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# Vascular cell adhesion molecule-1 (VCAM-1) plays a central role in the pathogenesis of severe forms of vasculitis due to hepatitis C-associated mixed cryoglobulinemia

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***Background/Aims*: To better characterize the molecules involved in leukocyte tissue infiltration during hepatitis C-mixed cryoglobulinemia (HCV-MC)-associated vasculitis.**

***Methods*: The involvement of ELAM, ICAM-1 and VCAM-1 was evaluated in 36 patients with HCV-MC vasculitis using three different approaches: concentrations of soluble forms by specific ELISA, tissue expression by immunohistochemistry on patients nerve biopsies, endothelial expression by FACS analysis, on cells activated in vitro by cryoprecipitates purified from HCV-MC patients.**

***Results*: Concentrations of sVCAM-1 were significantly elevated in the serum of HCV-MC patients compared to HCV patients without MC, the highest concentrations being found in severe vasculitis. VCAM-1 expression was detected on blood vessels from nerve biopsies performed in patients with severe vasculitis. When added to endothelial cells in vitro, HCV-MC patients cryoprecipitate induced VCAM-1 but also ELAM and ICAM-1 expression possibly through a mechanism due to the C1q complement fraction interaction with endothelial cells, since C1q was consistently present in the cryoprecipitates.**

***Conclusions*: VCAM-1 is mainly involved in the pathogenesis of HCV-MC-associated severe vasculitis and may be a potential interesting therapeutic target.**



***Keywords*: Hepatitis C; Mixed cryoglobulinemia; VCAM-1; Vasculitis**

## 1. Introduction

Mixed cryoglobulinemia (MC) is the most frequent extrahepatic manifestation associated with hepatitis C virus (HCV) chronic infection, being detected in 56% of the patients [1,2]. HCV-MC may be clinically asymptomatic or induce various kinds of immune complex (IC) vasculitis [3–5]. The most frequent is a chronic disease, histologically characterized by a perivascular mononuclear cell infiltrate, without vessel wall necrosis, affecting small-size veins and arterioles [5]. Clinical presentation is limited to cutaneous purpura and arthralgia, but may be more severe including subacute distal sensory polyneuropathy and glomerulopathy [4,5]. Another type of HCV-MC associated vasculitis appears to be very similar to periarteritis nodosa (PAN), being clinically characterized by acute onset, poor general condition, severe multifocal sensorimotor mononeuropathies, high blood pressure, central nervous system or digestive tract vasculitis and is histologically associated with medium-size arteries

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necrotizing vasculitis and a mixed polymorphonuclear and mononuclear cell infiltrate [5].

The Arthus phenomenon and its equivalents are considered good experimental models for IC-vasculitis and MC [6] and are histologically characterized by the association of tissue oedema, hemorrhage and neutrophil infiltration [6]. Neutrophilic inflammation in this model has been reported to be secondary to interactions between the leukocyte adhesion molecules L-selectin (CD62L) and β2 integrins (CD11a/CD18, CD11b/CD18) and their endothelial ligand, notably endothelial leukocyte adhesion molecule-1 (ELAM, CD62E) and intercellular adhesion molecule-1 (ICAM-1, CD54) [7]. Adhesion molecules expressed on leukocytes and endothelium are necessary for the recruitment of blood leukocytes into tissues during inflammation. These molecules belong to different families, the more important being the selectins, the integrins and some members of the immunoglobulin superfamily. Among the selectins, ELAM is early synthesized and expressed by inflamed endothelium, and participates in the initial steps of leukocyte adhesion [7]. ICAM-1 and the vascular cell adhesion molecule-1 (VCAM-1, CD106) are both members of the immunoglobulin superfamily and bind β2 integrins and α4 integrins, respectively, allowing leukocyte stable adhesion which precludes tissue migration. ICAM-1 is ubiquitously expressed and its endothelial expression increased after stimulation by proinflammatory agents, whereas VCAM-1 expression is limited to inflamed endothelium and podocytes [7].

HCV-MC vasculitis appears to be at least in part, histologically different from the Arthus model. In order to better characterize HCV-MC pathogenesis, we studied the involvement of ELAM, ICAM-1 and VCAM-1 in this disease.

