Evaluation of cell surface markers by flow cytometry
Avaliação dos marcadores da superfície celular por citometria de fluxo

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In this issue Vieira LM et al present an improved methodology to identify tissue factor (TF) expression in peripheral blood monocytes from healthy individuals. Cells were stimulated in vitro with LPS and analyzed by flow cytometry for the expression of surface markers such as CD14 (monocytes) and TF. The group concluded that age was not the major factor altering TF expression in monocytes from healthy individuals. They also suggest that further studies are needed to compare TF expression in monocytes from healthy individuals and patients in order to determine the role of TF in both physiologic and pathophysiologic conditions.

Tissue factor (TF), a 47-kDa transmembrane glycoprotein, is the cellular receptor for coagulation factor VII/VIIa. TF is the main initiator of the coagulation cascade and also plays an important role in angiogenesis and tumor metastasis. TF is expressed by a diversity of cells such as smooth muscle cells and fibroblasts (constitutively), monocytes, lymphocytes, granulocytes, platelets and endothelial cells (induced). TF induction in monocytes can occur by direct contact with other cells or engagement and cross-linking of the counter-receptors for several adhesion molecules. Inflammatory mediators such as interleukin 6 (IL-6), IL-8, monocyte-chemoattractant protein 1 (MCP-1), and tumor necrosis factor-A can also induce TF on monocytes. The endogenous inhibitor of TF, tissue factor pathway inhibitor-1 (TFPI-1), is also expressed on monocytes and may inhibit procoagulant activity of circulating leukocytes.

It is accepted that TF is the primary cellular initiator of the procoagulant activity (PCA) of monocytes. PCA of monocytes has implications in several pathologic conditions: in infections, elevated plasma tissue factor in patients with trauma and sepsis gives rise to thrombin generation, followed by intravascular coagulation. In inflammation, it is commonly accepted that a period of hypercoagulability after trauma or surgery is due to a TF increase in monocytes. In oncology, a procoagulant condition is frequent and enhanced TF expression in monocytes without stimulation or after LPS stimulation has been described. Benagiano et al. studied the antigen specificity and functional profile of in vivo activated T lymphocytes that infiltrate atherosclerotic plaques. They highlighted a possible role for activated T lymphocytes and their cytokines in driving the up-regulation of TF production by monocytes within atherosclerotic plaques, thus contributing to the thrombogenicity of lesions. In diabetes, microangiopathy is related to an increased expression of TF on monocytes.

TF antigen expression does not always correlate with PCA because other molecules, such as tissue factor protein inhibitor (TFPI), or the phospholipid composition of the membrane, can modulate the activity of the prothrombinase complex. Recalde et al. showed by in vitro assays that the number of monocytes with PCA forming fluorescent fibrin is roughly from a fifth to a third of the number of monocytes bearing TF antigen. This finding could indicate that only a fraction of monocytes with the TF antigen are functionally important while the remaining cells are inactive or have a level of PCA insufficient to form fibrin.

There are several disposable laboratory assays to identify TF antigen and PCA of monocytes from cells, urine, blood and plasma. Flow cytometry
(FACS) was born in Herzenberg’s group in 1968, and since then monoclonal antibodies (mAbs) and FACS emerge as truly complementary tools whose remarkable synergy created the basis for routine clinical diagnostic assays that now range from leukemia classification to monitoring CD4 T-cell loss as HIV disease progresses.12 Lindmark et al22 demonstrated by flow cytometry that activated platelets cause the rapid appearance of surface TF expression on monocytes without detectable mRNA formation. This indicates that TF may be stored intracellularly in these cells and can be exposed on the surface independent of de novo protein synthesis. On the other hand, Ott et al.24 evaluated patients with acute myocardial infarction (AMI) using flow cytometry, PCR, and ELISA techniques. They showed that the increased expression of TF (mRNA) and surface protein expression (flow cytometry) was a cause for the increased procoagulant activity of mononuclear cells in AMI. As mentioned above a marked increase of TF expression has been associated with thrombotic complications, and it is therefore of great importance to further study the regulation of monocyte TF. Flow cytometry assay, in addition to its diagnostic potential could be valuable for evaluating the humoral and cellular factors that regulate TF expression.

Bibliographic References


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