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IFN-γ Inhibits Double-Stranded RNA-Induced E-Selectin Expression in Human Endothelial Cells[1]

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IFN-γ plays a role in immune regulatory functions as well as in viral defense. We show in this study that IFN-γ treatment down-regulates the induction by a viral mimetic, polyinosinic-polycytidylic acid (poly(I:C)), of the endothelial cell-specific leukocyte adhesion protein, E-selectin. The inhibitory effect of IFN-γ on poly(I:C)-induced E-selectin was concentration and time dependent and was specific for dsRNA, in that the induction of E-selectin by TNF-α, IL-1β, thrombin, or LPS was not inhibited significantly by this pretreatment. IFN-γ pretreatment reduced poly(I:C)-induced E-selectin mRNA in a protein synthesis-independent manner. Poly(I:C)-induced E-selectin mRNA $t_{1/2}$ was reduced slightly by IFN-γ treatment, while the message for VCAM-1 was stabilized. Transient transfection of endothelial cells with an E-selectin promoter-driven reporter gene construct revealed that poly(I:C) stimulation of E-selectin promoter activity was decreased significantly by IFN-γ pretreatment. Poly(I:C)-induced nuclear factor-κB activation following IFN-γ pretreatment was unaffected, as shown by electrophoretic mobility shift analysis. These results indicate a novel role for IFN-γ in the regulation of E-selectin gene expression in response to dsRNA by a transcriptional mechanism independent of nuclear factor-κB, as well as by a minor decrease in message stability. ***The Journal of Immunology,*** **1997, 159: 3989–3994.**

Endothelial cell (EC)[3] activation and subsequent expression of leukocyte adhesion molecules are initial events in multiple physiologic and pathologic processes. An initial step in the development of an inflammatory response involves the adherence of blood-borne leukocytes to activated EC (reviewed in Refs. 1 and 2), which is mediated through the expression of leukocyte adhesion proteins on the EC surface. Three specific proteins that mediate leukocyte adhesion to vascular endothelium are E-selectin, VCAM-1, and ICAM-1 (3). These and other adhesion proteins are induced in EC in response to certain inflammatory response mediators, including IL-1, TNF-α, LPS, IFN-γ, and thrombin (4–6). It also has been shown that viral dsRNA induces EC adhesion protein expression and leukocyte adhesion to EC (7–9).

dsRNA is an intermediate within the replication cycle for RNA viruses and some DNA viruses, and has been demonstrated in many viral infected cells (10–12). A viral cause for atherosclerosis has been investigated for many years (7, 12–16). Etingin et al. have reported that virus-induced vascular injury initiates events leading to inflammation, thrombosis, and atherosclerosis (14). Intracellular dsRNA from virus infection of a cell induces gene expression that can be mimicked by exogenous dsRNA (17, 18). Polyinosinic-polycytidylic acid (poly(I:C)) is a synthetic dsRNA that is often used to simulate the viral infected state when added exogenously to cells. Poly(I:C) has been reported recently to induce leukocyte adhesion to EC through the expression of the EC adhesion proteins E-selectin, VCAM-1, and ICAM-1 (19–22).

IFN-γ is a pleiotropic cytokine that is involved in viral defense, cell growth and differentiation, and immune response (reviewed in Refs. 23 and 24). It is produced primarily by lymphocytes; however, IFN-γ receptors are expressed on nearly every cell type. In addition to its antiviral effects, IFN-γ has been shown to modulate the expression of certain adhesion proteins (either alone or in conjunction with cytokine induction) in EC (25–32). It was for these reasons that we thought to investigate whether the induction of leukocyte adhesion molecules in response to a synthetic dsRNA, poly(I:C), was modulated by IFN-γ in human vascular EC.

Materials and Methods

Materials

The mAbs against E-selectin, VCAM-1, ICAM-1, and rIFN-γ ($\geq 1 \times 10^7$ U/mg) were purchased from Genzyme Corp. (Cambridge, MA). The cDNA probes for E-selectin and VCAM-1 were generous gifts from Dr. Walter Newman of Otsuka Pharmaceutical Co. (Rockville, MD). Heparin and all media components, as well as other reagents not specifically mentioned, were purchased from Sigma Chemical Co. (St. Louis, MO). Poly(I:C) was purchased from Pharmacia Biotech (Piscataway, NJ) and freshly prepared for each experiment according to the recommendations of the manufacturer. ^{125}I-labeled streptavidin was purchased from Amersham Corp. (Arlington Heights, IL). [^{32}P]dCTP and [^{51}Cr]sodium chromate were the products of DuPont NEN (Boston, MA). All tissue culture plasticware was purchased from Costar Corp. (Cambridge, MA). Biotin-conjugated, affinity-purified F(ab′)$_2$ fragment goat anti-mouse IgG$^+$ IgM (H + L) was obtained from Jackson ImmunoResearch Laboratories (West Grove, PA). Nytran membranes used for Northern analysis were purchased from Schleicher & Schuell (Keene, NH). All reagents used in cell culture were tested for endotoxin contamination using the *Limulus* endotoxin detection assay kit from BioWhittaker (Walkersville, MD). The lack of significant endotoxin contamination was confirmed by measuring the experimental end points in the presence or absence of polymyxin B. The observations reported were not sensitive to polymyxin B addition.

