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EDITORIAL COMMENT

Counterregulation Rules in Atherothrombosis*

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The last decades have witnessed immense progress in understanding the mechanistic links between risk factors for atherosclerosis and the pathophysiology of atherothrombosis. Processes such as inflammation and oxidation have joined the traditional canon of risk factors that includes hyperlipidemia, hypertension, and hypercoagulability. The more we learn, the more overlap we appreciate in the mechanisms that mediate the atherothrombotic risk of these factors and processes.

Shared mechanisms link risk factors for atherosclerosis. Until recently, for example, most viewed hypertension as a disorder of vasomotion and/or of salt and water balance. We now appreciate that hypertension goes hand in hand with oxidative stress. Most notably, angiotensin II potently regulates the oxidases that give rise to the pro-oxidant species superoxide anion (1). Hypertension also links to inflammation through innate and adaptive immunity. Angiotensin II can stimulate production of cytokines such as interleukin-6 and monocyte chemotactic protein-1, and may augment the recruitment of leukocytes to the artery wall (2,3). More recent observations show the participation of adaptive immunity in hypertension, as T cells modulate the development of hypertension in mice (4).

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Moreover, the links between thrombosis and fibrinolysis and inflammation have become ever more compelling (5). Thrombin, in addition to its pro-coagulant properties, can provoke the production and release of pro-inflammatory cytokines from a variety of cell types involved in atherothrombosis. Inflammatory mediators, ultimately through the mediation of interleukin-6, augment the hepatic production of fibrinogen. The acute-phase inflammatory response likewise boosts levels of the major endogenous inhibitor of fibrinolysis, plasminogen activator inhibitor-1. Thus, in both hypertension and thrombosis, inflammation provides a mechanism that explains the effects of the classical risk factors on the arterial wall.

Oxidation of lipoproteins links lipids with oxidative stress. Most schemata of atherogenesis posit a primary role of oxidation of low-density lipoprotein (LDL) (6). The wealth of pre-clinical evidence supporting this view, and the relative weakness of competing concepts of the initiation of atherogenesis, has rendered the oxidation hypothesis of atherogenesis ascendant. The extrapolation to human disease of this hypothesis, despite abundant pre-clinical information and clinical associations, remains incomplete (7). Yet, the previous discovery that lipoprotein(a) (Lp(a)) furnishes a "sink" for the oxidized phospholipid components of oxidized lipoproteins opened a new link between thrombosis and lipoproteins, as individuals with elevated Lp(a) have a heightened risk for atherothrombotic events (8).

Oxidized lipids and atherothrombosis: a new link. In this issue of the *Journal*, Leibundgut et al. (9) expand the links between constituents of oxidized lipoproteins and the mechanisms that underlie atherothrombosis. Lp(a) consists of an LDL particle containing an apolipoprotein B molecule, to which apolipoprotein(a) [apo(a)] has bound covalently. Apo(a) contains multiple kringle repeats, a structural motif shared by plasminogen. Indeed, clinical correlations have long linked high levels of Lp(a) with heightened risk of atherothrombotic events. The structural similarity between apo(a) and plasminogen suggested to the La Jolla investigators that, like Lp(a), plasminogen might also serve as a "sink" for oxidized phospholipids in the blood compartment. They present an elegant and broad series of experiments in support of this innovative hypothesis. They used a combination of biochemical and chemical techniques to demonstrate that oxidized phospholipids bind to epsilon amino groups of certain lysine residues in plasminogen by forming Schiff bases. This covalent modification appears peculiar to plasminogen, among the other kringle-containing participants in coagulation and fibrinolysis.

The La Jolla team further probed the functional consequences of this derivatization. They produced plasminogen free of oxidized phospholipid by treatment with phospholipase in vitro, and then examined the effect of both the oxidized phospholipid-derivatized plasminogen and of unmodified plasminogen on thrombolysis in vitro. Curiously, they found that removal of the oxidized phospholipid moiety actually *prolonged* the clot lysis time. That is, the oxidative modification actually seems to promote the thrombolytic potential of plasminogen. This in vitro assay, while commonly used, may not extrapolate directly to humans. Yet, if the modification of products of lipoprotein oxidation of plasminogen enhanced its thrombolytic ability in vivo, it would provide yet another example of an unexpected beneficial effect of a mechanism usually considered a

