Livedo Vasculopathy vs Small Vessel Cutaneous Vasculitis

Cytokine and Platelet P-Selectin Studies

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Objective: To assess the role of platelets and lymphocyte-related immunological mechanisms in livedo vasculopathy (LV) and cutaneous small vessel vasculitis (CSVV). Livedo vasculopathy is thought to be related to the thrombotic occlusion of small and medium-sized dermal vessels. Cutaneous small vessel vasculitis comprises a heterogeneous group of disorders in which the main pathogenetic events could be modulated by circulating cytokines.

Design: Case series study of 2 groups of patients affected respectively with LV and CSVV.

Setting: A large clinical and research institute for the study and treatment of cutaneous diseases.

Patients: Consecutive patients with clinically and histologically proved idiopathic LV (n = 8) and CSVV (n = 20) were studied and compared with healthy donors (n = 20). Patients with potentially correlated systemic diseases were excluded.

Main Outcome Measures: Surface expression of platelet P-selectin and circulating level of interleukin (IL) 1β, tumor necrosis factor α (TNF-α), IL-8, IL-2, and soluble IL-2 receptor.

Results: The IL-2 and soluble IL-2 receptor levels were significantly higher in serum samples from patients with both LV (1.24 ± 0.46 IU/mL [mean ± SD] vs 0.46 ± 0.24 IU/mL, P < .001; 899 ± 368 IU/mL vs 628 ± 132 IU/mL, P < .02) and CSVV (0.91 ± 0.57 IU/mL, P < .02; 1087 ± 451 IU/mL, P < .001) than in those from the healthy controls. The serum levels of IL-1β, TNF-α, and IL-8 were higher in patients with CSVV than in controls (7.53 ± 6.7 pg/mL vs 4.58 ± 2.72 pg/mL; 23.7 ± 12.6 pg/mL vs 10.82 ± 2.46 pg/mL, P < .001; 37.8 ± 46 pg/mL vs 8.25 ± 3.53 pg/mL, P < .02, respectively). No significant difference in the serum levels of IL-1β (7.2 ± 4.9 pg/mL), TNF-α (12.9 ± 3.47 pg/mL), and IL-8 (5.9 ± 4.13 pg/mL) was observed in patients with LV compared with controls. An increased expression of platelet P-selectin was also detected in patients with LV in comparison with controls and patients with CSVV. The mean ± SD percentage of positive cells for P-selectin was 43% ± 5% in the patients with LV, 5.1% ± 2% in the controls (P < .001), and 5.3% ± 2% in the patients with CSVV (P < .001).

Conclusions: Taken together, these data demonstrate that different pathogenetic mechanisms are operative in LV and CSVV. In fact, platelet and lymphocyte activation is present in LV, whereas the levels of inflammatory mediators are in a normal range. In CSVV, the high serum levels of proinflammatory cytokines suggest that they are actively involved in the pathogenesis of cutaneous vasculitis.

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LIVEDO vasculopathy (LV) is an uncommon clinicopathologic condition also known as livedo vasculitis,1,2 atrophie blanche,3 and PURPLE (painful purpuric ulcers with reticular pattern of the lower extremities)4 in which the histological finding of leukocytoclasia is usually absent. It is clinically characterized by early, focal, painful purpuric lesions of the lower extremities that frequently ulcerate and slowly heal, leaving white, stellate, atrophic scars (atrophie blanche), telangiectasias, hemosiderinic livedolike hyperpigmentation, and livedo reticulonodularis (Figure 1). Histologically, it shows segmental hyalinization, endothelial swelling, and thromboses of dermal vessels (Figure 2). A variable number of perivascular lymphocytes are usually observed. The prevalence is estimated around 1:100 000 per year and is more frequent in young women (1:3).5 On the basis of the clinical evolution, the improvement during fibrinolytic and anti-
PATIENTS AND METHODS

