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INVESTIGATIVE REPORT

Cytokine Gene Polymorphisms in Patch-stage Mycosis Fungoides

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Cytokine production is under genetic control and certain allelic variants of cytokine genes are associated with lower or higher cytokine production *in vitro* and *in vivo*. The general concept is that a shift from a Th1 to a Th2 cytokine profile accompanies disease progression from patch-stage mycosis fungoides to tumour stage, although the results of the studies carried out have not been entirely conclusive. We aimed to investigate whether certain cytokine polymorphisms might represent a risk factor for developing patch-stage mycosis fungoides. Genotyping for IFN-γ (Th1 cytokine), IL-6, IL-10 (Th2 cytokines), TNF-α and TGF-β1 was undertaken for 33 patients with patch-stage mycosis fungoides and the results were compared with those in a control group. Genotype distribution showed no significant differences between the patients and the controls for any of the five cytokines studied. Our study suggests that patch-stage mycosis fungoides is not determined by a specific genotype polymorphism. However, further studies on larger numbers of cases are needed before definite conclusions can be drawn. *Key words: cutaneous T-cell lymphoma; cytokines; gene polymorphism.*

Mycosis fungoides (MF), the most common cutaneous T-cell lymphoma, is characterized by a CD4+ (T helper) clonal expansion in the skin, admixed with reactive lymphocytes composed largely of CD4+ cells and to a lesser extent CD8+ T cells, both most probably mediating an antitumour response (1). The aetiopathogenesis is still unknown. It has been suggested that MF arises from a state of chronic antigenic stimulation (2) that may be involved in the evolution from a reactive process to the malignant transformation of skin-homing T cells in a genetically susceptible individual.

Cytokines are considered to be of major importance in the development of MF. The general concept is that a shift from a Th1 (IFN-γ) (3, 4) to a Th2 (IL-4, IL-5, IL-10) (3, 5) cytokine profile in the skin lesions accompanies disease progression from patch stage to tumour stage. However, some investigators have not been able to find any clear polarization in cytokine production in patch-stage MF (6). Recently it was found that the neoplastic cells in MF (7, 8), as in Sezary syndrome (9), display a Th2-type cytokine profile, with deficient production of Th1-related cytokines (7, 8). In contrast, reactive CD4+ cytotoxic T lymphocyte clones isolated from skin lesions of early MF were found to express a Th1 cytokine profile (7). Taken together, these findings could support the importance of cytokines in tumour progression of MF. However, the role of cytokines in the process leading to malignant transformation of T-cells is still unknown. The possibility that this malignant transformation may be facilitated in a permissive cytokine environment should be considered. It is of interest that most studies of peripheral blood have shown that despite the very low number of circulating malignant T cells detected in the early stage of MF (9), stimulated mononuclear cells have a Th2 cytokine profile (10–13).

Regulation of cytokine levels has been shown to be under genetic control through genetic polymorphism in their coding and promoter sequences. A potential association with allelic variations in certain cytokine genes has been reported for several diseases, including autoimmune diseases (14, 15), infectious diseases (16, 17), allograft rejection (18, 19), T-cell-mediated diseases of the skin (20, 21) and lymphoproliferative malignancies (22–24).

The aim of the present study was to investigate whether a particular cytokine polymorphism might represent a risk factor for developing patch-stage MF.

MATERIALS AND METHODS

Subjects

The study population comprised 33 patients (23 men, 10 women; M:F=2:3) aged 17–80 years (mean 52 years) who had been diagnosed as having patch-stage disease on the basis of clinical, histopathological and immunohistochemical findings, according to the EORTC criteria (25). All patients were in stage 1A or 1B according to the Bunn-Lamberg staging system (26) and were relatively newly diagnosed patients with a follow-up of no more than 4 years. None had a history of atopic disease, autoimmune disorder or other malignancy, and none progressed to the advanced stage of the disease during this short follow-up period. Serving as a control group were 48

Table I. *Phenotype correlations of cytokine genotypes*

Cytokine locus	High production genotype	Intermediate production genotype	Low production genotype
IFN-γ (+874)	T/T	T/A	A/A
IL-10 (−1082, −819, −592)	GCC/GCC	GCC/ACC or GCC/ATA	ACC/ACC or ACC/ATA or ATA/ATA
IL-6 (−174)	GIG or G/C	–	C/C
TNF-α (−308)	A/A or G/A	–	G/G
TGFβ1 (+10 and +25)	T/T G/G or T/G G/G	T/C/ G/C/ or C/C/ G/G or T/T/ G/C	C/C G/C or C/C C/C or T/T/ C/C or T/C C/C

healthy age- and sex-matched individuals. The study was approved by the local ethical committee.

