FEBS Letters 372 (1995) 194–198 FEBS 16048

Interleukin-4 induces endothelial vascular cell adhesion molecule-1 (VCAM-1) by an NF-κb-independent mechanism

John M. McCarty[a,*], Esther K. Yee[a], Theresa A. Deisher[b], John M. Harlan[a], Kenneth Kaushansky[a]

[a]*Department of Medicine, Division of Hematology, 357710 University of Washington, Seattle, WA 98195-7710, USA*
[b]*Repligen Corporation, One Kendall Square, Cambridge, MA 02118, USA*

Received 2 August 1995

Abstract While all features of the inflammatory response induced by IL-1 are not observed following IL-4 stimulation, suboptimal concentrations both cytokines result in synergistic VCAM-1 expression in HUVEC. We have shown that, while IL-1 stimulated HUVEC express GM-CSF, tissue factor and VCAM-1, only VCAM-1 is detectable after exposure to IL-4. While kB was found essential for both basal and IL-1-mediated activity of VCAM-1, IL-4 induction was kB-independent. Inducible kB-binding proteins were identified in IL-1-, but not IL-4-stimulated nuclear extracts. Our results indicate that IL-4 exerts its transcriptional effects on the VCAM-1 gene through element(s) which do not require kB.

Key words: VCAM-1; IL-4; Endothelial cell; Gene regulation

1. Introduction

Endothelial cells undergo morphological and biochemical changes in response to diverse inflammatory stimuli. This inflammatory phenotype involves the expression of multiple cytokines, coagulation and fibrinolytic factors and cytoadhesion molecules. Cytokines, such as IL-1, TNF-α and IL-4, act on endothelium to give rise to functional sets of proteins which initiate and support the inflammatory response. While the sets of genes induced in endothelium by stimulation with either TNF-α, IL-1 or LPS are quite similar, treatment with IL-4 results in a different response. For example, IL-4 downregulates a number of endothelial genes activated by either IL-1 or TNF-α, including tissue factor [1], ICAM-1 [2] and E-selectin [3]. In addition, IL-4 acts in synergy with IL-1 or TNF-α to express MCP-1 [4], IL-6 [5,6] and VCAM-1 [7,8]. These findings suggest that IL-4 acts through endothelial mechanisms distinct from those induced by IL-1 or TNF-α.

An important aspect of the endothelial response to inflammatory stimuli is the expression of VCAM-1 on the luminal surface. By providing a mechanism for adherence of lymphocytes, monocytes and eosinophils, VCAM-1 serves to both establish and amplify the inflammatory process [3,7–12] VCAM-1 is induced on endothelial surfaces by stimulation with LPS, IL-1, TNF-α [13] or IL-4 [7,8,14]. Previous studies have shown that, while the protein is expressed at the cell surface by 4–6 h, peaks at 24 h and declines toward baseline after 72 h of stimulation by IL-1 or TNF-α [15], its expression after IL-4 stimulation is delayed. Thornhill [8] demonstrated induction of VCAM-1 expression no earlier than 12–14 h, with peak expression at 24 h, depending on the concentration of IL-4 used.

Costimulation experiments performed by Thornhill [8] and Masinovsky [7] showed that submaximal levels of IL-1 and IL-4 synergistically induced the expression of VCAM-1 on HUVEC. Iademarco et al. [14] showed by flow cytometry (FACS) analysis of HUVEC stimulated by TNF-α and IL-4 that VCAM-1 expression increased to levels above that seen with either agent alone; in addition, the combination prolonged VCAM-1 protein at the cell surface beyond that seen with TNF-α alone. Together, these observations suggest that separate but cooperative pathways exist for the transcriptional activation of the VCAM-1 promoter by these cytokines.

