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Ethnic differences in allelic distribution of IFN-g in South African women but no link with cervical cancer

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Abstract

Background: The failure of specific types of human papillomaviruses (HPV) to raise effective immune responses may be important in the pathogenesis of cervical cancer, the second most common cancer in South African women. Polymorphisms of a number of cytokine genes have been implicated in inducing susceptibility or resistance to cancers caused by infectious agents owing to their role in determining host immune response. Polymorphisms of IL-10 and IFN-γ genes are believed to influence the expression and/or secretion levels of their respective cytokines.

Methods and Results: In this study, women with histologically proven cancer of the cervix (n = 458) and hospital-based controls (n = 587) were investigated for bi-allelic -1082 (A/G) polymorphisms of IL-10 and the bi-allelic +874(A/T) polymorphisms of IFN-γ. In addition, the distributions of the allelic frequencies were stratified in both the African and mixed race population groups of South Africa. We found striking differences in the allele distribution of IFN-γ (X^2 = 0.02) among the two ethnic groups. A significant increase in the allele distribution of the IFN-γ AA genotype was found in the African group compared to the mixed population group (OR, 0.5; 95% CI, 0.2–1.0). For IL-10 there were no significant allelic differences between the two South African ethnic groups. Furthermore, when the ethnic groups were combined the IL-10 allelic frequencies in the combined South African data were similar to those observed in an Oriental population from Southern China and in an Italian population. However, the allele frequencies of the IFN-γ genotype among the two South African ethnic groups were different when compared to an Italian Caucasoid group. While crude analysis of these data showed both statistically significantly increased and diminished risks of cervical cancer among high producers of INF-γ and low producers of IL-10 respectively, these associations were no longer significant when the data were adjusted for confounding factors.

Conclusion: These findings demonstrate a clear correlation between ethnicity and IFN-γ polymorphism across different population groups. However, these differences in ethnicity and gene polymorphisms in the aforementioned cytokines are suggested not to influence the development of invasive cervical cancer but may represent an important susceptibility biomarker for other diseases and should be explored further.

Background

The major cause of cervical cancer is due to infection with specific high-risk types of HPV. [1–3] In South Africa invasive cancer of the cervix is the second most common cancer among women, with an age-standardized incidence rate of 15 for Asians, 26.5 for Africans, 17.7 for mixed race and 10.8 for Caucasians.[4] Although the incidence of genital HPV infection in various population groups is high most of these regress without intervention.[5] Investigating genetic host factors and immune responses could help to understand the association between genital HPV infection and carcinogenesis. In particular, cell-mediated immunity (CMI) is important in controlling both HPV infections and HPV-associated neoplasms. [6] CMI is regulated by cytokines that are secreted primarily by T helper (Th) cells and macrophages; Th cells are classified into two distinct subsets of cells according to their cytokine pattern. Th1 modulates cellular (type 1) and Th2 humoral (type 2) immune responses.[7] Th1 cells produce interferon-gamma (IFN-γ) and interleukin 2 (IL-2), which are immuno-stimulatory and are associated with the clearance of HPV infection and regression of cervical intraepithelial neoplasia.[8] Th2 cells produce IL-4 and IL-10, which are immuno-inhibitory and are capable of stimulating tumor growth.[9] Several studies have identified polymorphisms in cytokine gene regulatory regions that correlated to intra-individual variations in cytokine production. [10–12] In addition, cytokine gene polymorphisms have been implicated in various neoplastic and non-neoplastic human pathologies[13] and in a number of diseases including Epstein-Barr virus associated gastric carcinoma,[14] Alzheimer's,[15] rheumatoid arthritis[16] and in patients presenting with severe sepsis after trauma.[17] Others have documented the relationship of an increased type 2 and decreased type 1 cytokine profile to a range of tumours[9,18–20] and demonstrated the association of cytokine polymorphism with the risk and prognosis of cervical cancer. [21–23] Such efforts have generated many candidates and have shown that a decreased IFN-γ transcription and increased IL-10 transcription in the sub-epithelial tissues in patients with HPV-16 positive cervical intraepithelial neoplasia (CIN) is reported to play a role in the development and progression of HPV-16 associated cervical precancer.[24] Consequently the potential importance of type 1 and type 2 polymorphism of cytokines such IL-10 and IFN-γ in cervical cancer are of great interest.

