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Polymorphonuclear Leukocyte Integrins in Deep Venous Thrombosis

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Summary: The polymorphonuclear leukocytes (PMN) have a role in the pathophysiology of deep venous thrombosis (DVT). We examined the phenotypical expression of PMN beta$_2$-integrins (CD l l a, CDl l b, CD 11c) in a group of 19 subjects with leg DVT. PMN cells were incubated with fluorescent monoclonal antibodies against CD11a, CD11b, CD11c, and the evaluation was made by flow cytofluorimetry. The same integrins were determined after *in vitro* activation with 4-phorbol 12-myristate 13-acetate (PMA) and N-formyl-methionyl-leucyl-phenylalanine (fMLP). In DVT subjects, at baseline, the phenotypical expression of CD11b was decreased and that of CD11c increased when compared with normal controls. In normal subjects PMN activation with PMA and fMLP led to a constant increase of all PMN adhesion molecules, while in DVT subjects the CDl l a did not show any change. These data might have therapeutical applications, especially with the aim of preventing post-thrombotic deterioration of vein function.

Key Words: Deep venous thrombosis—Polymorphonuclear leukocyte integrins—Polymorphonuclear leukocyte activation.

Several papers (1–5) have underlined the role of leukocytes in deep venous thrombosis (DVT). During thrombogenesis in deep veins, an inflammatory response develops, and both processes involve complex interactions between circulating cells and vessel wall, primarily endothelial cells. The initial reversible interaction between circulating leukocytes and endothelium is mediated by L-selectin (constitutively present on non-activated leukocytes) and endothelial E-selectin, while firm polymorphonuclear leukocyte (PMN) adhesion to the endothelial surface and their subsequent transendothelial migration require PMN expression of β_2-integrins, that increases after cell activation. Cell activation is stimulated by thrombin itself and by a local production of pro-inflammatory cytokines (tumor necrosis factor-α [TNF-α], interleukin-6, etc.) that establish a chemotactic gradient toward the vein wall.

In animal models of venous thrombosis (6,7), a partial inhibition of leukocyte infiltration in the vein wall has been obtained through immunization against TNF-α, β_2-integrins (CD18), intercellular adhesion molecule-1 (ICAM-1), and P-selectin. The latter, expressed by activated platelets and endothelium, seems to have an essential role in the interrelated processes of thrombosis and inflammation. Its inhibition, in animal venous thrombosis, induced a decrease of leukocyte infiltration in the vein wall and a reduced collagen synthesis (8,9).

Clinical studies suggest that in human DVT there is a similar involvement of inflammatory cells and mediators (10,11), and some adhesion molecules have been proposed as markers of thrombosis onset in patients at high risk (12).

We previously examined, in DVT subjects, the behavior of some parameters reflecting the func-

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tional activity of the PMNs (membrane fluidity and cytosolic Ca^{2+} content), before and after in vitro activation with 4-phorbol 12-myristate 13-acetate (PMA) and N-formyl-methionyl-leucyl-phenylalanine (fMLP), demonstrating a systemic PMN functional abnormality (13). To our knowledge, there are few studies concerning the PMN integrin profile in venous diseases (14).

Our group recently determined, in 19 subjects (11 men and 8 women; mean age 59.4 ± 15.8 years) with acute leg DVT, demonstrated by echo-color-Doppler, the PMN integrin pattern (CD11a, CD11b, CD11c) obtained by using a flow cytofluorimetry (FACscan, Becton Dickinson) before and after activation (prolonged for 15 minutes) with PMA and fMLP. Venous blood samples were drawn from the arm of each subject within 3 days from diagnosis.

In DVT subjects, at baseline, the phenotypical expression of CD11b was decreased and that of CD11c was increased in comparison with normal controls; no difference was found in CD11a expression (Table 1). PMN activation with PMA and fMLP led to a constant and significant increase of CD11a, CD11b, and CD11c in normal subjects while in DVT subjects only an increase of CD11b and CD11c was observed with both agents (Table 1).

While the baseline decrease of CD11b observed in DVT subjects might depend on its consumption, the baseline increase of CD11c might be correlated to a spontaneous PMN activation. In DVT subjects after activation only the expression of CD11b and CD11c showed a trend similar to that found in normal subjects while CD11a, constitutively expressed on the PMN surface, did not show any change.

Although these results are preliminary, we think that the PMN integrin expression in DVT deserves attention, considering that in the future the therapeutic use of specific antibodies directed toward PMN integrins, with the aim of attenuating inflammation in the vein wall, might favorably influence the clinical outcome of the disease and prevent the deterioration of vein function (4).

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TABLE 1. Means ± S.D. of the PMN Adhesion Molecules in Normal Subjects and in DVT Subjects, At Baseline and After Activation with PMA and fMLP

	Baseline	PMA	fMLP
Normal subjects			
CD11a	105.0 ± 12.3	130.2 ± 43.4‡	181.8 ± 75.5§
CD11b	158.2 ± 26.4	263.4 ± 57.9§	265.4 ± 29.9§
CD11c	63.9 ± 17.4	98.0 ± 13.7§	115.2 ± 32.8§
DVT			
CD11a	99.6 ± 42.8	118.4 ± 54.17	112.1 ± 58.6
CD11b	71.1 ± 41.3†	193.2 ± 84.7§	210.9 ± 99.1§
CD11c	83.7 ± 31.2*	103.8 ± 18.0‡	142.9 ± 43.0§

*$p < 0.05$.

†$p < 0.001$ vs normal subjects.

‡$p < 0.05$.

§$p < 0.001$ vs baseline.

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