Initial reports by Iademarco [13,16] and Neish [17] have identified some of the elements required for the expression of VCAM-1 in IL-1 and TNF-α-stimulated HUVEC. From these studies, octamer motifs, paired GATA sites and, perhaps most importantly, a pair of κB sequences were found to be essential for the transcriptional activation of the VCAM-1 gene in response to IL-1 and TNF-α. Particular attention has been paid to members of the *c-rel* family of transcription factors in the regulation of genes involved in the inflammatory response. Functional analysis of the promoters of the GM-CSF [18], tissue factor [19], M-CSF [20], IL-6 [21], E-selectin [22,23], ICAM-1 [24] and VCAM-1 [13,16,17] genes have demonstrated that NF-kB-binding sites are essential for the transcriptional regulation of these inflammatory mediators in endothelium, lymphocytes and monocytes. In contrast, little is known about the promoter elements required for IL-4-induced VCAM-1 expression.

In this paper, we report on the differential effects of IL-1 and IL-4 stimulation on the expression of VCAM-1 in HUVEC. Optimal concentrations of IL-1 and IL-4 for the induction of VCAM-1 protein were selected by a sensitive ELISA. In addition, ELISA and functional assays for GM-CSF and tissue factor were performed in order to determine whether IL-1 and IL-4 also differentially regulated the expression of these endothelial cell proinflammatory proteins. Both IL-1 and IL-4 were found to induce VCAM-1, but in contrast to IL-1, neither GM-CSF nor tissue factor were induced at any concentration of IL-4. These results reinforce the assertion that the endothelial cell activation pathways for IL-1 and IL-4 differ. Because the NF-κB complex has been found to be necessary for full expression of the VCAM-1 gene, we have focused on the functional role of these sequences in response to IL-4. Reporter

*Corresponding author. Fax: (1) (206) 685-3062.

Abbreviations: VCAM-1, vascular cell adhesion molecule-1; HUVEC, human umbilical vein endothelial cells; GM-CSF, granulocyte macrophage colony-stimulating factor; MCP-1, monocyte chemotactic protein-1; ICAM-1, intercellular adhesion molecule-1; ELISA, enzyme-linked immunosorption assay; EMSA, electrophoretic mobility shift assay.

SSDI 0014-5793(95)00976-0

gene analysis revealed that both constitutive and IL-1-mediated induction of VCAM-1 was dependent on κB. In contrast, IL-4-mediated induction of VCAM-1 was found to be independent of κB. This latter result was confirmed by EMSAs which demonstrated that, although κB-binding proteins could be detected in quiescent endothelial cell nuclei and their levels were increased by IL-1 stimulation, nuclear translocation of NF-κB was not seen in response to IL-4 stimulation. Thus, IL-4 induction of VCAM-1 represents a distinct activation mechanism in HUVEC, a finding which warrants further study.

2. Materials and methods

2.1. ELISA of HUVEC

Second passage HUVEC were grown to confluence using culture conditions previously described [25] in duplicate gelatin-coated 96 well plates. After stimulation with cytokines, the supernatants were removed from each plate and assayed for GM-CSF by ELISA activity as previously described [26]. One plate was assayed for VCAM-1 by ELISA using mAb 4B9 as described [9]. The second plate was assayed for tissue factor activity in the method described below.

2.2. Procoagulant activity assay

Tissue factor activity was assayed by the method of Suprenant [27]. Following stimulation with cytokines, HUVEC grown in 96 well plates were decanted of media and excess was blotted off with paper towels. 100 μl of 0.02% saponin in medium was added to each well and incubated at room temperature for 10 min. After decanting the saponin-containing medium, 50 μl of a mixture of 212 μg/ml S-222 (KBI Pharmacia, Franklin, OH) and 10 U/ml Factor VII-Proplex T solution, in HUVEC medium without phenol red was added, and allowed to incubate at 37°C for 2 h. The activity was read using a Biokinetic plate reader (Biotek Instruments, Winsooki, VT) at 405nm at 1 and 2 h.

2.3. Reporter gene assay

Reporter gene constructs were assembled by linking the proximal 504 bp upstream of the ATG site of the VCAM-1 gene to chloramphenicol acetyl transferase (CAT) as a reporter gene. Construct WTCAT contains the wild-type promoter sequences; the NF-κB consensus binding sequences have been substituted with random sequences (−79 CTCTATGACCTCAATTGCTCTGCAGTTCTCCGCCTCTGCCTCCG −49) in κBμCAT. After transfection into cultured HUVEC by electroporation, reporter gene assays were performed as previously described [28].

