Cytokine-induced Down-regulation of zfm1/Splicing Factor-1 Promotes Smooth Muscle Cell Proliferation*

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One hallmark of inflammation is the proliferation of bystander cells such as vascular smooth muscle cells (SMC), a process governed by growth factors and cytokines. Whereas cytokine induction of gene products promoting inflammation and proliferation is well characterized, little is known about the concomitant down-regulation of potentially counter-regulatory gene products in these cells. By employing the suppression subtractive hybridization-PCR technique, RNA isolated from rat aortic SMC treated with the cytokines interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα) was subtracted from RNA of control cells. Eleven genes were identified, the expression of which fell by 44–77%. One, the transcripional repressor splicing factor-1 or zfm1, was characterized further. Antisense oligonucleotide hybridization mimicked the stimulatory effects of IL-1β and TNFα on SMC proliferation and expression of the chemokine MCP-1 and the vascular cell adhesion molecule-1. Moreover, in an in vivo mouse model of atherosclerosis, zfm1 abundance was decreased in proliferating arterial SMC. These findings suggest a role for zfm1 in controlling both proliferation and expression of pro-inflammatory gene products in SMC. Therefore, cytokine-induced down-regulation of zfm1 expression may contribute to the pathogenesis of hyperproliferative inflammatory diseases.

Pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα) play an important role both in acute and chronic inflammation. The consequences of a prolonged exposure of bystander cells to these cytokines are best characterized for vascular smooth muscle cells (SMC) in atherosclerosis (1) with several animal models demonstrating dedifferentiation of SMC upon cytokine exposure. Thus, pro-vascular SMC treated with IL-1β undergo phenotype changes in vivo very much like that of neointimal SMC in atherosclerotic lesions (2). In hypercholesterolemic rabbits, blockade of TNFα by exogenously applied neutralizing antibodies prevents coronary artery stenosis following heart transplantation (3). Moreover, pro-inflammatory cytokines such as IL-1β and TNFα are highly expressed in humans during various inflammatory conditions such as atherosclerosis (4), wound healing (5), or asthma (6).

The pro-inflammatory effect of these cytokines seems to involve the up-regulation of various gene products such as the chemokine monocyte chemoattractant protein-1 (MCP-1 (7)) or the vascular cell adhesion molecule-1 (VCAM-1 (8)), capable of recruiting circulating leukocytes to the site of inflammation. The infiltrating leukocytes in turn activate adjacent bystander cells resulting in increased mitogenesis. Moreover, MCP-1 not only acts as a chemoattractant for leukocytes but also can directly induce proliferation and de-differentiation of vascular SMC (9, 10). Although MCP-1 and VCAM-1 play an important role in cytokine-induced inflammation as, e.g. in atherosclerosis, it is unlikely that they are the only important components of the pro-inflammatory response to IL-1β plus TNFα.

Therefore, in addition to the many known genes induced by IL-1β plus TNFα, it is important to identify gene products, which help to maintain the phenotype of bystander cells in inflamed tissue, but are down-regulated upon exposure to pro-inflammatory cytokines. To identify such gene products, cultured vascular SMC were used as a model system. Subtractive hybridization of cdNA derived from rat aortic cultured SMC, which were exposed to IL-1β plus TNFα, against cdNA isolated from quiescent SMC combined with suppression PCR analysis (11) was performed, followed by cloning and sequencing of the differentially expressed gene products.

MATERIALS AND METHODS

Cell Culture—Rat aortic SMC were isolated by the explant technique and serially cultured up to passage 4 as previously described (12). Culture media were exchanged 1 h before the start of the incubations in the absence or presence of IL-1β (60 units/ml) plus TNFα (1000 units/ml), as indicated in the “Results” section.

Cell Quantification—Determination of cell number was performed with the CyQuant kit (Molecular Probes via MoBiTec, Göttingen, Germany) according to the manufacturer’s instructions.

