Tumor Necrosis Factor-α- or Lipopolysaccharide-induced Expression of the Murine P-selectin Gene in Endothelial Cells Involves Novel κB Sites and a Variant Activating Transcription Factor/cAMP Response Element*

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Tumor necrosis factor-α (TNF-α) or lipopolysaccharide (LPS) increases expression of the P-selectin gene in murine, but not in human, endothelial cells. These mediators augment expression of a reporter gene driven by the murine, but not the human, P-selectin promoter in transfected endothelial cells. The regions from −593 to −474 and from −229 to −13 in the murine P-selectin promoter are required for TNF-α or LPS to stimulate reporter gene expression. Within these regions, we identified two tandem κB elements, a reverse-oriented κB site and a variant activating transcription factor/cAMP response element (ATF/CRE), that participate in TNF-α or LPS-induced expression. The tandem κB elements bound to NF-κB heterodimers and p65 homodimers, the reverse-oriented κB site bound to p65 homodimers, and the variant ATF/CRE bound to nuclear proteins that included activating transcription factor-2. Mutations in each individual element eliminated binding to nuclear proteins and decreased by 20–60% the TNF-α or LPS-induced expression of a reporter gene driven by the murine P-selectin promoter in transfected endothelial cells. Simultaneous mutations of all elements further decreased, but did not abolish, induced expression. Co-overexpression of p50 and p65 enhanced murine P-selectin promoter activity in a κB site-dependent manner. These data indicate that the κB sites and the variant ATF/CRE are required for TNF-α or LPS to optimally induce expression of the murine P-selectin gene. The presence of these elements in the murine, but not the human, P-selectin gene may explain in part why TNF-α or LPS stimulates transcription of P-selectin in a species-specific manner.

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§ The abbreviations used are: TNF-α, tumor necrosis factor-α; ALLN, N-acetyl-leucinyl-leucinyl-norleucinal-H; ATF, activating transcription factor; BAEC, bovine aortic endothelial cells; CRE, cAMP response element; IL, interleukin; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAP, mitogen-activated protein; PDTC, pyrrolidine dithiocarbamate.
two API sites and a xB site that are conserved in at least four different mammals (11). TNF-α-induced expression of the E-
selectin gene is mediated by an enhancerosome that contains three xB sites, a variant ATF/CRE, and four A/T-rich sequences
that bind to the architectural HMG I(Y) proteins (32, 33). All these elements are required for TNF-α or LPS to maximally induce transcription, and they are conserved in both the hu-
man and murine E-selectin genes (34).

By contrast, TNF-α or LPS increases expression of the P-
selectin gene in murine endothelial cells but not in human
endothelial cells (35–37). In the preceding paper (38), we dem-
onstrated that these mediators augment expression of a re-
porter gene driven by the murine, but not the human,
P-selectin promoter in transfected endothelial cells. Further-
more, the sequences from −583 to −474 and from −229 to −13
in the murine P-selectin promoter are required for TNF-α or
LPS to stimulate expression of the reporter gene (38). To dis-
sect the molecular basis for this unusual species-specific gene
activation event, we employed pharmacologic agents, DNA-
binding experiments, transfection studies, and mutational
analysis to characterize the regulatory elements and their cog-
nate proteins that contribute to TNF-α- or LPS-induced expres-
sion of the murine P-selectin gene. We identified two tandem
xB sites, a reverse-oriented xB site and a variant ATF/CRE,
that participate in TNF-α- or LPS-induced expression. These
elements are not present in the corresponding regions of
the human P-selectin gene, which may account in part for the
species-specific response of the P-selectin gene to TNF-α or
LPS.

