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Immunogenetic studies in SARS: developing a clinical prognostic profile for severe diseases

Key Messages

1. No major gene was found to influence the course and outcome of illness secondary to SARS-CoV infection.
2. Phenotypic variation in IP-10 expression was not caused by any of the genetic factors investigated in this study.
3. Phenotypic measurements, instead of genetic markers, may be useful in future clinical applications.

Introduction

The 2003 outbreak of SARS in Hong Kong greatly affected the health care system. More than 80% of patients recovered while the remainder suffered a severe disease leading to respiratory failure and admission to the intensive care unit. The course of the disease and outcome were markedly heterogeneous and we hypothesised that these were partly determined by differences in the intensity of host reaction toward the infection.

Our previous investigation of the 1997 avian influenza A (H5N1) outbreak showed that patients who died of the disease had lymphoid depletion associated with marked elevation of circulating concentrations of cytokines, including interleukin-6, interleukin-2 receptor and interferon-gamma. There are many similar features between influenza H5N1 infection and SARS. It is likely that hypercytokinaemia together with associated systemic and local reactions play a key role in the lung damage and determine the disease outcome in patients with SARS.

There is no good predictor of disease outcome after SARS infection. Old age, higher neutrophil count, and serum lactate dehydrogenase were the only markers associated with subsequent admission to the intensive care unit. However, by themselves these are only certainly surrogate markers of disease intensity and immunocompetence. The underlying determinants remain unknown. The ability to predict the disease course may strongly influence the choice of treatment regimen, especially if severe disease could be anticipated early after admission to hospital.

IP-10 expression levels after SARS-CoV infection might be associated with disease prognosis.¹ It was uncertain whether such phenotypic variation was caused by a genetic difference (variation) among individuals or by environmental factors (such as the extent of virus exposure or other host factors). We studied the immune response at the genetic level in SARS patients. Genomic polymorphisms of inflammatory mediators accounting for variations in the intensity of an individual's immune reaction against a pathogen and circulating cytokines levels were studied as prognostic markers in patients who developed SARS infection. We hypothesised that patients with a particular high-risk genotype might have a more intense inflammatory response.

Aims and objectives

1. To characterise the inflammatory (particularly chemokine) responses in SARS-CoV infection.
2. To evaluate whether the differences in disease outcome between patients were related to genetic factors.

Methods

Study design

This was a genetic association study. A total of 677 SARS patients (including 500 controls) were studied to determine the genetic polymorphisms between

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groups of patients. In a subgroup analysis, SARS patients with adverse outcomes were compared with SARS patients who recovered (controls).

Genotyping of candidate genes

Genotypes of selected candidate genes were determined from DNA extracted from blood samples using a commercial DNA extraction kit. Representative variations (commonly single nucleotide polymorphisms [SNPs]) were genotyped by an established protocol. The frequencies of genotypes and alleles of each SNP were compared between SARS patients who had adverse outcomes and patients who recovered. As the sample size of both groups was limited, we also compared the frequencies of the alleles in the case group with those found in the population. We also estimated the population allele frequencies of each SNP.

Genotyping results were determined under stringent quality control procedures that included repeat genotyping of 1-2% of samples determined for each genotype, false positive polymerase chain reaction results and inclusion of standard samples in all batches of reactions.

Statistical analysis

Hardy-Weinberg equilibrium of alleles of individual genes was assessed by exact tests using a population genetics software (GENEPOP). Comparison of genotype frequencies between cases and controls were analysed by Chi squared tests. Univariate analysis was carried out to identify genotypes that were associated with adverse outcomes. The correlation between genotypes and inflammatory response/disease outcome was analysed by linear/logistic regression.

Results

Genetic variations in both forms of ACE genes (ACE1 and ACE2) were not a risk factor for severe disease prognosis after SARS infection. Genetic polymorphisms in the L-SIGN gene, another putative receptor for the virus, were also not associated with prognosis or disease susceptibility. Notably, genetic factors affecting both chemokine and cytokine genes were not associated with prognosis.

Discussion

Chemokine expression levels (particularly IP-10) was an important factor associated with disease prognosis, but the cause of such phenotypic variation was not certain. It may be due to a genetic difference between individuals or differences in environmental factors, such as extent of viral exposure, concurrent medical conditions, or other factors. This study explored a number of candidate genes considered important in the pathogenesis of infection and showed that differences between them were not related to differences in prognosis after SARS infection.

