In vivo electrotransfer of interleukin-10 cDNA prevents endothelial upregulation of activated NF-κB and adhesion molecules following an atherogenic diet

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ABSTRACT. ***Objectives.*** **Interleukin (IL)-10 has anti-atherogenic properties. However, the molecular mechanisms involved in IL-10 protection against atherosclerosis *in vivo* remain poorly understood. In this study, we examined the effect of IL-10 cDNA *in vivo* electrotransfer on diet-induced, endothelial activation.** ***Methods.*** **C57BL/6J mice were fed an atherogenic diet for 10 days. Expression of VCAM-1 and ICAM-1 was examined in the aortic sinus, a region predisposed to atherogenesis in mice, using immunohistochemistry. NF-κB activation was examined using a monoclonal antibody that selectively reacts with the activated form of the p65 subunit.** ***Results.*** **We detected a low basal expression of activated NF-κB, VCAM-1 and ICAM-1 in the endothelium of the aortic sinus. Endothelial expression of activated NF-κB, VCAM-1 and ICAM-1 was markedly increased after 10 days on the atherogenic diet ($p < 0.001$). *In vivo* electrotransfer of a murine IL-10-encoding plasmid completely prevented diet-induced endothelial upregulation of activated NF-κB, VCAM-1 and ICAM-1 ($p < 0.01$).** ***Conclusion.*** ***In vivo*** **electrotransfer of IL-10 cDNA prevents diet-induced endothelial activation. These results suggest that the protective effects of IL-10 may already occur in the very early stages of atherogenesis.**

Keywords: atherosclerosis, endothelial factors, gene therapy, inflammation

Endothelial cell activation with the consequent expression of cell adhesion molecules on the endothelial cell surface, is the primary event in the cascade leading to the development of atherosclerotic lesions [1, 2]. Induction of endothelial cell adhesion molecules, particularly vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1, is triggered by a variety of pathophysiologically relevant stimuli, including abnormal blood flow hemodynamics [3-6], oxidized LDL (oxLDL) [7-9] and atherogenic diets [10], as well as proinflammatory cytokines [11]. This induction is highly modulated by the activation of the proinflammatory nuclear transcription factor NF-κB [12-15]. Interleukin (IL)-10 is a potent anti-inflammatory cytokine expressed in the atherosclerotic lesion [16, 17], and has been shown to exert a major protective role against the development of atherosclerosis in C57BL/6 mice [18-20]. Different studies have demonstrated the beneficial role of IL-10 through various experimental models of atherosclerosis. IL-10 deficiency in C57Bl/6 mice fed an atherogenic diet has been shown to enhance early lesion formation [18, 19]. More recently, similar observations were obtained in apolipoprotein E (apoE)/IL-10 double-knockout (KO) mice fed a standard diet [21], and in LDL receptor-KO mice with bone marrow deficiency in IL-10 [22]. In parallel, other studies confirmed the anti-atherogenic effect of IL-10 by overexpressing it in LDLr- or apoE- deficient mice [20, 23]. Although these consistent data emphasize the anti-atherogenic properties of IL-10, the molecular mechanisms involved in IL-10-mediated protection against atherosclerosis *in vivo* remain poorly understood. *In vivo* studies have demonstrated that atherosclerotic lesions of IL-10-deficient mice showed increased infiltration of inflammatory cells [18, 22]. *In vitro* studies have revealed that IL-10 inhibited the interaction between activated endothelial cells and monocytes in an atherogenic context [19]. Based on these data, we hypothesized that at least some of the beneficial effects of IL-10 in atherosclerosis may involve a protective role against the up-regulation of cell adhesion molecules and the recruitment of inflammatory cells into the vessel wall. IL-10 has been shown to inhibit NF-κB activation *in vitro* and *in vivo* [24, 25] although no study has reported an inhibitory effect in endothelial cells. With regard to expression of cell adhesion molecules, contradictory results have been reported *in vitro* [26, 27], whereas lack of IL-10 was associated with increased VCAM-1 and ICAM-1 expres-

sion in a model of colitis *in vivo* [28]. *In vivo* electrotransfer of plasmid DNA has emerged as a highly efficient method of ensuring a sustained and significant delivery of specific molecules with no or minimal side effects [29-31]. Therefore, we designed this study to examine the effects of a single *in vivo*, intramuscular IL-10 cDNA electrotransfer on endothelial NF-κB activation and expression of VCAM-1 and ICAM-1 in the aortic sinus of C57BL/6 mice fed an atherogenic diet. As previous studies showed that induction of endothelial cell adhesion molecules following an atherogenic diet is rapid, and precedes the recruitment of circulating mononuclear cell into the vessel wall [32, 33], the time course of this study was limited to 10 days.