MATERIALS AND METHODS
Reagents and Antibodies—Recombinant human TNF-α and ALLN
calpain inhibitor I) were purchased from Boehringer Mannheim.
PDTC, cycloheximide, anisomycin, actinomycin D, and LPS from Sul-
amonella typhosa were obtained from Sigma. A stock solution of ALLN
was made in dimethyl sulfoxide (American Type Culture Collection) at
a concentration of 50 mM. Stock solutions of cycloheximide, anisomycin,
and actinomycin D were made in ethyl alcohol (Quantum Chemical Co.,
Tuscola, IL) at a concentration of 5 mg/ml. Antibodies against p65, p52,
p65, c-Rel, RelB, ATF-2, c-Jun, and c-Fos were obtained from Santa
Cruz Biotechnology (Santa Cruz, CA). Expression plasmids encoding
p50 or p65 were a generous gift from Dr. Craig Rosen (39).

Gel Mobility Shift Assay—Nuclear extracts from murine bEnd.3 en-
dothelial cells or BAEC were prepared as described (40). Gel mobility
shift assays were performed as described (15). The sequences of the
dioligonucleotides used in gel mobility shift assays are shown in the
figures. The dioligonucleotides were 5’-end-labeled by [γ-32P]ATP using
T4 polynucleotide kinase.

Construction of Chimeric Luciferase Expression Vectors—Plasmids
mp1379LUC, p1379B1I, and p0LUC were described in the preceding
paper (38). Plasmids mpMutxB, mpMutRxB, mpMutATF, mpMut-
Double, and mpMutTriple, which carry mutations in xB and/or variant
ATF/CRE as indicated in the text, were constructed in the following two
steps. 1) The KpnI-PstI or PstI-Sacl fragment in p1379B1I was replaced
with respective PCR products generated according to an overlap exten-
sion protocol (41, 42). 2) The KpnI-HindIII fragment (from −1379 to
−13) that carried each mutation was excised and inserted between the
KpnI and HindIII sites of p0LUC. All constructs were confirmed by
restriction mapping, and the fidelity of the PCR-generated cassettes
was verified by sequencing.

Cell Culture, Transfection, and Stimulation—Murine bEnd.3 cells
and BAEC were cultured as described (37, 42). Preparation of plasmids,
transfections and co-transfections, and luciferase assays were described
previously (15). The total amount of DNA for each co-transfection was
held constant at 8 μg/dish of cells by adding an appropriate amount of
a plasmid with a lacZ insert driven by a cytomegalovirus promoter. For
cell stimulation, culture medium containing the indicated concentra-
tion of recombinant human TNF-α, LPS, ALLN, PDTC, cycloheximide,
anisomycin, or actinomycin D was added to cells for the indicated time.

Northern Blot Analysis—Total RNA was prepared from bEnd.3 cells
by acid guanidinium thiocyanate/phenol/chloroform extraction (43).
Northern blot analysis was performed as described (43), using previ-
ously characterized probes (37).

RESULTS

TNF-α-induced expression of P-selectin in murine endothelial
cells Is Prevented by the Proteasome Inhibitor ALLN or the
Antioxidant PDTC but Superinduced by the Translation Inhibi-
tor Cycloheximide or Anisomycin—In the preceding paper (38), we
showed that TNF-α or LPS requires the sequences from −593 to −474 and from −229 to −13 in the 5’-flanking region of
the murine P-selectin gene to induce expression of a reporter
gene in transfected endothelial cells. Inspection of these regions
revealed several putative xB elements and a variant
ATF/CRE site, suggesting that NF-xB/Rel proteins and ATF-2/
c-Jun heterodimers may participate in inducible expression. As
an initial test of this hypothesis, we used the proteasome
inhibitor ALLN or the antioxidant PDTC that blocks activation of
NF-xB, and the translation inhibitor cycloheximide or aniso-
mycin that prevents reactivation of IxB-α and activates JNK/
p38 MAP kinases (44–46). We incubated murine bEnd.3 endo-
thelium cells with TNF-α or LPS in the absence or presence of
a pharmacologic agent for various times. Levels of P-selectin
mRNA from each group of cells were then measured by North-
ern blot analysis.

