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COMMENTARY

MODULATION OF ENDOTHELIAL FUNCTION BY INTERLEUKIN-1

A NOVEL TARGET FOR PHARMACOLOGICAL INTERVENTION?

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Host defense and inflammation involve close interactions between immunocompetent cells and the vessel wall [1, 2]. Leukocytes must adhere to and pass through endothelial linings in order to extravasate and localize at inflammatory sites. In the presence of cell-mediated immune reactions, vasodilation and proliferation of capillary endothelial cells have been documented [3]. Soluble products released by lymphocytes and macrophages are potent regulators of various functions of vascular cells, such as proliferation, migration, production of colony-stimulating factors and expression of class II histocompatibility (Ia) antigens [4–8]. The symbiotic relationship between leukocytes and vascular cells may open new avenues for therapeutic intervention in diseases affecting the vessel wall. It is therefore of interest to establish which molecules and pathways are involved in the two-way interaction between endothelial cells and lymphomononuclear cells. Interferon-γ (IFN-γ) has been shown to regulate the expression of Ia antigens in various cells including vascular endothelium [8], but the nature of the products involved remains elusive. We have shown recently that lympokines regulate arachidonate metabolism in vascular cells and have identified interleukin 1 (IL-1) as the mediator of this effect [9, 10].

Here we summarize our current understanding of the regulation of vascular cell function, endothelial in particular, by molecules with IL-1 activity [11], emphasizing the complexity and ambivalence of the responses elicited by this lymphokine. Although this complexity, suggestive of a finely regulated interactive network, defies any simple therapeutic approach, the IL-1-dependent pathway of regulation of endothelial cell (EC) function could offer clues for new pharmacological strategies.

INDUCTION OF PROSTACYCLIN (PGI_2) SYNTHESIS BY IL-1

We and other groups [9, 10, 12, 13] have shown recently that mononuclear cell products induce PGI_2 synthesis in EC from human umbilical vein and aortic smooth muscle cells. The active mediator in the supernatant fractions was characterized as IL-1 [10]. PGI_2 is the major metabolite of arachidonic acid in vascular cells and is a very potent vasodilator and platelet-antiaggregating agent [14]. Modulation of its synthesis is a very important therapeutic target in thrombotic diseases and vasospasm. We observed that IL-1 required a relatively long interaction with vascular cells (> 6 hr) to increase PGI_2 synthesis and this stimulation lasted several hours [9, 10].

This pattern of stimulation distinguishes IL-1 from previously described stimulators of PGI_2 synthesis such as thrombin, arachidonic acid, or the calcium ionophore H23187 [15, 16]. These agents induce a burst-like stimulation of PGI_2 with a peak of activity in about 2–3 min, rapidly declining to control values in the next 5–10 min.

The differences among these agents in their time course of PGI_2 stimulation probably reflect different mechanisms of action. IL-1 stimulation of PGI_2 in vascular cells does, in fact, require protein synthesis, being completely blocked by cycloheximide or emetine [10]. In contrast, thrombin or ionophore A23187 activity is not modified by protein synthesis inhibitors. It has been shown that fast-acting PGI_2 stimuli induce phospholipase(s) activation, release of endogeneous arachidonic acid from membrane phospholipids and its further conversion to PGI_2 [17]. More complex and still undefined is the mechanism of action of a "slow-acting" agent such as IL-1. Hypothetically it might act through *de novo* synthesis of the enzymes of the PGI_2 synthesis pathway (as has been shown for other slow-acting stimuli on other cell types [18, 19]) or through a slow increase in intracellular calcium influx.

Whatever the mechanism of action, a lasting increase in PGI_2 synthesis and release by vascular cells could have important pathophysiological implications. This prostaglandin has a very short half-life in the circulation (a few minutes) [14], and its biological activity depends on "how much" and for "how long" it can be produced by the vascular cells. Sustained stimulation of PGI_2 synthesis (as by IL-1) might prevent the fast disappearance of its biological activity.

