCP-1* -2518A/G were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). *RANTES* -403A/G, *RANTES* In1.1T/C, *IP-10* nt1811A/G, *IP-10* nt2867C/A and *Mig* nt367A/G were genotyped using the MassARRAY system (Sequenom, CA, US).

Statistical analysis

A two-step analysis was used to determine the association of polymorphisms with SARS. The genotype frequencies and allele frequencies of all the genes were compared between SARS patients and controls using a 3x2 Chi squared test and a 2x2 Chi squared test respectively. Logistic regression was then used to calculate the odds ratios (OR) [95% confidence interval (CI)] and corresponding P values of different genotype frequencies among SARS patients and controls by adjusting for age and sex as co-variables. Association with SARS infection outcomes (death vs survival) was then tested by comparing the genotype frequencies and allele frequencies of all the genes between the death group and the survival group of SARS patients using a 3x2 Chi squared test and a 2x2 Chi squared test respectively. The genotype frequencies of all the single nucleotide polymorphisms (SNPs) were tested for Hardy-Weinberg equilibrium (HWE) separately in SARS patients and controls using the Chi squared test. The significant P value for multiple testing was adjusted with Bonferroni's correction and all statistical analyses were performed using SAS, version 8.02 and SAS/Genetics (SAS Institute, NC, US).

Table 2. Genotype frequencies and allele frequencies of chemokines in SARS patients and controls

Genotype	SNP	No. (%)		OR (95% CI)*	P value*
		SARS (n=495)	Control (n=578)		
RANTES -403	AA	54 (10.9)	56 (9.7)		NS†
	AG	223 (45.0)	262 (45.3)		
	GG	218 (44.0)	260 (45.0)		
RANTES -28	CC	316 (63.8)	491 (84.9)	Reference	<0.0001
	CG	154 (31.1)	73 (12.6)	3.28 (2.32 - 4.64)	
	GG	25 (5.0)	14 (2.4)	3.06 (1.47 - 6.39)	
RANTES In1.1	CC	54 (10.9)	54 (9.3)		NS
	CT	217 (43.8)	257 (44.5)		
	TT	224 (45.3)	267 (46.2)		
IP-10 nt1811	AA	1 (0.2)	0 (0)		NS
	AG	38 (7.7)	29 (5.0)		
	GG	456 (92.1)	549 (95.0)		
IP-10 nt2867	AA	1 (0.2)	0 (0)		NS
	AC	38 (7.7)	31 (5.4)		
	CC	456 (92.1)	547 (94.6)		
Mig nt367	AA	1 (0.2)	0 (0%)		NS
	AG	38 (7.7)	34 (5.9)		
	GG	456 (92.1)	544 (94.1)		
		SARS (n=478)	Control (n=421)		
MCP-1 -2518	AA	115 (24.1)	113 (26.8)		NS
	AG	225 (47.1)	213 (50.6)		
	GG	138 (28.9)	95 (22.6)		
Allele					
RANTES -403	A	331 (33.4%)	374 (32.4%)		NS
	G	659 (66.6%)	782 (67.7%)		
RANTES -28	C	786 (79.4%)	1055 (91.3%)	2.80 (2.11 - 3.71)	<0.0001
	G	204 (20.6%)	101 (8.7%)		
RANTES In1.1	C	325 (32.9%)	365 (31.6%)		NS
	T	665 (67.2%)	791 (68.4%)		
IP-10 nt1811	A	40 (4.0%)	29 (2.5%)		NS
	G	950 (96.0%)	1127 (97.5%)		
IP-10 nt2867	A	40 (4.0%)	31 (2.7%)		NS
	C	950 (96.0%)	1125 (97.3%)		
Mig nt367	A	40 (4.1%)	34 (2.9%)		NS
	G	938 (95.9%)	1122 (97.1%)		
MCP-1 -2518	A	455 (47.6)	439 (52.1)		NS
	G	501 (52.4)	403 (47.9)		

* P value and OR (95% CI) were calculated with the use of logistic regression models, adjusted for sex and age. After correction by Bonferroni method, the significant P value should be less than 0.003

† NS denotes not significant

Results

Our case-control study for cytokines genotyped the SNPs *IFN-γ* +874A/T, *IL-10* -1082G/A, *IL-10* -592A/C, *TNF-α* -308G/A, *IL-12B* promoter 318/322, *IL-12B* intron 2 218/221, *IL-12B* intron 4 268/272 and *IL-12B* 3'UTR A/C in Chinese patients with SARS and in healthy controls. All SNPs were in HWE (P>0.05) in SARS patients and controls using the Chi squared test and the genotype distributions and allele frequencies of these SNPs are shown in Table 1. The *IFN-γ* +874A allele was overrepresented in the SARS patients (83.1%) when compared with the controls (66.3%) [P<0.001]. It was also significantly associated with susceptibility to SARS in a dose-dependent manner (P<0.001), ie individuals with *IFN-γ* +874 AA and AT genotype had OR of 5.19 (95% CI, 2.78-9.68) and 2.57 (95% CI, 1.35-4.88) of developing SARS respectively. The SNPs of *IL-10, TNF-α*, and *IL-12* SNPs were chosen for their potential regulation of protein expression levels. Nonetheless we found no significant association between these SNPs and SARS (Table 1). We also compared the genotype and allele frequencies of all the polymorphisms of the SARS patient death and survival groups but no significant associations were established.