As demonstrated previously (36, 37), P-selectin mRNA was
detected in unstimulated bEnd.3 cells, and the mRNA level
was markedly increased in cells treated with TNF-α for 4 h
(Fig. 1A). The proteasome inhibitor ALLN or the antioxidant
PDTC prevented the TNF-α-induced increase of P-selectin
mRNA but did not affect the levels of mRNA for CHO-B, a

FIG. 1. The proteasome inhibitor ALLN or the antioxidant
PDTC prevents, whereas the translation inhibitor cyclohexi-
mide superinduces, TNF-α-induced expression of P-selectin in
bEnd.3 cells. A, confluent bEnd.3 cells were preincubated for 1 h in the
presence or absence of 25 mM ALLN or 50 mM PDTC. The cells were
then incubated for 4 h with TNF-α (100 units/ml) in the continued
presence of the respective pharmacologic agent. Total RNA was isolated
and analyzed by Northern blotting with labeled cDNA probes for mu-
nine P-selectin or CHO-B. B, confluent bEnd.3 cells were incubated in
the presence or absence of TNF-α (100 units/ml), cycloheximide (CHX,
10 μg/ml), and/or actinomycin D (Act. D, 5 μg/ml). After the indicated
time, total RNA was isolated and analyzed by Northern blotting as in A.

![Fig. 1](http://www.jbc.org/)

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Fig. 2. The two tandem κB sites in the murine P-selectin gene, but not the corresponding region of the human P-selectin gene, bind to inducible nuclear proteins in a κB-dependent manner. A, sequence comparisons among selected κB sites. The putative κB motifs in each sequence are in boldface, and the mutated nucleotides are in italics. The numbering of the sequences derived from the murine P-selectin gene is relative to the translation start site. The listed sequences also encode the upper strands of the oligonucleotides used as probes and competitors in gel shift assays. B, the labeled tandem κB probe from the murine P-selectin gene was incubated with nuclear extracts from bEnd.3 cells incubated in the absence or presence of TNF-α or LPS for 3 h. The arrow marks the position of the major inducible DNA-protein complex. Inducible complexes of more rapid mobility are also present; these complexes were less consistently observed and may represent degradation products. C, the labeled tandem κB probe was incubated with nuclear extracts from TNF-α-stimulated BAEC in the absence or presence of a 100-fold excess of the indicated unlabeled competitor.
kB and ATF/CRE Sites in Murine P-selectin Gene

constitutively expressed transcript that is not affected by TNF-α or LPS (37, 47). By contrast, the translation inhibitor cycloheximide superinduced P-selectin mRNA levels in cells stimulated with TNF-α (Fig. 1B) or LPS (data not shown). Actinomycin D blocked the induction or superinduction of P-selectin mRNA, which verified that TNF-α or LPS induced expression of P-selectin mRNA through a transcriptional mechanism. The translation inhibitor anisomycin, which also activates JNK/p38 MAP kinases, elicited the same superinduction of P-selectin mRNA (data not shown). These data are consistent with the notion that activation of NF-κB/Rel proteins and JNK/p38 MAP kinases may participate in TNF-α- or LPS-induced expression of the murine P-selectin gene.

Characterization of Two Tandem kB Sites and a Reverse-oriented kB Site in the Murine P-selectin Promoter—To identify sites for binding to NF-κB, we synthesized double-stranded oligonucleotide probes encompassing each putative kB site from −593 to −13 in the 5′-flanking region of the murine P-selectin gene (Fig. 2A). Each labeled probe was assessed for binding to nuclear proteins from endothelial cells incubated with or without LPS or TNF-α.

