Etiopathogenesis of Systemic Sclerosis: role of Parvovirus B19 infection.

Michele Colaci  
Tutor: Prof. Clodoveo Ferri

Systemic Sclerosis (SSc) is a chronic autoimmune disease characterized by diffuse microangiopathy and fibrosis of the skin and visceral organs. Its pathogenesis is almost unknown: toxic and/or infectious triggers could induce an activation of B- and T-cells in a susceptible host, leading to an autoimmune response.

Parvovirus-B19 (PV-B19) DNA is frequently found in tissues and organs involved in several autoimmune disorders, such as vasculitis, arthritis, myocarditis, aplastic anaemia, interstitial lung disease. In fact, persistence of viral antigens, cytokines induction by NS1 viral protein, and molecular mimicry (i.e. cross-reaction between capsid peptide VP1 and collagen type II) may cause an autoimmune response.

Aim of our study is to investigate the role of PV-B19 in the pathogenesis of SSc: we suppose that a persistent PV-B19 infection could induce an impairment of the level of endothelial progenitor cells (EPCs), derived from stem cells of bone marrow (BM), and an overproduction of connective tissue by fibroblasts.

Our study protocol consists in three steps: an etiologic (1), a pathogenetic (2) and a therapeutic step (3). The study is carried out using BM and skin biopsies performed in SSc patients and subjects without autoimmune disorders (as controls).

Here, I present the progresses achieved in the first year of my Doctorate School:

**Step 1** (research of PV-B19 DNA): 7/8 BM specimens and 5/6 skin specimens of SSc patients were positive for PV-B19 by means of nested-PCR; these data are consistent with previous our findings.

[in collaboration with prof. A. Azzi - Department of Public Health, University of Florence]

**Step 2a** (dosage of EPCs in BM): 10 BM from SSc subjects (4 PV-B19+; 6 still unknown) and 10 BM from controls have been studied. EPCs were identified by means of immune-histochemistry for CD133 and KDR (receptor of VEGF type 2).
In comparison with the normal controls, there was a significant decrease in CD133+ cells (0.35%±0.4 vs 1%±0.74, P=0.0088) and in KDR+ cells (0.47%±0.24 vs 1.2%±0.53, P=0.0018). Moreover, we found a significant decrease of CD133/KDR co-expression in SSc BM cells compared to controls (13.7%±5.3 vs 47.7%±5.52, P=0.024).

[in collaboration with Dr N. Del Papa - Department of Rheumatology, G. Pini Hospital, Milan]

**Step 2b (dosage of chemokines in skin fibroblasts cultures):** 8 cultures from SSc patients’ fibroblasts were carried out; 2 PV-B19+, 1 PV-B19-, others still unknown. Up to date, data are still not available. Preliminarily, an increased production of CXCL10 (T helper 1 response) and CCL2 (T helper 2 response) chemokines in the supernatant of SSc cultures compared to controls have been observed. These findings are consistent with the significant increase of serum levels of the same chemokines in SSc patients versus controls (published data in Antonelli A et al. Rheumatology 2008; 47: 45-9).

[in collaboration with Dr A. Antonelli - Department of Internal Medicine, University of Pisa]

**Step 3 (experimental therapy – a case report):** a 69 years-old man affected by SSc complicated by chronic multiple non-healing skin ulcers was treated with erythropoietin. The latter is known to stimulate the proliferation and differentiation of EPCs. Treatment protocol was: 6000 UI weekly for the first 8 weeks, then tapered to 3000 UI/week for 4 weeks, and to 3000 biweekly for other 14 weeks, for a total of six months. An improvement of the ulcers was observed soon after the first month of treatment, with complete healing within 6 months. Before and after the treatment BM studies were carried out to evaluate EPCs. Basally, In comparison with the normal controls, there was a significant decrease in CD133+ cells (0.1% vs 0.47%±0.15), in CD34+ cells (0.7% vs 1.28%±0.6) and in KDR+ cells (0.64% vs 1.2%±0.53). After the treatment, the CD133 expression raised from 0.1% to 0.21%, CD34% from 0.7% to 1.58% and KDR from 0.64% to 0.95%. The BM is PV-B19+.