RT-PCR Analysis—Isolation of total RNA from the cells and semi-quantitative RT-PCR analysis was performed as previously described (12). The following primers were used for PCR: MCP-1: 5'-ACCTGCT-GCTACTCTTCAACTC-3', 5'-CATCTTGACTAATGAGATTCT-3' (product spans 1 intron in the rat MCP-1 gene, cDNA length is 454 bp); VCAM-1: 5'-TGGAGCGAGAAATAATTGATG-3', 5'-CACATGCT-AGGAGATGTGAGAC-3' (product spans 1 intron in the human VCAM-1 gene, cDNA length is 1104 bp); EF-2: 5'-GACATGCT-GCTACTCTTCAACTC-3', 5'-GAGCTACCAAGAAAAGGTTG-3' (no intron-spanning product, cDNA length is 220 bp); NOS: 5'-ATGGCTTCGCCCTTGGCAA-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF329825, AF329826, AF329828, and AF329827.

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‡ The abbreviations and trivial names used are: IL-1β, interleukin-1β; EF-2, elongation factor-2; IL-6, interleukin-6; apoE, mouse, mouse strain deficient in the apolipoprotein E gene; as-ODN, antisense oligodeoxynucleotide; sc-ODN, scrambled oligodeoxynucleotide; HMG-17, high mobility group-17; INOS, inducible nitric oxide synthase; IFNγ, interferon-γ; MCP-1, monocyte chemoattractant protein-1; FNCA, proliferating cell nuclear antigen; PDGF-BB, platelet-derived growth factor (BB-homodimer); RPA, replication protein-1; RT, reverse transcriptase; SMC, smooth muscle cells; SSSH, suppression subtractive hybridization; TNFα, tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule-1; RACE, rapid amplification of cDNA ends.

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Role of zfml in Smooth Muscle Cells

In Vivo Model of Vascular Inflammation—Housing and treatment of the animals was performed according to the German legislation on the protection of animals with a 12-h day and night rhythm. Mice deficient in apolipoprotein E (apoE®) mice, Jackson Laboratories, Bar Harbor, ME) and backcrossed 10 times to their genetic background (C57Bl/6j x 129S5/SvEv) were fed a high fat diet (21.2% fat, 0.15% cholesterol; Harlan Teklad TD88137) for 22 weeks. At the age of 30 weeks, a 10% (w/v) ferric chloride solution was applied for 3 min to the adventitia of the left common carotid artery to induce arterial thrombosis and neointima formation as described previously (16). After this procedure, wounds were closed and animals kept under standard conditions. Ferric chloride-induced injury resulted in the formation of a 2- to 3-mm-long thrombus and consecutive neointima formation (7–21 days post injury). Mice were killed after the times indicated by an overdose of Metofane (Schering-Plugen, Kenilworth, NJ), and the carotid arteries were excised, fixed for 4 h in 4% zinc formalin (Shandon, Frankfurt, Germany), and subjected to immunohistochemistry analysis.

Immunohistochemistry—Formalin-fixed segments of the mouse carotid artery were embedded in paraffin blocks of which 5- to 7-μm-thick sections were cut. After paraffin removal (boiling of the glass-fixed sections six times for 5 min in 10 ml sodium citrate, pH 6.0), they were inactivated in 10 ml Tris buffer, pH 7.6, containing 3% (w/v) hydrogen peroxide followed by blocking of nonspecific binding sites for 20 min at room temperature with 10% bovine serum albumin in Hanks’ balanced salt solution (Invitrogen). The secondary rabbit polyclonal anti-zfml antibody (15) was diluted 1:100 in 10 ml Tris buffer, pH 7.6, and incubated with the mounted sections for 2 h at room temperature. After thorough rinsing with 10 ml Tris buffer, pH 7.6, a peroxidase-coupled secondary antibody (anti-rabbit, Envision, Dako, Hamburg, Germany) was added and incubated for 30 min at room temperature. Thereafter, the sections were rinsed again, incubated for 30 min at room temperature with the ABC chromogen solution (Dako), counterstained with hematoxylin if suitable (Merck, Darmstadt, Germany), rinsed for 10 min with water, and finally mounted with Aquamount sealing medium (Gurr, Hanau, Germany). The stained sections were analyzed by light microscopy with brown nuclei counted as zfml-positive. In some experiments, monoclonal antibodies against the replication protein-1 (RPA; Calbiochem-Novabiochem, Bad Soden, Germany) and/or the DNA-polymerase subunit PCNA (proliferative cell nuclear antigen; Dako) were used in essentially the same manner as described above.