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[3] Abbreviations used in this paper: EC, endothelial cell; poly(I:C), polyinosinic-polycytidylic acid; EMSA, electrophoretic mobility shift analysis; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NF-κB, nuclear factor-κB.

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Cell culture

HUVEC and bovine aortic EC were isolated by collagenase treatment, as previously described (6). The human EC were cultured in medium MCDB 107 supplemented with heparin (90 μg/ml), 15% FBS (v/v), and EC growth supplement (150 μg/ml) derived from bovine hypothalamus, and the bovine EC were grown in DME/F12 medium. Culture plates were coated with fibronectin (1 $\mu g/cm^2$). Primary cultured EC or EC between passages 1 and 4 were used where specified. All cell types were grown under 5% CO_2 at 37°C.

EC surface expression of leukocyte adhesion proteins

EC were plated in 48-well cluster plates. EC were washed twice with medium containing 1% BSA and incubated for 1 h at 4°C with mAbs (1 μg/ml). The wells were washed three times, and biotin-conjugated, affinity-purified $F(ab')_2$ fragment goat anti-mouse IgG^+ IgM (H + L) (1/1000 dilution) was added and the plate was incubated at 4°C for 30 min. After three washes, ^{125}I-labeled streptavidin (0.25 μCi/well) was added to each well and the cells were incubated at 4°C for 15 min. Subsequently, the wells were washed four times, the cells were lysed with 1% Triton X-100, and the radioactivity was quantitated. In all surface expression assays, the adhesion of an unrelated Ab, mouse IgGIκ Ab (MOPC), was measured as an indication of nonspecific binding. These control measurements were consistently <1% of total binding.

RNA isolation and Northern analysis

EC were treated and total RNA was extracted using the TRIZOL reagent from Life Technologies (Grand Island, NY), per manufacturer's instructions. Total RNA (20 μg) was electrophoresed through 1% formaldehyde agarose gels in the presence of ethidium bromide and capillary blotted to Nytran membranes. High specific activity probes were prepared by labeling cDNAs corresponding to VCAM-1, E-selectin, γ-actin, and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) following the random primer labeling method of Feinberg and Vogelstein (33) using [α-^{32}P]dCTP (3000 Ci/mmol). The incorporated counts were separated from free label by gel filtration using a spin column (Sepharose CL-4B), and total cpm were determined from Cerenkov counts. The Nytran membranes were prehybridized and hybridized according to the manufacturers' instructions for at least 4 h at 42°C. The filters were washed serially in 2× SSC (20× = 3 M sodium chloride, 0.3 M sodium citrate), 0.5% SDS, and 0.2× SSC, 1% SDS at 42°C for 1 h. Loading efficiency was determined both by ethidium bromide staining as well as by intensity of γ-actin or GAPDH message for each sample. The relative intensity of message expression for either E-selectin or VCAM-1 was normalized using the expression of either housekeeping gene.

Nuclear extract preparation and electrophoretic mobility shift analysis (EMSA)

Nuclear proteins from EC were prepared using a modification of a previously described method (34). Briefly, cells were washed with iced PBS and then scraped and spun down. The cell pellets were then resuspended in 500 μl of buffer A (10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT, and 1 mM PMSF) and spun down. The pellet was then resuspended in buffer A (80 $\mu l/10^7$ cells), to which 0.1% Triton X-100 was added. After a 10-min incubation on ice, the samples were spun at 3500 rpm for 4 min at 4°C. The supernatant was removed and the nuclear pellet was washed in buffer A (500 μl). The nuclear pellet was then resuspended in 50 μl of buffer C (20 mM HEPES, 25% glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM DTT, and 1 mM PMSF). Following a 30-min incubation at 4°C, the samples were spun at 20,000 × *g* for 10 min. The supernatant containing nuclear proteins was stored at −70°C until used for EMSA. Protein concentration of the nuclear extracts was determined using the bicinchoninic acid assay method (Pierce, Rockford, IL). The sequence of the 28-residue oligonucleotide probe (E-selectin NF-κB) was taken from the consensus NF-κB binding domain of the E-selectin promoter (5′-AGGCCATTGGGGATTTCC-TCTTTACTGG-3′) (35). The oligonucleotide probes were annealed and labeled by a filling-in reaction, using a [^{32}P]dCTP, unlabeled dTTP, dATP, dGTP, and the Klenow fragment of DNA polymerase 1. Nuclear extracts (5 μg protein) were incubated for 20 min at room temperature in 20 μl total reaction mixture containing: 5 × 10^4 cpm ^{32}P-labeled probe (~1 ng), 225 μg/ml BSA, 0.1 mg/ml poly(dI:dC), and binding buffer (12 mM HEPES, pH 7.9, 4 mM Tris, 60 mM KCl, 1 mM EDTA, 12% glycerol, 1 mM DTT, and 1 mM PMSF). Competition analysis was performed to verify the specificity of the shifted band using 100- to 200-fold excess of unlabeled NF-κB-specific and nonspecific oligonucleotide that was coincubated with the nuclear extracts in the binding reaction for 15 min at room temperature before the radiolabeled NF-κB oligonucleotide probe was added. Samples were loaded on nondenaturing 4% polyacrylamide gels in 1× Tris-glycine buffer, pH 8.5. Electrophoresis was performed at 15 V/cm. The gels were then dried and analyzed by autoradiography.