IFN-γ plays a pivotal role in defense against viruses and intracellular pathogens and in the induction of immune-mediated inflammatory responses.[25] Pravica *et al*., 2000 noted a novel single nucleotide polymorphism (SNP), T to A, located at the +874 position from translation start site in the first intron of IFN-γ gene, which coincides with a putative NF-κB binding site that could play a fundamental role in the induction of constitutively high IFN-γ production. The association of +874 alleles T to A with a low (AA), medium (AT) and high (TT) cytokine production was shown *in vitro*.[26] The same group reported a number of polymorphisms in the IL-10 gene of which the SNP situated at the -1082 position of the promoter region of the IL-10 gene plays an important role in determining high, medium and low production of IL-10.[27] The association of G/A SNP at position -1082 has been associated with low (AA), medium (AG) and high (GG) cytokine production were shown *in vitro*.[27]

Several studies have demonstrated that ethnicity and cytokine polymorphism plays a significant role in the susceptibility to a wide range of diseases [28–30] including cervical cancer [21,22]. We thus determined the allelic and genotype frequencies of +874 IFN-γ and -1082 IL-10 genes using the ARMS-PCR methodology among two South African ethnic groups and investigated their relationship to the development of cervical cancer. Using previously collected data on known and suspected risk factors to adjust for confounding,[31] we found a significant difference in the distribution of the bi-alleleic +874 IFN-γ gene among the black and mixed population groups in South Africa. Similarly, we found a pronounced difference in the frequency of IFN-γ when compared to published data from three other groups of different ethnic origin. Interestingly, we did not observe a significant association between cytokine polymorphisms and susceptibility or resistance to developing cervical cancer. We postulate that the non-association between cervical cancer and the aforementioned cytokine gene polymorphisms is multi-factorial and may be a consequence of biologic factors, ethnicity, socioeconomic risk factors and life style. In addition, we reasoned that while others have generated strong correlation between cytokine polymorphism and the development of cervical cancer,[32,33] these studies might have been designed to have insufficient statistical power to examine these relationships adequately. These results also suggest that the disparity observed in the ethnic groups in South Africa may influence immune responses in other diseases as reported in several South African studies. [34,28]

Methods

Patients and Control populations

Individuals were selected from a cancer case-control study conducted in the Western Cape Province of South Africa, from January 1998 to December 2001.[31] Data on known risk factors and potential confounders was collected using a detailed questionnaire administered by trained nurse interviewers. Blood samples from 458 patients with cervical cancer and 587 hospital-based controls were analysed for IL-10 and IFN-γ gene polymorphisms. All of the patients and hospital based controls

with conditions unrelated to the use of hormonal contraception and no history and evidence of cervical disease were from the mixed race and African population groups residing in the Western Cape Province of South Africa.

DNA extraction

Genomic DNA was extracted from whole blood using the QiAamp Spin Blood Kit (QIAGEN, Valencia, CA) in accordance with manufacturer's instructions.

Cytokine genotyping

The A and T alleles at position +874 in the first intron of the IFN-γ gene and the A and G alleles at position -1082 in the promoter region of the IL-10 gene were identified using the amplification refractory mutation system polymerase chain reaction (ARMS-PCR) methodology as previously described.[35] The amplified ARMS-PCR products were then visualized by electrophoresis in 2 % agarose gel stained with ethidium bromide.

Statistical analysis

All statistical analyses were performed using PC SAS version 6.12 (SAS Institute Inc., Cary NC). Allelic frequencies between patients and control groups and ethnic populations were compared using the X^2 test. Unconditional logistic regression was used to estimate odds ratios (OR's) for developing cancer of the cervix in relation to the cytokine polymorphisms. These OR's were adjusted for the following confounding factors; ethnic group, 5 year age group, years of education, age at first sexual intercourse, number of sexual partners, urban/rural living, number of Pap smears, injectable/oral contraceptive use, smoking, and parity, IL-10 and IFN-γ.

Results

To investigate the reproducibility of the assigned genotypes thirty samples were randomly chosen and their genotypes confirmed by repeating the ARMS-PCR.