Statistical Analysis—Unless indicated otherwise, results are expressed as means ± S.E. of n observations with cells obtained from the aortas of different animals. A one sample t test with a two-sided p value or one-way analysis of variance followed by a Dunnett post test was used where appropriate to determine statistically significant differences between the means and control with p < 0.05 considered significant.

RESULTS

Differential Gene Expression—To characterize genes down-regulated upon cytokine exposure (IL-1β (60 units/ml) plus TNFα (1000 units/ml) for 6 h), a subtractive hybridization followed by suppression PCR (SSH-PCR) was performed (11), and 15 clones derived from this procedure were analyzed. To confirm their differential expression in the cytokine-treated SMC, Northern blot, RT-PCR, or Western blot analyses (depending on antibody availability) were performed for these gene products. According to Northern blot analysis, an average decrease in e.g. zyxin, HMG-17, and zfml mRNA expression of 60%, 55%, and 51% was determined after 6-h exposure of the SMC to IL-1β plus TNFα (Fig. 1, Table I). An identical decrease in mRNA expression was also detected by RT-PCR analysis (Fig. 1b) and, in the case of zyxin and zfml, correlated with the concomitant decrease in protein abundance (60% and 56% decrease, respectively), as judged by Western blot analysis (Fig. 1c).

Of the 15 clones analyzed, two were cloning artifacts, down-regulation by IL-1β/TNFα of one gene product (KIAA0067) could not be confirmed, and one clone could not be unambiguously identified using GenBank™ and was therefore omitted from further analysis. Thus, 11 of 15 clones represented truly down-regulated gene products (Table I). Although partly unknown, all identified gene products either have a high degree of homology to already known genes (e.g. HSR1 homologue) or to
homologues from other species, which are well characterized (e.g. zyxin; cf. Table I). Moreover, the inhibitory effect of IL-1β and TNFα on the expression of these genes, at least for zfm1 and zyxin, appeared to occur at the level of transcription, as confirmed by nuclear run-on analyses (Fig. 2A).

Role of zfm1—The transcriptional repressor splicing factor-1 or zfm1 was chosen for further analysis on a functional basis. First, zfm1 protein was characterized. The predominant protein recognized by the antiserum (15) revealed a molecular mass of 70 kDa in the Western blot analysis, corresponding to the splice variant zfm1A. No band with a molecular mass of 55 kDa was detected, indicating that the other splice variant capable of interfering with transcription, zfm1E (13), is not expressed by the cultured SMC. Isoform-specific PCR analysis confirmed that only zfm1A but no zfm1E mRNA is expressed by the cultured SMC (Fig. 2B).

Second, the effects of IL-1β and TNFα on zfm1 expression were monitored over time. Cytokine-induced down-regulation of zfm1 mRNA in the cultured SMC was detectable for at least 18 h (Fig. 2C). Although the inhibitory effect of the

| Gene product (length of clone) | Function and homology to known genes from rat or other species | Level of expression |
|-------------------------------|---------------------------------------------------------------|--------------------|
| CaBP-1 (410 bp)               | Redox-active subunit of endoplasmic 4-hydroxyprolylhydrodase (100% to rat) | 51 ± 11a |
| Cathepsin B (360 bp)          | Secretory protease (99% to rat)                               | 43 ± 7a |
| D54 (410 bp)                  | Member of a protein family involved in calcium signalling (65% to human) | 49 ± 7a |
| Fln-29 (580 bp)               | Putative human TRAF-interacting zinc finger protein (84% to human) | 33 ± 21 |
| HMG-17 (330 bp)               | High mobility group transcriptional regulator family (88% to mouse) | 49 ± 14 |
| Osteoprotegerin (400 bp)      | Hormone involved in bone metabolism (100% to rat)            | 23 ± 12a |
| Protein disulfide isomerase (620 bp) | ER enzyme involved in the processing of secretory proteins (100% to rat) | 38 ± 6a |
| TCTP-21 (210 bp)              | Cell growth and tumor-related calcium binding protein (82% to mouse) | 35 ± 15a |
| zfm1 (300 bp)                 | Transcriptional repressor/splicing factor (100% to rat)       | 44 ± 8a |
| Zyxin (520 bp)                | Cytoskeleton-associated zinc finger protein (92% to mouse)     | 40 ± 12a |
| GTP-binding protein HSR1 (230 bp) | Not known, similar to the human HSR1 gene (81%)               | 44 ± 10a |