Cytokine gene polymorphism

DNA was extracted from fresh peripheral blood mononuclear cells (PBMC) using a High Pure PCR Template Preparation Kit (Roche Molecular Biochemical, Mannheim, Germany). Single nucleotide mutations were analysed in five different cytokines, leading to assignment of genotype and phenotype, as described previously (19) (Table I). Coding sequence mutation was analysed for IFN-γ at position +874 (T versus A). It has been shown *in vitro* that a homozygous T is associated with the ability to produce high levels of IFN-γ; the heterozygous T/A is an intermediate producer and the homozygous A genotype generates only a small amount (27). IL-6 promoter was studied for the presence of a single nucleotide modification in position −174: both the G/G and G/C genotypes are known to correlate *in vivo* with a high-production phenotype, whereas the C/C genotype is a low producer of IL-6 (15). Three different polymorphisms were surveyed for the IL-10 promoter: position −1082 (G vs A), position −819 (C vs T) and position −592 (A vs C); the first and the third positions are in strong linkage disequilibrium with each other (28). The phenotypes attributed to these polymorphisms are a result of a combination of all the three polymorphic regions. For example, GCC corresponds to G in position −1082, C in position −819, and C in position −592. Thus an individual who is homozygous for this polymorphism will have a high IL-10 production phenotype. An intermediate phenotype corresponds to either GCC/ACC or GCC/ATA. A low IL-10 production phenotype corresponds to ACC/ACC, ACC/ATA or ATA/ATA. These phenotypes have been found *in vitro* and *in vivo* (28, 29).

For TNF-α the presence of a G or A nucleotide in position −308 of the promoter region generates three potential genotypes corresponding to two different phenotypes in vitro (30). The A/A and G/A genotypes represent the potential to produce high levels of TNF-α whereas the G/G genotype is associated with low production of this pro-inflammatory cytokine. Two single nucleotide mutations were analysed for TGF-β1, both in the coding region: codon +10 can be either T or C, and codon +25 either C or G. Potentially there are nine different combinations of these two isolated mutations that give rise to three different secretion phenotypes *in vitro* (31) – high, intermediate and low producers of TGF-β1.

Polymerase chain reaction with sequence-specific primers

Polymerase chain reaction (PCR) amplification was carried out according to the manufacturer's recommendations (One Lambda, Inc., Canoga Park, CA, USA). Briefly, after the addition of the appropriate primer pairs, salts, buffer and Taq polymerase, the samples were subjected to 30 cycles of PCR as follows: one cycle of 130 s at 96℃, dropping to 63℃ for another 60 s; 9 cycles of 10 s at 96℃ and 60 s at 63℃; the final 20 cycles included a three-temperature ramp-annealing for 10 s at 96℃, hybridization for 50 s at 59℃ and an extension step of 30 s at 72℃. PCR products were then loaded onto an agarose gel and photographed using an ultraviolet transilluminator. Positive lanes were analysed on a specific working sheet.

Statistical analysis

To compare the genotype distribution of cytokines as well as frequencies of genotype variations among the patients with MF with those among healthy controls, either the χ^2 test or the Fisher's exact test were used, as appropriate. p values of $\leqslant 0.05$ were considered statistically significant.

