Modulation of Endothelial Cell Functions by Different Molecular Species of Interleukin 1


Different molecular species of interleukin 1 (IL-1) were examined for the spectrum of responses elicited in human endothelial cells (HEC), including synthesis of prostacyclin (PGI2), tissue-type plasminogen activator (TPA), platelet activating factor (PAF), and plasminogen activator inhibitor (PPI). The IL-1 preparation utilized for the present study included a natural, partially purified IL-1, a preparation purified to homogeneity with extensive homology with the derived amino acid IL-1β (p17) sequence designated “22k factor.” Murine recombinant IL-1α, human recombinant IL-1α (p15) and β (p17). Natural, partially purified IL-1, a mixture of α and β species, induced the entire spectrum of responses in HEC. Production of PAF was elicited by all forms of IL-1 tested. PGI2 and PAF were elicited by “22k factor” and by human recombinant IL-1β and α but not by murine recombinant IL-1α. PAF synthesis was stimulated by murine and human recombinant IL-1α but not by human recombinant IL-1β and 22k factor. Thus the available different molecular forms of IL-1 elicit largely but not completely overlapping patterns of responses in HEC. The IL-1 pathway of regulation of HEC functions might provide a basis for novel strategies in therapeutically oriented research on vessel wall disorders.

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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. Specifically, the activated monocyte product interleukin 1 (IL-1) has been reported to induce multiple responses in human endothelial cells (HEC). Others and our group have recently shown that this monokine induces prostacyclin (PGI2) and platelet activating factor (PAF) synthesis in endothelial cells (EC) from human umbilical vein. Bevilacqua et al. established that IL-1 stimulates expression of a tissue factor-like procoagulant activity (PCA); increase in plasminogen activator inhibitor (PA-I) production by HEC after IL-1 was also observed.

IL-1 is a family of polypeptides, biochemically heterogeneous but with some common biological properties. At least two dissimilar human gene products have been cloned with limited homology, denominated α(p15) or β(p17). One murine IL-1 molecular species was also cloned and appeared to be closely related to the corresponding human α IL-1 form, with 62% matching and 93% conserved sequences. IL-1 has pleiotropic effects, its targets including immunocompetent cells, liver, central nervous system (CNS), synovia, bone, and muscle. The multiplicity of the biological activities attributed to IL-1 and the heterogeneity of the components of the IL-1 family raise the question of whether distinct molecules have distinct biological roles. Attempts to relate different molecular species to the diverse effects of IL-1 on a series of tissues and cellular targets have largely failed. Wood and co-workers were able to dissociate the effects of human IL-1α vs IL-1β on the stimulation of PGE2 production by porcine synovocytes. However, intraspecies differences on the activities of the different molecules of IL-1 have not been described. Here we tested the activity of the series of IL-1 molecules so far available, including recombinant preparations, on HEC. We report that the different molecules elicit a largely but not completely overlapping spectrum of responses on these cells.

Materials and Methods

IL-1 preparations. IL-1 preparations utilized for the present study included:

1. A commercially available purified preparation obtained from Staphylococcus albus-stimulated human monocytes and purified by affinity chromatography employing immobilized rabbit antibodies and gel filtration (UltraPure IL-1, Genzyme, Boston); we will refer to this material as human natural IL-1 (hIL-1).
2. A pure preparation from mitogen-stimulated human mononuclear cells, purified to homogeneity; the N-terminal amino acid sequence of this material, referred to as “22k factor,” has been described and shows extensive homology with the derived amino acid IL-1β sequence.
3. Murine recombinant IL-1 (lot 14430-21) from the P388 D1 cell line was a kind gift of Dr LoCMDAC (Roche Institute, New York). Since this clone shows extensive homology with the human IL-1 gene designated α, we will refer to this material as murine recombinant IL-1α (mrIL-1α). This preparation had a specific activity of 105.

From the Istituto di Ricerche Farmacologiche “Mario Negri,” Milano, Italy; the Cattedra di Chimica e Propedeutica Biochimica, Università di Torino, Torino, Italy; Tufts University, School of Medicine, Boston, and the Laboratory of Virology, The Rega Institute, Faculty of Medicine, University of Leuven, B-3000 Leuven.

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Address reprint requests to Dr Elisabetta Dejana, Istituto di Ricerche Farmacologiche “Mario Negri,” Via Eritrea 62, 20157 Milano, Italy.

