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From the Denver VA Medical Center and University of Colorado Health Sciences Center.

Correspondence to Jane E.B. Reusch, MD, Denver VA Medical Center Endo 111-H, 1055 Clermont St, Denver, CO 80220. E-mail jane.reusch@uchsc.edu

(Circulation 2003;108:1164-1166.)

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Circulation is available at http://www.circulationaha.org
DOI: 10.1161/01.CIR.0000084296.45158.50

Focused Perspective

Cyclic AMP Response Element-Binding Protein in the Vessel Wall

Good or Bad?

Jane E.B. Reusch, MD; Dwight J. Klemm, PhD

Atherosclerosis and postangioplasty restenosis are leading causes of death in the Western world. Recent advances in our understanding of vascular biology would suggest that targeting specific signals in the post–balloon injury vessel or in persons prone to development of atherosclerosis could decrease lesion formation and in the long term decrease cardiovascular events. In light of this, numerous laboratories around the world are investigating the functional activities of the various cellular components of the atherosclerotic plaque, including vascular smooth muscle cells (SMCs), endothelial cells (ECs), and monocyte/macrophages.

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One of the strategies for examining vascular wall response to injury in animal models has been the use of the balloon injury model. In this model, a balloon catheter is used to cause an injury and denuding of the endothelium. The animals are monitored serially over 7, 14, and 28 days for narrowing of the blood vessel lumen after injury. This model traditionally has been used to examine activation of SMCs and their proliferative response. In response to balloon injury, vascular SMCs (which are normally contractile and quiescent [nonproliferative]) are released into a proliferation phase by the injury and loss of the endothelium.

Immunostaining studies have suggested that the majority of the cells that cause luminal narrowing are SMCs. However, recent studies have also demonstrated the presence of inflammatory cells in this model. Macrophages are observed both in the balloon injury model and in balloon injury/stent models. Thus, interfering with either SMC function or macrophage activation would be expected to have an impact on restenosis.

Agents that can be delivered either pharmacologically or directly at the time of angioplasty to decrease neointimal proliferation are considered to have therapeutic promise. The most useful are agents that decrease SMC proliferation. In the present issue of Circulation, Tokunou et al3 present a series of experiments that indicate that delivery of a DNA-binding dominant negative isoform of the transcription factor CREB (cyclic AMP response element-binding protein) at the time of balloon injury leads to a decrease in neointimal thickening. At first glance, this study may appear to contradict our recent reports that CREB restrains mitogen-stimulated SMC proliferation and migration. In the present commentary, we will review the available data on CREB in macrophages and SMCs and offer our view of the studies by Tokunou et al.

CREB in Vascular Tissues

CREB is a 43-kDa nuclear transcription factor in the beta leucine zipper family of transcription factors, and it has been shown to have important functions in differentiation of numerous target tissues, including adipocytes, vascular SMCs, neurons, and cardiac myocytes. One additional function of CREB is the prevention of apoptosis. This has been carefully outlined in neuronal cells, fat cells, and most recently, beta cells. Additional unpublished studies suggest that CREB may also play a pivotal role in terminal macrophage differentiation (C.K. Glass, MD, Professor of Medicine, University of California San Diego, personal communication, 2003). No published studies have examined CREB in ECs. Given the potent impact of CREB on numerous vascular cell types, CREB would be expected to have pleiotropic effects on the vessel wall.

SMC proliferation is the primary end point for most investigators studying the balloon injury/restenosis model. The question is what the expected impact of CREB on vascular SMC proliferation and migration would be. Our laboratory has demonstrated both in vivo and in vitro that CREB content correlates negatively with proliferation. In addition, expression of active CREB decreases mitogen-stimulated proliferative capacity and migratory capacity. Expression of dominant negative CREB augments the ability of platelet-derived growth factor to stimulate proliferation and migration in vitro. CREB decreases expression of numerous cell cycle–regulatory proteins, as well as expression of the growth factor receptors endothelin, endothelin 1 receptor, and platelet-derived growth factor receptor α. In the study by Tokunou et al in the present issue of Circulation, the authors demonstrate in vitro that adenoviral delivery of dominant negative CREB into SMCs in culture leads to increased smooth muscle apoptosis and no appreciable change in entry through cell cycle.

