ORIGINAL ARTICLE

Cytokine gene polymorphisms associated with symptomatic parvovirus B19 infection

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Background: The immune system has been implicated in the pathogenesis of certain clinical manifestations of parvovirus B19 infection, including rash and arthralgia. Cytokines feature in the pathogenesis of parvovirus B19 infection, so inherited variability in cytokine responses to B19 infection might have a bearing on the symptomatology of parvovirus B19 infection.
Aims: To investigate the possible role of cytokine gene polymorphisms in the clinical manifestations of parvovirus B19 infection.
Methods: Thirty six patients with a variety of symptoms at acute infection and follow up (mean, 22.0 months) and controls (99–330, depending on the locus) were examined for the following cytokine polymorphisms: tumour necrosis factor α (TNFα), –308; interferon γ (IFN-γ), +874; interleukin 6 (IL-6), –174; IL-10, –592, –819, and –1082; and transforming growth factor β1 (TGFβ1), +869 (codon 10) and +915 (codon 25).
Results: The TNFα –308*A allele occurred in 13.9% of the parvovirus group compared with 27.0% of the control group (odds ratio (OR), 0.44; p = 0.02). The TGFβ1 CG/CG haplotype was more frequent in the parvovirus group than in the controls (16.7% *v* 5%; OR, 4.8; p = 0.02). Within the B19 infected group, the TGFβ1 +869 T allele was associated with skin rash at acute infection (p = 0.005), whereas at follow up the IFN-γ +874 T allele was associated with the development of anti-B19 non-structural protein 1 antibodies (p = 0.04).
Conclusions: The results of the present study suggest that inherited variability in cytokine responses may affect the likelihood of developing symptoms during parvovirus infection.

The immune system has been implicated in the pathogenesis of certain clinical manifestations of parvovirus B19 infection, including rash and arthralgia,[1] and we have recently shown that symptomatic infection may be HLA-DRB1 restricted.[2] Because cytokines are known to feature in the pathogenesis of parvovirus B19 infection,[3] we hypothesised that inherited variability in cytokine responses to B19 infection might have a bearing on the symptomatology of parvovirus B19 infection.

MATERIALS AND METHODS

Genomic DNA from 36 previously characterised patients with acute B19 infection were studied.[2] Normal healthy controls were also enrolled in our study. Both test and control groups were from the north west of England and almost all were white.

Human genomic DNA was tested for single nucleotide polymorphisms (SNPs) affecting cytokine gene transcription by amplification refractory mutation system–polymerase chain reaction using primers described previously. The following SNPs were analysed: tumour necrosis factor α (TNFα), –308[4]; interferon γ (IFN-γ), +874[5]; interleukin 6 (IL-6), –174[6]; IL-10, –592, –819, and –1082[5]; and transforming growth factor β1 (TGFβ1), +869 (codon 10) and +915 (codon 25).[5] Internal control primers, which amplified the human growth hormone gene, were used throughout.[4]

Data on allele frequencies in normal controls in north west England for the TNFα, IFN-γ, TGFβ, and IL-10 genes were taken from Perrey *et al*.[5] Data on allele frequencies in normal controls in north west England for IL-6 were obtained by testing 99 healthy normal controls from the School of Biological Sciences, University of Manchester.

RESULTS

Patients with symptomatic parvovirus B19 infection

Thirty six patients with acute B19 infection (serum anti-B19 IgM positive) were studied. These patients had an age range of 9 to 52 years, with a mean of 31.4, and a female to male ratio of 6.2 : 1. Table 1 shows the symptoms, B19 markers, and autoantibody results. All 36 of these patients were contacted after a follow up period of two to 37 months (mean, 22.0) (in 33 of these patients the follow up period was at least seven months). At this time, 15 patients were found to have symptoms that began at the time of acute infection and which persisted throughout the follow up period. These symptoms, together with B19 markers and autoantibodies are shown in table 1.