Transient transfection and luciferase assay

The E-selectin promoter-driven luciferase reporter vector was constructed by cloning DNA corresponding to the first 540 bp 5′ from the transcription initiation start site of the human E-selectin promoter sequence (35) into the *SmaI* restriction site of the pGL3-Basic luciferase reporter vector from Promega Corp. (Madison, WI). The DNA was generated by PCR amplification of human genomic DNA. Bovine aortic EC were grown in six-well plates to ~60% confluence and transfected for 18 h in OptiMEM with 15 μg/ml lipofectin reagent (Life Technologies, Gaithersburg, MD), 1 μg/ml E-selectin promoter-driven luciferase reporter vector, and 250 ng/ml SV40 promoter-driven β-galactosidase reporter vector. HUVEC were transfected in one 150-mm plate for 6 h in OptiMEM with 10 μg/ml lipofectin reagent and 1 μg/ml E-selectin promoter-driven luciferase reporter vector without the SV40 promoter-driven β-galactosidase reporter vector. Following transfection, the cells were allowed to recover overnight and then trypsinized and replated into six-well plates for cell treatment with IFN-γ and poly(I:C). Both cell types were washed twice following transfection and treated with IFN-γ (10–100 ng/ml) for 24 h. Following this incubation period, the cells were induced with poly(I:C) for 6 h. Cells were then washed twice in PBS and harvested by scraping in 300 μl of lysis buffer (Promega Corp.). Cell debris was pelleted by centrifugation, and the supernatant was assayed for luciferase and β-galactosidase activity and protein content. Luciferase activity was determined by combining 75 μl of cell extracts with 150 μl of Luciferin reagent (Promega Corp.), and measuring luminescence using a microplate luminometer. β-Galactosidase activity was determined using the Galacto-Light Plus assay kit from Tropix (Bedford, MD). Transfections were conducted in triplicate and the data were corrected for transfection efficiency using β-galactosidase activity in the case of the bovine aortic EC. Since the HUVEC were transfected in a single tissue culture plate and then passaged into six-well plates before test treatments, these data did not require normalization for transfection efficiency and were only corrected for protein content. Protein content of the cell extracts was measured using the bicinchoninic acid assay method (Pierce).

Results

Prolonged pretreatment with IFN-γ inhibits dsRNA-induced surface expression of E-selectin. IFN-γ treatment, either alone or together with cytokine induction, has been shown to regulate the expression of certain leukocyte adhesion proteins (25–32). We wished to determine whether IFN-γ treatment would affect poly(I:C)-induced leukocyte adhesion protein expression in vascular EC. We treated HUVEC with IFN-γ simultaneously with poly(I:C) or for varying periods of time before poly(I:C) treatment and measured surface expression of E-selectin and VCAM-1. When IFN-γ was added simultaneously with poly(I:C), there was no significant effect on the level of these proteins in HUVEC (Fig. 1). However, when IFN-γ was added to the HUVEC 24 h before poly(I:C) induction, there was a significant reduction (50%) of the dsRNA-induced E-selectin (Fig. 1), while VCAM-1 levels were augmented (data not shown). Prolonged exposure (72 h) of EC to maximal doses of IFN-γ (100 ng/ml) had no effect on protein synthesis (data not shown). We measured endothelial surface expression of adhesion proteins in response to poly(I:C) in the presence and absence of IFN-γ pretreatment in increasing concentrations. Figure 2 shows that IFN-γ pretreatment dose dependently reduced the level of E-selectin expression in response to poly(I:C) with an $IC_{50} \approx$ 0.5 ng/ml. We also measured VCAM-1 and ICAM-1 expression and found that IFN-γ treatment induced both of these proteins, as previously shown (25–29, 32), and had an additive effect with poly(I:C) treatment (data not shown). The inhibitory effect of IFN-γ pretreatment on E-selectin expression was specific for the induction in response to poly(I:C) in that the level of E-selectin induced by TNF-α, IL-1β, thrombin, and LPS was unaffected or slightly augmented (Fig. 3). Pretreatment of HUVEC with IFN-γ decreased poly(I:C)-induced levels of E-selectin on the cell surface