## 2. Material and methods

### 2.1. Patients

Thirty-six patients (aged 36–81 years) with HCV-MC, 18 patients with HCV chronic infection without MC (HCV, aged 26–76) and 18 healthy volunteers (aged 25–71) were included in this study. Informed consent was obtained for each patient and the study conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Chronic HCV infection was defined as follows: alanine aminotransferase values more than twice the upper limit for more than 6 months, anti-HCV antibodies detected by third generation ELISA, HCV RNA detected by polymerase chain reaction amplification techniques, and histological lesions compatible with chronic hepatitis C on liver biopsy. Patients were considered as having HCV-MC if they had evidence of HCV infection associated with clinical symptoms of vasculitis and the presence of serum cryoprecipitates in addition to complement consumption. All patients with HCV-MC were untreated and clinically active. All patients and controls were negative for the human immunodeficiency and hepatitis B viruses.

Clinical manifestations were classified as follows:

- group 1: low-grade severity MC limited to chronic cutaneous purpura and peripheral arthritis, mononuclear cell dermal vasculitis on skin biopsy, in the absence of renal or neurological involvement;
- group 2: high-grade severity MC consisting in cutaneous purpura associated with either kidney (creatininemia > 120 μmol/l and /or proteinuria > 1 g/24 h and histological findings related to cryoglobulinemia on renal biopsy), or peripheral nerves involvement (clinical symptoms and abnormal electromyography consistent with distal sensory polyneuropathy associated with mononuclear cell perivascular infiltrates on neuromuscular biopsy);
- group 3: PAN-like vasculitis consisting in extensive cutaneous purpura associated with peripheral nerve involvement (clinical symptoms and abnormal electromyography consistent with severe multifocal mononeuropathies associated with mixed polymorphonuclear and mononuclear cell necrotizing vasculitis on neuromuscular biopsy) in the context of poor general condition (fever > 38.5°, weight loss > 5 kg), associated with either the digestive tract, or the central nervous system involvement (ischemic clinical manifestations associated with vasculitis on biopsy and/or microaneurisms/non atheromatous occlusions on aortoarteriography), or acute onset high blood pressure (systolic > 160 mmHg and/or diastolic > 95 mmHg).

Several biological parameters were studied, including rheumatoid factor (quantitative determination, Laboratoire Fumouze, Levallois-Perret, France), cryoglobulin, haemolytic complement CH50, complement fraction C4, C-reactive protein and fibrinogen concentrations.

### 2.2. Immunohistological studies

All patients with neuropathy due to high-grade severity HCV-MC or PAN-like diseases underwent a full-thickness open biopsy of the superficial peroneal nerve and peroneous brevis muscle in the most affected limb. Part of the specimen was paraffin-embedded and both transversal and longitudinal sections were stained with either haematoxylin-eosin, PAS or congo red. In each case, the presence of inflammatory vascular lesions was evaluated in both muscle and nerve samples by examination of paraffin-embedded specimens on serial sections. For immunohistochemistry studies, consecutive serial sections with inflammatory vascular lesions were analyzed. Paraffin-embedded nerve sections were labelled using anti-VCAM, anti-ICAM mAb (murine IgG1, Dako laboratories), or an anti-IgG Ab as a negative control, and revealed by the immunoperoxydase method. The serial sections in paraffin-embedded used for hematoxylin-stain, anti-VCAM-1 or anti-ICAM-1 labelling were consecutive.

### 2.3. Detection, isolation and characterization of cryoglobulin

Cryoglobulin was precipitated from serum as previously described [8]. Immunoglobulin various isotypes and C1q concentrations were measured by nephelemetry (IMMAGE, Beckman-Coulter, Marseille, France). For in vitro experiments, the cryoprecipitate was prepared in sterile conditions without sodium azide, its concentration was measured using bicinchonimic acid (detection range: 20–200 μg/ml, Pierce Perbio, Paris, France), and stored at −80 °C until using. Before addition to endothelial cells, the cryoprecipitate was dissolved by heating 2 h at 37 °C and diluted either in complemented or decomplemented AB human serum (ABS, Etablissement Français du Sang, Marseille, France) or in decomplemented fetal calf serum (FCS, Gibco Invitrogen, Cergy-Pontoise, France).

### 2.4. Soluble adhesion molecule measurements

Blood was obtained by venipuncture and serum aliquoted and stored at −80°C until assayed. sELAM, sICAM-1 and sVCAM-1 were measured using specific ELISA from R&D Systems (Abingdon, UK). Interassay and intrassay variations were less than 8%.

### 2.5. Cell culture

Human umbilical vein endothelial cells (HUVEC) were obtained, grown and used on passage 3–5, as previously reported [9]. When confluent, HUVEC were cultured in M199 containing either ABS or FCS, then stimulated for 6 or 16 h with various concentrations of cryoprecipitate