Allelic frequencies were compared using the X^2 test and confirmed for their fit to the Hardy-Weinberg equilibrium test. The allele frequencies for IFN-γ +874 in the cases and controls among the two ethnic groups are reported in Table 1. The allelic distribution for IFN-γ was significantly different (X^2 = 0.02) between the two ethnic groups. Specifically, the frequency of the IFN-γ AA genotype was significantly higher among the African population and was associated with a decreased risk of cervical cancer and this association was of borderline significance when the data was adjusted for IL-10 (OR, 0.5; 95% CI, 0.2–1.0). Moreover, in the combined South African data the distribution of the IFN-γ AA genotype was significantly different when adjusted for ethnicity and IL-10 however this difference was longer significant when the data was adjusted for known risk factors and confounding factors. There were no significant differences in the distribution of IFN-γ allelic polymorphism in the controls and cases with cervical cancer.

The allelic frequencies for the polymorphism at position -1082 of the IL-10 promoter gene in the cases with cervical cancer and controls are shown in Table 2. Borderline significance in the association between the distribution of -1082 IL-10 alleles and susceptibility or resistance to cervical cancer was noted in the crude data (OR, 0.6; 95% CI, 0.3–1.0) and when it was adjusted for IFN-γ (OR, 0.6; 95% CI, 0.3–1.0). However, when the data was adjusted for potential confounding factors, no significant association was observed (OR, 0.6; 95% CI, 0.3–1.2). Similar results were obtained when the cases and controls were stratified into the two ethnic population groups (data not shown).

Logistic regression estimates for developing cancer of the cervix in relation to gene polymorphisms of -1082 IL-10 and +874 IFN-γ are shown in Table 3. Since associations of borderine significance were observed in the risk for developing cervical cancer in the high and low producing alleles for INF-α (Table 1) and IL-10 (Table 2) when compared to the medium producing alleles, we aimed at further investigating the risk associated for the respective high and low producing alleles for the aforementioned cytokines. These estimates are shown in Table 3. There was a significant difference in the allelic distribution of IFN-γ T allele in the combined data which leads to an increased risk of cervical cancer but only when adjusted for the IL-10 data (OR, 3.7; 95% CI, 1.2–11.0). Similarly, for IL-10 borderline significance was observed in the distribution of the IL-10G allele in the combined data while adjusting for the IFN-γ data (OR, 0.5; 95% CI, 0.2–1.0). There were no significant associations between these cytokine polymorphisms and susceptibility or resistance to cervical cancer when the risk estimates were adjusted for known risk factors and possible confounding factors.

Table 4 shows the allele frequencies of IL-10 and INF-γ in our two control ethnic groups and compares them to other population studies. Allele frequencies for the combined South African data for IL-10 were similar to those reported in an Italian[36] and not significantly different from two British Caucasoid groups.[37,38] There was an increased frequency of the IL-10 A allele and decrease in the G allele in the Chinese population[39] when compared to the South African data and other population groups. There was an increased frequency of the IFN-γ A allele in the African and mixed race groups, compared with that found in an Italian Caucasian population. It is difficult to compare our data to the Italian Caucasoid data since we do not have the raw data from their study, but it is clear that 61% and 77% are significantly greater propor-

Table 1: Odds Ratios and 95% confidence intervals for the development of cervical cancer in relation to INF-γ +874 alleles among the cervical cancer cases and controls in two South African ethnic populations.

Race	Genotype	CaCx n (%)	Controls* n (%)	OR[1] (95% CI)	OR[2] (95% CI)	OR[3] (95% CI)
1. All#		(n = 261)	(n= 405)			
	TT	19 (7)	33 (8)	0.7(0.4–1.3)	1.0(0.4–2.3)	0.8(0.3–2.1)
	AA	146 (56)	260 (64)	0.6(0.5–0.9)	0.6(0.4–0.9)	0.7(0.4–1.1)
	AT	96 (37)	112 (28)	1.0	1.0	1.0
2. Mixed race		(n = 165)	(n= 265)			
	TT	14 (8)	26 (10)	0.7(0.3–1.4)	1.2(0.4–3.2)	1.0(0.3–3.1)
	AA	85 (52)	158 (59)	0.7(0.4–1.0)	0.7(0.4–1.2)	0.7(0.3–1.3)
	AT	66 (40)	81 (31)	1.0	1.0	1.0
3. African		(n = 96)	(n = 140)			
	TT	5 (5)	7 (5)	0.7(0.2–2.6)	0.7(0.2–3.0)	0.4(0.2–2.3)
	AA	61 (64)	102 (73)	0.6(0.3–1.1)	0.5(0.2–1.0)	0.6(0.3–1.4)
	AT	30 (31)	31 (22)	1.0	1.0	1.0