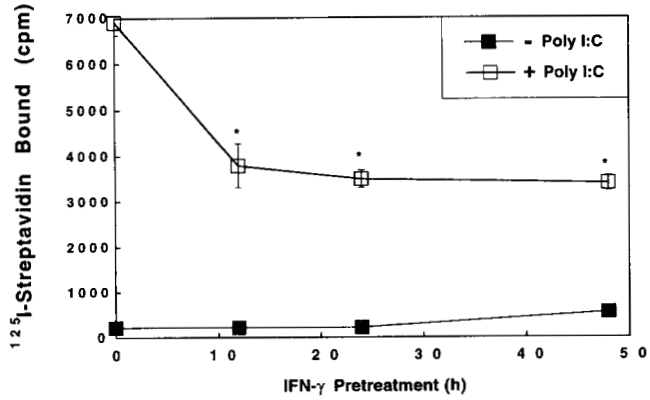

FIGURE 1. Prolonged pretreatment with IFN-γ is required for inhibition of dsRNA-induced surface expression of E-selectin. HUVEC were pretreated with IFN-γ (10 ng/ml) for the indicated time, followed by 6-h poly(I:C) (100 μg/ml) treatment. The 0-h time point reflects simultaneous treatment (i.e., no pretreatment) of IFN-γ and poly(I:C) for 6 h. Surface expression of E-selectin protein was quantitated, as described in *Materials and Methods*. The Ab control, MOPC (mouse IgG1κ Ab), showed the nonspecific Ab binding to be insignificant. Data are expressed as means ± SE (n = 3) and are representative of four similar experiments. *$p \leq 0.05$.

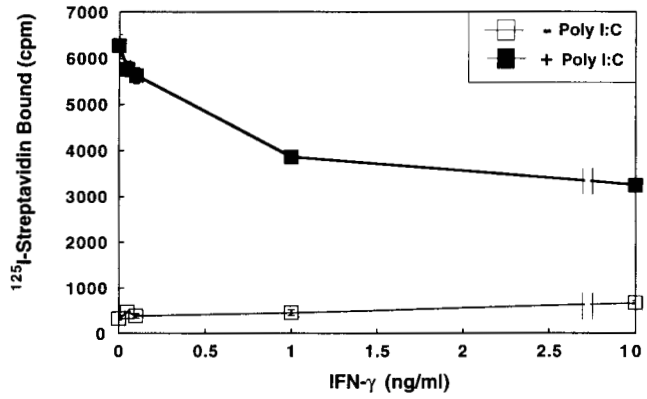

FIGURE 2. Concentration response curve for the effect of IFN-γ pretreatment on surface expression of E-selectin in response to poly(I:C). HUVEC were pretreated with IFN-γ for 24 h in the absence or presence of the subsequent 6-h poly(I:C) (100 μg/ml) treatment. E-selectin expression on the cell surface was determined as described in *Materials and Methods*. Data are presented as means ± SE (n = 3). Data represent one of four similar experiments.

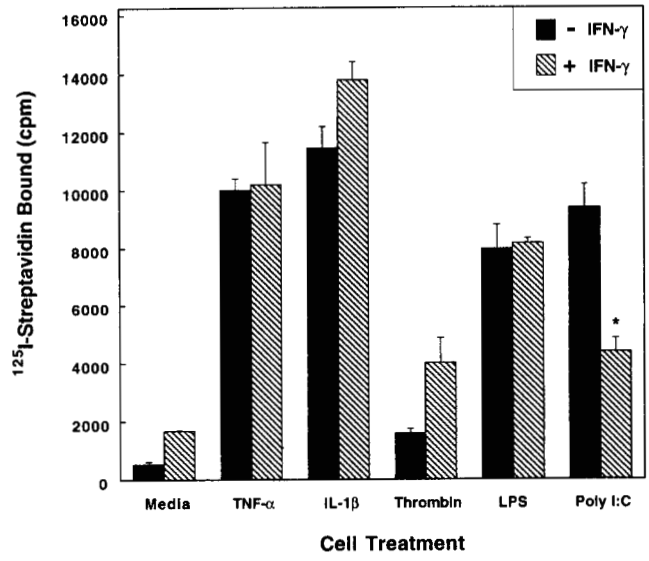

FIGURE 3. Inhibitory effect of IFN-γ on the surface expression of E-selectin on EC is specific for dsRNA. HUVEC were treated with TNF-α (10 ng/ml), IL-1β (10 ng/ml), thrombin (30 U/ml), LPS (10 ng/ml), or poly(I:C) (100 μg/ml) with or without 24-h pretreatment of IFN-γ (100 ng/ml). Surface expression of E-selectin was determined as described in *Materials and Methods*. Data are presented as means ± SE (n = 3) and represent one of two similar experiments. *$p \leq 0.05$.