A labeled probe encompassing the murine P-selectin tandem kB elements formed a DNA-protein complex with nuclear extracts from TNF-α- or LPS-stimulated, but not unstimulated, bEnd.3 cells (Fig. 2B). The labeled probe also formed a complex of identical mobility with extracts from TNF-α-stimulated but not unstimulated BAEC. Complex formation was sequence-specific, as it was prevented by addition of a 100-fold molar excess of the unlabeled probe, but not of a 100-fold excess of a probe containing the corresponding sequence from the human P-selectin gene (Fig. 2C). Complex formation required the kB elements, because it was prevented by addition of a 100-fold excess of an unlabeled kB probe from the murine H-2Kb gene (48). Furthermore, a murine P-selectin tandem kB probe with mutations in the kB elements failed to bind to inducible proteins (Fig. 2D).

A labeled probe containing the reverse-oriented kB element formed a TNF-α- or LPS-inducible DNA-protein complex with nuclear extracts from BAEC and bEnd.3 cells (Fig. 2B). Again, complex formation was sequence-specific, as it was prevented by addition of a 100-fold excess of the unlabeled probe but not of a probe containing the corresponding sequence from the human P-selectin gene. Binding required the kB sequence, because it was prevented by addition of a 100-fold excess of the unlabeled H-2Kb kB sequence or by introduction of mutations into the reverse-oriented kB site (Fig. 2B).

Probes encompassing four other putative kB sites (termed sequence I to IV, respectively) were tested for binding to nuclear proteins from unstimulated or TNF-α-stimulated BAEC. The sequence I probe formed a specific DNA-protein complex with extracts from TNF-α-stimulated but not unstimulated BAEC. The mobility of the complex was identical to that formed with the tandem kB elements, but the labeling intensity was much weaker under the same experimental conditions. The sequence II, III, and IV probes did not form detectable complexes.

To identify the proteins in these inducible complexes, we preincubated nuclear extracts from TNF-α-stimulated BAEC or bEnd.3 cells with antibodies to NF-κB/Rel proteins prior to the gel shift assay. Antibodies to p50 partially inhibited binding of nuclear proteins from TNF-α-stimulated BAEC to the tandem kB probe (Fig. 4A) or the sequence I probe (data not shown).
The two tandem \( \kappa B \) elements in the murine P-selectin gene bind to NF-\( \kappa B \) heterodimers and p65 homodimers, whereas the reverse-oriented \( \kappa B \) element binds preferentially to p65 homodimers. These data indicate that the tandem \( \kappa B \) elements bind to both p50/p65 heterodimers and p65 homodimers, whereas the reverse-oriented \( \kappa B \) site binds preferentially to p65 homodimers. Sequence I binds to NF-\( \kappa B \) heterodimers and p65 homodimers but with apparently low affinity.

Characterization of a Variant ATF/CRE in the Murine P-selectin Promoter—A putative ATF/CRE in the murine P-selectin promoter is identical in sequence to the ATF/CRE in the E-selectin gene and deviates one nucleotide from the CRE consensus sequence (27, 28, 49). To test whether the P-selectin ATF/CRE competes with the other two elements for binding to common nuclear proteins, we synthesized oligonucleotide probes encompassing the murine P-selectin ATF/CRE, the human E-selectin ATF/CRE, or a CRE consensus sequence (Fig. 5A). A labeled probe encompassing the P-selectin ATF/CRE formed a DNA-protein complex when incubated with extracts from unstimulated bEnd.3 cells (Fig. 5B) or TNF-\( \alpha \)-stimulated bEnd.3 cells (data not shown). Relatively little complex formation was detected, consistent with a possibly lower affinity of the variant ATF/CRE for nuclear proteins. Complex formation was sequence-specific, as it was prevented by addition of a 100-fold molar excess of the unlabeled probe but not of an unrelated GATA element. Complex formation also required the ATF/CRE sequence, because it was prevented by addition of a 100-fold excess of an unlabeled probe encoding the E-selectin ATF/CRE or a CRE consensus sequence. The labeled CRE consensus sequence probe formed two complexes, A and B, when incubated with nuclear extracts from bEnd.3 cells (Fig. 5C). Formation of each complex was sequence-specific, as it was prevented by addition of a 50–200-fold excess of the unlabeled probe. Formation of complex A but not B was significantly diminished by addition of a 50–200-fold excess of an unlabeled probe encoding the P-selectin or E-selectin ATF/CRE. An unlabeled probe containing mutations in the murine P-selectin ATF/CRE or a probe containing the corresponding sequence from the human P-selectin gene did not inhibit complex formation (Fig. 5C). These data suggest that the murine P-selectin ATF/CRE, the E-selectin ATF/CRE, and a CRE consensus sequence bind to common nuclear factors in complex A.