INDUCTION OF TISSUE PROCOAGULANT ACTIVITY (PCA) IN ENDOTHELIAL CELLS

In normal conditions, vascular EC act to inhibit coagulation and thrombosis. However, it has been

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reported that in some circumstances EC can be induced *in vitro* to express tissue factor PCA, thereby activating coagulation via the extrinsic pathway [20]. Bevilacqua *et al.* [21] showed that exposure of EC to IL-1 leads to a marked increase of their PCA. The activity was expressed on the surface of intact EC, suggesting that it would be accessible to the plasma clotting system *in vivo*. Tissue factor activity on the EC surface would lead to activation of factor VII, then of factors IX and X, propagating a procoagulant pathway on the cell surface to generate thrombin [22].

In contrast to PGI_2 stimulation, PCA elevation is transient and is followed by hyporesponsiveness to fresh IL-1 [21]. The time courses of PGI_2 and PCA expression are somehow different. When PCA starts to decline (after 8 hr of exposure with IL-1 [21]), PGI_2 begins to increase and remains high for at least 24 hr [9, 10]. This suggests that different activities may be expressed by EC at different times after IL-1 exposure.

IL-1-INDUCED INCREASE OF LEUKOCYTE ADHESION TO EC

Leukocyte adhesion to the endothelium is a key event in inflammation and in the pathogenesis of a series of vascular diseases. Polymorphonuclear leukocyte (PMN) adhesion to EC is a characteristic phenomenon at sites of acute inflammation, while monocyte interaction with EC has been observed in chronic inflammatory reactions [1, 23]. In addition, under conditions of tissue ischemia [24, 25] or hypercholesterolemia [26] in animals, PMNs and monocytes adhere to the EC lining of large vessels. This process is followed by egress of these cells from the vascular space, frequently associated with EC damage and death. This series of events has been indicated as an early step in the development of atheroschlerotic plaques [26, 27]. The mechanism of the leukocyte–EC interaction remains to be fully elucidated, and the importance of EC and leukocyte activation in this process is still a matter of debate.

Very recently, Bevilacqua *et al.* [28] reported that IL-1 acts directly and selectively on EC, markedly increasing the adhesion of PMNs, monocytes and the related lines HL60 and U937 to these cells. The effect of IL-1 on leukocyte adhesion was protein synthesis dependent and followed the same time course as PCA expression, peaking at 4 hr and declining to basal level within 12 hr. IL-1 activity appears to be mediated by *de novo* expression of cell associated structures, not released in the culture media [28]. Preliminary data suggest that these structures are proteins which are recognized by specific monoclonal antibodies able to substantially inhibit IL-1-induced leukocyte adhesion to EC. These membrane proteins appear to be expressed only after IL-1 stimulation of EC and not in unstimulated EC or in other vascular cell types. In addition, they specifically mediate leukocyte but not platelet or erythrocyte adhesion to these cells [28].

OTHER EFFECTS OF IL-1 ON THE ENDOTHELIUM

We have shown recently [29] that IL-1 increases "platelet activating factor" (PAF, 1-*O*-alkyl-2-acetyl-su-glycero-3-phosphocholine) production by EC. This phospholipid is a potent mediator of inflammation and cell-to-cell interactions (see Ref. 30 for review). It causes activation of platelets, neutrophils and monocytes and *in vivo* induces broncho-constriction and vasospasm and increases vascular permeability. IL-1 stimulation of EC induces a marked increase in cell-associated PAF with a small release of this material in the culture medium [29]. Like other activities mediated by IL-1, PAF synthesis by EC required a long interaction with the cells (> 2 hr), lasted for several hours, and was inhibited by protein synthesis inhibitors. In contrast, all the other stimuli of PAF production in EC are rapid, their effects last for only a few minutes, and they do not require protein synthesis to be active [31, 32].

The biological role of the IL-1 mediated EC expression of PAF remains to be characterized. The range of biologically active PAF concentrations is very close to those reached in EC after IL-1 stimulation [29]. In addition, the lasting effect of IL-1 could counteract the short biological half-life of this substance *in vivo*.