*a p < 0.05 versus control.
Role of zfm1 in Smooth Muscle Cells

The rate of proliferation of the cultured SMC by 40% over a 24-h period (Fig. 4). Down-regulation of zfm1 protein expression (48-h pre-treatment with the as-ODN) resulted in a virtually identical increase in the rate of SMC proliferation. Pre-treatment with the as-ODN plus exposure to IL-1β and TNFα did not result in a further increase in the rate of proliferation whereas the sc-ODN alone had no appreciable effect (Fig. 4A).

Interestingly, interferon-γ (1000 units/ml) and platelet-derived growth factor (PDGF-BB, 0.5 ng/ml), when compared with IL-1β and TNFα, exerted a similar effect on SMC proliferation (Fig. 4A) and zfm1 expression (Fig. 4B) whereas other cytokines such as IL-6 (0.5 ng/ml) or IL-10 (1 ng/ml) or another growth factor-like agent, insulin (10 nm), had no such effect (Fig. 4B).

Finally, the consequences of the decrease in zfm1 protein abundance for MCP-1 and VCAM-1 expression were analyzed. Pre-treatment of the cultured SMC with the as-ODN for 24 h resulted in an increase of ~2-fold in expression of these pro-inflammatory gene products, while the sc-ODN had no such effect (Fig. 5B). In comparison, exposure of the SMC to IL-1β plus TNFα for 6 h triggered a 5- to 6-fold increase in MCP-1 and VCAM-1 gene expression and, in addition, induced expression of the inducible isoform of nitric oxide synthase (iNOS) in these cells. The latter effect was not mimicked by pre-treating the SMC with the zfm1 as-ODN (Fig. 5A). Exposure of the SMC to IL-1β plus TNFα after down-regulation of zfm1 expression by the as-ODN had no additional or even synergistic effect on MCP-1 or VCAM-1 expression when compared with the effects of the cytokines alone (Fig. 5B).

**Regulation of zfm1 Expression in Vivo**—In addition to the cultured SMC, zfm1 expression was monitored in a model of vascular lesion formation triggered by pro-inflammatory cytokines in vivo. Ferric chloride-induced injury of the carotid artery of cholesterol-fed apoE−/− mice was accompanied by a transient down-regulation of zfm1 protein in the SMC of the media and the developing neointima. Only after completion of neointima formation and structural stabilization of the vessel wall at ~3 weeks, zfm1 expression re-occurred in the nuclei of neointimal and medial SMC (Fig. 6A). At this stage, i.e. when SMC proliferation was impeded, SMC expressing high amounts of zfm1 protein and cells almost devoid of zfm1 could be observed in parallel. Interestingly, there was no nuclear co-expression of zfm1 and PCNA, a marker for proliferation, at this time point, as exemplified by a cross-section of a carotid artery 3 weeks post ferric chloride injury (see Fig. 6B). Virtually the same inverse immunostaining was observed with another proliferation marker, RPA (not shown).

**DISCUSSION**

**Inflammation and Proliferation**—Inflammation is an often beneficial response of the immune system and affected tissues to injury. Proliferation of normal bystander cells such as fibroblasts and SMC plays a central role in the course of such reactions to fully reconstitute the affected tissue both structurally and functionally (17). A prerequisite for SMC to proliferate is a de-differentiation, which is frequently induced by the mixture of cytokines, growth factors, chemokines, and other stimuli present in inflamed tissue. In contrast to these well-characterized stimulatory factors, virtually nothing is known about the fate of gene products that are expressed in these cells to maintain an anti-proliferative and differentiated phenotype following tissue injury.

**IL-1β/TNFα-repressed Gene Products**—Of the 11 gene products characterized to be down-regulated in the cultured SMC upon exposure to IL-1β plus TNFα, the majority may indeed

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2 K. Schäfer, unpublished observation.
directly or indirectly be associated with cell growth, gene transcription, and signal transduction.

Besides the transcriptional repressor zfm1 that is discussed in detail below, rat-Fln29, a TNFα-receptor-associated factor-interacting zinc finger protein with a high homology to the cytosolic inhibitory protein A20 (18), and HMG-17, a member of the high mobility group (HMG) family of transcription coordinating proteins (19), may be of interest for changes in SMC phenotype that are characteristic for the early phase of inflammation.

