

Effects of tumor necrosis factor antagonist treatment on hepatitis C-related immunological abnormalities

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ABSTRACT. *Background.* Chronic hepatitis C infection is frequently associated with a mixed cryoglobulinaemia and circulating auto-antibodies, especially anti-smooth muscle cells (SMA) and anti-liver/kidney/microsome type 1 (LKM-1) anti-tissue antibodies. Treatments with TNF antagonists favour the emergence of auto-antibodies, and particularly anti-dsDNA antibodies. *Objective.* To determine the impact of TNF antagonists on hepatitis C-related immune abnormalities. *Methods.* We prospectively monitored for 14 weeks, six patients with actively replicating chronic hepatitis C, initiating an anti-TNF treatment for an associated rheumatoid arthritis. *Results.* Anti-nuclear and anti-dsDNA antibodies were induced in two and three patients, respectively. Treatment had no impact on the production of antibodies against extractable nuclear antigens, and it did not induce anti-tissues antibodies in any patient. Cryoglobulinaemia appeared in 2/6 patients, and it persisted in 2 others. No patient developed any new signs of autoimmunity. HCV viraemia remained unchanged. *Conclusions.* Induction of auto-antibodies by TNF antagonist treatments does not involve anti-tissues antibodies, even in patients with actively replicating chronic hepatitis C prone to produce anti-SMA and anti-LKM-1 antibodies. In contrast, TNF antagonists may favour emergence of cryoglobulinaemia in such patients.

Keywords: TNF antagonists, chronic viral hepatitis, auto-antibody, cryoglobulinaemia

Chronic hepatitis C infection is frequently associated with the presence of circulating auto-antibodies. Clifford *et al.* reported the presence of rheumatoid factors and anti-smooth muscle cell (anti-SMA) antibodies in as many as 66% and 76% of patients, respectively [1]. Although the incidence of auto-antibodies was lower in the study of Cassani *et al.* [2], these authors observed auto-antibodies in 30% of patients, with antinuclear antibodies (ANA), anti-SMA antibodies and anti-liver/kidney microsomes type 1 (anti-LKM1) antibodies in 9%, 20% and 6% of chronic hepatitis C patients, respectively. In this study, high serum alanine aminotransferase (ALT) levels and female gender were independently associated with the presence of auto-antibodies. A high prevalence of mixed cryoglobulinaemia was also observed in patients with chronic hepatitis C (reviewed in [3]).

Treatments with tumor necrosis factor (TNF) antagonists have been recently introduced for a number of inflammatory and auto-immune conditions, including rheumatoid arthritis (RA), spondylarthropathies, Crohn's disease and psoriasis. Emergence of auto-antibodies is a common ob-

servation in patients treated with TNF antagonists. ANA corresponding to anti-double stranded (ds) DNA antibodies of the IgM class were induced in 30% to 70% of patients, depending on the assay and on the underlying disease [4-7]. TNF antagonists also favour emergence of anti-cardiolipin IgM auto-antibodies. In contrast, they have no effect on the development of auto-antibodies recognizing extractable nuclear antigens (ENA), including anti-SSA, -SSB, -ribonucleoprotein (RNP), -topoisomerase 1 (Scl-70) and -thyroid peroxidase antibodies [6, 7]. In most cases, appearance of auto-antibodies in patients treated with TNF antagonist remains silent, with no associated auto-immune clinical manifestations [4, 5, 7]. Rare cases of clinical lupus have been observed, most often benign and regressing after withdrawal of the drug [8].

Because of the frequency of chronic hepatitis C, a number of patients with this condition may require treatment with TNF antagonists for an associated inflammatory disorder. Two recent studies have shown that TNF antagonists had no adverse effects on liver inflammation in patients with

chronic hepatitis C and no effect on the level of hepatitis C virus (HCV) replication [9, 10]. Whether TNF antagonists favour development of immune abnormalities associated with chronic hepatitis C infection has not been addressed so far. In this study, we prospectively monitored six patients with actively replicating chronic HCV infection and with RA requiring initiation of TNF antagonist treatment. HCV viral load, aspartate aminotransferase (AST), ALT, presence of auto-antibodies and of cryoglobulinaemia were tested at inclusion and at week 14 of treatment. This study suggests that TNF antagonists favour the emergence of cryoglobulinaemia, but not of chronic hepatitis C-related auto-antibodies.