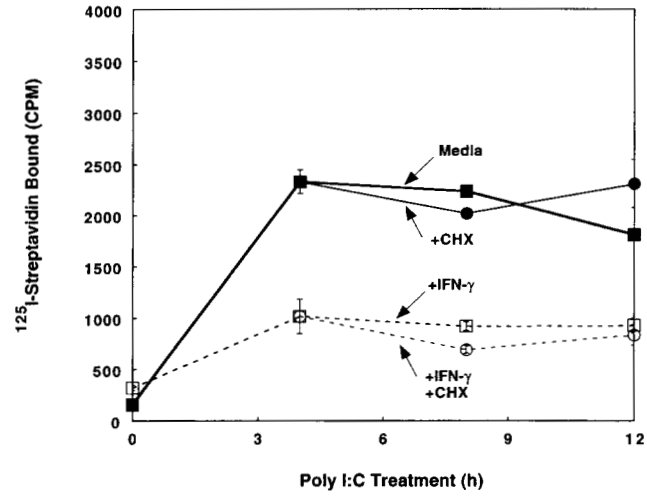

FIGURE 4. IFN-γ pretreatment does not affect the $t_{1/2}$ of poly(I:C)-induced surface expression of E-selectin on EC. HUVEC were treated with poly(I:C) (100 μg/ml) for increased periods of time with or without 24-h IFN-γ pretreatment. Following 4-h poly(I:C) treatment, cells were treated with cycloheximide (10 μg/ml) for 4 to 8 h in the presence or absence of IFN-γ pretreatment. Surface expression of E-selectin was determined as described in *Materials and Methods*. Data are presented as means ± SE (n = 3) and represent one of two similar experiments.

without affecting the $t_{1/2}$ of the protein (Fig. 4). HUVEC were treated with poly(I:C) for 4 h, after which time cycloheximide, a potent inhibitor of protein synthesis, was added to stop further translation of protein. Figure 4 shows that poly(I:C)-induced E-selectin was expressed stably on the EC surface for up to 12 h whether or not the cells were pretreated with IFN-γ. Therefore, the reduced levels of poly(I:C)-induced E-selectin that occurred with IFN-γ pretreatment were a result of a decrease in the initial amount of E-selectin on the cell surface, rather than an effect of IFN-γ on the protein $t_{1/2}$.

IFN-γ pretreatment decreases poly(I:C)-induced E-selectin message. We performed Northern analysis to determine whether IFN-γ pretreatment would affect steady state levels of E-selectin mRNA in response to poly(I:C). Figure 5 shows that the E-selectin message induced by poly(I:C) was down-regulated (30–40% normalized for GAPDH message) by IFN-γ pretreatment. This inhibitory effect of IFN-γ on the poly(I:C)-induced E-selectin mRNA was not dependent on protein synthesis, since the decrease in message was still evident when cycloheximide (10 μg/ml) was present during the pretreatment period (Fig. 5). As seen for other dsRNA-inducible genes (36), cycloheximide treatment superinduced the poly(I:C)-induced E-selectin mRNA (Fig. 5), while cycloheximide plus IFN-γ treatment together did not induce E-selectin message in the absence of poly(I:C), nor did either treatment induce the message

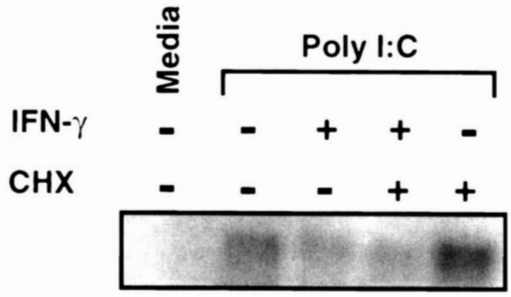

FIGURE 5. IFN-γ pretreatment decreases E-selectin message levels in response to poly(I:C). HUVEC were treated with poly(I:C) (100 μg/ml) for 2 h with or without 16-h IFN-γ (100 ng/ml) pretreatment in the presence or absence of 16-h cycloheximide (10 μg/ml) treatment, as indicated. Northern analysis was performed as described in *Materials and Methods*. Message levels were quantitated via phosphor imaging analysis and reported normalized for levels of GAPDH mRNA in the same experiment. Data represent one of two similar experiments.

alone (data not shown). The E-selectin message $t_{1/2}$ was also studied to determine whether IFN-γ acted post-transcriptionally to affect the stability of poly(I:C)-induced mRNA. Peak steady state message for E-selectin in response to poly(I:C) occurred at approximately 2 h (Fig. 6*A*). The $t_{1/2}$ of the message was determined by detecting the remaining poly(I:C)-induced message at different times following treatment of cells with actinomycin D, a potent inhibitor of additional transcription. Figure 6*B* indicates that IFN-γ pretreatment reduced the $t_{1/2}$ of poly(I:C)-induced message for E-selectin from 2.8 to 2 h, while it stabilized the message for VCAM-1 (Fig. 6*C*).