2.4. EMSA

Using nuclear extracts from IL-1- and IL-4-stimulated HUVEC prepared in the manner described by Dignam [29], EMSA were performed using duplex oligonucleotides containing the κB consensus binding sequences from VCAM-1 (AATTCCCTGGTTTCCCCTTGAAGGGATTTCCCTCC) and from the human immunoglobulin Igκ enhancer (AATTTCCCCAGAGGGGGATTTCCAAGAGGCC) as previously described [30].

2.5. Reagents

The IL-1 and IL-4 used in this study were generous gifts of S. Gillis (Immunex, Seattle, WA). Anti-VCAM-1 monoclonal antibodies were prepared as described [9].

3. Results

3.1. IL-4 induces VCAM-1 protein

As shown in Fig. 1, IL-1 induced endothelial cell expression of VCAM-1, GM-CSF and tissue factor in a concentration-dependent manner. In contrast, IL-4 induced VCAM-1, but not GM-CSF or tissue factor. A difference in the time course of IL-1- and IL-4-induced VCAM-1 expression was also apparent. VCAM-1 was induced by IL-1 within 4 h and declined after 24 h. In contrast, while IL-4 induction of VCAM-1 was also detectable within 4 h, expression continued to increase at 48 h.

3.2. Functional assessment of VCAM-1 promoter activity

Reporter gene assays were performed to evaluate the relative functional contribution of κB sequences in the regulation of VCAM-1 expression. The WTCAT construct was chosen for study as it contains the two κB sites and in preliminary experiments was found to be a highly active reporter, a finding consistent with that of Iademarco [16] and Neish [17]. A mutant construct in which the κB motifs were altered to random sequences, κBμCAT, was used to test the contribution of κB to IL-1 and IL-4 induction of VCAM-1. As shown in Table 1, stimulation of the WTCAT construct by either IL-1 or IL-4 resulted in a 1.4–2.4-fold induction in CAT expression. Compared with the wild-type vector, constitutive reporter gene activity of the κBμCAT construct was substantially reduced. However, despite this lower baseline activity, culture with IL-4, but not with IL-1, resulted in a 2–3-fold induction of reporter gene activity. While differences in reporter gene activity between the two constructs were seen up to 48 h after stimulation, the most marked difference was observed at 24 h.

3.3. EMSA demonstrates no IL-4-inducible κb-binding proteins

In order to confirm our results with the reporter gene assays, we next performed EMSAs using oligonucleotide probes con-

Table 1
Relative CAT activity of the VCAM-1 promoter comparing WTCAT and κBμCAT constructs

	24 h			48 h		
	Fold	induction	S.E.M.	Fold	induction	S.E.M.
WTCAT						
None	(1.00)		(0.00)	(1.00)		(0.00)
IL-1 0.5 U/ml	1.57		0.14	2.40		0.01
IL-1 20 U/ml	2.00		0.00	1.43		0.10
IL-4 500 U/ml	1.92		0.25	1.59		0.02
IL-4 5000 U/ml	2.32		0.13	1.36		0.13
κBμ CAT						
None	0.26	(1.00)	0.06	0.38	(1.00)	0.11
IL-1 0.5 U/ml	0.26	1.00	0.06	0.46	1.21	0.05
IL-1 20 U/ml	0.13	0.50	0.00	0.39	1.00	0.11
IL-4 500 U/ml	0.83	3.20	0.15	0.79	2.07	0.05
IL-4 5000 U/ml	0.63	2.42	0.17	0.24	1.58	0.05

Results are reported as fold induction relative to unstimulated WTCAT (data columns 1 and 4) or to unstimulated κBμCAT (data columns 2 and 5). S.E.M. of nine experiments reported in data columns 3 and 6.