METHODS

Six patients (one woman and five men, 44.2 ± 8.6 year old (mean \pm SD)) were enrolled between January 2003 and January 2004, in a multicentric, prospective study. They met the criteria of the American College of Rheumatology for the diagnosis of RA; they were seropositive for anti-HCV antibodies in an ELISA assay, and HCV viraemia was detected by PCR. AST and ALT were within the normal range in all patients. In three patients (patient 1, 3 and 6), liver biopsy had been performed and HCV disease activity scored as A1F1, A1F2 and A1F2, respectively, using the MetaVir score system. No patient had received specific therapy for HCV infection. All patients were naive for TNF antagonists and received immunosuppressive drugs, including methotrexate, at inclusion. These treatments remained unchanged during the 14 weeks of the study. Five patients (1 to 5) were treated with infliximab (3 mg/kg at week 0, 2 and 6), whereas patient 6 was treated with etanercept (25 mg twice a week). HCV viral load was measured by quantitative PCR assay using COBAS AM-

PLICOR HCV MonitorTM (Version 2.0) kit. ANA were tested using Hep2 cells as a screening test (KallestadTM, BioRad, Redmond, WA, USA). If the titre of ANA was $> 1:80$, detection of anti-dsDNA and anti-ENA antibodies was performed. Antibodies against ENA were tested by ELISA (BindazymeTM anti-ENA screening assay, Binding Site, Birmingham, UK). When they were detected, the antigen recognized (SSA, SSB, Sm, RNP, Scl-70, Jo-1) was determined using an ENA-LISA detection kit (BMD, Marne-la-Vallée, France). The presence of anti-dsDNA antibodies was tested for using the Crithidia luciliae test (BMD). Anti-tissues antibodies (anti-mitochondria, -SMA, -LKM1 and -parietal cell antibodies) were tested using the triple tissue (rat liver/kidney/stomach) IIF Kit (BMD).

RESULTS

Plasma HCV viral load was tested at inclusion and during TNF antagonist treatment. No change was observed: HCV viraemia was 5.9 ± 1.1 (mean \pm sem), 6.0 ± 1.0 and $6.0 \pm 0.9 \log_{10}$ copies/mL at inclusion and at weeks 6 and 14 of treatment, respectively. TNF antagonist treatment also had no effect on AST and ALT serum concentrations, which remained within the normal range in all patients throughout the course of the study.

At inclusion, patient 3 had ANA with a homogeneous pattern, still detected at week 14 of treatment, with an increase of the ANA titre from 320 to 1280 (*table 1*). In this patient, anti-dsDNA antibodies appeared during treatment, with a 320 titre at week 14. Patient 1 had ANA with a speckled pattern at inclusion, attributed to anti-ENA antibodies, with low titres of anti-SSA and anti-SSB antibodies. In this patient, the titre of anti-SSA and anti-SSB did not increase during treatment, but anti-dsDNA antibodies appeared. In patient 2, ANA antibodies appeared during

Table 1
Evolution of immunological abnormalities

Patient	ANA ^a				anti-dsDNA ^b Ads				Anti-ENA Abs ^c						Cryoglobulin	Anti-tissu antibodies	
	Week	Presence	IFI Pattern ^d	Titre	(IFI C, luciliae)		Elisa		SSA SSB Sm/RNP Scl-70 Jo-1								
					Presence	Titre	Presence	Value (IU/ml)	Presence								
1	0	+	Spec	160	-	-	-	<20	+	84	43	-	-	-	-	-	-
	14	+	Ho+Spec	640	+	320	-	<20	+	87	48	-	-	-	-	+	-
2	0	-	-	-	-	-	-	<20	NT							-	-
	14	+	Ho+Nuc	320	-	-	+	67.5	-							-	-
3	0	+	Ho	320	-	-	-	<20	-							+	-
	14	+	Ho	1280	+	320	-	<20	-							+	-
4	0	-	-	-	NT	-	-	<20	NT							-	-
	14	-	-	-	NT	-	-	<20	NT							-	-
5	0	-	-	-	NT	-	-	<20	NT							+	-
	14	-	-	-	NT	-	-	<20	NT							+	-
6	0	-	-	-	NT	-	-	<20	NT							-	-
	14	-	-	-	NT	-	+	33.5	NT							+	-

NT = not tested, Ho = homogeneous, spec = speckled, Nuc = nucleolar.