PATIENTS

The study included 8 patients (5 women and 3 men; mean age, 32.5 years) showing the typical clinical and histological features of LV and 20 patients (12 women and 8 men; mean age, 46 years) with clinical and histological manifestations of CSVV who were hospitalized at Istituto Dermopatico Immacolata, Rome, Italy, during the last 4 years. Twenty healthy donors matching the patients in age and sex distribution were evaluated as a control group. We excluded all patients with both LV and CSVV and potentially related systemic diseases. The LV lesions were exclusively localized on the lower part of the legs and the dorsum of the feet, whereas the CSVV purpuric manifestations involved not only the legs but also, in some cases, the thighs and glutei. No patients were receiving therapy when a blood sample and a skin biopsy specimen of a recent active lesion were obtained. Laboratory and immunological studies, including determinations of antinuclear antibodies, antibodies to DNA, antiendothelial antibodies, antineutrophil cytoplasmic autoantibodies, anticytokines, lupus anticoagulant, serum complement levels (C3, C4, and CH50), circulating immune complexes, cryoglobulins, cryofibrinogen, rheumatoid factor, and circulating lymphocyte subpopulations, were routinely performed in all patients.

All patients consented to participate in the study, which was approved by the Ethical Committee of the institution. Representative clinical and histological findings of LV are shown in Figures 1 and 2 and the typical clinical appearance and histological signs of CSVV are shown in Figures 3 and 4.

SERUM SAMPLES

Serum samples were collected and stored frozen at −80°C until assayed for cytokines. For flow cytometric studies, the platelets were prepared from blood samples obtained from an antecubital vein through a 19-gauge butterfly needle with no tourniquet in an attempt to minimize platelet activation during blood collection. After the first 2 mL of blood was discharged, 4.5 mL was collected into a plastic syringe containing 200 µL of 1% glutaraldehyde solution and a 0.1 mL polycarbonate filter (pore size, 3 µm) and placed in a tube containing 3 mL of buffer (137 mM NaCl, 5 mM KCl, pH 7.4) in a refrigerator at 4°C. Platelet-rich plasma was obtained by centrifugation of blood at 1200g for 15 minutes produced a platelet-containing pellet, which was washed and resuspended in phosphate-buffered saline (pH 7.4).

IMMUNOENZYMATIC ASSAY

Serum levels of IL-1β, TNF-α, IL-8, IL-2, and sIL-2R were measured by enzyme-linked immunosorbent assay test kits (Medgenics Diagnostic, Fleurus, Belgium [IL-1β, TNF-α, and IL-2]; R&D Systems, Minneapolis, Minn [IL-8]; and Cell Free, T cell Science, Cambridge, Mass [sIL-2R]). All assays were based on the dual immunometric sandwich principle and were performed according to the manufacturer’s instructions.

FLOW CYTOMETRIC ANALYSIS

A 1-mL platelet suspension containing 5×10⁶ cells was incubated in the dark with 20 µL of anti–P-selectin fluorescein isothiocyanate–labeled monoclonal antibody (mAb) for 30 minutes at room temperature. At the end of incubation, the platelets were washed 3 times in phosphate-buffered saline. Nonspecific binding of mAb was assessed by incubating platelets with fluorescein isothiocyanate–labeled anti-CD13 mAb. The results of fluorescence were analyzed in a flow cytometer (FACscan, Becton-Dickinson, Rutherford, NJ). All measurements were monitored after excitation of cells passing through the beam of an air-cooled argon ion laser (488-nm excitation, 15-mW output). Parameters collected included forward-angle light scatter (FSC), 90° light scatter, and 530-nm green fluorescence. A gate was set around the platelets, and 5000 cells were analyzed for fluorescent isothiocyanate fluorescence to quantify the amount of the platelet-bound mAb. The binding of the antibody was expressed as the percentage of platelets positive for antibody. Anti–P-selectin (platelet activation–dependent granule external membrane [PADGEM]) (IgG1 and mouse) and anti–CD13 (pammyeloid, IgG1, and mouse) fluorescein isothiocyanate–labeled mAbs were commercially obtained (Immunotech, Marseille, France).