**Physiological Significance of zfm1**—zfm1 was chosen for further analysis, because, according to data retrieved from the literature, it appeared to be the most promising candidate to be involved in SMC phenotype control. To our knowledge, this is the first demonstration of zfm1 as a modulator of SMC proliferation and pro-inflammatory gene expression.

Besides its function as a co-factor of mRNA splicing events (15), zfm1 has been shown to act as a transcriptional repressor of genes under control of the transcriptional activator EWS and homologous proteins by directly interacting with these proteins (13, 20). Whereas several zfm1 splice variants seem to be predominantly involved in RNA splicing, two naturally occurring isoforms, A and E, were reported to be inhibitors of EWS-like proteins (13). The cultured SMC only express zfm1A.

zfm1-mediated repression directly targets the basal transcription machinery, because EWS is part of the constitutive transcription complex, which binds and activates the transcription factor TFIID. Interestingly, several EWS-like proteins exist in mammals (20), all of which have been characterized to be activators of transcription of as yet unknown physiological significance. One attractive hypothesis is, therefore, that these factors modulate the cellular response to specific stimuli, e.g. cytokine signaling to the nucleus. Indeed, two proteins, hTAFIII and TLS, both homologous to EWS, have been characterized as co-activators of the cytokine-inducible transcription factor NF-κB (21, 22). This finding, on the other hand, suggests a mechanism for the putative anti-inflammatory effects of zfm1 through inhibition of such co-activators.

**Unmasking the Effects of zfm1 on Proliferation and Gene Expression**—According to run-on analysis, down-regulation of zfm1 expression by IL-1β plus TNFα occurred at the level of transcription and was maintained for at least 18 h. To mimic this effect without potentially confounding cytokine effects, a specific as-ODN was employed. Down-regulation of zfm1 protein expression by the as-ODN indeed resulted in effects very similar to those of the cytokines, i.e. an up-regulation of both MCP-1 and VCAM-1 gene expression and, in addition, an increase in the rate of SMC proliferation.

**When considering how zfm1 inhibits basal transcription (see above), it may become intelligible as to why its down-regulation exerted a weaker effect on MCP-1 or VCAM-1 expression than IL-1β plus TNFα stimulation of the SMC. Although constituting an important step in cytokine-induced gene expression, zfm1 suppression thus cannot account for full activation of these genes, because it affects the basal transcription machinery but does not induce gene expression per se. This notion is...**
supported by the apparent lack of effect of down-regulating zfm1 on iNOS expression. Transcription of this gene must be induced, i.e., the iNOS gene remains silent despite de-blockade of the basal transcription machinery as long as cytokine-inducible transcription factors such as NF-κB are inactive (24).

This assumption is also corroborated by the finding that as-ODN-based down-regulation of zfm1 protein before addition of the cytokines does not alter their stimulatory effect on MCP-1 or VCAM-1 expression. On the other hand, this finding may be interpreted as indicating that lowering zfm1 protein below the level obtained with the as-ODN has no further effect on gene expression in the SMC.

In contrast, as-ODN lowering of zfm1 protein accelerated the rate of proliferation of the SMC to the same extent as the cytokine exposure, whereas the combination did not result in a greater effect. This apparent discrepancy is best explained by a moderate increase in expression of several constitutively active genes associated with cell cycle control following down-regulation of zfm1, which already suffices to maximally induce SMC proliferation (cf. the comparable effect of PDGF and that of the as-ODN on SMC proliferation). Such subtle changes in gene expression triggering the entry of eukaryotic cells into the cell cycle are well described in the literature (see Ref. 25 for review).

Does an increase in zfm1 expression consequently suppress SMC proliferation? For various reasons this question is difficult to address experimentally. Moreover, when considering that lowering the zfm1 abundance below a certain threshold unmasks its effects on SMC proliferation, affirmation of this question is not at all certain. Perhaps, maintaining zfm1 expression despite the presence of pro-inflammatory cytokines constitutes a more feasible approach to further substantiate its anti-proliferative capacity.

