Functions of the cytokines in relation oral lichen planus-hepatitis C

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ABSTRACT

Background

Lichen planus (LP) is a chronic common mucocutaneous inflammatory disorder of uncertain aetiology. An association between hepatitis C virus (HCV) infection and LP has been recognised, particularly in Italy, Spain and Japan.

The pathogenesis of such an association is unclear, but it may be due to cell-mediated cytotoxicity to an epitope shared by HCV and damaged keratinocytes. Recent studies using *in situ* hybridization suggest that HCV may replicate in the oral mucosa.

Objectives

The aim of the present study was to examine the oral *epithelium* of patients with oral LP for evidence of HCV-RNA by polymerase chain reaction (PCR) and to examine the relationship to cytokines including interferon (INF-γ), interleukins (IL-1, IL-2, IL-4, IL-6, IL-8 , and IL-10), tumour necrosis factor (TNF-α) and transforming growth factor (TGFβ-1).

Patients/Methods

We selected 100 Italian patients, and divided them into 4 groups. Group A consisted of 25 HCV+ve patients with erosive oral LP. Group B was a control group constituted by 25 healthy HCV -ve subjects with no LP. Group C consisted of 25 HCV-ve patients with oral reticular LP and Group D was made of 25 HCV-ve patients with oral erosive LP. The patients of group A (test group) were submitted to oral biopsy with 2 samples of epithelium, lesional and non-lesional, and a 10 ml peripheral blood sample was taken. The patients of group B (negative control), C and D (comparison groups) were submitted to oral epithelial biopsy and a 10 ml peripheral blood sample was collected.

PCR was used to search for HCV-RNA in biopsy material. Cytokines INF-γ ,IL-1, IL-2, IL-4, IL-6, IL-8 , IL-10 and TNF-α _ and TGFβ-1 were assayed in serum.

Results and Conclusions

PCR did not detect the viral genome in oral epithelium of the patients with oral LP and HCV+ve (group A), but there was an increase in levels of TNF-α and a reduction of IL-1, INF-γ and IL-8 compared to patients who had oral reticular LP but HCV-ve and to patients who had oral erosive LP but HCV - ve, and compared to negative controls.

The results indicate that patients of group A showed a reduction of pro-inflammatory but an increase in immunomodulant cytokines.

The results suggest the possibility that HCV exerts an indirect effect, mediated possibly by the induction of cytokines and lymphokines.

Keywords: *Lichen, HCV, viral, cytokines*.

INTRODUCTION

Lichen planus (LP) is a common inflammatory mucocutaneous condition. Estimates of the prevalence vary among different populations, but the condition does not appear to exhibit a racial predilection (1-3).

The aetiology of LP is unclear in most instances, but in some patients drugs or infective agents are implicated (4). Some patients with LP have an increased prevalence of hepatitis C virus (HCV) infection. In such patients, liver abnormalities frequently occur, especially chronic active hepatitis (4,5). Therefore, HCV is thought to contribute to the development of LP and it has also been estimated that HCV-infected patients have at least twice the risk of developing LP than the general population (6). Furthermore, cases of concomitant LP and HCV have been reported (7-9). It has also been suggested that LP could be a possible marker of HCV infection, but the link between these diseases has not been consistent (4,10).

Different studies have confirmed that keratinocytes are not only the main targets of immune attack in oral LP (OLP) but may play a critical role through production of cytokines; the TIMC (tissue-infiltrating mononuclear cells) are stimulated in situ to differentiate to produce a range cytokines characteristic of

LP, and the inflammation is modulated by the local cytokine network (11-18).

The present study aimed to investigate the possible aetiopathogenic association between LP and HCV in an Italian population, and the relationship with various cytokines.

PATIENTS AND METHODS

The study group consisted of 100 Italian patients seen at the odontological clinic University of Naples. Informed consent to the study was obtained and the protocol approved by the local research ethics committee. Oral LP was diagnosed on the basis of the recognised typical clinical features and histological findings. Serum antibodies to HCV were determined by Enzyme-Linked Immunosorbent Assay (Elisa II), corroborated by Recombinant Immunoblot Assay (RIBA II) and confirmed with Polymerase Chain Reaction (PCR). Patients who were anti-HCV +ve together with serum HCV-RNA confirmed by PCR , were termed HCV+ve.

Patients receiving haemodialysis, and patients hospitalized for organ transplantation had been excluded.

The 100 patients were subdivided into 4 groups, Thus:

• Group A; 25 HCV+ve patients with erosive OLP (16 females and 9 males).

• Group B; a negative control group of 25 otherwise healthy patients undergoing surgery during the same period. These patients were HCV- and did not have OLP.