Variation in levels of serum inflammatory mediators

reflects phenotypic differences in host inflammatory reactions during an infection. The intensity of immune response might also be genetically determined. The differences in genetic makeup between individuals are mostly accounted for by single-base differences known as SNPs. Many studies show an association between SNPs and predisposition to adult respiratory distress syndrome (ARDS) and survival after sepsis or other insults.^{2,3} In the context of predisposition to ARDS after trauma, the interaction between circulating concentrations of interleukin-1, tumour necrosis factor and plasminogen activator inhibitor-1 and the genotype for plasminogen activator inhibitor-1 (PAI-1) have been studied.⁴ In addition to PAI-1, other genetic polymorphisms were also associated with a predisposition to and/or severity and outcomes of ARDS, including angiotensin-converting enzyme,² CD14,⁵ surfactant protein,⁶ and HLA genotypes.⁷ The association between alleles of the two ACE genes (ACE and ACE2) and severity of ARDS after SARS infection revealed negative results.^{8,9} In addition, a study on novel SNPs identified by resequencing of the ACE2 gene also yielded no association with SARS infection.¹⁰

Several other immunogenetic studies have been reported in association with SARS infection, two of which suggested such association with HLA genotype. Among 37 Taiwan SARS patients, HLA-B*4601 was associated with both a predisposition to infection as well as the severity of infection.¹¹ However, the association of this allele could not be replicated in another study in Chinese SARS patients using a larger sample size.¹² In contrast, HLA-B*0703 was found to be a predisposition allele. However, this rare allele is found in about 3% of the general population and could not account for a major predisposition factor for SARS infection.¹² Genetic variation in the L-SIGN gene (CLEC4M) was also not associated with disease severity.¹³ It is clear that immunogenetics is an important field in SARS research. However, none of the genes studied so far appear to be important or major determinants of disease outcome.

Conclusions

No major genetic risk factors for disease susceptibility or disease prognosis were determined in this study. Phenotypic determination by assay of chemokine expression levels (eg serum concentration of chemokines) may be important independent risk factors useful in future clinical applications. We should increase awareness of the importance of chemokines in immune responses and review the facilities for measuring them in clinical practice.

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1. Chan KC, Tang NL, Hui DS, et al. Absence of association

between angiotensin converting enzyme polymorphism and development of adult respiratory distress syndrome in patients with severe acute respiratory syndrome: a case control study. *BMC Infect Dis* 2005;5:26.

2. Chiu RW, Tang NL, Hui DS, et al. ACE2 gene polymorphisms do not affect outcome of severe acute respiratory syndrome. *Clin Chem* 2004;50:1683-6.
3. Tang NL, Chan PK, Hui DS, et al. Lack of support for an association between CLEC4M homozygosity and protection against SARS coronavirus infection. *Nat Genet* 2007;39:691-2.

References

1. Tang NL, Chan PK, Wong CK, et al. Early enhanced expression of interferon-inducible protein-10 (CXCL-10) and other chemokines predicts adverse outcome in severe acute respiratory syndrome. *Clin Chem* 2005;51:2333-40.
2. Marshall RP, Webb S, Bellington GJ, et al. Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2002;166:646-50.
3. Villar J, Flores C, Mendez-Alvarez S. Genetic susceptibility to acute lung injury. *Crit Care Med* 2003;31(4 Suppl):S272-5.
4. Menges T, Hermans PW, Little SG, et al. Plasminogen-activator-inhibitor-1 4G/5G promoter polymorphism and prognosis of severely injured patients. *Lancet* 2001;357:1096-7.
5. Gibot S, Cariou A, Drouet L, Rossignol M, Ripoll L. Association between a genomic polymorphism within the CD14 locus and septic shock susceptibility and mortality rate. *Crit Care Med* 2002;30:969-73.
6. Lin Z, Pearson C, Chinchilli V, et al. Polymorphisms of human SP-A, SP-B, and SP-D genes: association of SP-B Thr131Ile with ARDS. *Clin Genet* 2000;58:181-91.
7. Takatsuka H, Takemoto Y, Mori A, Okamoto T, Kanamaru A, Kakishita E. Common features in the onset of ARDS after administration of granulocyte colony-stimulating factor. *Chest* 2002;121:1716-20.
8. Chan KC, Tang NL, Hui DS, et al. Absence of association between angiotensin converting enzyme polymorphism and development of adult respiratory distress syndrome in patients with severe acute respiratory syndrome: a case control study. *BMC Infect Dis* 2005;5:26.
9. Chiu RW, Tang NL, Hui DS, et al. ACE2 gene polymorphisms do not affect outcome of severe acute respiratory syndrome. *Clin Chem* 2004;50:1683-6.
10. Itoyama S, Keicho N, Hijikata M, et al. Identification of an alternative 5'-untranslated exon and new polymorphisms of angiotensin-converting enzyme 2 gene: lack of association with SARS in the Vietnamese population. *Am J Med Genet A* 2005;136:52-7.
11. Lin M, Tseng HK, Trejaut JA, et al. Association of HLA class I with severe acute respiratory syndrome coronavirus infection. *BMC Med Genet* 2003;4:9.
12. Ng MH, Lau KM, Li L, et al. Association of human-leukocyte-antigen class I (B*0703) and class II (DRB1*0301) genotypes with susceptibility and resistance to the development of severe acute respiratory syndrome. *J Infect Dis* 2004;190: 515-8.
13. Tang NL, Chan PK, Hui DS, et al. Lack of support for an association between CLEC4M homozygosity and protection against SARS-coronavirus infection. *Nat Genet* 2007;39:691-2.