Symposium on Recent Progress in Research of Atherosclerosis; from the View Point of Vascular Biology¹⁾

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1. Cytotoxic Effect of Activated Platelets on Endothelial Cells

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Since Ross proposed a response to the injury hypothesis in 1976 and in 1986, the injury of the endothelial cell and the following responses of various types of cells in blood and the vessel wall have been focussed upon as the initiating phenomena of atherosclerosis (1, 2).

The damage of endothelial cells due to hyperlipidemia, inflammation, hypertension, smoking, stress and other so-called risk factors causes plasma infiltration into the vessel wall with which various inflammatory substances, immunoreactive molecules, and/or even large particles of lipoprotein could be associated (3, 4).

Ross, who found platelet-derived growth factor (PDGF) in 1975, described in his first paper that platelet adhesion to damaged endothelial cells could be the first reaction by which the damage is accelerated and the release of PDGF induces the migration of smooth muscle cells from media into subendothelial layers and progression to atherosclerotic changes.

However, immunohistochemical studies using monoclonal antibodies against macrophages revealed very early adhesion of macrophages on damaged but seemingly normal endothelial cells and their invasion into the subendothelial layer of the arterial wall (3). Based on these observations, Ross proposed a second "response to injury hypothesis" in 1986 (2). He stressed

macrophages to be the initiating factor of vascular injury before platelets adhere to the vessel wall. He described that the entry of macrophages into the arterial wall exaggerate the damage of endothelial cells and platelet adhesion follows. Aggregated platelets on the damaged endothelial cells initiate thrombus formation, releasing various types of vasoactive substances such as thromboxane A₂, serotonin, histamine and PDGF. We demonstrated that the intraaortic flush of thromboxane A₂ induces typical vascular damage in both coronary and aortic wall of rabbits (4, 5).

Here, in vitro study we confirm that platelets once activated, primarily damage endothelial cells, and describe the changes which occur in the damaged endothelial cells.

Cytotoxicity of activated platelet

To study whether or not activated platelets cause damage to endothelial cells, we measured the percentage of [³H] adenine released from endothelial cells into culture media 3 hours after the addition of platelets activated by collagen. Cultured endothelial cells taken from bovine fetal aorta had been preincubated with [³H] adenine. Human platelets activated by collagen or sonicated platelets cause a significant release of [³H] adenine from endothelial cells which dose-dependently

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increases according to the number of activated platelets and to the co-cultured incubation time (6).

These data suggest that activated platelets could damage endothelial cells which, in turn, causes plasmal infiltration and/or infiltration of monocytes into vessel wall.

Regarding the changes in endothelial cells treated with activated platelets, the activated platelets induced Ca^{++} entry into endothelial cells.

The intracellular Ca^{++} concentration in cultured endothelial cells was measured by Fura II method. An immediate response of a Ca^{++} concentration increase and then gradual decrease were recorded in endothelial cells when cocultured with collagen-induced activated platelets. They maintained a higher level than before coculture.

The intracellular IP3 level also exhibited a significant increase when cocultured with activated platelets, suggesting Ca^{++} mobilization from the storage site. This increased concentration of intra-cellular Ca^{++} concentration could be a characteristic of injured endothelial cells causing contraction and induction of plasmal infiltration.

Thin-layer gas chromatography studies revealed that 80% of [^3H] adenosine is stored in endothelial cells as [^3H] ATP, when [^3H] adenosine is preincubated with endothelial cells. When cocultured with activated platelets, the intracellular percentage of ATP decreased statistically significantly and in turn ADP increased, and thus a statistically significant decrease of adenylate charge was recorded as compared with the changes in controls. This data suggests that a remarkable energy discharge occurs in injured endothelial cells (7).

The cAMP level in endothelial cells exhibited a statistically significant decrease when cocultured with activated platelets, as compared with that in controls. This change is also a characteristic feature of injured endothelial cells.

Recently we confirmed that this change could be due to the decreased adenylate cyclase activity. (not yet published).

Ultrascope examination demonstrated vacuolation of mitochondria in endothelial cells cocultured with activated platelets, suggesting a characteristic feature of damaged endothelial cells.

Decreased NO production in injured endothelial cells

Injured endothelial cells suffer not only impaired barrier functions but also disturbed physiological functions. Recently we devised a new method to measure the amount of NO produced in endothelial cells. Endothelial cells were cocultured with platelets which had been incorporated with [^3H] ATP. NO, which was produced and released from endothelial cells, stimulates the activity of guanylate cyclase in platelets which, in turn, results in the production of [^3H] cGMP. Thus, the amount of NO released from endothelial cells could be determined by measuring [^3H] cGMP (8).

Using this new device, we confirmed the decreased NO production of injured endothelial cells as compared with that in control cells.

This in vitro experiment may suggest impairment of the physiological functions of endothelial cells by activated plate-

lets.

Endothelial cell protection therapy

These findings offer insight into the prevention and treatment of atherosclerosis. Recent studies have revealed important roles of endothelial cells in the initiation of vascular injury and the progression of atherosclerosis. Thus a new viewpoint of the prevention and treatment of atherosclerosis is derived, endothelial cell protection therapy.

The correction of risk factors which has been clinically the traditional therapy for patients with atherosclerotic disorders means the protection of endothelial cells from the damage induced by risk factors. Our data reveals that antiplatelet therapy is also a form of endothelial cell protection.

In fact, the prevention of platelet activation demonstrated the protection of endothelial cells from activated platelet induced injury (9). Mixture of dbcAMP in the media of cultured endothelial cells showed a preventative effect against adenine release and/or the reduction of NO production by activated platelets. The protection of endothelial cells from various injuries will now be the main focus of therapy for atherosclerotic changes.

Summary

Modern science has made clear the important roles of endothelial cells in both physiological and biochemical functions of vessel wall and their impairment initiate the process of atherosclerotic changes.

Activated platelets also injure endothelial cells which were characterized with energy discharge, the decrease of cAMP content, the Ca^{++} entry, and dysfunction of NO production.

These data suggest the protection of endothelial cells from the injury could be the important protective factor from atherosclerosis. From this point of view, antiplatelet therapy is not only for antithrombotic but also for endothelial cell protective therapy.

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