• Group C; 25 HCV -ve (no HCV) patients (14 females) with reticular OLP.

• Group D;25 HCV -ve(no HCV) ve patients (12 females) with erosive OLP.

All patients had a 10 ml sample of peripheral blood collected.

The patients of group A were submitted to oral mucosal biopsy, taking 5 mm of lesional and 5 mm of non-lesional epithelium, trying to avoid collecting lamina propria (in order to minimise contamination with non-epithelial cells). The patients of groups B (negative controls), C and D were submitted to oral mucosal biopsy (groups C and D had lesional biopsy), taking 5 mm of epithelium without lamina propria. All biopsies were washed in 0.9% saline for 5 minutes and after 30 min incubation at 37°C, were treated with 3 U/ml elastase to complete separation of epithelium from connective tissue, resulting in an "epithelial biopsy" (19). The epithelial biopsies from patients of group A were examined for HCV by using RNAzol (liquid mixture of guanidine and isothiocyanate) to precipitate RNA pellets for synthesis of cDNA through the use of a reverse transcriptase. The cDNA was amplified via PCR with specific HCV primers. For positive controls, RNA was extracted from the peripheral blood mononuclear cells from HCV+ patients.

Total RNA was extracted from the epithelial biopsy samples and from lymphocytes isolated from peripheral blood by density gradient sedimentation (Ficoll High Pure RNA Isolation;Roche Diagnostics). Subsequently the RNA was back-transcripted using a standard protocol.

Peripheral blood mononuclear cells were used as RNA positive control since the presence of HCV viral in tissue lymphocytes of HCV +ve patients is uncertain.

Two μl of the synthesized cDNA was amplified in a mixture containing, in a final volume of 50 μl, Tris-HCl 10mM pH 8.3, MgCl2 1.5 mM, KCl 50mM, dNTP 200M, 2.5 Taq DNA polymerase (Boehringer Mannheim) 0.5 μM sense and antisense INF-gamma ,IL-1, IL-2, IL-4, IL-6, IL-8 , IL-10 and TNFγ, ___TGFβ-1 (Maxim-Biotec) primers. The products of PCR were analyzed by agarose gel electrophoresis to 1.2% in Tris-Borate-EDTA (TBE), coloured with ethidium bromide. PCR using specific primers for INF-γ ,IL-1, IL-2, IL-4, IL-6, IL-8 , IL-10, TNF-α __and_ TGFβ-1 was carried out on all biopsies and blood (lymphocyte) using a semi-quantitative assessment based on band density. The amplification cycle profile was as follows: denaturation at 94°C for 10 s, annealing at 60°C for 20 s, and extension at 72°C for 30 s. The durations of denaturation in the first cycle and extension in the last cycle were extended for 7 min. After 30 and 35 cycles of amplification for each cytokine, each PCR product was electrophoresed on a 2% agarose gel, visualized by ethidium bromide staining, and photographed.

RESULTS

Patients of group A (HCV+ve patients with OLP) had HCV-RNA detectable by PCR in peripheral blood lymphocytes. The HCV viral genome was not detected the oral epithelium from these patients with OLP. Patients of groups B, C and D were HCV-RNA negative both in serum and biopsies.

There was an increase in TNF-α, and a reduction of IL-1, INF–γ and IL-8 mRNA (Table 1) in the HCV+ve patients with OLP (group A).

DISCUSSION

HCV RNA was not demonstrable in oral epithelium from patients with oral lichen planus (OLP), irrespective of their hepatitis C virus (HCV) status. However, there was an increase in TNF-γ (which factor inhibits keratinocyte proliferation), and a reduction of IL-1, INF-γ and IL-8 (factors inducing keratinocyte growth, neutrophil activation and lymphocyte growth respectively) in the HCV+ve patients with OLP (group A), in comparison with HCV -ve cases of OLP (group C and D) and HCV-ve and OLP negative controls (group B) (Table 1). Thus the HCV viral genome was not detected in oral epithelium of HCV+ve patients with OLP but there was an increase of TNF-α and a reduction of IL-1, INF-γ and IL-8 in comparison to HCV –ve patients with OLP, and to negative controls.

Recent reviews (20,21) have focused on a hypothetical relationship between LP and chronic HCV infection , but epidemiological results (22,23) are controversial. OLP is the form most commonly reportedly related to chronic liver disease (CLD) (24).