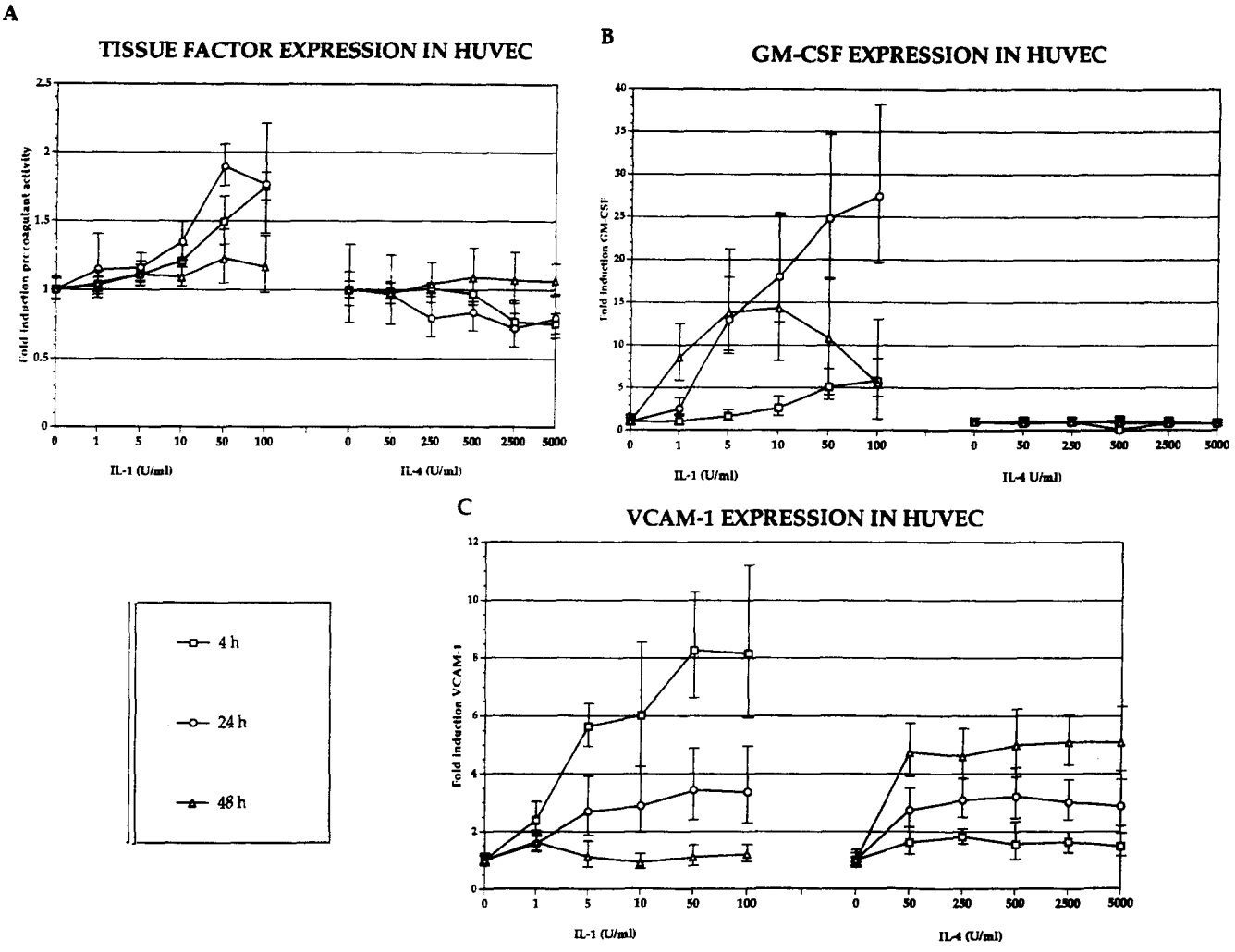

Fig. 1. Induction of tissue factor, GM-CSF and VCAM-1 by IL-1 and IL-4 in cultures HUVEC. Tissue factor was assayed by procoagulant activity assay and GM-CSF and VCAM-1 were detected by sensitive ELISA. *Y*-axis scales vary for each graph. Dose–response curves for IL-1 and IL-4 are shown for 4, 24 and 48 h after stimulation. Error bars represent 2 S.D. of the mean.

taining the VCAM-1 κB sites. As shown in Fig. 2, both high and low concentrations of IL-1 induced nuclear proteins which specifically bound to the VCAM-1 κB motifs. In contrast, κB-binding activity above that of unstimulated cells was not detected with either oligonucleotide probe using nuclear extracts from endothelial cells stimulated with both concentrations of IL-4. Additional assays performed using an oligonucleotide containing the immunoglobulin NF-κB-binding sequences also failed to demonstrate specific binding to κB sequences from IL-4-stimulated HUVEC nuclear extracts (data not shown). The use of an Oct-1 consensus binding motif probe confirmed the integrity of nuclear extracts used in this assay.