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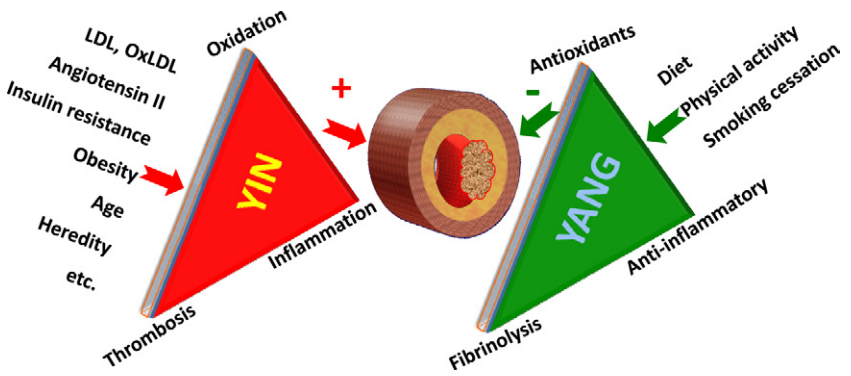

Figure 1 **Schematic Depiction of Some Counterregulatory Mechanisms That Operate in Atherothrombosis**

The **text on the left** lists risk factors for atherothrombosis; the processes denoted at the apices of the **red triangle** represent linked mechanisms that promote atherothrombosis. The **text on the right** lists behaviors that may mitigate the risk for atherothrombosis; the processes denoted at the apices of the **green triangle** represent some linked endogenous mechanisms that limit atherothrombotic risk. LDL = low-density lipoprotein; OxLDL = oxidized low-density lipoprotein.

culprit in atherothrombosis. Indeed, the more we learn, the more counterregulatory processes seem the rule rather than the exception in the control of atherothrombosis, as in other biological processes.

The La Jolla group translated their in vitro findings to some pilot observations on patient-derived blood specimens. In one of their key preliminary findings, they describe an increase in the plasminogen derivatized by oxidized phospholipid in the blood of individuals who have sustained an acute coronary event. This rise in modified plasminogen following acute coronary syndromes might augment fibrinolytic potential, if indeed the in vitro results presented pertain to humans. Perhaps the well-known early peak in recurrent events in the months following acute coronary syndromes would be worse, were it not for this unexpected action of plasminogen derivatized with oxidized phospholipids. Furthermore, as the authors point out, the novel mechanisms that they have uncovered might help to explain the increase in experimental atherogenesis previously reported in mice with loss of plasminogen function.

The yin and yang of atherothrombosis. The results reported here situate in a broader context of counterregulatory balances that apply to all major pathways implicated in atherothrombosis (Fig. 1). In adaptive immunity, for example, we now appreciate the tug of war between mediators elaborated by T lymphocytes of the T helper 1 (Th1) versus the T helper 2 (Th2) and regulatory T cell (T_{reg}) subpopulations (7). We also recognize that B1 lymphocytes can ameliorate atherogenesis, while B2 cells aggravate it. Humoral immunity, as exemplified by antioxidized LDL antibodies, may mitigate atherogenesis. In the province of innate immunity, we now appreciate the participation of pro-inflammatory populations of monocytes (those bearing high levels of the surface marker Ly6c in mice) and their less inflammatory counterparts, and of pro-inflammatory classically activated M1 macrophages and alternatively activated M2 macrophages in this disease (10).

We have long appreciated the balance between procoagulants and fibrinolytics that operate in thrombogenesis and regulate clot stability. In the lipoprotein field, we have long observed the particularly pro-atherogenic effects of certain fractions of LDL, and the counterbalancing effects of high levels of high-density lipoprotein in association with the risk for atherothrombosis. We understand now that even modification by phospholipids can exert a counterregulatory effect in this context.

The deeper we dig, the less support we garner for our initially simplistic assumptions about the culprits and protectors in atherothrombotic risk. For most "yins," a "yang" provides balance. Most pathophysiologic pathways have plusses and minuses in the ultimate generation of clinical events. The more we probe, the more complexity emerges in the web of links that intertwines our traditional rubrics of lipids, oxidation, thrombosis, and inflammation. The double-edged sword with which these processes cut, and the links that unite them, highlight the challenge we face in finding new targets for interventions that will tip the overall balance toward benefit. Our ever-growing recognition of these complexities indicates how our pre-clinical prejudices and experiments focused on one side of the scale may cause us to underestimate the countervailing influences, and thus lead us astray. Provocative observations such as those presented by the La Jolla group remind us to check our preconceptions at the door of experiment. We must resist the urge to compact the rich and multidimensional complexity of the biology of atherothrombosis into the narrow space of our personal preoccupations.

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