The reports associating LP with HCV reveal marked geographic variation (table 2) (25-31). In countries with a low prevalence of HCV, LP appears un-associated with HCV (30,31) and one interesting Spanish study (32) showed HCV-positivity was more frequent in the groups of patients with LP-CLD (78%) and CLD (42.8%) than in patients with LP alone (3.1%). This suggests that chronic liver disease is a prerequisite for LP to develop in patients with serum antibodies against HCV. This virus may play a role in development of oral lesions in HCV-infected patients

Table 1. *Cytokine responses in LP*

		CYTOKINES								
		INF-γ	IL-1	IL-2	IL-4	IL-6	IL-8	IL-10	TNF-α	TGFβ-1
Group A HCV+ve Erosive LP	Epithelium non lesional	-+	++-	-+	+	+	+-	++-	+++	+
	Epithelium lesional	+	+	+	-+	-+	++-	+++	+++	+++
	Blood	+-	++-	-+	-	-+	+	+	+	+++
Group B Healthy controls	Epithelium	+	+++	-+	-	-	+++	-	+-	+++
	Blood	-	-	-	-	-	-	-	+	-
Group C Disease controls HCV-ve Reticular LP	Epithelium lesional	+++	+++	-	-	-	+++	+-	+	+
	Blood	-+	+	-	-	+	-+	-	+	-
Group D Disease controls HCV-ve Erosive LP	Epithelium lesional	+++	++-	-	-	+	+++	+-	+++	+-
	Blood	-+	+-	-	-	+	-+	-	+	-

Scoring:

+++	highly represented in all samples
++-	represented in majority of samples
+	weakly present in all samples
+-	weakly present in majority of samples
-+	weakly present in few samples
-	represented not in all samples

Table 2. *Geographic patterns of association of HCV with.*

Lichen Planus			Control group			
Country	Patients N°	% HCV+ve	Patients N°	% HCV+ve	Control	References
Japan	45	62				(Nagao Y et al, 1998)
U.S.	22	55	40	25	Psoriasis	(Chuang TY et al,1999)
Italy	70	27	70	4,3	Other keratoses	(Carrozzo M et al , 1996)
Spain	78	20	82	2,4	Skin disorders	(Sánchez-Pérez J et al, 1996)
Germany	84	16	87	1,1	Skin disorders	(Imhof M et al, 1997)
Netherlands	55	0				(Van der Meij EH et al, 2000)
U.K.	45	0				(Tucker SC et al, 1999)

(27,33-35), but any mechanism is still not clear. One possibility is that different HCV genotypes may have different effects.

Although Imhof and colleagues found a high prevalence of HCV genotype 1B in their study, this probably reflects the fact that LP affects older patients (29). In an Italian study of LP, the prevalence of different HCV genotypes was similar to that in a population with CLD without LP (36) . The prevalence of autoantibodies was not higher in HCV+ve than in HCV- ve patients, but HCV+ve patients had higher levels of serum immunoglobulins, which could be related to cryoglobulinaemia (35). This is not in accord with this work. The value of antiviral treatment effective against HCV has not been proven; indeed to the contrary, there are many reports of interferon inducing or aggravating LP (37-39).

Several recent studies have confirmed that HCV is an important correlate in patients with LP, in Southern Europe and Japan (5, 26,36). However, a much low percentage of HCV infection has been found in LP patients in England and northern France (31,40) . There are also significant differences in levels of HCV RNA between the genotypes of HCV and between LP patients and controls (36). For this reason, it may be that the genetic make-up of the host rather than viral factors, is important. Indeed, the HLA-DR6 allele can influence infection and could explain the geographical heterogeneity of the association between HCV and LP (29,36). Previous studies have suggested that HCV may persist and replicate in the diseased oral mucosa (5,35). but other studies have shown no HCV transcripts in the epithelium in cutaneous LP (41).

Our results confirm this in that HCV RNA was not demonstrable in oral epithelium in OLP.

However, it is possible that this difference could be related to the method used for collecting biopsy samples, since we deliberately excluded as much as possible of the vascular connective tissue and examined only oral epithelium. In any event, others have shown that HCV replicates in epithelia both from lesions and elsewhere (42).

Our results suggest that the stimulation of TNF-α and repression of IL-1, INF-γ and IL-8, may be more important in producing cytokine changes which might influence the transformation of reticular to erosive LP, possibly by impairing the efficacy of repair mechanisms of keratinocyte damage, as hypothesised by others (43,44).

CONCLUSION

The results indicate that patients of group A (erosive LP and HCV+ve) showed a reduction of pro-inflammatory but an increase in immunomodulant cytokines in lesional tissue, in comparison to non-lesional tissue and to circulating cytokines, and in comparison to the tissue from patients of groups B, C and D. HCV RNA was not demonstrable in oral epithelium.

These results suggest that the hepatitis C virus exerts an indirect effect, mediated possibly by the modulation of these cytokines and lymphokines in the pathogenesis of oral erosive lichen planus. HCV may have no direct viral responsibility, but the possibility remains that this result is spurious.

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