Complex A was previously demonstrated to contain the protein ATF-2 in nuclear extracts from other cells (50). To confirm that complex A from bEnd.3 cell nuclear extracts contained ATF-2, we preincubated bEnd.3 extracts with antibodies to ATF-2, c-Jun, or other nuclear proteins prior to the gel shift assay. As shown in Fig. 5D, preincubation with antibodies to ATF-2, but not to c-Jun, c-Fos, p50, or p65, significantly diminished formation of complex A. These data indicate that the murine P-selectin ATF/CRE, the E-selectin ATF/CRE, and a CRE consensus sequence bind to ATF-2 in bEnd.3 cells.

The Tandem \( \kappa B \) Sites, the Reverse-oriented \( \kappa B \) Site, and the Variant ATF/CRE Are Required for TNF-\( \alpha \) or LPS to Maximally Induce Expression of a Reporter Gene Driven by the Murine P-selectin Promoter in Transfected Endothelial Cells—To determine whether the tandem \( \kappa B \) sites, the reverse-oriented \( \kappa B \) site, and the variant ATF/CRE allowed TNF-\( \alpha \) or LPS to induce expression of murine P-selectin, we mutated these elements, individually or in combination, in a reporter construct driven by the murine P-selectin promoter. The mutations were the same as those made in the mutant probes that eliminated binding to nuclear proteins. Following transfection of the wild-type construct or each mutant construct into BAEC, the cells were incubated in the absence or presence of TNF-\( \alpha \) or LPS for 4.5 h and then harvested for assay of luciferase activity. Mutations in each individual element decreased TNF-\( \alpha \)- or LPS-induced expression by 20–60% relative to that of the wild-type construct (Fig. 6). Combined mutations in the re-
The variant ATF/CRE in the murine P-selectin (mP-sel) gene competes with the human E-selectin (hE-sel) ATF/CRE and a CRE consensus sequence for binding ATF-2 and other nuclear proteins. A, comparisons among selected ATF/CRE (27, 28). The ATF/CRE sites are in boldface, and the mutated bases are in italics. The listed sequences also encode the upper strands of the oligonucleotides used as probes or competitors in gel shift assays. B, the labeled murine P-selectin ATF/CRE probe was incubated with bEnd.3 nuclear extracts in the absence or presence of a 100-fold excess of the indicated competitor. The arrow indicates the specific DNA-protein complex formed. C, the labeled CRE consensus sequence probe was incubated with bEnd.3 nuclear extracts in the absence or presence of a 50–200-fold excess of the indicated competitor. D, the labeled CRE consensus sequence probe was incubated with bEnd.3 nuclear extracts in the absence or presence of the indicated antibodies.
verse-oriented kB site and the variant ATF/CRE or in all three elements further decreased, but did not abolish, TNF-α- or LPS-induced expression. These data demonstrate that the tandem kB sites, the reverse-oriented kB site, the variant ATF/CRE, and still uncharacterized elements are required for TNF-α or LPS to maximally induce expression of a murine P-selectin reporter gene in transfected endothelial cells.