It has been observed* that IL-1 induced a marked increase of plasminogen activator inhibitor in EC. These cells can synthesize different forms of plasminogen activator(s) and inhibitor(s) [33].

Plasminogen activator(s) mediates conversion of plasminogen to plasmin which then is responsible for dissolution of thrombi and clearance of fibrin. The final fibrinolytic activity of EC is determined by the balance between the plasminogen activator(s) and inhibitor(s) activities they produce. An increase in plasminogen activator inhibitor such as that induced by IL-1 might reduce the ability of EC to lyse intravascular thrombi and therefore limit their antithrombotic properties.

Preliminary data also show that IL-1 can stimulate EC release of von Willebrand factor from preformed stores [34]. This protein is known to be elevated in plasma during the acute response phase in inflammatory reactions [35]. Direct stimulation by IL-1 of von Willebrand factor release by EC might be the mechanism of this phenomenon.

Also worth noting is a recent report that IL-1, acting synergistically with γ-interferon, induces dramatic changes in EC shape and cytoskeletal organization and stimulate the synthesis of highly structured extracellular matrix [36, 37]. IL-1 does not seem to induce an increase in EC proliferation [38].

IMPLICATIONS OF IL-1-INDUCED MODIFICATIONS OF EC FUNCTIONS

As summarized in Table 1, IL-1 induces a wide range of biological activities in EC. One important observation is that all of them have been seen in cultured EC *in vitro*, but the biological relevance of these modifications *in vivo* has still to be established. Several naturally occurring polypeptide inhibitors of IL-1 activity have been found in plasma and urine

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Table 1. IL-1 activities on endothelial cells

Stimulation of:
Prostacyclin
Tissue procoagulant activity
Plasminogen activator inhibitor
Platelet-activating factor
von Willebrand factor release
Changes in
Shape
Matrix composition
Increase in adhesion of:
Polymorphonuclear leukocytes
Monocytes
Related leukocyte cell lines

[39–41], suggesting that these substances may systemically counterbalance the biological activity of IL-1. However, EC are able to synthesize IL-1 after stimulation with LPS or thrombin [22]. This released IL-1 might then act "locally" by an autocrine mechanism on the endothelium and could possibly avoid inactivation by plasma inhibitors.

Another important consideration is that, in the past, endothelium has always been considered to play a passive role in hemostatic and inflammatory phenomena. Recent observations, however, suggest that EC participate actively in the responses of the inflammatory and coagulation system. Immunological processes *in vivo* are often associated with local or disseminated intravascular coagulation and decrease in fibrinolytic activity. The observed stimulation by IL-1 of EC-procoagulant activity and of plasminogen activator inhibitor suggests that EC may play an important role in these processes. In general, it was always assumed that EC was a "non-thrombogenic surface", not reactive to platelets, leukocytes or the coagulation system. The IL-1 interaction with EC appears dramatically to change these constitutive properties. After IL-1 stimulation, EC can promote coagulation, synthesize and expose PAF which is a potent activator of platelets and leukocytes, and become reactive to leukocyte adhesion. This might lead to increased reactivity of the vessel wall to thrombus formation. The subsequent increase in PGI_2 release by EC may later counterbalance this condition by inhibiting platelet aggregation and thrombus formation.

An important pharmacological target could therefore be the selective modulation of IL-1-induced EC modifications. IL-1 is a family of polypeptides, biochemically heterogeneous with some common biological properties [11]. At least two dissimilar gene products have been cloned with limited homology [42–44] (denominated α and β) [44]. These molecules, though biochemically different, share common activities in different systems. Preliminary results with EC, collected by our group, indicate that the two molecules elicit different responses. IL-1-α can stimulate PAF synthesis without affecting PGI_2 production, whereas IL-1-β stimulates PGI_2 but not PAF.

These preliminary results support the possibility of selective stimulation or inhibition of IL-1-induced EC activities.