STATISTICAL ANALYSIS

Statistical analysis was performed using a Student t test for unpaired data, and P values of less than .05 were considered significant. The analysis of the fluorescence intensity histogram was performed using the Kolmogorov-Smirnov 2-sample test. Only values of α<.001 were considered significant.

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Small vessel vasculitis (SVV), according to Jorizzo’s recent classification of vasculitis, includes a wide group of diseases (eg, necrotizing, urticarial, Henoch-Schönlein purpura, essential mixed cryoglobulinemia, and septic vasculitis). They refer to specific inflammatory changes of venules, arterioles, and capillaries. A pathogenetic role of circulating immune complexes and autoantibodies (eg, antineutrophil and antiendothelial antibodies) in these syndromes is widely accepted. The skin is a preferential target organ of SVV mostly resulting in clinical features of palpable purpura (Figure 3) and histological changes of leukocytoclastic vasculitis (Figure 4). Cutaneous SVV (CSVV) can be limited to the skin or can represent the cutaneous involvement in the case of systemic vasculitis.

The P-selectin (a 140-kd protein) is stored in the α granules of platelets and in the Weibel-Palade bodies of
endothelial cells. This protein is rapidly redistributed to the plasma membrane during cell activation and degranulation. P-selectin expression is enhanced on a pathophysiologically modified surface on activated endothelial cells, such as those found overlying atherosclerotic lesions, or on activated platelets. The enhancement of P-selectin expression leads to the rolling of polymorphonuclear cells on the endothelium or to the adhesion of neutrophils to the spread platelets. Moreover, P-selectin has been demonstrated to have an important role in the recruitment and transmigration of specific T-lymphocyte subsets to inflammatory sites. Thus, P-selectin mediates a number of interactions between the neutrophils, the activated endothelium, and the activated platelets.

Cytokines are known to be released by a number of cells during inflammation, and they can modulate several vascular tissue and circulating cell functions. The pattern of released cytokines and related endothelial cell changes may play a critical role in cutaneous vascular disorders.

We investigated patients with typical cutaneous lesions and histopathological findings of LV (n = 8) and CSVV (n = 20) for the presence of circulating proinflammatory cytokines, such as interleukin (IL) 1β, tumor necrosis factor α (TNF-α), and IL-8, and serum levels of IL-2 and soluble IL-2 receptor (sIL-2R) as markers of lymphocyte activation. Moreover, the P-selectin expression on the platelet surface was studied in both groups of patients: those with LV and those with CSVV.

### RESULTS

#### LABORATORY AND IMMUNOLOGICAL STUDIES

Antinuclear antibodies (1:80, homogeneous pattern), circulating immune complexes (C1q binding system), and antinuclear antibodies, respectively, were detected in 3 different patients with LV. In the CSVV group, 3 patients with antinuclear antibody positivity with a homogeneous pattern, 4 patients with circulating immune complexes, 3 patients with low C3 levels, 5 patients with increased circulating IgA levels, 1 patient with antineutrophil cytoplasmic autoantibodies (typical perinuclear antineutrophil cytoplasmic autoantibody), and 3 patients with antinuclear antibody positivity (IgG) were found. There was no significant correlation between the latter results and those of the cytokine study.

The mean ± SEM erythrocyte sedimentation rate was 15 ± 10 mm/h in the group of patients with CSVV and 7 ± 8 mm/h in the group of patients with LV. Cytofluorimetric analysis of circulating lymphocyte subsets showed...
an increased value of CD4+ T cells in percentage and absolute number in both the patients with LV (CD4/CD8 mean ratio, 2.5) and the patients with CSVV (CD4/CD8 mean ratio, 2.2) (reference range, 1.2-1.9).