METHODS

Mice

Female C57BL/6J (5 to 9 mice per group), 7-8 weeks old were fed either a standard "A04" diet, which contained 3 % fat (UAR, France) or were placed on an atherogenic diet for 10 days. The atherogenic diet was generated by addition of 15 % cacao butter, 1.25 % cholesterol and 0.5 % sodium cholate to the standard diet. Mice were kept in accordance with standard animal care requirements, housed 4-5 per cage and maintained on a 12-hour light-dark cycle. Water and food were given *ad libitum*. We chose the C57BL/6J model in order to control the induction of high blood cholesterol levels starting from normal baseline values. The 10-day period was chosen in order to study the precise role of IL-10 on endothelial activation, before any invasion of the arterial wall by inflammatory cells.

Immunohistochemical studies

After 10 days on the atherogenic diet, mice were killed by CO_2 overdose. The basal half of the ventricles and the ascending aorta were removed, embedded in OCT compound (tissue Tek), frozen in isopentane, and stored at - 70 °C until processing. Serial 10 µm sections of the aortic sinus with valves (80-90 per mouse) were cut on a cryostat. Each slide contained 6 to 10 sections that were representative of the whole aortic sinus. Frozen sections were fixed in acetone before immunostaining. They were incubated with either a primary goat polyclonal antibody against mouse VCAM-1 (Santa Cruz), a primary goat polyclonal antibody against mouse ICAM-1 (Santa Cruz), or a primary mouse monoclonal antibody against NF-κB, p65 subunit (Boehringer Mannheim). This latter antibody recognizes the IκB binding region on the p65 DNA binding subunit and therefore selectively reacts with p65 in the activated form of NF-κB. This antibody has been useful in numerous studies for immunohistochemical detection of activated NF-κB in specific cell types [34]. Immunostains were visualized after incubation with the corresponding pre-adsorbed, secondary biotinylated antibodies (Vector Laboratories) and the use of avidin-biotin horseradish peroxidase visualization systems (Vectastain ABC Kit) (Vector Laboratories). We took advantage of the InnoGenex Mouse-to-Mouse Iso-IHC Kit to abrogate any background staining that may have resulted from the use of the mouse primary anti-NF-κB antibody on mouse tissues. Irrelevant immunoglobulins were used for negative controls. Six to ten sections per animal (representative of the whole aortic sinus) were analyzed for each immunostaining.
A quantitative analysis of the percentage of positive cells for each immunostaining was performed at a magnification of x 400 in a strictly blinded manner.

Systemic delivery of murine IL-10 by intramuscular injection of expression plasmid DNA

To assess the effects of IL-10 supplementation on *in vivo* endothelial NF-κB activation and expression of VCAM-1 and ICAM-1, mice placed on the atherogenic diet were injected at day 0 with either murine IL-10 expression plasmid, pCor-IL10, or an empty plasmid as control, as previously described [18]. IL-10 or control expression plasmid (15 µg) was injected into both tibial cranial muscles of anesthetized mice. Briefly, transcutaneous