IFN-γ pretreatment inhibits poly(I:C)-induced E-selectin promoter activity. To determine whether the regulation by IFN-γ on poly(I:C)-induced E-selectin expression occurred at the transcriptional level, we measured E-selectin promoter activity. EC were transfected transiently with an E-selectin promoter-driven luciferase reporter gene construct, as described in *Materials and Methods*. Following the transfection period, the EC were either left untreated or treated with IFN-γ for 24 h before the 6-h poly(I:C) treatment and assayed for luciferase activity. The data, corrected for transfection efficiency, showed that IFN-γ pretreatment reduced poly(I:C)-induced E-selectin promoter activity by ≥70%, $p \leq 0.05$ in both HUVEC (Fig. 7*A*) as well as bovine aortic EC (Fig. 7*B*). These results indicated that the effect of IFN-γ on the induction of E-selectin expression in response to poly(I:C) occurred at the transcriptional level.

IFN-γ pretreatment has no effect on poly(I:C)-induced NF-κB activation in vascular endothelium. We used EMSA to investigate whether the decrease in E-selectin promoter activity was the result of reduced transcription factor availability. Using an oligonucleotide (E-selectin NF-κB), corresponding to the consensus NF-κB binding domain of the E-selectin promoter, we noted that poly(I:C) as well as TNF-α-induced NF-κB activation was unaffected by IFN-γ pretreatment (Fig. 8). Competition studies using unlabeled E-selectin NF-κB oligonucleotide (Fig. 8), as well as nonspecific oligonucleotide in molar excess, verified the specificity of the shifted band within the EMSA.

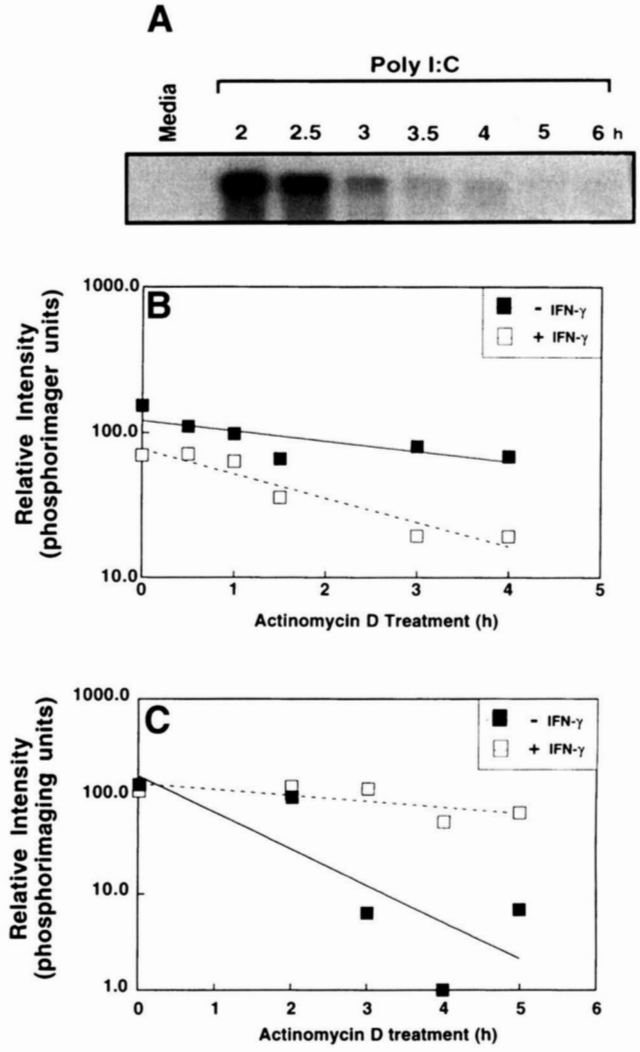

FIGURE 6. IFN-γ pretreatment affects the message $t_{1/2}$ of poly(I:C)-induced genes. *A*, HUVEC were either left untreated or treated with poly(I:C) (100 μg/ml) for the indicated times. Northern analysis using total RNA (20 μg/lane) was performed, and the membrane was probed for E-selectin mRNA, as described in *Materials and Methods*. HUVEC were treated with poly(I:C) (100 μg/ml) for 2 h in the presence or absence of IFN-γ (100 ng/ml) pretreatment, followed by actinomycin D (10 μg/ml) treatment for the indicated times. Northern analysis was then performed as described in *Materials and Methods*, and the membrane was probed for E-selectin mRNA (*B*) or VCAM-1 mRNA (*C*). The data were quantitated using phosphor imaging analysis, normalized for GAPDH expression within the same experiment.