4. Discussion

IL-4 appears to play an important role in the physiology of the endothelial response to inflammatory and immune-reactive processes. IL-4 activation of endothelium may contribute to the recruitment of eosinophils and mononuclear leukocytes in chronic inflammatory diseases ranging from asthma to atherosclerosis. In contrast to activation by IL-1, TNF-α or LPS, activation by IL-4 results in the expression of VCAM-1, but not ICAM-1 or E-selectin [2] on endothelial surfaces. This selectivity results in the preferential adherence of eosinophils and mononuclear leukocytes rather than neutrophils. Other features of the inflammatory response produced by IL-1 and TNF-α, such as induction of procoagulant activity [1] and secretion of GM-CSF, were not observed with IL-4.

In this study, we investigated the basis of IL-4 induction of VCAM-1 expression in HUVEC. While many reports have detailed mechanisms of HUVEC gene regulation following stimulation with IL-1, TNF-α and LPS, there have been fewer reports on the effects of IL-4. By demonstrating synergy of IL-1 and IL-4 in VCAM-1 induction, studies by Thornhill [1,8] and by Masinovsky [7] suggested that the IL-4 regulatory pathway may be distinct. Additional studies have reported that IL-4 modulated the expression of endothelial cell inflammatory genes induced by IL-1. For example, while IL-4 downregulates the IL-1-mediated induction of tissue factor [1], it counters the IL-1 downregulation of thrombomodulin [31]. More recent studies by Kapiotis [32] show that coincubation of HUVEC with pyrogens (IL-1, TNF-α or bacterial lipopolysaccharide) and IL-4 differentially modulated the expression of endothelial cell adhesion molecules. While this combination of cytokines

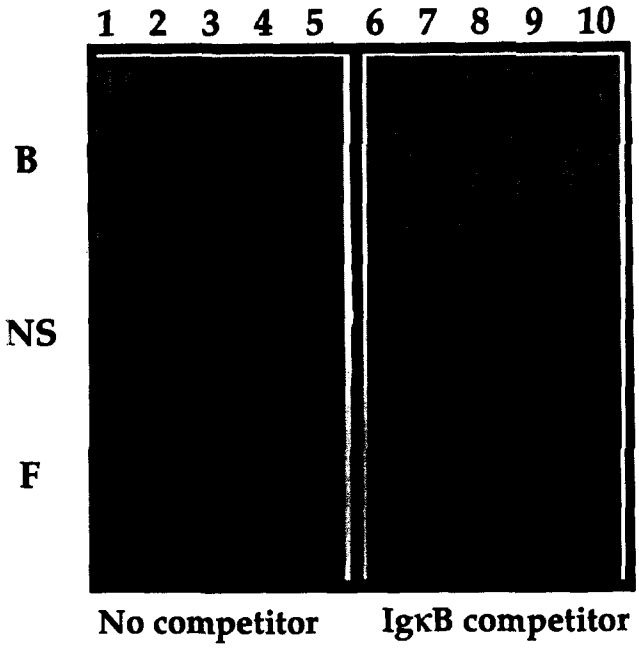

Fig. 2. EMSAs of VCAM-1 κB motifs with HUVEC nuclear extracts. Lane numbers correspond to HUVEC nuclear extracts stimulated under the following conditions: 1,6) unstimulated; 2,7) IL-1 0.5 U/ml for 4 h; 3,8) IL-4 500 U/ml for 4 h; 4,9) IL-1 0.5 U/ml for 24 h; 5,10) IL-4 500 U/ml for 24 h. Reactions in lanes 6–10 also contain unlabeled immunoglobulin κB motifs at a 50-fold relative concentration as a competitor. B, specifically binding shifted band; NS, non-specific bands; F, free probe.