For the study on chemokines, *RANTES* -28C/G, *RANTES* -403A/G, *RANTES* In1.1T/C, *IP-10* nt1811A/G, *IP-10* nt2867C/A, *Mig* nt367A/G, and *MCP-1* -2518 A/G were genotyped. Their genotype and allele frequencies are shown in Table 2. The *RANTES* -28 CG and GG genotypes were significantly associated with SARS susceptibility with OR of 3.28 (95% CI, 2.32-4.64) and 3.06 (95% CI, 1.47-6.39) respectively (P<0.0001) [Table 2]. The *RANTES* -28 G allele was also significantly increased in SARS patients (P<0.0001, OR=2.80, 95% CI, 2.11-3.71) [Table 2]. Our data did not show any significant association with the SNPs of *IP-10*, *Mig* and *MCP-1* (Table 2). All genotype frequencies of the chemokine polymorphisms in SARS patients and controls were in HWE except for the *RANTES* -28C/G frequency in the controls. To confirm that there was no genotyping error that may have contributed to the HWE observation, direct DNA sequencing was performed on 20 to 30 samples for each SNP. No ambiguous results were obtained. We further compared the genotype and allele frequencies of the *RANTES* -28C/G between the SARS death and survival groups. The *RANTES* -28 G allele was associated with death from SARS in a gene-dosage dependent manner (P=0.014), with -28 CG and GG individuals having a 2.12-fold (95% CI, 1.11-4.06) and 4.01-

Table 3. Genotype and allele frequencies of *RANTES* -28C/G among death and survival groups in SARS patients

RANTES -28C/G	No. (%)		OR (95% CI)*	P value*
	Death (n=57)	Survival (n=438)		
Genotype				0.014
CC	26 (45.6)	290 (66.2)	Reference	
CG	25 (43.9)	129 (29.5)	2.12 (1.11 - 4.06)	
GG	6 (10.5)	19 (4.3)	4.01 (1.30 - 12.4)	
Allele				0.002
C	77 (67.5)	709 (80.9)		
G	37 (32.5)	167 (19.1)	2.10 (1.30 - 3.39)	

* P value and OR (95% CI) were calculated with the use of logistic regression models, adjusted for sex and age

fold (95% CI, 1.30-12.4) increased risk of death from SARS respectively (Table 3). No association with death from SARS was detected for the other chemokine genes studied.

Discussion

It has been reported previously that the *IFN-γ* +874A allele is associated with infectious diseases, revealing its potential role in host defences against microbial infections. The mechanism by which the *IFN-γ* +874A/T allele influences susceptibility to SARS may depend on its role in the regulation of IFN-γ production. The T allele of *IFN-γ* +874A/T provides a binding site for the transcription factor nuclear factor-kB (NF-kB), which is able to regulate IFN-γ expression. It is possible that low IFN-γ production may impair the anti-viral response to SARS-CoV, rendering these individuals more susceptible to infection with this virus. Our observation that the *IFN-γ* +874A allele was significantly associated with SARS-CoV infection suggests a genetic risk factor for SARS. The role of IFN-γ in the antiviral response to SARS-CoV has also been supported by recent studies showing that IFN-γ can inhibit the replication of SARS-CoV in combination with IFN-β in vitro.

RANTES is responsible for the recruitment of eosinophils, lymphocytes, monocytes and basophils at the site of inflammation and is involved in many viral infections. We found that the -28 G allele of *RANTES* was associated with susceptibility to and death from SARS. *RANTES* -28C/G is located at the NF-κB binding site, suggesting that this SNP may be involved in the regulation of RANTES expression. Further in vitro studies have shown that the *RANTES* -28 G allele enhances NF-κB binding that leads to elevation of promoter activity and increases RANTES expression in CD8+ T cells, CD4+ T cells and monocytes/macrophages. Together with our observation that the -28 G allele is associated with SARS, we conclude a high level of RANTES may predispose individuals to developing SARS. Too high a level of RANTES may intensify lung inflammation and lead to lymphopaenia, increasing the chance of secondary infection and hence the death rate among SARS patients. Therefore, we speculate that the *RANTES* -28 G allele that associates with higher levels of RANTES may enhance inflammation and lead to severe clinical outcomes in SARS. Indeed, the *RANTES* -28 G allele did show a strong association with death in Hong Kong Chinese patients with SARS (Table 3).

We did not find a significant association between the SNPs of *IL-10, TNF-α, IL-12, IP-10, Mig,* and *MCP-1* and SARS. Nevertheless, we cannot entirely exclude the roles of these cytokines and chemokines in susceptibility to SARS, because other SNPs in these genes may also be involved in gene expression regulation. Further studies on other SNPs able to alter gene expression levels are required to ascertain the relationship of these SNPs with SARS.

Conclusions

We demonstrated that the *IFN-γ* +874A allele is significantly associated with SARS susceptibility in a dose-dependent manner.[5] Due to its role in regulating IFN-γ expression, this allele may be involved in the pathogenesis of SARS by altering IFN-γ production. In addition, we demonstrated that the *RANTES* -28 G allele, which correlates with high RANTES production, was associated with SARS susceptibility.[6] It was also associated with adverse SARS outcomes, suggesting that a high RANTES level may play a role in the pathogenesis of SARS.[6]

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