Cytokine allele frequencies for B19 patients versus normal controls

A comparison between cytokine allele frequencies of B19 infected patients and controls revealed no significant difference except in the case of TNFα at position –308. In this case, the A allele was 13.9% in the parvovirus group compared with 27.0% in the normal group (odds ratio (OR), 0.44; 95% confidence interval (CI), 0.21 to 0.89; p = 0.02). A comparison of haplotype frequencies for IL-10 in parvovirus infected patients versus controls revealed no significant difference (table 2). However, for TGFβ1, the frequency of the CG/CG haplotype was 16.7% in the parvovirus group compared with 5% in the controls (OR, 4.8; 95% CI, 1.01 to 16.9; p = 0.02) (table 2). This

Abbreviations: CI, confidence interval; IFN-γ, interferon γ; IL, interleukin; NS1, non-structural protein 1; OR, odds ratio; SNP, single nucleotide polymorphism; TGFβ, transforming growth factor β; Th1, T helper cell type 1; TNFα, tumour necrosis factor α

Table 1 Parvovirus B19 infected patients; clinical details, B19 virus markers, and associated cytokine alleles

	Acute infection		Follow up	
Clinical manifestation, B19 marker	No.	Allele	No.	Allele
Skin rash	15	TGFβ1 +869*T†	0	
Arthritis	24		10	
Fatigue	18		11	
Thrombocytopenia	2		2	
Transient aplastic crisis, hereditary spherocytosis	1		0	
Lymphadenopathy	2		1	
Myalgia	1		0	
Mother with fetal death	2		NA	
Serum anti-B19 VP1/2 IgM	36		0	
Serum anti-B19 VP1/2 IgG	35		35	
Serum B19 virus DNA	34		9	IL-6 –174*G‡
Serum anti-B19 NS1 IgG	6		14	IFNγ +874*T§ IL-10 –819/–592*TA¶ TGFβ1 +869*C††

†Odds ratio (OR), 4.83; 95% confidence interval (CI), 1.70 to 13.76; p=0.005; ‡OR, 3.44; 95% CI, 0.89 to 13.30; p=0.12; §OR, 2.75; 95% CI, 1.03 to 7.37; p=0.04; ¶OR, 2.89; 95% CI, 0.81 to 11.36; p=0.11; ††OR, 2.19; 95% CI, 0.76 to 6.62; p=0.10.
IFNγ, interferon γ; IL, interleukin; NS1, non-structural protein 1; TGFβ1, transforming growth factor β1; VP, viral protein.

Table 2 IL-10 and TGFβ gene haplotype frequencies in 36 symptomatic B19 infected patients compared with controls

Cytokine	Locations of single nucleotide polymorphisms	Haplotype (transcriptional level)	Haplotype frequency in B19 group	Haplotype frequency in controls*
IL-10	–1082/–819/–592	GCC/GCC	12 (33.3%)	99 (30.0%)
		GCC/ACC	7 (19.4%)	68 (20.6%)
		GCC/ATA	8 (22.2%)	70 (21.1%)
		ACC/ACC	4 (11.1%)	27 (8.2%)
		ACC/ATA	3 (8.3%)	41 (12.4%)
		ATA/ATA	2 (5.5%)	25 (7.6%)
			Total=36	Total=330
TGFβ1	+869(c10)/+915(c25)	TG/TG	14 (38.9%)	44 (41%)
		TG/CG	11 (30.6%)	38 (36%)
		TG/CC	3 (8.3%)	13 (12%)
		CG/CG (intermediate)	6 (16.7%)†	5 (5%)
		TG/TC	0 (0%)	0 (0%)
		CG/CC	2 (5.6%)	6 (6%)
		TC/TC	0 (0%)	0 (0%)
		TC/CC	0 (0%)	0 (0%)
		CC/CC	0 (0%)	1 (1%)
			Total=36	Total=107

*Data taken from Perrey *et al*.[5] †Odds ratio, 4.8; 95% confidence interval, 1.01 to 16.9; p=0.02 (non-significant; p=0.18 after correction for multiple testing).
IL-10, interleukin 10; TGFβ1, transforming growth factor β.

result should be treated with caution because significance was lost after correction for multiple testing.