#All (1) = Mixed race (2) and African (3) population groups combined OR[1] = crude odds ratio adjusted for ethnic group in the combined ethnic group analysis OR[2] = adjusted for IL-10 OR[3] = adjusted for ethnic group (only in the analysis combining Mixed race and African groups), 5 year age group, education, age at first sex, number of sexual partners, urban/rural living, number of pap smears, injectable/oral contraceptive use, smoking, parity and IL-10

Table 2: Odds Ratios and 95% confidence intervals for cervical cancer in relation to -1082 IL-10 alleles in South African cervical cancer cases (CaCx) and controls.

Genotype	CaCx* (n = 197) n (%)	Controls* (n = 182) n (%)	OR[1] (95% CI)	OR[2] (95% CI)	OR[3] (95% CI)
GG	29 (15)	41 (23)	0.6(0.3–1.0)	0.6(0.3–1.0)	0.6(0.3–1.2)
AA	88 (45)	76 (42)	0.9(0.6–1.5)	1.0(0.6–1.6)	1.0(0.6–1.6)
AG	80 (41)	65 (36)	1.0	1.0	1.0

*Data given as percentages (As there were no differences in the distribution of IL-10 alleles between the mixed race and African groups the data was combined) OR1 = crude odds ratio OR2 = crude OR adjusted for IFN-γ OR3 = adjusted for ethnic group, 5 year age group, years of education, age at first sexual intercourse, number of sexual partners, urban/rural living, number of Pap smears, injectable/oral contraceptive use, smoking, parity, and IFN-γ.

tions than 32%. There was a significant difference in the distribution of the INF-γ A allele when the mixed race group and African groups were compared ($p < 0.007$) and no significance in the distribution for the TT ($p < 0.06$) and TA (0.07) genotypes.

Discussion

Several studies have investigated the possible role of cytokine gene polymorphisms and the prevalence of HPV-induced cervical cancer.[32,40] This impairment of host factors might result in susceptibility or resistance to tumor progression. Other studies have included ethnicity as a factor in identifying impairments where the distribution of alleles across populations may influence the outcome of disease.[21,33,41,42] In this study we noted significant differences in the distribution of +874 IFN-γ alleles compared to the distribution for -1082 IL-10 alleles among the African and mixed race groups in South Africa.

Although our study did not find a correlation between IFN-γ and IL-10 gene polymorphisms and the risk for developing cancer of the uterine cervix, it is powered by strong statistical analysis. We adjusted for all known risk factors and possible confounding factors for the development of cervical cancer including IL-10 and IFN-γ. As the aforementioned cytokines are important immuno-modulators and contribute to the development and progression of cervical cancer, [9,24] it was important to adjust each one against the other as a potential confounder. In addition, IL-10 has anti-inflammatory capabilities that can down-regulate the production of IFN-γ, [28] thus polymorphisms of these cytokines may interact with each other and influence disease progression.

To the best of our knowledge this is the first study investigating the genetic association of polymorphisms in the +874 IFN-γ gene with cervical cancer among two racially

Table 3: Odds Ratios and 95% confidence intervals CI for developing cancer of the cervix in relation to genotypes considered as high or low producers of 1. -1082 IL-10 and 2. +874 IFN-γ.