Discussion

IFN-γ plays multiple roles in immune regulatory functions as well as in viral defense. Due to its antiviral properties, we thought to investigate whether IFN-γ may be involved in the induction of EC leukocyte adhesion molecules in response to the viral mimetic, poly(I:C). Previously others have shown that IFN-γ treatment up-regulated the expression of the leukocyte adhesion molecules, VCAM-1 and ICAM-1, either alone or synergistically with other cytokines (25–29, 32). Additionally, it has been shown that short-term IFN-γ treatment accelerated and prolonged TNF-α-induced E-selectin expression on the EC surface (31, 37); however, no significant effect has been reported for the induction of E-selectin either by IFN-γ alone or with cytokine induction.

We observed that IFN-γ pretreatment significantly inhibited poly(I:C)-induced surface expression of E-selectin, even though there was a concomitant up-regulation of VCAM-1 and ICAM-1. IFN-γ pretreatment reduced poly(I:C)-induced E-selectin gene expression at the transcriptional level through the down-regulation of E-selectin promoter activity, as well as at the post-transcriptional level by destabilizing E-selectin message. IFN-γ has been shown

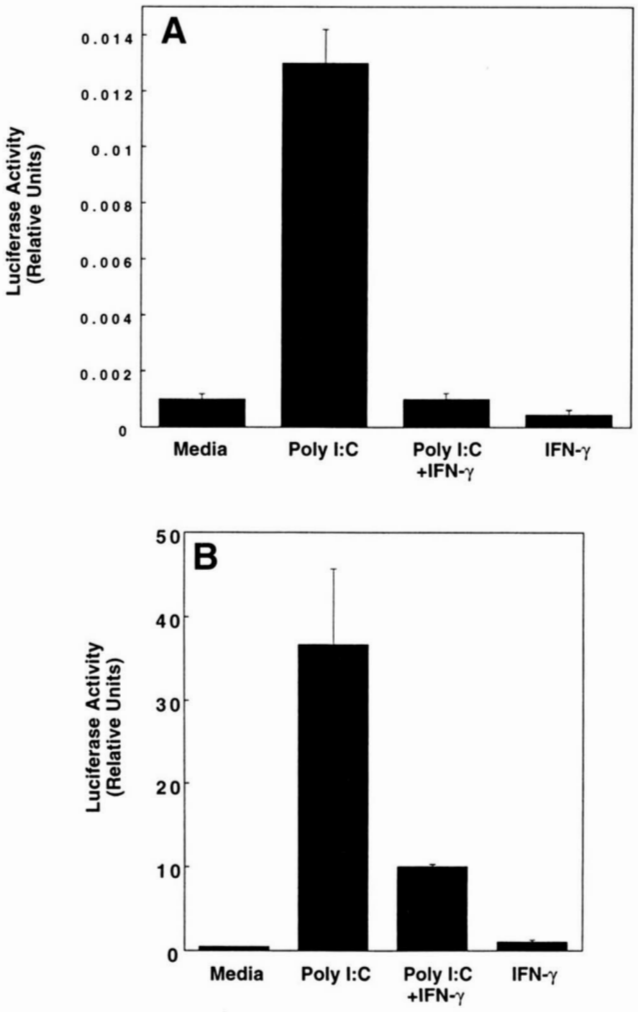

FIGURE 7. IFN-γ pretreatment inhibits E-selectin promoter activity in response to poly(I:C). *A*, HUVEC were transfected transiently with E-selectin promoter-driven luciferase reporter gene constructs and assayed for luciferase activity in response to poly(I:C) (100 μg/ml) treatment for 6 h in the presence or absence of 24-h IFN-γ pretreatment (10–100 ng/ml) and corrected for transfection efficiency, as described in *Materials and Methods*. *B*, Bovine aortic EC were transfected transiently with both E-selectin promoter-driven luciferase reporter gene constructs and the β-galactosidase reporter constructs. E-selectin promoter activity was measured following 6-h treatment of poly(I:C) in the presence or absence of 24-h IFN-γ pretreatment and normalized as described in *Materials and Methods*. These results are one of three similar experiments in which data represent means ± SE (n = 3).