A Reporter Gene Driven by the Murine P-selectin 5'-Flanking Region Recapitulates Post-inductional Repression—Since translocation of p65-containing NF-κB/Rel proteins into the nucleus is transient because of feedback inhibition by newly synthesized IκB proteins (20, 21), NF-κB-dependent transcription usually declines after its initial induction. We measured the activity of a luciferase reporter gene driven by the murine P-selectin promoter in transfected BAEC that were stimulated with TNF-α for various times. Because luciferase protein and mRNA levels turn over rapidly, the kinetics of luciferase activity accurately reflect the kinetics of transcriptional activity of the reporter gene (51). Luciferase activity increased at 3 h, reached a maximum at 4.5 h, then declined rapidly and returned to a basal level by 13 h (Fig. 7). These data are consistent with post-inductional repression of transcription of the murine P-selectin gene.

Co-overexpression of p50 and p65 Augments κB-dependent Expression of a Reporter Gene Driven by the Murine P-selectin Promoter—To test directly the role of NF-κB/Rel proteins in inducing expression of murine P-selectin, we co-transfected BAEC with plasmids encoding the NF-κB/Rel protein p50 or p65 with a reporter gene driven by the wild-type P-selectin promoter or the promoter with mutations in the κB and ATF/CRE. Co-expression of p50 alone slightly decreased promoter activity of the wild-type or mutant construct. However, co-expression of increasing amounts of p65 with a fixed concentration of p50 markedly increased promoter activity of the wild-type construct but not of the mutant construct (Fig. 8). Co-expression of p65 alone also increased expression of the wild-type reporter gene, but to a lesser extent than that elicited by co-expression of p65 with p50 (data not shown). These data demonstrate that p65-containing NF-κB/Rel proteins regulate κB-dependent expression of the murine P-selectin gene.

DISCUSSION

We identified and characterized two tandem kB sites, a reverse-oriented kB site and a variant ATF/CRE site, that are required for TNF-α or LPS to optimally induce expression of the murine P-selectin gene. The presence of these elements in the murine, but not human, P-selectin gene may help explain why TNF-α or LPS increases expression of P-selectin in mu-
other experiment. Similar results were obtained in an independent transfection. In the murine gene are important for TNF-α/Rel proteins. The unique sequence flanking or overlapping the gene that binds p50 or p52 homodimers (15, 42) and the three A/T-rich sequences for binding to the architectural HMG I(Y) proteins (53). In the E-selectin gene, binding of HMG I(Y) proteins to A/T-rich sequences enhances binding of NF-κB heterodimers to the κB sites and facilitates interaction of NF-κB heterodimers with ATF-2/c-Jun proteins that bind to an adjacent ATF/CRE. These interactions contribute to formation of a highly organized enhanceosome (2, 32, 33). By analogy, the binding of HMG I(Y) proteins to A/T-rich sequences in the murine P-selectin gene may enhance binding of NF-κB to the tandem P-selectin κB sites and may facilitate binding of NF-κB to the weak κB element located 13 base pairs 3' to the tandem κB sites. Proteins binding to the P-selectin tandem κB sites and the A/T-rich sequences may also cooperate with other transcription factors to form an enhanceosome.

The reverse-oriented κB site in the murine P-selectin gene binds preferentially to p65 homodimers and is required for optimal TNF-α- or LPS-induced expression of a reporter gene driven by the murine P-selectin promoter. To our knowledge, this is the first reported example of an asymmetric κB element with an opposite orientation relative to the transcriptional start site. This element may function because it binds to symmetrical p65 homodimers that do not require a specifically oriented κB sequence to transactivate gene expression. The identification of this unusual κB site further supports the notion that p65 homodimers and NF-κB heterodimers have distinct biological functions (54–56).

Mutation of the variant ATF/CRE in the murine P-selectin gene eliminated binding to ATF-2 and other nuclear proteins, and it also decreased the TNF-α- or LPS-induced expression of a reporter gene driven by the murine P-selectin promoter. This suggests that activation of the JNK/p38 MAP kinases, which phosphorylate ATF-2, is required for TNF-α or LPS to optimally induce expression of P-selectin. Combined mutations of the κB sites and the variant ATF/CRE further decreased, but did not abolish, the TNF-α- or LPS-inducible expression, indicating that other regulatory elements also participate. Candidate elements include three putative AP1 sites identified in the preceding paper (38) and the low affinity κB site identified in this study. Deletional analysis did not reveal a role for these elements in TNF-α- or LPS-inducible expression of the murine P-selectin reporter gene. However, deletions may alter other regulatory elements that modulate the function of the deleted element.