IL-1 IN INTERACTIONS INVOLVING ENDOTHELIUM

Available information strongly suggests that IL-1 can dramatically influence a variety of vessel wall functions and that it plays a major role in leukocyte–EC cell interactions. A diagramatic representation of the possible role of IL-1 in the interactions between blood vessels and immunocompetent cells or tissues is presented in Fig. 1. Besides responding to IL-1, endothelial cells have also been reported to produce it [22]. Hence, one might speculate that IL-1 may well be the "endogenous" mediator responsible for at least some of the effects of agents such as endotoxin on vascular cells. The IL-1-producing capacity of EC, together with the expression of Ia antigens [8], could integrate these cells in immunological circuits, and EC do, in fact, have the potential to act as antigen presenting cells [45]. The immune response, once activated with the cooperation of vascular endothelium, would lead to release of IL-1 from monocytes, natural killer (NK) [46, 47] cells and B-cells [48] acting, in turn, on vessel wall function.

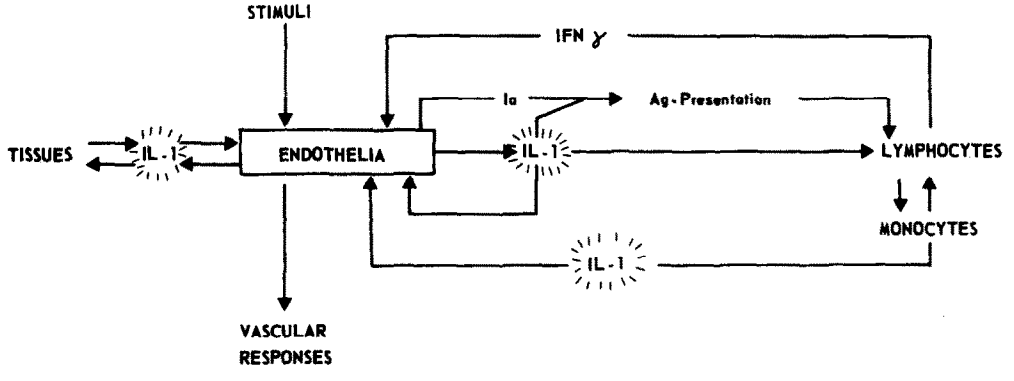

Fig. 1. Hypothetical central role of IL-1 in communication between endothelium and leucocytes or tissues. Endothelial cells can act as accessory cells (expression of Ia and production of IL-1) and initiate immune responses. These, in turn, via leukocyte IL-1 and interferon-γ, can influence EC functions. More in general, IL-1 could serve as a communication signal between vessel walls and extravascular tissues.

Cell types of various origins, including epithelial, mesenchymal and central nervous system cells, have the potential to secrete IL-1 [11, 35]. This mediator class is characterized by its pleiotropic effect, its targets including liver, central nervous system, synovia, bone and muscle [11, 35]. IL-1 may therefore serve as a common communication signal in the interactions between blood vessels and surrounding tissues (Fig. 1). In this view, IL-1 molecules would play a pivotal role in signaling between vessel walls and tissues.

CONCLUDING REMARKS

Published observations and work in progress summarized here show that molecules with IL-1 activity profoundly alter the functional status of EC, affecting the production of PGI_2, PAF, PCA and PAI. IL-1 has been shown to affect the expression of adhesive molecules on vascular endothelium [28]. Since vessel walls are involved in such a variety of pathological processes, the novel IL-1-mediated pathway of regulation of vascular function merits careful scrutiny. For instance, considerable efforts have been made to selectively alter arachidonate metabolism in platelets and vascular cells as an approach to the prevention of thrombosis; IL-1-induced synthesis of PGI_2 could provide a new target for pharmacological effects in this area.

Molecules with IL-1 activity are biochemically heterogeneous and different genes with limited homology have been shown to encode for molecules with IL-1 activity (see Ref. 11 for review). IL-1 has pleiotropic effects and elicits a range of responses in different systems [35].

Although previous attempts to relate different molecular species to the diverse effects of IL-1 have failed [49], preliminary analysis using recombinant IL-1 has revealed that different molecules with IL-1 activity elicit a range of distinct responses in vascular endothelium. These preliminary observations indicate that the IL-1 pathway of regulation of vascular function is amenable to selective modulation and should provide the impetus for pharmacological research in this area.