^a Anti-nuclear antibodies.

^b double-strander DNA.

^c Extractable nuclear antigens (normals values : 30 U/ml).

^d IFI pattern on hep2 cells.

treatment, attributed to anti-dsDNA antibodies, which were detected by ELISA. No patient scored positive at any time for anti-Sm, -RNP, -Scl-70 and -Jo-1 antibodies, nor for anti-tissues antibodies (including anti-mitochondria, -SMA, -LKM1 and -parietal cell antibodies). None of the patients develop clinical manifestations of lupus, Sjögren's syndrome or any new signs of autoimmunity. Two patients (patient 3 and 5) had cryoglobulinaemia at inclusion, which was still present at week 14. Cryoglobulinaemia developed during the follow-up in two other patients (patients 1 and 6) without any clinical abnormalities.

DISCUSSION

In this prospective study of six patients with actively replicating chronic hepatitis C and treated with TNF antagonists for RA, we observed no effect of TNF antagonists on the level of HCV replication or on serum aminotransferase levels. This confirms findings observed in two retrospective studies involving 5 and 24 patients, respectively [9, 10]. Our study extends these previous reports by showing the impact of TNF antagonists on autoimmune abnormalities, with a special attention being paid to those related to HCV infection. As in patients with no HCV infection, we observed a significant induction of ANA and anti-dsDNA antibodies by TNF antagonists. ANA developed in 2/6 patients, and their titre increased in a third patient in whom they were already present at inclusion. In two of the three patients, ANA were related to the presence of anti-dsDNA antibodies. This observation is consistent with the results obtained in non-HCV-infected patients [4-7]. In one patient, titres of anti-SSA and anti-SSB antibodies, already detected at inclusion, remained unchanged during treatment. Anti-SSA, -SSB or other -ENA antibodies were not detected in the other patients either at inclusion or 14 weeks later. This result is consistent with a prospective study of 53 patients with Sjögren's syndrome and treated with infliximab, where no induction of anti-SSA or anti-SSB was observed in patients negative at inclusion [11]. Of note, no patient experienced any sign of Sjögren's syndrome, lupus or any new signs of autoimmunity. Mixed cryoglobulinaemia is associated with both RA and chronic HCV infection. It was present at inclusion and persisted in 2/6 patients and it appeared in two other patients. Emergence of cryoglobulinaemia has not been reported in patients with RA treated with TNF antagonists, and, as for the appearance of anti-SSA and anti-SSB, these features could be favoured by HCV infection. None of these four patients had cryoglobulinaemia-related clinical symptoms at any time during the study. Interestingly, if TNF blockers have been reported as a possible promising treatment in some cases of vasculitis, the only three published cases of HCV-associated cryoglobulinaemia and vasculitis were disappointing, with one very partial and transient improvement [12] and two failures [13]. Moreover, the only controlled study of a TNF blocker (etanercept) in vasculitis (Wegener disease) was negative [14]. In contrast to cryoglobulinaemia, we observed no induction of anti-tissue auto-antibodies, and especially of anti-SMA and anti-LKM1 antibodies, which are frequently associated with chronic hepatitis C. Even the three patients in whom TNF antagonist treatment led to the emergence of ANA and/or anti-dsDNA auto-antibodies, had no anti-

tissues antibodies. This underlines the specificity of auto-antibodies induced by TNF antagonists, already outlined in the absence of chronic HCV infection. Interestingly, the auto-antibodies induced by such treatments (anti-dsDNA, rheumatoid factors, anti-cardiolipin), are usually of the IgM class, and HCV-related mixed cryoglobulinaemia usually includes an IgM component. This may be a clue to understanding why TNF antagonist treatment induced cryoglobulinaemia in our study, whereas it had no or only rare effects on IgG auto-antibodies, including anti-SMA and anti-LKM1 antibodies. Auto-antibodies emerging during TNF antagonist treatment are usually clinically silent, possibly due to the low avidity of antibodies for their antigen. This may explain why no cryoglobulinaemia-associated symptoms developed in any of our patients. Further prospective studies involving larger numbers of patients should confirm the selectivity of effects of TNF antagonists on immune abnormalities in HCV-infected patients, as well as the absence of related clinical symptoms.

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