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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 (CD11a, CD11b, CD11c) 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 cytometry. 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 CD11a did not show any change. These data might have therapeutic applications, especially with the aim of preventing post-thrombotic deterioration of vein function.

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

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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  $\beta_2$ -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- $\alpha$  [TNF- $\alpha$ ], 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- $\alpha$ ,  $\beta_2$ -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  $59.4 \pm 15.8$  years) with acute leg DVT, demonstrated by echo-color-Doppler, the PMN integrin pattern (CD11a, CD11b, CD11c) obtained by using a flow cytometry (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).

## REFERENCES

1. Stewart GJ. Neutrophils and deep venous thrombosis. *Haemostasis* 1993;23:127.
2. Whiston RJ, Hallett MB, Davies EV, et al. Inappropriate neutrophil activation in venous disease. *Br J Surg* 1994;81:695.
3. Downing LJ, Strieter RM, Kadell AM, et al. Neutrophils are the initial cell type identified in deep venous thrombosis induced vein wall inflammation. *ASAIO J* 1996; 42:M677.
4. Wakefield TW, Strieter RM, Prince MR, et al. Pathogenesis of venous thrombosis: A new insight. *Cardiovasc Surg* 1997;5:6.
5. Reiter M, Bucek RA, Koca N, et al. Deep vein thrombosis and systemic inflammatory response: A pilot trial. *Wien Klin Wochenschr* 2003;115:111.

**TABLE 1.** Means  $\pm$  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 $\pm$ 12.3	130.2 $\pm$ 43.4 <sup>‡</sup>	181.8 $\pm$ 75.5 <sup>§</sup>
CD11b	158.2 $\pm$ 26.4	263.4 $\pm$ 57.9 <sup>§</sup>	265.4 $\pm$ 29.9 <sup>§</sup>
CD11c	63.9 $\pm$ 17.4	98.0 $\pm$ 13.7 <sup>§</sup>	115.2 $\pm$ 32.8 <sup>§</sup>
DVT			
CD11a	99.6 $\pm$ 42.8	118.4 $\pm$ 54.17	112.1 $\pm$ 58.6
CD11b	71.1 $\pm$ 41.3 <sup>†</sup>	193.2 $\pm$ 84.7 <sup>§</sup>	210.9 $\pm$ 99.1 <sup>§</sup>
CD11c	83.7 $\pm$ 31.2 <sup>*</sup>	103.8 $\pm$ 18.0 <sup>‡</sup>	142.9 $\pm$ 43.0 <sup>§</sup>

\*p < 0.05.

†p < 0.001 vs normal subjects.

‡p < 0.05.

§p < 0.001 vs baseline.

6. Wakefield TW, Strieter RM, Wilke CA, et al. Venous thrombosis-associated inflammation and attenuation with neutralizing antibodies to cytokines and adhesion molecules. *Arterioscler Thromb Vasc Biol* 1995;15:258.
7. Wakefield TW, Strieter RM, Downing LJ, et al. P-selectin and TNF inhibition reduce venous thrombosis inflammation. *J Surg Res* 1996;64:26.
8. Myers DD Jr, Henke PK, Wroblewski SK, et al. P-selectin inhibition enhances thrombus resolution and decreases vein wall fibrosis in a rat model. *J Vasc Surg* 2002;36:928.
9. Thanaporn P, Myers DD, Wroblewski SK, et al. P-selectin inhibition decreases post-thrombotic vein wall fibrosis in a rat model. *Surgery* 2003;134:365.
10. Hogevoid HE, Lyberg T, Kahler H, et al. Expression of beta-2-integrins and L-selectin by leukocytes and changes in acute-phase reactants in total hip replacement surgery. *Eur Surg Res* 1996;28:190.
11. Quarmby J, Smith A, Collins M, et al. A model of in vivo human venous thrombosis that confirms changes in the release of specific soluble cell adhesion molecules in experimental venous thrombogenesis. *J Vasc Surg* 1999;30:139.
12. Smith A, Quarmby JW, Collins M, et al. Changes in the levels of soluble adhesion molecules and coagulation factors in patients with deep vein thrombosis. *Thromb Haemost* 1999;82:1593.
13. Caimi G, Canino B, Ferrara F, et al. Leukocyte rheology before and after chemotactic activation in some venous diseases. *Eur J Vasc Endovasc Surg* 1999;18:411.
14. Saharay M, Shields DA, Porter JB, et al. Leukocyte activity in the microcirculation of the leg in patients with chronic venous disease. *J Vasc Surg* 1997;26:265.