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REFERENCES

1. J. M. Harlan, *Blood* **65**, 513 (1985).
2. W. M. Baldwin III, *Immun. Today* **3**, 267 (1982).
3. P. J. Polverini, R. S. Cotran and M. M. Sbolley, *J. Immun.* **118**, 529 (1977).
4. B. M. Martin, M. A. Gimbrone, Jr., E. R. Unanue and R. S. Cotran, *J. Immun.* **126**, 1510 (1981).
5. P. J. Polverini, R. S. Cotran, M. A. Gimbrone, Jr. and E. R. Unanue, *Nature, Lond.* **269**, 804 (1977).
6. M. C. Cohen, P. T. Picciano, W. J. Douglas, T. Yoshida, D. L. Kreutzer and S. Cohen, *Science* **215**, 301 (1982).
7. G. C. Bagby, Jr., E. McCall, K. A. Bergstrom and D. Burger, *Blood* **62**, 663 (1983).
8. J. S. Pober, M. A. Gimbrone, Jr., R. S. Cotran, C. S. Reiss, S. J. Burakoff, W. Fiers and K. A. Ault, *J. exp. Med.* **157**, 1339 (1983).
9. E. Dejana, F. Breviario, G. Balconi, V. Rossi, G. Remuzzi, G. de Gaetano and A. Mantovani, *Blood* **64**, 1280 (1984).
10. V. Rossi, F. Breviario, P. Ghezzi, E. Dejana and A. Mantovani, *Science* **229**, 174 (1985).
11. C. A. Dinarello, *J. clin. Immun.* **5**, 287 (1985).
12. C. R. Albrightson, N. L. Baenziger and P. Needleman, *J. Immun.* **135**, 1872 (1985).
13. K. S. Callahan, W. J. Wallis and J. M. Harlan, *Thromb. Haemostas.* **54**, 110 (1985).
14. S. Moncada and J. R. Vane, *Pharmac. Rev.* **30**, 293 (1978).
15. B. B. Weksler, C. W. Ley and E. A. Jaffe, *J. clin. Invest.* **62**, 923 (1978).
16. E. Dejana, G. Balconi, C. De Castellarnau, B. Barbieri, M. Vergara-Dauden and G. de Gaetano, *Biochim. biophys. Acta* **750**, 261 (1983).
17. R. F. Irvine, *Biochem. J.* **204**, 3 (1982).
18. P. J. Whiteley and P. Needleman, *J. clin. Invest.* **74**, 2249 (1984).
19. A. J. R. Habenicht, M. Goerig, J. Grulich, D. Rothe, R. Gronwald, U. Loth, G. Schettler, B. Kommerell and R. Ross, *J. clin. Invest.* **75**, 1381 (1985).
20. M. Colucci, G. Balconi, R. Lorenzet, A. Pietra, D. Locati, M. B. Donati and N. Semeraro, *J. clin. Invest.* **71**, 1893 (1983).
21. M. P. Bevilacqua, J. S. Pober, G. R. Majeau, R. S. Cotran and M. A. Gimbrone, Jr., *J. exp. Med.* **160**, 618 (1984).
22. D. M. Stern, I. Bank, P. P. Nawroth, J. Cassimeris, W. Kisiel, J. W. Fenton II, C. Dinarello, L. Chess and E. A. Jaffe, *J. exp. Med.* **162**, 1223 (1985).
23. P. C. Wilkinson and J. M. Lackie, *Curr. Topics Path.* **68**, 47 (1979).
24. K. M. Mullane, N. Read, J. A. Salmon and S. Moncada, *J. Pharmac. exp. Ther.* **228**, 510 (1984).
25. R. G. Schaub, C. A. Simmons, M. H. Koets, P. J. Romano II and G. J. Stewart, *Lab. Invest.* **51**, 218 (1984).
26. A. Faggiotto, R. Ross and L. Harker, *Arteriosclerosis* **4**, 323 (1984).
27. R. G. Gerrity, *Am. J. Path.* **103**, 181 (1981).
28. M. P. Bevilacqua, J. S. Pober, M. E. Wheeler, R. S. Cotran and M. A. Gimbrone, Jr., *J. clin. Invest.* **76**, 2003 (1985).
29. F. Bussolino, F. Breviario, C. Tetta, M. Aglietta, A. Mantovani and E. Dejana, *J. clin. Invest.* **77**, 2027 (1986).
30. F. Snyder, *Med. Res. Rev.* **5**, 107 (1985).
31. G. Camussi, M. Aglietta, F. Malavasi, C. Tetta, W. Piacibello, F. Sanavio and F. Bussolino, *J. Immun.* **131**, 2397 (1983).
32. S. M. Prescott, G. A. Zimmerman and T. M. McIntyre, *Proc. natn. Acad. Sci. U.S.A.* **81**, 3534 (1984).
33. E. G. Levin, *Proc. natn. Acad. Sci. U.S.A.* **80**, 6804 (1983).
34. A. E. Schorer, C. F. Moldow and M. E. Rick, *Blood* **66** (Suppl. 1), 358a (1985).
35. C. A. Dinarello, *New Engl. J. Med.* **311**, 1413 (1984).
36. R. Montesano, L. Orci and P. Vassalli, *J. cell. Physiol.* **122**, 424 (1985).
37. R. Montesano, A. Mossaz, J-E. Ryser, L. Orci and P. Vassalli, *J. Cell Biol.* **99**, 1706 (1984).
38. P. Libby, D. J. Wyler, M. W. Janicka and C. A. Dinarello, *Arteriosclerosis* **5**, 186 (1985).
39. J. G. Cannon and C. A. Dinarello, *Science* **227**, 1247 (1985).