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described below, and contained 0.23 U/μg.

4. Human recombinant IL 1 (pIL7) expressed in Escherichia coli from amino acid 71-269 homologous to a subsequently isolated clone designated α. We will refer to this as human recombinant IL 1α (hrIL 1α). The specific activity of this preparations was 2 x 10^4 U/μg.

5. Human recombinant IL 1α (hrIL 1α) was kindly supplied by Dr Gillis (Immunex, Seattle). The specific activity was 10^5 U/μg.

IL 1 preparations contained no detectable endotoxin as assessed by the negative Limulus assay on samples containing 100 U/mL of IL 1. IL 1 activity was evaluated in a costimulatory assay with C3H/HeJ thymocytes as responding cells and phytohemagglutinin (0.5 μg/mL) as stimulus. A partially purified IL 1 preparation was assigned a value of 1,000 U/mL and used as standard in each assay. Results from triplicate cultures were plotted on a linear regression against the standard preparation and expressed as half maximal units of IL 1 for the various preparations were calculated relative to an IL 1 preparation utilized in one of the collaborating laboratories (AE and AM). Subsequent comparisons were made on the basis of the concentrations of these agents, which were equally active in the mouse thymocyte costimulatory assay. It has to be mentioned that as evaluated from this test the specific activity of hrIL 1α was 10^5 U/μg protein; of hrIL 1α, 10^4 U/μg; and of hrIL 1β, 2 x 10^4 U/μg. Thus we had to use 50 times more hrIL 1β to obtain concentration equally active on the thymocyte costimulatory assay. We have previously reported that hrIL 1 increased PGI2 synthesis in vascular cells at concentrations ranging from 1 to 10 U/mL. These observations were now extended to homogenous natural human IL 1 (“22K factor”), hrIL 1β, and hrIL 1α (Fig 1). In contrast, hrIL 1α at concentrations as high as 100 units/mL (corresponding to 1 ng/mL) did not elicit production of PGI2 in HEC, as shown by the experiment presented in Fig 1.

A similar pattern was observed when PCA was examined (Fig 2). The homogeneous “22K factor,” hrIL 1β, and hrIL 1α stimulated synthesis of PCA as efficiently as hrIL 1α, whereas hrIL 1α was ineffective at concentrations as high as 100 U/mL.

We have recently observed that hrIL 1 and mrIL 1α elicited PAF production in HEC. As shown in Fig 3, besides hrIL 1 and mrIL 1α, hrIL 1α was also an effective inducer of cell-associated PAF in HEC, whereas the “22K factor” had no such activity up to 60 U/mL and hrIL 1β showed statistically significant activity only at concentrations as high as 100 U/mL (corresponding to 500 ng/mL). HnIL 1, mrIL 1α, “22K factor” stimulated production of PA-I in HEC in a comparable way (Fig 4). Since mrIL 1α failed to induce PGI2 production and PCA in HEC (see above), it was important to assess whether this preparation contained inhibitors of IL 1-elicted response.

Therefore we incubated HEC for four hours at 37°C with 50 U/mL of MrIL 1α mixed with 5 U/mL of 22K factor and measured the PCA produced by these cells. When the cells

![Fig 1](https://example.com/fig1.png)

**Fig 1.** Effect of hnlL 1, mrlL 1α, hrIL 1α, “22K factor,” and hrIL 1β on 6-keto-PGF1α production by HEC. The numbers below the columns indicate the U/mL of the IL 1 forms, as calculated from the thymocyte costimulatory assay as described in the text. The black column reports the control mean value of 6-keto-PGF1α produced in the absence of IL 1. The values are means ± SEM of four separate experiments. In each experiment all the IL 1 molecular species were tested on the same cell culture. *P < 0.01 v control values by Dunnett’s test.
were stimulated by the "22K factor" alone, the PCA produced was 22.8 ± 3 U/10^6 cells; mrIL 1α induced 1.4 ± 0.1 U/10^6 cells and the combination of the two agents 18.9 ± 3 U/10^6 cells (the values are means ± SEM of four replicates, and the results were similar in one additional experiment). It is concluded that the mrIL 1α preparation does not interfere with "22K factor" activity on PCA production. In addition, since mrIL 1α contained 0.23 μg of guanidine per unit, HEC were incubated with up to 12 μg/mL guanidine alone or in combination with 10 U/mL of hnIL 1; no effects on HEC production of PG12, PAF, PCA, or PA-1 were observed.

