FULL PAPER

Differential cytokine genotype frequencies among Canadian Aboriginal and Caucasian populations

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Genetic diversity related to the human immune response is a key factor in individual and population survival throughout human history. Population diversity in disease susceptibility and resistance have been identified and linked to differences in cytokine mRNA and protein expression levels. Polymorphisms in the regulatory regions of cytokine genes can influence gene transcription levels and they have been associated with susceptibility to, and/or severity of, autoimmune disorders such as rheumatoid arthritis, meningococcus and sepsis. It is reported here that in two study populations, Canadian Aboriginal individuals have a higher frequency of cytokine single-nucleotide polymorphisms favouring a low production of TNF α , IFN γ and IL-10 and high production of IL-6 as compared to a Caucasian population. We postulate that the evolution of this unique cytokine genotype profile may be linked to the Aboriginal adaptation to selection pressures related to an environment in which helminthic, parasitic and fungal infections predominated.

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Introduction

Infectious pathogens such as *Mycobacterium tuberculosis* and Respiratory Syncytial Virus (RSV) continue to have a devastating effect on certain North American subpopulations.^{1–6} For example, in Canada, 43% of all new active and relapsed cases of tuberculosis occur among Aborigines, 37% occur among foreign-born individuals and 20% among Canadian born nonAboriginals.² While most studies focus on socioeconomic differences,^{7–9} little attention has been paid to the genetic differences that may exist between human populations that could regulate the quality of the immune response to a given pathogen. In contrast, studies of inbred mice have clearly demonstrated that the genetic background plays a key role in determining the quality of the cytokine response (eg T_H1 vs T_H2), which in turn affects the host's ability to eradicate an infectious agent (ie *Leishmania major*, *Toxoplasma gondii*, *M. tuberculosis*).^{10–14}

With the completion of the human genome project, it has become apparent that single-nucleotide polymorphisms (SNPs) are common and depending on location may affect gene transcription levels.¹⁵ In the context of the host's immune response to an infectious agent, the identification of differences in cytokine SNP profiles may have important implications for the host's resistance or

susceptibility to that particular pathogen. The current study describes the frequency of SNPs, which are known to correlate with differential gene expression for the IL-6, IL-10, TNF α , IFN γ and TGF- β genes, in Caucasian, Canadian Aboriginal and Filipino cohorts and postulates why such distinct cytokine SNP profiles may have evolved at a population level.^{16–23}

Results

In two independent studies, it was found that ethnic populations have statistically different cytokine SNP profiles. It was initially observed that cytokine genotypes of North American Aborigines and Filipinos with ESRD had significantly different cytokine genotypes as compared to a Caucasian with end-stage renal disease (ESRD) cohort (Table 1). A second study, which involved normal control populations of Caucasian and Aboriginal individuals, validated the initial observations (Table 2).

As compared to the Aboriginal and Filipino cohorts, Caucasians maintain a higher frequency of the TNF α (–308) and IFN γ allele SNPs whose phenotypic expression are associated with enhanced production of these cytokines.^{16–19,24,25} In contrast to the Caucasian cohort, individuals of Aboriginal and Filipino descent had a high frequency of the IL-6 (–174) allele, associated with a higher production of this cytokine.^{22,23} The Aboriginal individuals maintained a statistically higher frequency of the 'G' allele at the TNF α (–308) loci and would therefore tend to be low producers of this cytokine as compared to

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Table 1 Cytokine phenotype frequencies by percent for patients with ESRD

Cytokine	Caucasian ESRD (H, I, L)	Aboriginal ESRD (H, I, L)	Filipino ESRD (H, I, L)
TNF α	3, 29, 68	1, 10, 88*	0, 4, 96*
IFN γ	19, 46, 35	6, 24, 69*	8, 31, 62*
IL-10 (-1082)	20, 47, 33	9, 37, 53*	0, 4, 96*
IL-10 (-592)	58, 32, 10	37, 42, 20*	11, 35, 54*
IL-10 (-819)	58, 32, 10	37, 42, 20*	11, 35, 54*
IL-6	38, 45, 17	82, 17, 1*	96, 0, 4*
TGF β codon 25	87, 12, 1	97, 3, 0*	96, 4, 0
TGF β codon 10	35, 50, 15	33, 49, 18	31, 38, 31

* $P < 0.005$ when compared to Caucasian population.