40. Z. Liao, R. S. Grimshaw and D. L. Rosenstreich, *J. exp. Med.* **159**, 126 (1984).
41. J-M. Dayer and J-F. Balavoine, in *Physiologic, Metabolic and Immunologic Actions of Interleukin-1* (Eds. M. Kluger, J. J. Oppenheim and M. C. Powand). Alan R. Liss, New York, in press.
42. P. T. Lomedico, U. Gubler, C. P. Hellmann, M. Dukovich, J. G. Giri, Y-C. E. Pan, K. Collier, R. Semionow, A. O. Chua and S. B. Mizel, *Nature, Lond.* **312**, 458 (1984).
43. P. E. Auron, A. C. Werb, L. J. Rosenwasser, S. F. Mucci, A. Rich, S. M. Wolff and C. A. Dinarello, *Proc. natn. Acad. Sci. U.S.A.* **81**, 7907 (1984).
44. C. J. March, B. Mosley, A. Larsen, D. P. Cerretti, G. Braedt, V. Price, S. Gillis, C. S. Henney, S. R. Kronheim, K. Grabstein, P. J. Conlon, T. P. Hopp and D. Cosman, *Nature, Lond.* **315**, 641 (1985).
45. H. Hirschberg, O. J. Bergh and E. Thorsby, *J. exp. Med.* **152**, 249s (1980).
46. G. Scala, P. Allavena, J. Y. Djeu, T. Kasahara, J. R. Ortaldo, R. B. Herberman and J. J. Oppenheim, *Nature, Lond.* **309**, 56 (1984).
47. A. Rambaldi, G. Alessio, V. Rossi, M. B. Donati, N. Semeraro and A. Mantovani, *Scand. J. Immun.* **22**, 363 (1985).
48. G. Scala, Y. D. Kuang, R. E. Hall, A. V. Muchmore and J. J. Oppenheim, *J. exp. Med.* **159**, 1637 (1984).
49. D. D. Wood, E. K. Bayne, M. B. Goldring, M. Gowen, D. Hamerman, J. L. Humes, E. J. Ihrie, P. E. Lipsky and M-J. Staruch, *J. Immun.* **134**, 895 (1985).