Cytokine	Race	*Genotype	CaCx n (%)	Controls n (%)	OR[1] (95% CI)	OR[2] (95% CI)	OR[3] (95% CI)
1. IL-10	All#		n = 117	n = 117			
		GG	29 (25)	41 (35)	0.6(0.3–1.0)	0.5(0.2–1.0)	0.6(0.3–1.2)
		AA	88 (75)	76 (64)	1.0	1.0	1.0
2. IFN-γ	All#		n = 165	n = 299			
		TT	19 (12)	33 (11)	1.2(0.6–2.2)	3.7(1.2–11.0)	1.1(0.5–2.7)
		AA	146 (88)	266 (89)	1.0	1.0	1.0
	Mixed		n = 99	n = 184			
		TT	14 (14)	26 (14)	1.0(0.5–2.2)	3.7(0.9–15.6)	1.5 (0.4–4.8)
		AA	85 (86)	158 (86)	1.0	1.0	1.0
	African		n = 66	n = 109			
		TT	5 (8)	7 (6)	1.6	3.2(0.6–18.0)	0.8(0.2–4.2)
		AA	61 (92)	102 (94)	(0.4–6.3) 1.0	1.0	1.0

#All = Mixed race and African population groups (As there were no differences in the distribution of IL-10 alleles between the two South African ethnic populations, the data was combined) *Genotype = (TT and GG associated with high cytokine secretion and the AA associated with low cytokine secretion) OR[1] =crude odds ratio (high secreting allele vs low secreting allele) OR[2] = crude odds ratios, IFN-γ data adjusted for IL-10 and IL-10 data adjusted for IFN-γ OR[3] = fully adjusted for ethnic group, 5 year age group, education, age at first sex, number of sexual partners, urban/rural living, number of pap smears, injectable/oral contraceptive use, smoking, parity and IFN-γ data adjusted for IL-10 and IL-10 data adjusted for IFN-γ

Table 4: Frequencies (%) of 1. IFN-γ genotype and 2. IL-10 (estimated by the Hardy-Weinberg Equilibrium) alleles in African and mixed race ethnic control groups and other populations.

Allele	South African		#Italian Caucasians	+South-east England	†Manchester, UK	§China
	African	Mixed race				
1. IFN-γ	(n = 103)	(n = 188)	(n = 363)			
+874T/T	4	10	21.2	-	-	-
+874T/A	19	29	46.8	-	-	-
+874A/A	77	61	32.0	-	-	-
2. IL-10	*(n = 182)		(n = 726)	(n = 152)	(n = 660)	(n = 166)
-1082A	60		63	47.4	51	94
-1082G	40		37	52.6	49	6.0

*Combined African and mixed race control groups in this study (As there were no differences in the distribution of IL-10 alleles between the two South African ethnic populations, the data was combined to compare allele frequencies to other populations). Data reported by #Poli *et al.* (2002); +Reynard *et al.* (2000); †Perrey *et al.* (1998); §Mok *et al.* (1998)

different ethnic groups within South Africa. We found a significant difference in the distribution of the IFN-γ AA genotype in the combined South African data when adjusted for ethnicity and IL-10. However, when the data was adjusted for known risk factors and potential confounders the association between IFN-γ gene polymorphism and the risk for developing cancer of the uterine cervix was no longer significant. In addition, we observed a pronounced increase in the distribution of IFN-γ A allele among the African group compared to the mixed population group, which correlated with a decreased risk of developing cervical cancer when adjusted for IL-10. This result is of interest as several studies have reported that the IFN-γ A allele is associated with the development of disease. [43–45] Nevertheless, as this is the first IFN-γ gene polymorphism association study there are no comparative data with which to compare our results. We observed no significant association between IFN-γ and IL-10 gene polymorphisms and cancer of the uterine cervix when the analyses included control for confounding factors.

Our findings are discordant with those reported by Stanczuk *et al*., 2001, who also investigated the association of -1082 IL-10 gene polymorphism with cervical cancer. They demonstrated that women who were predisposed to producing high or medium levels of IL-10 were more likely to develop cervical cancer than subjects who were predisposed to producing low levels of IL-10.[32] However, their study was based on a small sample size and no adjustment for known risk factors for cervical cancer was made. Previous studies on the level of IL-10 production have also been conflicting.[27,46] It was found that the -1082 G and A alleles were correlated with low (AA), medium (AG) and high (GG) levels of IL-10 production as measured in *in vitro* stimulated peripheral blood lymphocytes.[27] Similarly, in an association study of IL-10 haplotypes and juvenile rheumatoid arthritis individuals that are homozygous for -1082A allele (ATA/ATA) produced less IL-10 than those without the ATA haplotype.[47] Nevertheless, the considered low producing haplotype was not associated with disease. However, other groups have reported that the -1082A allele was associated with high IL-10 production.[46,48] Helminen *et al*., 2001 investigated the association of IL-10 gene polymorphism and susceptibility to primary Eptein-Barr virus (EBV) infections. They reported that the -1082A allele was associated with a poorer clinical outcome[49] and measured spontaneous plasma levels by enzyme immunoassay. It was found that the ATA haplotype was associated with both high levels of IL-10 production and an increased risk for symptomatic disease.[49]