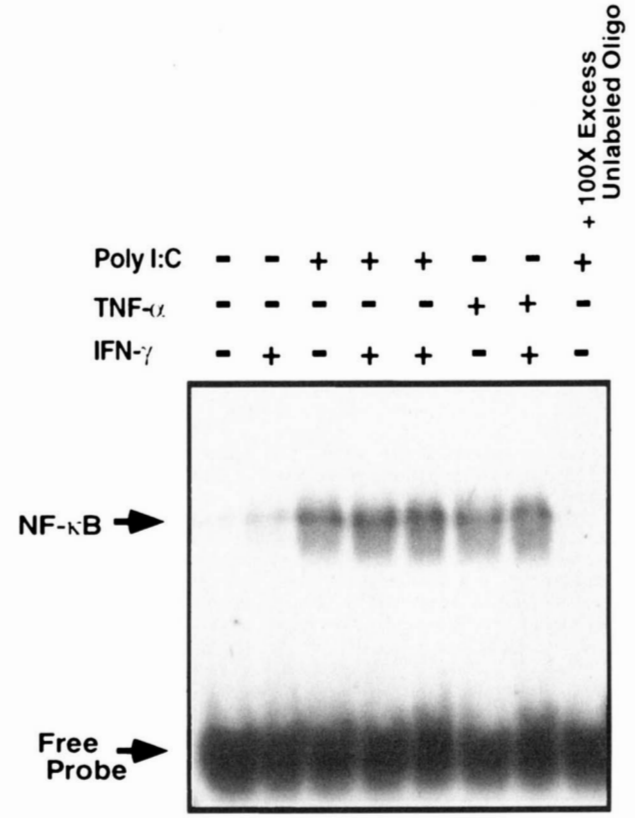

FIGURE 8. IFN-γ pretreatment does not affect poly(I:C)-induced NF-κB activation. HUVEC were treated with poly(I:C) (100 μg/ml) for 1.5 h or TNF-α (10 ng/ml) for 45 min, with or without the 24-h pretreatment of IFN-γ (10 ng/ml (*lane 4*) and 100 ng/ml (*lanes 2, 5,* and *7*)). Nuclear extracts were prepared and incubated with radiolabeled oligonucleotides corresponding to the NF-κB consensus binding domain of the E-selectin promoter and run on a polyacrylamide gel, as described in *Materials and Methods*. Autoradiography was done to visualize electrophoretic mobility shifts when nuclear protein binding occurred. Data represent one of four similar experiments.

to down-regulate the expression of multiple induced genes, including granulocyte-macrophage CSF, platelet EC adhesion molecule-1, c-*sis* (platelet-derived growth factor B chain), monocyte chemoattractant protein-1, and KC (GRO/melanoma growth-stimulating activity) (30, 38–40). The effect of IFN-γ on induced gene expression has been shown to occur at both the transcriptional (38, 41) and post-transcriptional level (30, 40, 42, 43). The fact that the down-regulation of E-selectin expression in response to dsRNA by IFN-γ was not sensitive to cycloheximide indicated that the mechanism by which it acts does not require newly synthesized proteins. This protein synthesis-independent gene regulation by IFN-γ is not unique; it has been reported that IFN-γ regulates the induction of other genes in a similar fashion (38).

We found that the transcriptional down-regulation of E-selectin in response to poly(I:C) by IFN-γ occurred through an NF-κB-independent pathway. Although the induction of E-selectin by some stimulators has been shown to be NF-κB dependent, it is known to be necessary, but not sufficient to induce this gene (44, 45). As suggested by previous reports from our group (44, 46), NF-κB binding to its consensus site on the E-selectin promoter exhibits marked differences in redox sensitivity from that of the binding to VCAM-1 promoter sequences. Since IFN-γ has been shown to produce oxygen intermediates (47), it may be altering the redox state within the cells, accounting for differential gene regulation. Alternatively, the activation of STAT1, in response to IFN-γ treatment, may be interfering with the poly(I:C)-induced E-selectin expression. This may be occurring since STAT1 has been shown recently to directly interact with the cAMP response element-binding protein/p300 family of transcriptional coactivators (48) that may also play a role in the E-selectin gene regulation through physical interactions with the transcription factors NF-κB and activation transcription factor-2 (ATF-2) (49–51). Hence, the inhibition of poly(I:C)-induced expression of E-selectin in response to IFN-γ treatment may be the result of competition for a necessary transcriptional coactivator protein.

In summary, our results demonstrate that IFN-γ inhibits the induction of E-selectin in EC in response to a viral mimetic, dsRNA. This inhibitory effect of IFN-γ occurs at the transcriptional level in an NF-κB-independent manner, as well as at a post-transcriptional

level via the destabilization of E-selectin message. These observations describe a novel inhibitory role of IFN-γ on leukocyte adhesion molecule expression in human vascular EC in response to dsRNA.

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