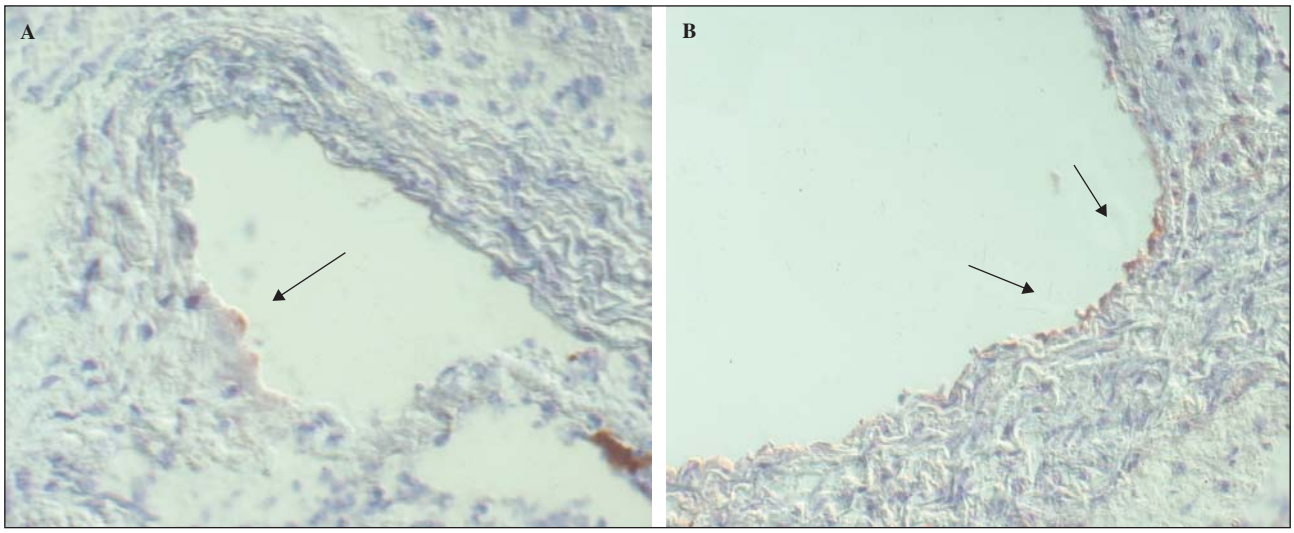

Figure 1
Representative photomicrographs of tissue sections from the aortic sinus of C57BL/6 female mice fed a standard diet and injected at day 0 with a control empty pCor plasmid. The sections were stained for activated p65 NF-κB (**A**) and VCAM-1 (**B**). Original magnifications: x 400.