Recently, various groups raised the issue of whether the risk of developing cervical cancer is influenced by the hosts' cytokine profile. Ghaderi *et al*., 2000 reported that the tumor necrosis factor (TNF) a-11 allele was more frequent in HPV16-seropositive cervical intraepithelial neoplasia (CIN) patients and in HLA-DR15-DQ6 positive patients among Swedish women with cervical cancer. It was suggested that the combination of these three markers may increase the risk for developing CIN. Additionally, several reports have investigated the serum levels of cytokines in women with cervical cancer. Increased levels of IL-6 and IL-8 in cervicovaginal secretions,[50] elevated serum levels of IL-8, IL-10, TNF-beta, TNF-alpha and GM-CSF[51] and a reduced IFN-γ transcription in both epithelial and sub-epithelial zones,[24] have been found in patients with cervical cancer. Furthermore, several studies have demonstrated the association of p53 with cancer of the uterine cervix, however, the functional relevance of this polymorphism is in contention. It was shown that the proline/proline genotype at codon 72 of p53 represented a significant risk factor for the development of cervical cancer, [41,52] while others have reported that the arginine/arginine genotype was associated with cervical cancer. [53–55] This disparity was suggested to be a manifestation of geographic or ethnic variation of the population study [41,56]

Several studies in cervical cancer survival have suggested that the higher mortality rates in black populations is explained primarily by the more advanced clinical stage at time of diagnosis.[57,58] Furthermore, this variation in cervical cancer mortality according to ethnicity may be attributed to the unavailability of screening services, less aggressive treatment patterns, greater clinical severity of disease and unfavorable socioeconomic status in black women with cervical cancer.[57,59] Similarly, in South Africa, cancer of the cervix is the most common cancer in African women (31.2%) compared to white women (2.7%) and the incidence rates are comparable to those found in the rest of Africa and other countries.[4]

The high degree of ethnic disparity in susceptibility to cancer of the cervix has sparked interest in identifying a reference population for investigating the distribution of cytokine allele frequencies in different normal population groups. When our control study groups were compared to those of other population groups (Table 4) the distribution of the allele frequencies for -1082 IL-10 were similar to those observed in three European Caucasoid groups but differed from that found in a Chinese population. For IFN-γ, the allele frequencies among the African and mixed population group were disparate when compared to an Italian Caucasoid population. These data highlight the possible variability of cytokine gene frequencies in different population groups and may be of relevance in other infectious diseases.

Conclusion

Polymorphisms within cytokine genes can serve as immunologic markers for identifying individuals at risk for disease. However, caution should be taken when association studies are considered as there are several potential confounding factors that may contribute to the progression of disease and cytokine polymorphisms should not be viewed in isolation. Nevertheless, in this study we conclude that the allele distribution of IFN-γ +874 gene polymorphism was significantly different among the South African and other ethnic population groups and this disparity might have a significant consequence in the clinical outcome of other diseases and may account for variation in host susceptibility. Although our study did not show an association between IL-10 -1082 and IFN-γ +874 gene polymorphisms and the risk of developing cervical cancer on the available data we question whether any particular cytokine can be responsible for the development of cancer of the uterine cervix. These data highlight the need for further studies to clarify the association of ethnic variation and cytokine polymorphism and the need for careful

study design and adequate sample sizes to ensure that spurious associations are not reported.

Authors' contributions

VAG assisted in design of study, carried out the study and drafted the manuscript. HROC performed the statistical analysis for the study and assisted in editing manuscript. JAS assisted in the presentation of the data, overall preparation of the manuscript, the editing and final proof-reading of the manuscript. MH participated in the design and coordination of the case control study. GAS participated in the design of the cytokine study. A-LW conceived of the study and participated in the design and coordination of the study. All authors read and approved the final manuscript.

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