NEFROLOGÍA. Vol. XX. Suplemento 1. 2000

Transcriptional regulation of the human INOS gene by IL-1β in endothelial cells

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The inducible isoform of the nitric oxide synthase (iNOS) is expressed in many tissues, including the vascular endothelium, after induction by various inflammatory stimuli. In view of the direct contact of endothelial cells with circulating blood, production of large amounts of nitric oxide by endothelial iNOS might occur under various clinical conditions characterized by entry of microorganisms and their products into the blood stream or a surge of cytokines, including sepsis, dialysis, cytokine therapy, graft rejection, or regional inflammation. A host of pathophysiologic effects might emanate from the resultant release of nitric oxide, such as profound vasodilation and hypotension, aggravation of inflammation, cytotoxicity, antimicrobial defense, and inhibition of adhesion and aggregation of leukocytes and platelets. The bulk of the work on the transcriptional regulation of the iNOS gene has involved the murine gene in macrophages and vascular smooth muscle (VSM) cells. Corresponding work in the rat iNOS gene has largely focused on macrophages, VSM cells, and mesangial cells. There is currently no information on the transcriptional regulation of the human iNOS gene (or that of other species) in endothelial cells. Previously, we demonstrated that a 1.1-kb human iNOS promoter/luciferase construct was induced strongly by LPS and only modestly by IL-1β in macrophages, but not in VSM cells. In this study, we examined the transcriptional regulation of IL-1β-induced expression of the human iNOS gene in rat pulmonary vein endothelial cells (PVEC). Transfection experiments demonstrated that this human iNOS promoter was strongly inducible by IL-1β (6-fold on average) in PVEC. Mutational analysis suggested that the putative *cis*-regulatory elements responsible for this induction are localized within the -205/+88 bp promoter fragment. Further mutational analysis and cotransfection experiments with expression vectors for different isoforms of NF-κB, IκB, and Iκb kinase (IKK) suggested that the NF-κB site located within this region (at position -115 to -106 bp) is important, but not sufficient, for full induction of the human iNOS promoter by IL-1β. We then identified four C/EBP binding sites within the -205/+88 bp region. Mutational analysis demonstrated that these C/EBP sites along with the NF-κB site are largely responsible for the induction of the human iNOS promoter by IL-1β. Overexpression of C/EBP isoforms revealed that C/EBP sites participate in the induction of the human iNOS promoter by IL-1β. Cotransfection with expression vector for liver-enriched transcriptional activator protein (LAP) activated the human iNOS promoter to the same level as IL-1β treatment, whereas expression vector for liver-enriched transcriptional inhibitory protein (LIP) resulted in strong downregulation of basal and IL-1β-induced promoter activity. Western blot analysis showed that C/EBPβ (LAP and LIP isoforms) was constitutively present in PVEC and was induced (~2-fold) by IL-1β. On the other hand, C/EBPδ was not constitutively expressed but was strongly induced by IL-1β. No other C/EBP isoforms were detectable. However, EMSA analysis demonstrated that in untreated cells C/EBPβ, although present by Western blot, did not bind to DNA. Supershift assays showed that in IL-1β-treated cells both C/EBPβ and C/EBPδ, but not C/EBPα or C/EBPε, participated in complex formation.

We conclude that the *cis*-regulatory elements responsible for the transcriptional activation of the human iNOS gene by IL-1β in endothelial cells are localized in the 5′-flanking region downstream of -0,2 kb. Further, our data demonstrate that such induction depends on transcription factors NF-κB, C/EBPβ, and C/EBPδ. These data provide new insight into the themes of complexity and tissue specificity that characterize the transcriptional regulation of the human iNOS gene.