TNF-α and LPS use strikingly similar mechanisms to induce expression of the murine P-selectin gene (this study) and the human and murine E-selectin genes (2). Both genes use κB sites and a variant ATF/CRE to transmit signals received from extracellular stimuli. The use of two or more signal-regulated elements may allow optimal adjustment of gene expression in response to a variety of challenges (12). The similar pathways by which TNF-α and LPS regulate the E-selectin gene and the murine P-selectin gene are consistent with the origin of the selectin gene family by gene duplication (57). This may partially explain the overlapping functions of P-selectin and E-selectin in the mouse (58–60). It also predicts that agents such as ultraviolet light and IL-1β, which activate NF-κB or JNK/ p38 kinases (2), will stimulate expression of the murine, but not the human, P-selectin gene.

Our studies have the inherent limitation that cultured endothelial cells may not contain the same transcription factors and signaling proteins found in microvascular endothelial cells in vivo. However, TNF-α or LPS clearly augments expression of both P- and E-selectin in murine endothelial cells in vivo (35, 36, 61, 62). By contrast, intradermal injection of LPS increases expression of E-selectin, but not P-selectin, in venules of nonhuman primates (63). Furthermore, E-selectin mRNA levels increase in the atria of patients after cardiopulmonary bypass, whereas P-selectin mRNA levels decline (64). These in vivo
studies further support a species-specific response of the P-selectin gene to TNF-α, LPS, or related mediators. Thus, the mechanisms for regulating the inducible transcription of the P-selectin gene appear to have evolved from mice to humans. This species-specific fine tuning of gene expression may extend to other inflammatory mediators and adhesion molecules. For example, either IL-4 or oncostatin M induces expression of P-selectin in murine and human endothelial cells. But induced expression in human cells is delayed and requires new protein synthesis, whereas induced expression in murine cells is more rapid and does not require new protein synthesis (37). IL-4 suppresses the TNF-α-inducible expression of E-selectin in human endothelial cells (65). IL-4 prevents E-selectin expression by activating Stat6, which binds to a DNA element that overlaps one of the xB sites in the human E-selectin gene, thereby competitively inhibiting binding of NF-κB (66). The Stat6 element, however, is not conserved in the murine E-selectin gene (34), suggesting that IL-4 may not suppress TNF-α-inducible expression of E-selectin in mice. Therefore, distinct mechanisms may be used to regulate expression of selectin genes in different species.

Our results also suggest that the function assigned to a selectin in a particular animal model may not necessarily apply to humans. In many rodent models of inflammation, tissue injury or other insults generate thrombin, histamine, oxygen-derived radicals, or other mediators that stimulate endothelial cells to redistribute P-selectin from Weibel-Palade bodies to the cell surface (67–69). Depending on the specific challenge, TNF-α, IL-β, or LPS may also be elaborated. These agents cause murine endothelial cells to increase synthesis of P-selectin, which may travel directly to the cell surface if the machinery that sorts proteins into Weibel-Palade bodies is saturated. Some models may induce expression of other cytokines such as IL-4 and oncostatin M, which can also increase synthesis of P-selectin. The relative contributions of these distinct pathways of inducible pathways may be difficult to distinguish in vivo. Our data suggest that mediators that activate p65-containing NF-κB dimers or ATF-2-containing dimers may increase transcription of the P-selectin gene in rodents, but not in humans. Close scrutiny of the mechanisms for inducing P-selectin expression in animal models may be necessary to interpret the relevance of the findings for human biology.

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