Effects of Other Cytokines—Apart from interferon-γ, which...
might have been expected, PDGF also repressed zfm1 gene expression to a similar extent as did IL-1β plus TNFα. PDGF is thought to play a crucial role in the development of atherosclerosis and possesses many properties of the cytokines analyzed in this study. Besides enhancing SMC proliferation, PDGF also induces expression of pro-inflammatory gene products such as MCP-1 in vascular SMC (26). Therefore, PDGF may be defined as a pro-inflammatory cytokine rather than a typical growth factor, the action of which is also amenable to zfm1 modulation.

Role of zfm1 in Vivo—Finally, the putative role of zfm1 as a factor capable of preventing inflammation-induced de-differentiation of vascular SMC was studied in an in vivo model of accelerated neointima formation. A summary of the relative amount of zfm1-positive nuclei in the media and neointima of carotid arteries from control mice (control), 1 week post injury (7 days), and 3 weeks post injury (21 days). All sections were either stained with hematoxylin and counted for zfm1-positive nuclei and total nuclei, respectively (n = 4–6; *, p < 0.05 as indicated). B, representative immunohistochemical analysis of zfm1 and PCNA protein expression. Comparison of the expression of the proliferation marker PCNA (left panel) and zfm1 (right panel) in two consecutive cross-sections of the same carotid artery 3 weeks post injury (original × 100). Corresponding nuclei, i.e. zfm1-positive nuclei not seen on the left and PCNA-positive nuclei not seen on the right are designated by arrows to highlight the inverse expression pattern of the two proteins. "L" marks the vascular lumen. Sections from three (control) to five animals were analyzed with virtually identical results.

Fig. 6. In vivo model for cytokine-dependent vascular inflammation. A summary of the relative amount of zfm1-positive nuclei in the media and neointima of carotid arteries from control mice (control), 1 week post injury (7 days), and 3 weeks post injury (21 days). Consecutive sections were either stained against zfm1 or with hematoxylin and counted for zfm1-positive nuclei and total nuclei, respectively (n = 4–6; *, p < 0.05 as indicated). B, representative immunohistochemical analysis of zfm1 and PCNA protein expression. Comparison of the expression of the proliferation marker PCNA (left panel) and zfm1 (right panel) in two consecutive cross-sections of the same carotid artery 3 weeks post injury (original × 100). Corresponding nuclei, i.e. zfm1-positive nuclei not seen on the left and PCNA-positive nuclei not seen on the right are designated by arrows to highlight the inverse expression pattern of the two proteins. "L" marks the vascular lumen. Sections from three (control) to five animals were analyzed with virtually identical results.

Role of zfm1 in Vivo—Finally, the putative role of zfm1 as a factor capable of preventing inflammation-induced de-differentiation of vascular SMC was studied in an in vivo model of accelerated neointima formation that appears to be mediated by pro-inflammatory cytokines such as IL-1β and TNFα (27). The observed transient down-regulation of zfm1 abundance in the media and in the ensuing neointima of the injured carotid arteries supports the notion of zfm1 acting as an endogenous inhibitor of pro-inflammatory and proliferative responses in the vessel wall. Interestingly, in the terminal phase of neointima formation, nuclei of SMC, which stained positively for zfm1, stained negatively for the proliferation markers PCNA or RPA (28) and vice versa, further supporting this hypothesis.

Conclusions—The present findings demonstrate that, among several cytokine down-regulated gene products, zfm1 was characterized as an inhibitor of pro-inflammatory gene expression and proliferation in vascular SMC. Because there is an increasing number of proteins with genetic and/or functional similarities to zfm1 and its counterpart EWS, these findings point toward a system of co-activators and matching repressors of the constitutive transcription machinery that modulate gene expression. Moreover, the stimulatory effects of pro-inflammatory cytokines on the initiation and progression of inflammatory responses can no longer be solely explained by an induction of pro-inflammatory genes (i.e. through activation of the corresponding transcription factors) but must also consider a complementary cytokine effect through attenuating the expression of negative regulatory factors such as zfm1. It might well be this additional effect, if prolonged, that contributes to the transition of an acute to a chronic inflammatory response, at least in the vessel wall.