RESULTS

Table II presents the phenotype expression deduced from the genetic polymorphism in five selected cytokines in patients with patch-stage MF and normal controls. As no statistically significant differences were found in

Table II. *Phenotypic expression (see Table I) of the selected five cytokines in patients with patch-stage mycosis fungoides (MF) (n=33) and in healthy controls (n=48)*

	Low n (%)	Intermediate n (%)	High n (%)	Overall p value
IFN-γ				
MF	8 (24)	16 (49)	9 (27)	
Control	18 (38)	24 (50)	6 (13)	
p value	0.20	0.8	0.09	0.18
IL-10				
MF	11 (33)	16 (49)	6 (18)	
Control	17 (36)	25 (52)	6 (13)	
p value	0.84	0.75	0.53	0.77
IL-6				
MF	2 (6)		31 (94)	
Control	1 (2)		47 (98)	
p value	0.35		0.56	0.56
TNF-α				
MF	27 (82)		6 (18)	
Control	42 (88)		6 (13)	
p value	0.53		0.53	0.53
TGF-β1				
MF	3 (9)	6 (18)	24 (73)	
Control	3 (6)	10 (21)	35 (73)	
p value	0.68	1.0	0.98	0.86

the genotype frequencies and distribution of patients with stage IA or IB MF, the patients were analysed as a single group. There were no statistically significant differences between the study population and the control group in the genotype distribution of any of the cytokines examined.

DISCUSSION

A potential association with allelic variations in certain cytokine genes has recently been reported for several T-cell-mediated disorders (20, 21) and lymphoproliferative malignancies (22–24). Although previously reported studies on the Th1/Th2 cytokine profile in early MF have not been entirely concordant, most found decreased expression of IFN-γ in the blood (10–13), increased expression in lesional skin (3, 4), and increased expression of IL-10 in the blood (12). The present study suggests that this Th1/Th2 imbalance cannot be explained on the basis of allelic variation leading to differences in the transcription of these specific genes. Thus the potential to produce a Th2 response, i.e. the secretion of higher levels of IL-10 (12) and lower levels of IFN-γ (10–13) by stimulated PBMCs, as reported for patients with early-stage MF, is probably not determined by a genotype consistent with high production of IL-10 or low production of IFN-γ. We should note that we did not study the genetic polymorphism of IL-4, the main inducer of Th2 development, found to be over-produced by stimulated PBMCs in early MF patients (10, 11, 13), as it is not included in the cytokine genotyping tray (One Lambda, Inc.) used in the present study.

TNF-α plays an important role in the development and function of normal lymphoid tissue. High-production polymorphisms in the promoter region of the TNF-α gene at position −308 have been associated with an increased susceptibility for the development of follicular lymphoma (24) and influenced the outcome of non-Hodgkin's lymphoma (23), suggesting a pathophysiological role for the genetic control of the immune response in lymphoid malignancies (23). In our study no specific polymorphism in this TNF-α locus was found to be associated with early pathogenesis of MF. However, other high-production polymorphisms have been identified in the promoter regions of the TNF-α, as at position −857, which was associated with increased susceptibility to development of adult T-cell leukaemia/lymphoma in HTLV-1 carriers (24), and at position −238, found in association with early onset of psoriasis (20). The importance of TNF-α in the pathogenesis of MF has recently been illuminated by cDNA microarrays analysis, which allowed the identification of an MF signature, including upregulation of 20 genes, half of which were directly implicated in the regulation of TNF-α signalling (32). The latter observation extends the results of previous studies showing an increased expression of TNF-α in skin lesions of MF (4, 33).

TGF-β1 is an immunosuppressive cytokine that inhibits the activity of both Th cell types in human subjects. In MF it has been shown to be secreted by reactive T cells as well as by the tumour cells (7). Our study comparing patients with patch-stage MF with normal controls detected no specific polymorphism of this cytokine. It is of interest that one of the main hypotheses is that MF is derived from chronic antigenic stimulation (2). For example, there are reports of the development of Sezary syndrome in patients with atopic dermatitis (34). In this context, it should be noted that very recently atopic dermatitis was found to be associated with a low-producer TGF-β1 cytokine genotype (21).

Although we did not find any specific cytokine gene polymorphism in association with early-stage MF, it is yet to be determined whether cytokine gene polymorphisms determine the tumour progression to the advanced stage of the disease. We preliminarily studied 10 patients with tumour-stage MF but failed to detect any significant differences in genotype distribution and frequencies between this group and patch-stage MF or normal controls (data not shown). However, the number of patients with tumour-stage MF was too small for a meaningful comparison.

In summary, our study suggests that the development of patch-stage MF is not determined by a specific genotype polymorphism. However, further studies on larger numbers of cases with early-stage as well as with advanced-stage MF are needed to better elucidate the role of cytokine gene polymorphism in the pathogenesis of MF.

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