Table 2 Cytokine phenotype frequencies by percent for normal controls

Cytokine	Caucasian normal controls (H, I, L)	Aboriginal normal control (H, I, L)
TNF α	3, 29, 68	0, 7, 93*
IFN γ	20, 53, 27	4, 20, 76*
IL-10 (-1082)	22, 56, 22	6, 32, 62*
IL-10 (-592)	61, 38, 1	34, 50, 16*
IL-10 (-819)	61, 38, 1	34, 50, 16*
IL-6	35, 48, 17	86, 14, 0*
TGF β Codon 25	85, 14, 1	94, 6, 0*
TGF β Codon 10	42, 48, 11	25, 61, 14

* $P < 0.005$ when compared to Caucasian population.

the Caucasian cohort. Similarly, the Aborigines maintained a statistically higher frequency of the 'A' allele at the IFN γ loci, which would be associated with low production of IFN γ . The allele frequencies of IL-10 (-819) and IL-10 (-592) were similarly distributed in a given population, which would suggest that these loci are in linkage disequilibrium. However, the Aborigines had IL-10 loci phenotypes skewed towards a lower production level of IL-10 as compared to the Caucasian cohort. In contrast, the Aboriginal group, as compared to the Caucasian cohort, had alleles associated with a higher expression level for IL-6 ('G' allele at -174) and TGF β_1 ('G' allele codon 25). There was no statistical difference between allele frequencies of the Aboriginal and Caucasian groups for TGF β_1 (codon 10).

The Filipino patients exhibited the same differences as seen in the Aboriginal patients in cytokine genotype frequencies relative to the Caucasian cohort (Table 1). Interestingly, the only difference observed between the Aboriginal and Filipino patients was in relation to the IL-10 promoter polymorphisms in which the Filipino group were skewed to be even lower IL-10 producers than the Aboriginal population.

Discussion

The novel observation of this study relates to the fact that cytokine SNP profiles were distinct between the ethnic populations examined. In particular, the genotype profile

observed in this study suggests that Caucasians might favour a T $_H$ 1 immune response, while the SNP frequencies in the Aboriginal and Filipino cytokine promoter regions would tend to favour a T $_H$ 2 immune response. In order to account for these genetic differences, one should consider the historical context in which these populations evolved in relationship to their microbial environments.

The Old World Caucasian populations, during the past 11 000-year period, traded a hunting and gathering lifestyle, to one that included the domestication of plants and animals, sedentism and subsequently urbanization. These changes in subsistence and settlement patterns resulted in profound changes in the microbial environment in the Old World.²⁶ With the advent of agriculture and urbanization, the so-called 'crowd infections' became prevalent and epidemics of smallpox, measles, plague and tuberculosis occurred throughout Europe.^{27,28} The high mortality rates associated with these pathogens likely exerted intensive selective pressures on the population.²⁷ For example, given the high prevalence of tuberculosis infection leading to premature death in Europe (ca. 18th century), survival would have in part favoured those individuals who could effectively mount a delayed-type sensitivity (DTH) immune response. This type of response is characterized at a cytokine level by IFN γ and TNF α expression.²⁹ Indeed, when anti-TNF α was used to treat patients with inflammatory bowel disease, it was associated with the occurrence of, or the reactivation of, tuberculosis confirming the important role of TNF α as an immune modulator or neutralizer for tuberculosis.^{30,31} To date, eight DNA variants have been identified in the TNF α promoter (-1031T/C, -863C/A, -857C/T, -575G/A, -376G/A, -308G/A, -244G/A and -238G/A) and some of these polymorphisms have been linked to ethnicity and disease susceptibility and outcome.³²⁻³⁵ Certain SNPs in the TNF α promoter region have been implicated in the pathogenesis of infectious diseases such as malaria. For example, cerebral malaria has been associated with the TNF α (-308) 'A' allele, while severe malarial anemia was associated with the TNF α (-238) 'A' allele.^{35,36} Studies have shown a higher TNF α production phenotype in carriers with the 'A' allele suggesting that polymorphisms in the TNF α promoter region may have a direct effect on the outcome of malaria and other infectious diseases.¹⁶⁻¹⁸