Cytokine allele frequencies for particular symptoms/markers in patients with parvovirus B19

At the time of acute B19 infection, the only significant correlation was that of the TGFβ1 +869 T allele with the occurrence of a skin rash (OR, 4.83; 95% CI, 1.70 to 13.76; p = 0.005) (table 1). At follow up, the only significant association was between the IFN-γ T allele and the development of anti-B19 non-structural protein 1 (NS1) antibodies (OR, 2.75; 95% CI, 1.03 to 7.37; p = 0.04). In addition, there were several trends that did not reach significance. For example, the association of the IL-6 –174 G allele with the presence of serum B19 DNA at follow up (OR, 3.44; 95% CI, 0.89 to 13.30; p = 0.12); the association of the IL-10 –819/–592 TA allele with the development of anti-NS1 antibodies at follow up (OR, 2.89; 95% CI, 0.81 to 11.36; p = 0.11); and the association of the TGFβ1 +869 C allele with development of anti-NS1 antibodies at follow up (OR, 2.19; 95% CI, 0.76 to 6.62; p = 0.10) (table 1).

DISCUSSION

A comparison of cytokine allele frequencies of B19 infected patients versus controls revealed that the TNFα –308 A allele occurred at a lower frequency in the parvovirus group than in the normal group. This is paradoxical because this allele has been associated with a high TNFα production phenotype, and TNFα appears to be an important mediator in parvovirus infections.[3] In addition, the TNFα signalling pathway has been shown to be important in apoptosis caused by parvovirus B19 infection.[7] The finding of a negative association with the high producing TNFα –308*A allele in acute infection, if confirmed, may suggest that an inability to produce initial high concentrations of this cytokine may increase the likelihood of symptoms by allowing viral replication, and the production of

clinical manifestations of infection. However, this conclusion may be difficult to prove without performing volunteer studies.

The TGFβ1 CG/CG haplotype, associated with the B19 group, has been associated with an intermediate level of transcription,[5] so any explanation based on the level of transcription of TGFβ1 may not be satisfactory. It is possible that this haplotype is linked with one or several other important loci, which might explain this association.

> "It seems plausible that the level and timing of cell mediated immunity achieved during acute infection may modify B19 virus replication in keratinocytes, with a consequent effect on the development of a skin rash"

The TGFβ1 +869 T allele was associated with the development of a skin rash at the time of acute B19 infection. This particular allele has been associated with high levels of transcriptional activity.[5] TGFβ suppresses the immune response by inhibiting the proliferation of T cells via downregulation of predominantly IL-2 mediated proliferative signals. It also inhibits the growth of natural killer cells in vivo, deactivates macrophages, and suppresses antibody production. This association cannot be explained by an inhibitory effect on antibody production because it is well documented that the occurrence of symptoms is coincident with specific IgG production.[1] B19 DNA and capsid protein have been detected in keratinocytes of the stratum basale of the epidermis,[8] and therefore direct infection has been suggested to account for the skin rash associated with parvovirus B19 infection. It seems plausible that the level and timing of cell mediated immunity achieved during acute infection may modify B19 virus replication in keratinocytes, with a consequent effect on the development of a skin rash.

Antibodies to the NS1 protein have in certain studies (albeit inconsistently) been associated with severe courses of B19 infection, including chronic B19 arthralgia,[9] and have been shown to neutralise viral infectivity.[10] In our study, the development of anti-NS1 antibodies was more frequent in patients with the IFN-γ +874 T allele, which has been associated with high production of IFN-γ. IFN-γ is required for the T helper cell type 1 (Th1) immune responses necessary to control viral infections. It may be that anti-NS1 antibodies reflect a Th1 immune response and one that is more likely to result in a more severe course in some individuals. This is consistent with the weaker associations with the IL-10 –819/–592*TA and TGFβ1 +869*C alleles, both of which have been associated with low transcriptional activity.[5]

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Take home messages

- The tumour necrosis factor α (TNFα) –308*A allele occurred in 13.9% of the parvovirus group compared with 27.0% of the control group, which was unexpected because this allele is associated with high TNFα production and TNFα is an important mediator in parvovirus infection
- The transforming growth factor β1 (TGFβ1) CG/CG haplotype was more frequent in the parvovirus group than in the controls
- Within the B19 infected group, the TGFβ1 +869 T allele, which is associated with high transcriptional activity, was associated with skin rash at acute infection
- At follow up the interferon γ (IFN-γ) +874 T allele, which is associated with high production of IFN-γ, was associated with the development of anti-B19 non-structural protein 1 antibodies

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