So how do we resolve these divergent and paradoxical results? First, we should consider the noncontroversial aspects of this work. The fact that interference with CREB
activity through a dominant negative isoform of CREB would augment apoptosis is an expected result, consistent with studies conducted in our laboratory (J.E.B. Reusch, MD, and P.A. Watson, PhD, unpublished data, 2003) and in numerous other cell types and cell culture systems. The second issue, the apparent discrepancy between acute CREB activation of prothrombotic genes (thrombin) and restraint of mitogen-stimulated proliferation, could be interpreted as follows. Loss of CREB function in unstimulated SMCs in culture does not affect proliferation in a robust way (observed by both groups). In contrast, in Figure 5 of their article, Tokunou et al. demonstrate a modest decrease in BrdU labeling in the intimal layer and a robust increase in apoptotic cell death in vessels treated with the dominant negative CREB. The authors interpret this decrease in proliferative index as evidence that dominant negative CREB decreases SMC proliferation. There is no clear histological evidence that the cells infected with the dominant negative CREB are nonproliferative (ie, double staining). Another explanation for decreased intimal proliferation could be loss of the stimulatory effects of macrophages and ECs. Interference with macrophage terminal differentiation, then you might expect to see less macrophage accumulation in these lesions and therefore less mitogen stimulation of SMC proliferation. The impact of CREB on ECs is unknown. In the balloon injury model, a large amount of virus is delivered to the endothelium. Dominant negative CREB could easily induce apoptosis in the ECs and thereby decrease EC cytokine production. This would be a reasonable interpretation of the studies presented, in light of the fact that this particular phosphorylation-deficient CREB mutant, CREB(M1), has normal DNA binding and does not appear to have an impact on proliferative capacity in the basal state (Figure 1).

**So, Is CREB Good or Bad in the Vessel Wall?**

Transcriptional regulation has an impact on SMC phenotype, in terms of modulation between a synthetic (active) and a contractile (quiescent) phenotype. It is a very complex system with numerous checks and balances. Russell Ross described cyclic AMP as the mitogenic gate, which was an inhibitor in most instances of smooth muscle proliferation, with the exception of a change in cellular context where agents such as endothelin and angiotensin 2 could drive SMC proliferation. Because of high cyclic nucleotide abundance in SMCs, most CREB that is present in SMCs is in the phosphorylated or active form. CREB content is very high in the vascular SMCs of young healthy animals, both rodents and monkeys (K.D. O’Brien and T.O. McDonald, unpublished data, 2002). Vascular CREB content is diminished in numerous rodent models of atherosclerosis disease–prone states, such as the insulin-resistant ob/ob mouse, Zucker rat, and most recently, the fat-fed LDL receptor–null mouse (J.E.B. Reusch, MD, and K.D. O’Brien, unpublished data, 2002). In addition, vascular CREB content is decreased with aging in rodent models. We have therefore postulated that CREB is a gatekeeper for protection from transition to the proliferative SMC phenotype. We have taken that hypothesis further to suggest that loss of CREB in the rodent models is a permissive step for SMC activation.

If CREB is an important vasculoprotective transcription factor, how would one explain CREB as a target of the vasculotoxic agents endothelin and angiotensin or as a prime regulator of the procoagulant factor thrombin? The simple answer is that we don’t yet know. The more speculative response (Figure 1) is that CREB is an immediate early gene, which responds in concert with AP1 factors and nuclear factor-κB to numerous toxic stimuli, not only in vascular SMCs, but also in other cell types such as neurons, beta cells, fat cells, and various lymphocytes. In the context of metabolic and cytokine stress, we have observed in vitro and in vivo that there is an acute and robust activation of CREB and numerous other inflammatory cytokines and immediate early genes. With time, chronic metabolic stress leads to loss of both acute signaling to CREB through the AKT signaling pathway and decreased CREB expression in numerous target tissues, including the heart, the vasculature, and the nervous system. An important study by Mabuchi et al. demonstrated that acute neuronal injury to the brain activated cyclic AMP response element (CRE)-responsive genes very robustly. This was interpreted to be a cytoprotective response. It is also likely that the acute activation and phosphorylation of CREB with balloon injury is a cytoprotective response and that CREB working in concert with other immediate early genes is an appropriate response to injury.

**Conclusions**

The article in the present issue of *Circulation* by Tokunou et al. demonstrates that adenoviral delivery of DNA-binding dominant negative CREB M1 at the time of balloon injury leads to decreased neointimal lesion formation and slows down restenosis. Our interpretation of these data is that dominant negative CREB acts by increasing apoptosis of ECs and vascular SMCs and interfering with recruitment and activation of macrophages. Because this CREB M1 vector decreases intimal lesions, it could be a reasonable pharmacological target. In contrast, the interpretation that adenoviral
delivery of that dominant negative CREB interferes with SMC proliferation is unlikely, according to their in vitro studies and the literature. Transgenic or SMC-specific adenoviral delivery of CREB would be necessary to clear up any remaining controversy. In order to leave the reader with a more general understanding of how CREB may be contributing to the vascular lesion, the model shown in Figure 2 is proposed.

Acknowledgments

This work is supported by VA Merit review and Research Enhancement Awards Program (Drs Reusch and Klemm); the National Institutes of Health (RO1DK53969 to Dr Klemm and PO1 HL 14985 to Drs Reusch and Klemm); the American Heart Association; the American Diabetes Association; and the Juvenile Diabetes Research Foundation (Dr Reusch).

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KEY WORDS: Editorials | protein, DNA-binding, cyclic AMP–responsive | atherosclerosis | restenosis | cells, smooth muscle, vascular
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Circulation. 2003;108:1164-1166
doi: 10.1161/01.CIR.0000084296.45158.50
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/108/10/1164

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