Similarly, IFN γ has an important role in a host's resistance to *M. tuberculosis* although IFN γ production cannot adequately control the infection without the presence of other critical cytokines.³⁷ While to date there is no evidence from population studies that common variants in the IFN γ receptor genes affect susceptibility to tuberculosis, there are cases of inactivating mutations in the IFN γ receptor, which was associated with susceptibility to usually nonpathogenic mycobacteria.^{29,38} In terms of other cytokines, evidence from animal models recently found that IL-6 expression by macrophages can prevent the effective containment of *M. tuberculosis* infection.¹⁴ Coming back then to the Caucasian population in this study, it is interesting to note that they maintain a relatively higher frequency of cytokine SNPs that would favour a delayed-type hypersensitivity response (ie IFN γ , TNF α) and a lower IL-6 expression level compared to the other cohorts. Given the aforementioned high prevalence of tuberculosis in Europe

since ca. 1700 to the early 1900s when effective antimicrobial therapy was developed, then this might account for the cytokine SNP profile observed today in the Caucasian population.

In contrast, Aboriginal North American populations experienced a unique set of evolutionary pressures because of their migration across the Bering Land Bridge (ca. 15–20 000 years ago) and their isolation from Old World populations until after ca. 1400 AD.^{39–44} The Aboriginals maintained an ancient mobile hunting and gathering lifestyle, which had existed in the Old World and that was conducive to the maintenance of diffuse settlements with low population densities.^{8,40,45–48} The analysis of pathologies from human skeletal remains of ancient Aboriginal individuals indicate that parasitic, fungal infections and malnutrition were present among the early populations.^{49–52} The consumption of raw or undercooked meat, living conditions that were confined during the winter months, and the pattern of collecting wild plants brought these people into close contact with fungi and parasites, and left them vulnerable to seasonal nutritional deficiency. This microbial environment would tend to favour positive selection for a T_H2 -type adaptive immune response in order to survive. Indeed, studies of South American Aboriginal families with *Shistosoma mansoni* infection have confirmed the protective role of T_H2 -type cytokines IL-4, IL-6 and IL-13 in protection against parasitic infection.⁵³ The overexpression of T_H2 -type cytokines (IL-6 and IL-10) induces hyperactivity of B cells, which is characteristic of systemic lupus erythematosus,⁵⁴ and the SNP at IL-6 (–174) was implicated in the age of onset of rheumatoid arthritis.⁵⁵ Polymorphisms in the promoter regions of TNF α , IFN γ , IL-10 and IL-6 are associated with differential expression levels of these cytokines and the maintenance of certain polymorphisms in a population may confer a selective advantage.^{16–23} In the context of the current study the fact that the Canadian Aboriginal cytokine SNP profile is skewed in favour of high IL-6 and relatively low TNF α and IFN γ production would support the concept of a parasitic environment, and the low prevalence of *M. tuberculosis* in this population prior to European contact.

The Filipinos cytokine SNP frequencies are more similar to that of the North American Aboriginal population than either of these groups is to the Caucasians (Table 1). The common Asian heritage of the ancestral Aboriginal and Filipino populations, and their parallel migration away from the European continent before 12 000 years ago,^{56,57} may account for these similarities.

Studies of infectious disease among Aboriginal populations have focused on socioeconomic factors contributing to disease resistance and susceptibility.^{1–3,58–60} In most of these studies, genetic differences between populations are acknowledged as contributing factors in disease susceptibility and resistance; however, the basis for this genetic disparity remains largely unexplored. Susceptibility to tuberculosis, for example, among the relatively isolated South American Aché is high. However, individuals with high nutritional and socioeconomic status within this group are equally at risk as those with low nutritional and socioeconomic status.⁶¹ It has been suggested that the high rates of antibody production and T_H2 -mediated activation by Aché individuals competes with the T_H1 -mediated defences required to effectively

fight against infectious diseases such as tuberculosis.⁶¹ Similarly, North American Aboriginal populations in Manitoba have the highest incidence of tuberculosis relative to other local populations.^{1–3} While the unequal risk of disease between ethnic populations maybe partly explained by environmental and socioeconomic differences, cytokine SNPs may also play a role in this variability. Our current ongoing studies are testing this hypothesis.

In summary, ethnic affiliation is likely a factor in the observed differences of cytokine SNP frequencies. These differences between the Caucasian, Aboriginal and Filipino populations may in part reflect the fact that these populations have evolved in response to the selective pressures of their respective microbial environments.