HISTOPATHOLOGIC FINDINGS

Biopsy specimens were obtained from active LV and CSVV skin lesions on the legs. All LV specimens showed endothelial thickening, focal hyalinization, extravasated erythrocytes, and a small perivascular lymphocytic infiltrate associated with a variable number of thrombi in the middermal and upper dermal vessels. No sign of fibrinoid necrosis of the dermal vessels was seen. Neutrophils and nuclear dust were also absent in the infiltrate. All CSVV specimens demonstrated fibrinoid necrosis, infiltration by neutrophils, and consistent signs of leukocytoclastic in the small dermal vessels.

DETECTION OF SERUM IL-1β, TNF-α, AND IL-8

Our results showed high levels of IL-1β, TNF-α, and IL-8 in patients with CSVV when compared with the same levels in healthy donors (7.53 ± 6.7 pg/mL [mean ± SD] vs 4.58 ± 2.72 pg/mL; 23.7 ± 12.6 pg/mL vs 10.82 ± 2.46 pg/mL, P<.001; 37.8 ± 46 pg/mL vs 8.25 ± 3.53 pg/mL, P<.02, respectively) (Figure 5). No significant difference in the serum levels of IL-1β (7.2 ± 4.9 pg/mL), TNF-α (12.9 ± 3.47 pg/mL), or IL-8 (5.9 ± 4.13 pg/mL) was observed between patients with LV and controls; however, a difference was noted in the serum levels of TNF-α (P<.05) and IL-8 (P<.002) between the LV group and the CSVV group (Figure 5).

DETECTION OF SERUM IL-2 AND sIL-2R

We found significantly higher levels of IL-2 and sIL-2R in serum samples from patients with LV (1.24 ± 0.46 IU/mL vs 0.46 ± 0.24 IU/mL, P<.001; 899 ± 368 IU/mL vs 628 ± 132 IU/mL, P<.02) and from patients with CSVV (0.91 ± 0.57 IU/mL, P<.02; 1087 ± 451 IU/mL, P<.001) (Figure 6). Seven of the 8 patients with LV showed IL-2 values higher than those of the healthy donors. Elevated serum levels of sIL-2R were also detected in 6 of the 8 patients with LV.

DETECTION OF MEMBRANE PLATELET P-SELECTIN

The number of platelets reactive with anti–P-selectin mAb was significantly higher in patients with LV than in patients with CSVV and controls (43% ± 5% [mean ± SD] vs 53% ± 3%, P<.001; and 43% ± 5% vs 5.1% ± 2%, P<.001, respectively). All tested patients with LV showed an increased expression of surface platelet P-selectin compared with the controls.

COMMENT

Patients with the diagnosis of LV were assessed clinically for the characteristic aspect of painful, reticulated, ulcerative lesions of the legs, which result in ivory atrophic areas, and histologically for the typical focal thrombi, segmental hyalinization, and a mild lymphocyte infiltration around the dermal vessels. We did not detect any significant sign of leukocytoclastic vasculitis.

Although various reports have revealed the deposition of immunoreactants in blood vessels of patients with...
LV, the “vasculitic” pathogenesis of this disease has never been sufficiently supported. Numerous investigators consider LV an occlusive vasculopathy. Clinical improvement obtained using fibrinolytic,13 anticoagulant,16 and antithrombotic therapies suggests this pathogenetic mechanism. The latter hypothesis is also supported by the good results of treatment with pentoxifylline17 and the presence of protein C deficiency as a potential pathogenetic factor in favor of a thrombotic origin of LV. We have not studied protein C in our patients; therefore, our study does not provide insight in this regard.

Our data showing an increased expression of membrane platelet P-selectin in all the patients with LV indicate a condition of platelet activation and seem to confirm the increased activity of platelets during LV,19 as has been demonstrated in other diseases.20 Recently, it was shown that activated platelets bound to circulating lymphocytes and that in high endothelial venules the extravasation phase of lymphocytes, or rolling, can be mediated by activated platelets through the expression of external membrane P-selectin in vivo.21 P-selectin-mediated platelet adhesion to the endothelium may also occur in acutely inflamed nonlymphoid vascular beds, where immunologically distinct L-selectin ligands have been observed.22 The low expression of P-selectin on the platelet surface in CSVV rules out a critical role for the platelets in this disease. We can imagine an interaction between neutrophils, endothelium, and lymphocytes in CSVV and a cross talk between platelets, lymphocytes, and endothelium in LV.