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REFERENCES
1. Nilsson, J. (1993) Cardiovasc. Res. 27, 1184–1190
2. Fukumoto, Y., Shimokawa, H., Ito, A., Kadokami, T., Yonemitsu, Y., Aikawa, M., Owada, M. K., Egashira, K., Sueishi, K., Nagai, R., Yazaki, Y., and Takeshita, A. (1997) J. Cardiovasc. Pharmacol. 29, 222–231
3. Clausell, N., Molossi, S., Sett, S., and Rabinovitch, M. (1994) Circulation 89, 2768–2779
4. Simon, A. D., Yazdani, S., Wang, W., Schwartz, A., and Rabban, L. E. (2000) J. Thromb. Thrombolysis 8, 217–222
5. Nath, C., and Gulati, S. C. (1998) Acta Haematol. 99, 175–179
6. Thomas, P. S. (2001) Immunol. Cell Biol. 79, 132–140
7. Gosling, J., Snaymaker, S., Gu, L., Tseng, S., Zlot, C. H., Young, S. G., Rollins, B. J., and Charo, I. F. (1999) J. Clin. Invest. 103, 773–778
8. Li, H., Cybulsky, M. I., Gimbrone, M. A., Jr., and Libby, P. (1993) Arterioscler. Thromb. Vasc. Biol. 13, 197–204
9. Porreca, E., Di Peibbo, C., Reale, M., Castellani, M. L., Baccante, G.,
Barbacane, R., Conti, P., Cuccurullo, F., and Poggi, A. (1997) J. Vasc. Res. 34, 58–65
10. Denger, S., Jahn, L., Wende, P., Watson, L., Gerber, S. H., Kuhler, W., and Kreuzer, J. (1999) Atherosclerosis 144, 15–23
11. Diatchenko, L., Lau, Y. F., Campbell, A. P., Chenchik, A., Moqadam, F., Huang, B., Lukyanov, S., Lukyanov, K., Gurakaya, N., Sverdlov, E. D., and Siebert, P. D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6025–6030
12. Cattaruzza, M., Wachter, R., Wagner, A. H., and Hecker, M. (2000) Br. J. Pharmacol. 129, 1155–1162
13. Zhang, D., and Childs, G. (1998) J. Biol. Chem. 273, 6868–6877
14. Macalma, T., Otte, J., Hensler, M. E., Bockholt, S. M., Louis, H. A., Kalff-Suske, M., Grzeschik, K. H., von der Ahe, D., and Beckerle, M. C. (1996) J. Biol. Chem. 271, 31470–31478
15. Guth, S., and Vale, C.C. (2000) J. Biol. Chem. 275, 38059–38066
16. Konstantinides, S., Schafer, K., Thines, T., and Loskutoff, D. J. (2001) Circulation 103, 576–583
17. Gharaee-Kermani, M., and Phan, S. H. (2001) Curr. Pharm. Des. 7, 1083–1103
18. Heyninck, K., and Beyaert, R. (1999) FEBS Lett. 442, 147–150
19. Bustin, M., Trieschmann, L., and Postnikov, Y. V. (1995) Semin. Cell Biol. 6, 247–255
20. Zhang, D., Paley, A. J., and Childs, G. (1998) J. Biol. Chem. 273, 18086–18091
21. Uranishi, H., Tetsuka, T., Yamashita, M., Asamitsu, K., Shimizu, M., Itoh, M., and Okamoto, T. (2001) J. Biol. Chem. 276, 13395–13401
22. Yamah-Hezi, A., and Dikstein, R. (1998) EMBO J. 17, 5161–5169
23. Lademery, M. (1997) Biosciences 10, 963–969
24. Hammermann, R., Dreissig, M. D., Messner, J., Fuhrmann, M., Berrino, L., Gohert, M., and Racke, K. (2000) Mol. Pharmacol. 58, 1294–1302
25. Tyson, J. J., Novak, B., Odell, G. M., Chen, K., and Thron, C. D. (1996) Trends Biochem. Sci. 21, 89–96
26. Bogdanov, V. Y., Poon, M., and Taubman, M. B. (1998) J. Biol. Chem. 273, 24392–24398
27. Elhage, R., Maret, A., Pieraggi, M. T., Thiers, J. C., Arnal, J. F., and Bayard, F. (1998) Circulation 97, 242–244
28. Dimitrova, D. S., and Gilbert, D. M. (2000) Exp. Cell Res. 254, 321–327
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