Materials and methods

Study populations

ESRD patients were initially enrolled, with informed consent and approval of the University of Manitoba Institutional Review Board, into a study to explore the relationship between cytokine genotypes and inflammatory events (eg infection). However, it was noted that cytokine genotype profiles appeared to correlate with ethnicity and hence the following groups were identified to formally evaluate this relationship between cytokine SNPs and ethnicity further: North American Aboriginal with ESRD ($n=78$), Caucasian with ESRD ($n=217$) and Filipinos with ESRD ($n=26$). To validate these initial observations and investigate host response to infectious disease, a separate study protocol was established and approved by the University of Manitoba Institutional Review Board. Herein, we report the results of the control groups consisting of Aboriginal ($n=130$) and Caucasian individuals without ESRD ($n=116$) that were consented. The age range of the cohort is approximately 18–60 years.

The North American Aboriginal cohort with ESRD was composed of self-proclaimed Status Indian individuals. Given the geographical location of the study centre in relation to the surrounding Aboriginal populations, the study group is likely Ojibwa or Cree. They are non-Caucasian and their respective heritages are considered to be native to North America. The Aboriginal control individuals are Oji-cree from a Reserve. In contrast, the Caucasian patients are of European descent. The Filipino population in Manitoba is largely derived from immigration from the Philippines that has occurred in the last 5–20 years.

Cytokine genotyping

Genomic DNA was extracted from buffy coat cells by absorption onto QIAamp silica-gel following QIAGEN protease digestion (Qiagen, Mississauga, Canada). After column elution, the purity and concentration of extracted DNA was determined by UV spectroscopy (BioRad, Mississauga, Canada). Single-nucleotide polymorphisms for IL-6, TNF α , IFN γ , IL-10, TGF β_1 codon 25 and TGF β_1 codon 10 were determined using a commercial PCR-SSP kit (One Lambda, Canoga Park, USA). The specific gene polymorphisms probed for were as follows: –174 (G/C)

Table 3 Reporting for cytokine genotypes and phenotypes

Cytokine polymorphisms	Genotype	Phenotype producer
TNF α (–308)	G/G	Low
	G/A	Intermediate
	A/A	High
TGF β ₁ (codons 25)	G/G	High
	G/C	Intermediate
	C/C	Low
TGF β ₁ (codons 10)	T/T	High
	T/C	Intermediate
	C/C	Low
IL-10 (–1082)	G/G	High
	G/A	Intermediate
	A/A	Low
IL-10 (–819)	C/C	High
	C/T	Intermediate
	T/T	Low
IL-10 (–592)	C/C	High
	C/A	Intermediate
	A/A	Low
IL-6 (–174)	G/G	High
	G/C	Intermediate
	C/C	Low
IFN γ (+874)	T/T	High
	T/A	Intermediate
	A/A	Low

in the IL-6 promoter, –1082 (G/A), –819 (T/C) and –592 (A/C) in the IL-10 promoter, –308 (G/A) in the TNF α promoter, codon 25 (G/C) and codon 10 (T/C) of the TGF β ₁ signal sequence and +874 (T/A) of intron 1 of IFN γ .

Each PCR reaction contained preoptimized sequence-specific primers, 100 ng of genomic DNA, and 0.25 U *Taq* polymerase (PE Biosystems, Mississauga, Canada). Following the initial denaturation steps, samples were subjected to an initial nine rounds of PCR consisting of 96°C for 10 s, 63°C for 60 s followed by 20 rounds of PCR consisting of 96°C for 10 s, 56°C for 30 s and 72°C for 30 s. To visualize the PCR products, 10 μ l of the amplified reaction was run in a 2.5% agarose gel containing 0.5% ethidium bromide at 150 V for 5 min. DNA bands were then visualized with UV light on a transilluminator and photographed for subsequent analysis. Individuals (or samples) were classified into the predicted high, intermediate or low producer phenotypes according to their genotypes (Table 3).

Statistical analyses

Statistical analysis was performed using SAS software (SAS Institute, Cary, NC, USA). Values are reported as percentages. The Fisher Exact test was used for comparison of the categorical variables. $P < 0.01$ are reported as significant. This higher degree of stringency was used given that multiple comparisons frequently lead a result to be significant at the $P < 0.05$ level.

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