The elevated levels of IL-2 and sIL-2R and the normal serum levels of inflammatory cytokines (IL-1β, TNF-α, and IL-8) indicate that an immunological process (specific lymphocyte subset activation?) is likely to be operative during LV. The association with platelet P-selectin findings suggests that these mechanisms may play a role in the process of thrombotic vasocclusion. Damle et al23 demonstrated that P-selectin mediates interactions of primed T cells and activated platelets and differentially modulates their production of proinflammatory cytokines.

The presence of anticardiolipin antibodies and of lupus anticoagulant activity has been recently emphasized in LV as a possible explanation for the vasocclusion.24 Anticardiolipin antibodies (IgG) were detected in only 1 patient in our study. Baccard et al25 hypothesized a heterozygous protein C deficiency as a potential pathogenetic factor in favor of a thrombotic origin of LV. We have not studied protein C in our patients; therefore, our study does not provide insight in this regard.

The data concerning circulating cytokines in our patients with CSVV and the histological aspect of leukocytoclastic vasculitis provide substantial evidence of the active role of proinflammatory cytokines (IL-1, TNF-α, and IL-8) in the pathogenesis of this group of diseases. In recent years, various mediators released during inflammation have been reported to promote endothelial cell activation, thromboses, and fibrosis.26,27 Interleukin 1 and TNF-α up-regulate the endothelial expression of adhesion molecules.28 An increased local production of IL-1β and TNF-α has been found in kidney lesions of Wegener granulomatosis and microscopic polyangiitis by in situ hybridization and reverse transcriptase polymerase chain reaction.29 Furthermore, Mulligan and Ward20 demonstrated that both IL-1 and TNF-α can enhance, with different severity, immune complex-induced lung and dermal vascular injury. Finally, on exposure to IL-1 and TNF-α, endothelial cells, fibroblasts, and other cell types produce IL-8, which is one of the main chemoattractants for neutrophils.30 The increased circulating levels of IL-1, TNF-α, and IL-8 in CSVV support the central role of inflammatory mechanisms initiated by immune complex deposition and recruitment and activation of neutrophils in the pathogenesis of CSVV. The discordance of the levels of cytokines and the erythrocyte sedimentation rates in our patients suggests that cytokines have a specific role in CSVV. The disregulation of the IL–2–sIL–2R system observed in CSVV confirms the results reported by Grau et al,32 who found a marked increase in serum IL–2 levels during active phases of systemic vasculitis. The activation of the lymphocytes, and their pathogenetic involvement, is a fundamental step in the development of some types of systemic vasculitis.

According to the literature, LV mostly represents an idiopathic disorder, occasionally described in association with systemic diseases.3 The most likely pathogenetic mechanism is mediated by platelet activation and/or a hypercoagulable state primary or is secondary to anticardiolipin antibody syndrome, protein C deficiency, and fibrinolytic anomalies. Clinically and histologically, LV differs from CSVV, in which palpable purpura and leukocytoclasia are usually present.

In conclusion, in light of the results of this study, we suggest that a basic immunological disorder (lymphocyte activation?) and activated platelet expression of P-selectin may have an important cooperative role in inducing LV. In CSVV, a specific pattern of cytokine changes is present during the active phase. It confirms the important pathogenetic role of cytokines in association with immune complex deposition and the consequent stimulation of neutrophils and lymphocytes.

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REFERENCES


Error in Chemical Name. In the vignette titled “Dramatic Cutaneous Psoriasis Improvement in a Patient With the Human Immunodeficiency Virus Treated With 2’3’-Dideoxycytidine and Ritonavir,” published in the April 1997 issue of the ARCHIVES (1997;133:531), the chemical name of a drug was printed incorrectly. The correct name should be 2’3’-dideoxy,3’-thyacytidine instead of 2’3’-dideoxycytidine.