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Cytokines and Soluble Cell Adhesion Molecules

Possible Markers of Inflammatory Response in Atherosclerosis

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INTRODUCTION

We hypothesize that cell-cell communication disturbance is an important pathogenetic mechanism in all steps of atherogenesis. The pathogenesis in most pathologic processes may be considered from a new point of view: as the result of disturbances of "habitual" cell-cell communications.[1] These "habitual" communications are made possible by four classes of mediators (messengers): cell adhesion molecules, extracellular matrix, cytokines, and protooncogens.[2]

The main goal of our research was the comparative analysis of cytokines, soluble cell adhesion molecule (sCAM) secretion after blood coagulation and high shear rate for studying the involvement of hemostasis in pathophysiology of atherosclerosis.

MATERIALS AND METHODS

The investigation studied 29 atherosclerotic patients. The original research technique has been developed. We based our study on the fact that thrombus generation and flow changes are adequate stimuli for fast cell inflammatory response.[3] The initial cytokines level [IL-1a, IL-1b, IL-6, IL-8, IL-10 (Immunotech, Prague)] and soluble cell adhesion molecules [sP- and sE-selectins, sICAM-1, sVCAM-1 (R&D, Abington Oxon, U.K.)] were established and their changes in response to coagulation and fibrinolysis (incubation of blood clot 6 h at 37°C) and to standardized viscosimetric flow using rotational viscosimeter (shear rate 100 1/sec. 60 sec at 37°C, samples incubation 6 h) were measured.

RESULTS

Measurement of initial cytokines concentration showed a high level of proinflammatory cytokines IL-1 (mainly IL-1b) and IL-6 (TABLE 1). In contrast IL-1a, the shear stress probe, induced excess secretion of IL-1b, dramatically exceeding the ini-

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TABLE 1. Cytokines level, blood coagulation, and high shear stress in patients with atherosclerosis[a]

Cytokine (ng/ml)	Initial level	After shear stress (+ 6-h incubation)	After blood coagulation (+ 6-h incubation)
IL-1a	12.90 ± 6.72	62.09 ± 51.63*	51.68 ± 48.54*
IL-1b	55.28 ± 14.09	680.95 ± 589.97*	133.98 ± 132.68*,**
IL-6	65.16 ± 55.66	1610.81 ± 650.78*	847.55 ± 676.21*,**
IL-8	0	60.25 ± 42.75*	51.98 ± 44.46*
IL-10	0.81 ± 1.40	158.14 ± 112.62*	9.72 ± 4.78*,**
ET-1 (pg/ml)	15.88 ± 15.83	35.46 ± 11.49*	17.89 ± 15.16 **

[a]Data are mean ± SD.
*Statistically significant in comparison to initial group.
**Statistically significant differences between responses to coagulation and high shear rate (*t* test).

TABLE 2. Concentration of sCAM in response to coagulation and high shear stress in patients with atherosclerosis

SCAM (ng/ml)	Initial level	After shear stress (+ 6-h incubation)	After blood coagulation (+ 6-h incubation)
sP-selectin	168.16 ± 127.62	589.67 ± 301.50*	118.54 ± 72.97*,**
sE-selectin	90.70 ± 47.93	80.87 ± 67.93*	91.85 ± 63.39
sICAM-1	515.65 ± 124.49	633.22 ± 105.63*	527.38 ± 137.83
sVCAM-1	1039.74 ± 528.87	1087.12 ± 300.13	1263.29 ± 239.91

*Statistically significant differences in comparison to initial means.
**Statistically significant differences between the both probes.

tial level and response to coagulation. A sharp increase of IL-6 in response to both probes was detected. However, its increase in response to shear activation exceeded the postcoagulation level. A similar tendency was observed for IL-10. Simultaneously, the IL-8 concentration changed in a manner similar to IL-1a with both probes.

Interestingly, ET-1 level increased in both probes (TABLE 2). The initial sCAMs concentration was unexpectedly high in all patients with atherosclerosis. The sE-selectin level after rheologic probes was statistically different from the initial level. The sP-selectin level after blood coagulation probes strongly increased. In contrast, after rheologic probes its concentration decreased significantly.

To reveal interrelationships between cytokines and sCAMs secretion we carried out the correlation analysis. This demonstrated the pleiotropy and regulatory manner of the processes. Initial IL-1b levels were closely related to ET-1 in coagulation and shear activation probes ($r = 0.61$ and 0.69, respectively, $p < 0.05$). Secretion of IL-1a and IL-1b correlated to other cytokines; IL-1b and IL-6 were closely correlated to IL-8 in both probes ($r = 0.83$ and 0.63, $N = 29$, $p < 0.02$). The initial sICAM-1 downregulated IL-1b, IL-8, and IL-10 secretion after viscosimetric flow ($r = -0.76$, -0.74 and -0.71, $p < 0.01$) and initial sVCAM-1 downregulated IL-1a and IL-8 secretion. Therefore, their

levels after probes were closely related (for sVCAM-1 and IL-1a $r = -0.44$, $p < 0.05$ in rheologic probe and $r = -0.56$, $p < 0.02$ in coagulation probe). Similarly, the sICAM-1 decreased IL-10 secretion in coagulation probe. In contrast, the close negative correlation between initial sE-selectin concentrations and ET-1 in coagulation and viscosimetric probes was detected ($r = -0.9$ and $r = -0.89$, respectively, $p < 0.001$). At the same time, the initial sE-selectin level was downregulated by the IL-1a ($r = -0.39$, $p < 0.05$). The level of sP-selectin was positive correlated to IL-8 secretion after coagulation ($r = 0.46$, $p < 0.02$), to IL-1a level after shear stress ($r = 0.56$, $p < 0.01$) and initial IL-6 level ($r = 0.39$, $p < 0.05$).

CONCLUSIONS

(1) The increased levels of IL-1b, IL-6, sP- and sE-selectins, sICAM-1, and sVCAM-1, were found even without functional probes in patients with atherosclerosis. This serves as a convincing confirmation of the inflammatory nature of atherosclerosis.

(2) This is still hypothetical in that the pathophysiologic role of sCAM (in counterbalance to cell adhesion molecules expressed on the cell surface) consists of cytokine-secretion inhibition by possible blocking of the juxtacrine activating pathway. Therefore, obtained results suggest that the sCAM are cytokines themselves, because they have as their main features solubility, short-distant action, pleiotropy, and excessive synthesis.

(3) The VCAM-1 and ICAM-1 antagonists (monoclonal antibodies or non-antibodies origin) may be useful as antithrombotics and drugs and may be able to prevent ischemia-reperfusion injury.

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