did not affect the expression of ELAM-1 in their study, a downregulation of ICAM-1 expression and and an enhancement of VCAM-1 expression was reported. The comparison of VCAM-1, GM-CSF and tissue factor in the present study is thus representative of the different sets of genes induced in each of the induced phenotypes. Whereas tissue factor, GM-CSF and VCAM-1 are all induced by IL-1, only VCAM-1 is induced by IL-4. Moreover, our results show that there are differences in both the magnitude and duration of VCAM-1 expression after IL-1 and IL-4 stimulation of HUVEC. In general, the time course of VCAM-1 gene expression induced by IL-4 can be characterized by a later and more sustained induction than that induced by IL-1. In contrast, a higher peak fold induction of VCAM-1 protein is seen after IL-1 stimulation. By ELISA, VCAM-1 cell-surface protein expression progressively increased over the 48 h of the time course studied at all concentrations of IL-4. Together, these findings point to an IL-4 regulatory pathway that is at least partly independent of that employed by IL-1. Because IL-4 stimulation of HUVEC gives rise to a select repertoire of inflammatory proteins, these mechanisms must be mediated by relatively restricted signals rather than by global cellular processes.

Previous studies by Iademarco et al. have investigated the mechanisms of IL-4-induced VCAM-1 expression [14]. They concluded that in contrast to the transcriptional activation induced by TNF-α, IL-4 increased mRNA stability but failed to show appreciable reporter gene activity. However, only very low levels of VCAM-1 were detected on endothelial cells stimulated for up to 72 h in that study. In contrast, while our ELISA studies did not extend beyond 48 h, high level expression was detected and VCAM-1 levels continued to increase throughout the time course of our study. The difference in VCAM-1 cell-surface expression in the two studies may reflect differences in tissue culture conditions which could account for the differences in transcriptional activity reported in the present study. Endothelial cells in culture are known to lose their ability to express proteins under select conditions or with serial passage, such as seen with P-selectin (J. Harlan, pers. commun.). In addition, compared with the WTCAT construct used in the present study, the 2180VCAMCAT reporter construct used in the studies of Iademarco contained additional upstream flanking sequences. Differences in transcriptional activity might also be due to the presence of *cis*-acting elements in the larger reporter construct which downmodulate the transcriptional activity mediated by our WTCAT promoter sequences. Despite these arguments, however, it is clear that transcriptional effects do not completely explain the mechanism of IL-4-mediated induction of VCAM-1 in HUVEC. Other mechanisms, such as the transcriptional contribution of other upstream *cis*-acting sequences, or the influence of posttranscriptional effects on VCAM-1 mRNA, may complete the pathway of VCAM-1 induction by IL-4.

The reporter gene data suggests that, while NF-κB is not required for VCAM-1 induction by IL-4, it is still important in the constitutive expression of the promoter. One explanation for the latter finding may be that nuclear proteins bind to κB sequences in the absence of cell stimulation and that these transcription factors modulate the constitutive expression of the gene. Constitutive expression of κB-binding proteins has been previously reported for TNF-α production in quiescent macrophages [33]. We report similar findings here for the VCAM-1 gene. However, examination of our reporter gene results show that the IL-4-stimulated transcriptional activity of the κB mutant is lower than that of the wild-type construct. This implies that factors occupying the VCAM κB complex are necessary for full IL-4-induced expression of the gene. At least four distinct proteins have been shown to bind to κB sequences, in both homo- and heterodimeric configurations [34]. DNA-protein crosslinking studies, cotransfection experiments with specific κB family members and antisense oligonucleotide inhibition experiments will help to identify the transcription factors mediating this effect more precisely.