**DISCUSSION**

The first aim of the present study was to assess whether the modulation of endothelial cell functions by IL 1, which was described by using crude or natural partially purified IL 1, was also effected by purified recombinant molecules. Human natural IL 1 elicited the entire spectrum of HEC responses examined in the present study, thus confirming previous observations.8,11,13 This preparation contains the different species, α (p15) and β (p17), of IL 1.21 In contrast, when recombinant IL 1 molecules (or natural material purified to homogeneity in the case of "22K factor") were examined, differential responses were elicited by the different molecular species examined. All the IL 1s tested induced synthesis of PA-1 in HEC. Murine recombinant IL 1α stimulated the synthesis of PAF in HEC but was consistently inactive in inducing PG12 and PCA. Murine recombinant IL 1α is highly homologous to the human α species.17,19 However, in contrast to the murine α species, human recombinant IL 1α stimulated PCA and PG12 synthesis (in the limited number of assays in which we could test this particular IL 1).

We examined the effects on HEC of two preparations of IL 1β, a natural purified material ("22K factor") and a recombinant preparation. Both IL 1β preparations elicited the entire spectrum of HEC responses examined with the possible exception of PAF: induction of PAF was only observed with recombinant IL 1β at a concentration of 100 U/mL, corresponding to 500 ng/mL. Under the same conditions murine recombinant IL 1α induced PAF at 1 U/mL corresponding to 0.01 ng/mL as previously reported.13 Thus IL 1β seems to be 100 (on a U/mL basis) or 50,000 (on an ng/mL basis) times less effective than the α species.

The apparently different response of HEC to α and β IL 1 species for PAF production is the first evidence of an intraspecies functional dissociation between these two IL 1 forms in the same cell type. The observation that hnIL 1 stimulates PAF synthesis excludes the possibility of a peculiar effect related to the recombinant nature of the two α species. Conversely the lack of effect of the β forms of IL 1 does not seem to be simply related to their lower activity on HEC, as demonstrated by their effectiveness on the other HEC functions tested. Thus the data presented here indicate that different molecular species of IL 1 elicit largely but not completely overlapping patterns of responses in HEC.

The in vivo response to partially purified IL 1 assessed by fever, neutrophilia, changes in plasma divalent cations, or fibrinogen concentrations, differs in various animal species29,30; furthermore, biochemically distinct IL 1 prepara-
tions can be differently affected by these species-related restrictions. It has not been elucidated whether these differences reflect the presence of target organ-specific inhibitors in the preparations, differences in the in vivo bioavailability, or different cell susceptibility. Through the analysis of the activities of four distinct preparations of human IL 1 separated by size or charge, Wood and co-workers observed no clear-cut differences among them, with the exception that the stimulation of PGE2 production by pig synoviocytes was only induced by the PI 5.5, 15,000 Kd mol wt IL 1 preparation. These authors considered the possibility of the presence of inhibitors in the inactive preparations. However, the slight inhibitory activity observed in some of the IL 1 preparations was not strong enough to completely account for their apparent inactivity.

In this paper the peculiar spectrum of responses elicited by mIL 1a, in respect to the hrIL 1a, might be related to the murine origin of this clone and suggests that slight differences in the molecular structure may greatly affect functional properties.

In conclusion, the results presented here show that molecules with IL 1 activity have some selectivity in effects on HEC functions and emphasize the potential complexity of the fine regulation of leukocyte–vessel wall interactions. The HEC activities considered in this study, i.e., PG12, PCA, PAF and PA-I, might be important in the modulation of vascular tone and thrombus formation. In particular, PAF produced by IL 1-stimulated HEC is mostly cell associated and appears to be appropriately located to interact with adjacent leukocytes and eventually platelets and to promote their activation. The IL 1 pathway of regulation of these HEC functions could thus provide a basis for novel strategies in therapeutically oriented research on vessel wall disorders.

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REFERENCES

factor is related to endogenous pyrogen and interleukin-1. Nature 314:266, 1985


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E Dejana, F Breviario, A Erroi, F Bussolino, L Mussoni, M Gramse, G Pintucci, B Casali, CA Dinarello and J Van Damme