Our results demonstrate that transcriptional response elements for IL-1 and IL-4 lie within 289 bp from the CAP site of the VCAM-1 gene. The reporter gene assays show that these elements support a 1.4- to 2.4-fold increase in promoter activity in response to cytokine stimulation. While this study found at least one of the IL-1 response elements to be κB, IL-4 induction of the mutant κB construct implies that an IL-4 response element lies outside of the κB motifs of VCAM-1. The EMSA studies performed in this report support our conclusions that NF-κB is not induced in IL-4-stimulated endothelial cells. Iademarco [14] also noted that immunolabeling experiments did not show differences in either the level or the localization of NF-κB or *rel* family members after IL-4 stimulation. Thus, these results begin to unravel the molecular basis for the synergistic induction of VCAM-1 protein expression on HUVEC observed with low concentrations of IL-4 and IL-1 [7,8].

Finally, although it is clear that the use of a truncation construct of the VCAM-1 promoter is artificial, we feel that our observations remain valid as these proximal sequences retain

functional activity consonant with the endogenous gene, demonstrating responsiveness to both IL-1 and IL-4. Furthermore, this construct confers tissue specificity as transfection into lymphocyte cell lines generated no basal or induced CAT activity (data not shown). Additionally, previously published functional analyses of the VCAM-1 promoter using constructs containing additional upstream sequences failed to demonstrate augmented basal or IL-1-stimulated promoter activity [13,16,17]. Induction of VCAM-1 protein expression on HUVEC by IL-4 appears to be an NF-κB-independent process. In contrast, full expression of VCAM-1 in response to inflammatory cytokines requires intact κB sequences. These findings suggest that a non-κB regulatory pathway acts in concert with κB to induce VCAM-1. Although the inflammatory response is likely due to the combined action of cytokines, understanding the activation mechanisms of each individual stimulus is necessary groundwork for studying coordinate induction. Future studies will be directed at the identification of the IL-4-responsive elements in the VCAM-1 which are responsible for the transcriptional upregulation of VCAM-1 gene expression induced by IL-4. Given the participation of IL-4 in the promotion of atopic processes [35] and the potential role of VCAM-1 in the recruitment of eosinophils in allergic reactions [36], a better definition of the molecular pathways involved in IL-4 induction of VCAM-1 may yield insights into the pathophysiology of allergic conditions.

Acknowledgements: This work was supported by National Research Service Award 5F32 DK087741–02, a Daland Fellowship from the American Philosophical Society (to J.M. McCarty), National Insitutes of Health Grants R01 CA31615 (to K. Kaushansky) and U01 HL47151 (to J.M. Harlan).

References

[1] Herbert, J.M., Savi, P., Laplace, M.C. and Lale, A. (1992) FEBS Lett. 310, 31–3.
[2] Renkonen, R., Mattila, P., Majuri, M.L., Paavonen, T. and Silvennoinen, O. (1992) Scand. J. Immunol. 35, 525–30.
[3] Bochner, B.S., Luscinskas, F.W., Gimbrone Jr., M.A., Newman, W., Sterbinsky, S.A., Derse-Anthony, C.P., Klunk, D. and Schleimer, R.P. (1991) J. Exp. Med. 173, 1553–7.
[4] Rollins, B.J. and Pober, J.S. (1991) Am. J. Pathol. 138, 1315–9.
[5] Paleolog, E.M., Aluri, G.R. and Feldmann, M. (1992) Cytokine 4, 470–8.
[6] Moutabarrik, A., Ishibashi, M., Namiki, M., Kameoka, H., Kokado, Y., Yoshizaki, K., Takano, Y., Yokokawa, K., Takahara, S., Seguchi, T. et al. (1992) Transplant Proc. 24, 2898–9.
[7] Masinovsky, B., Urdal, D. and Gallatin, W.M. (1990) J. Immunol. 145, 2886–95.
[8] Thornhill, M.H., Wellicome, S.M., Mahiouz, D.L., Lanchbury, J.S., Kyan-Aung, U. and Haskard, D.O. (1991) J. Immunol. 146, 592–8.
[9] Carlos, T.M., Schwartz, B.R., Kovach, N.L., Yee, E., Rosa, M., Osborn, L., Chi-Rosso, G., Newman, B., Lobb, R. et al. (1990) Blood 76, 965–70.
[10] Dobrina, A., Menegazzi, R., Carlos, T.M., Nardon, E., Cramer, R., Zacchi, T., Harlan, J.M. and Patriarca, P. (1991) J. Clin. Invest. 88, 20–6.
[11] Rice, G.E., Munro, J.M. and Bevilacqua, M.P. (1990) J. Exp. Med. 171, 1369–74.
[12] Schwartz, B.R., Wayner, E.A., Carlos, T.M., Ochs, H.D. and Harlan. J.M. (1990) J. Clin. Invest. 85, 2019–22.
[13] Iademarco, M.F., McQuillan, J.J. and Dean, D.C. (1993) Proc. Natl. Acad. Sci. USA 90, 3943–7.
[14] Iademarco, M.F., Barks, J.L. and Dean, D.C. (1995) J. Clin. Invest. 95, 264–71.
[15] Osborn, L., Hession, C., Tizard, R., Vassallo, C., Luhowskyj, S., Chi-Rosso, G. and Lobb, R. (1989) Cell 59, 1203–11.
[16] Iademarco, M.F., McQuillan, J.J., Rosen, G.D. and Dean, D.C. (1992) J. Biol. Chem. 267, 16323–9.
[17] Neish, A.S., Williams, A.J., Palmer, H.J., Whitley, M.Z. and Collins, T. (1992) J. Exp. Med. 176, 1583–93.
[18] Schreck, R. and Baeuerle, P.A. (1990) Mol. Cell Biol. 10, 1281–6.
[19] Mackman, N., Brand, K. and Edgington, T.S. (1991) J. Exp. Med. 174, 1517–26.
[20] Yamada, H., Iwase, S., Mohri, M. and Kufe, D. (1991) Blood 78, 1988–95.
[21] Libermann, T.A. and Baltimore, D. (1990) Mol. Cell Biol. 10, 2327–34.
[22] Montgomery, K.F., Osborn, L., Hession, C., Tizard, R., Goff, D., Vassallo, C., Tarr, P.I., Bomsztyk, K., Lobb, R., Harlan, J.M. et al. (1991) Proc. Natl. Acad. Sci. USA 88, 6523–7.
[23] Whelan, J., Ghersa, P., Hooft-van Huijsduijnen, R., Gray, J., Chandra, G., Talabot, F. and DeLamarter, J.F. (1991) Nucleic Acids Res. 19, 2645–53.
[24] Voraberger, G., Schafer, R. and Stratowa, C. (1991) J. Immunol. 147, 2777–86.
[25] Broudy, V.C., Kaushansky, K., Harlan, J.M. and Adamson, J.W. (1987) J. Immunol. 139, 464–8.
[26] Brown, C.B., Hart, C.E., Curtis, D.M., Bailey, M.C. and Kaushansky, K. (1990) J. Immunol. 144, 2184–9.
[27] Surprenant, Y.M. and Zuckerman, S.H. (1989) Thromb. Res. 53, 339–46.
[28] Kaushansky, K. (1989) J. Immunol. 143, 2525–9.
[29] Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. (1983) Nucleic Acids Res. 11, 1475–89.
[30] Shoemaker, S.G., Hromas, R. and Kaushansky, K. (1990) Proc. Natl. Acad. Sci. USA 87, 9650–4.
[31] Kapiotis, S., Besemer, J., Bevec, D., Valent, P., Bettelheim, P., Lechner, K. and Speiser, W. (1991) Blood 78, 410–5.
[32] Kapiotis, S., Quehenberger, P., Sengoelge, G., Partan, C., Eher, R., Strobl, H., Bevec, D., Zapolska, D., Schwarzinger, I. and Speiser, W. (1994) Circ. Shock 43, 18–25.
[33] Collart, M.A., Baeuerle, P. and Vassalli, P. (1990) Mol. Cell Biol. 10, 1498–506.
[34] Shu, H.B., Agranoff, A.B., Nabel, E.G., Leung, K., Duckett, C.S., Neish, A.S., Collins, T. and Nabel, G.J. (1993) Mol. Cell Biol. 13, 6283–9.
[35] Ricci, M. (1994) Clin. Exp. Allerg. 24, 801–12.
[36] Resnick, M.B. and Weller, P.F. (1993) Am. J. Respir. Cell Mol. Biol. 8, 349–55.