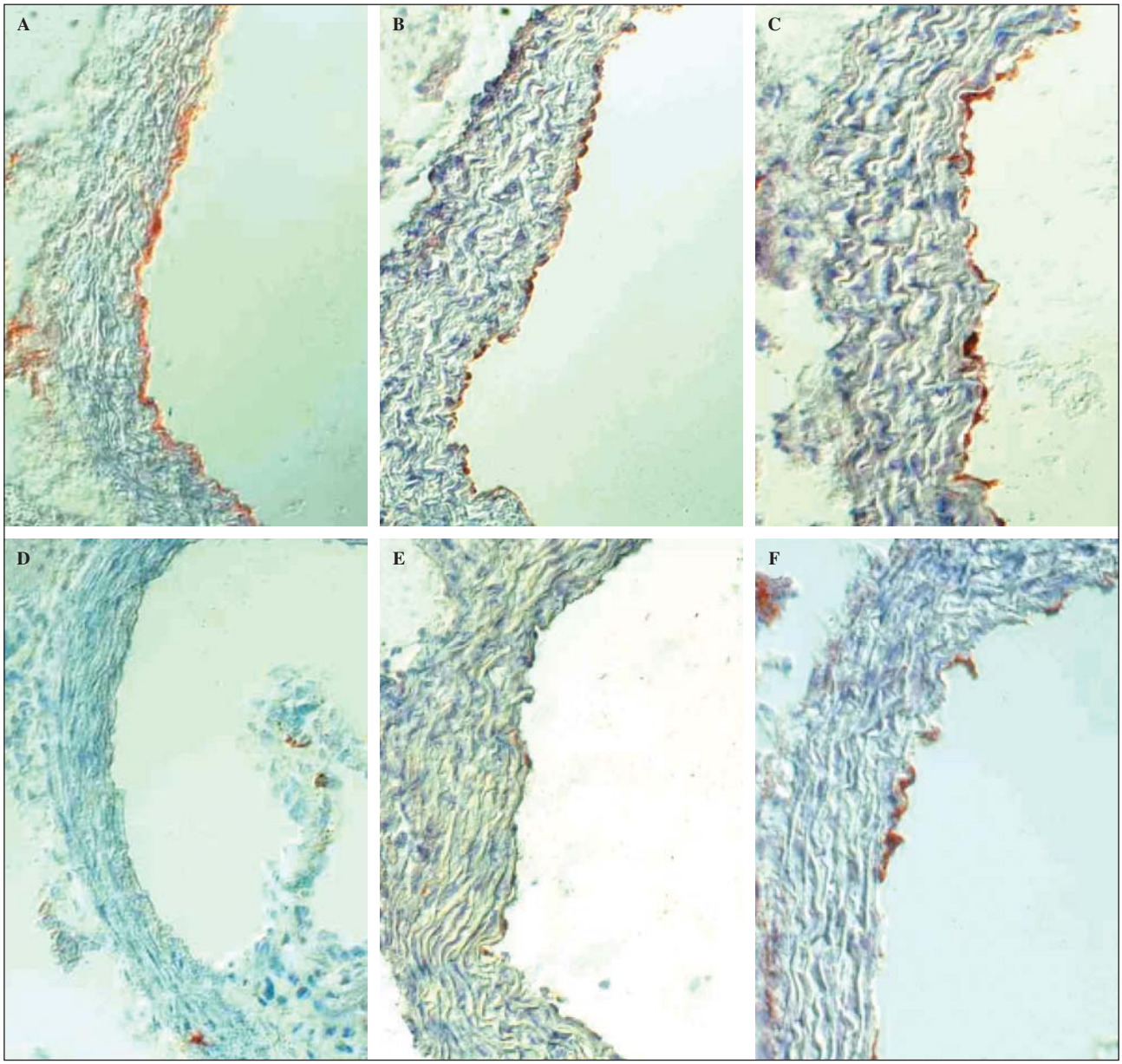

Figure 2
Representative photomicrographs of tissue sections from the aortic sinus of C57BL/6 female mice fed an atherogenic diet for 10 days, and injected at day 0 with either a control (**A-C**) or an IL-10-encoding plasmid (**D-F**). The sections were stained (in red) for activated p65 NF-κB (**A, D**), VCAM-1 (**B, E**) or ICAM-1 (**C, F**). We detected a significant activation of p65 NF-κB in the endothelium after 10 days on the atherogenic diet (**A**). *In vivo* transfer of IL-10 resulted in nearly complete inhibition of endothelial p65 NF-κB activation (**D**). We also detected a significant up-regulation of endothelial VCAM-1 (**B**) and ICAM-1 (**C**) after 10 days on the atherogenic diet. *In vivo* transfer of IL-10 significantly prevented the induction of both VCAM-1 (**E**) and ICAM-1 (**F**). Original magnifications: x 400.

Table 1
Quantitative analysis of the percentage of positive staining for activated p65 NF-κB, VCAM-1 and ICAM-1 in endothelial cells of the aortic sinus of C57BL/6 mice (n = 5 to 9 for each staining)

	Standard diet	**Atherogenic diet**	
		Control plasmid	**IL-10 plasmid**
% NF-κB	0.41 ± 0.19	23.9 ± 3.1[1]	1.9 ± 0.5[2]
% VCAM-1	5.5 ± 0.6	17.2 ± 1.5[1]	4.7 ± 1.0[2]
% ICAM-1	10.7 ± 1.2	30.8 ± 1.8[1]	11.7 ± 2.8[2]

[1]$p < 0.001$ for comparison between standard diet and control plasmid in the atherogenic diet.
[2]$p < 0.001$ for comparison between control and IL-10 plasmids in mice fed the atherogenic diet.

electric pulses (8 square-wave electric pulses of 200 V/cm, 20 ms each, at 2 Hz) were delivered by a PS-15 electropulsator (Genetronics) using two stainless steel plate electrodes placed 4.2 to 5.3 mm apart, each side of the leg. Blood samples were obtained by vein puncture at day 2 and day 10 after transfection. Circulating IL-10 levels were determined in the serum samples using of a highly sensitive ELISA kit specific for murine IL-10 (BioSource International).

Statistical analysis

Data are expressed as mean ± SEM. Simple regression analysis was performed to analyze the relation between expression of activated NF-κB and that of VCAM-1 and ICAM-1. The effects of the atherogenic diet or *in vivo* IL-10 cDNA electrotransfer on endothelial expression of activated NF-κB, VCAM-1 and ICAM-1 were determined using a t-test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Basal expression of activated p65, VCAM-1 and ICAM-1 in lesion-prone areas

A careful study of the aortic sinus revealed constitutive immunoreactivity for the activated form of NF-κB in occasional endothelial cells, exclusively located at atherosclerosis-prone areas (*figure 1A*). Otherwise, the staining was negative indicating that the antibody did not recognize the non-activated form of NF-κB. Negative staining using an irrelevant IgG3 further confirmed the specificity of the reaction (data not shown). As expected, we also observed constitutive VCAM-1 (*figure 1B*) and ICAM-1 expression in the endothelial cells of lesion-prone areas (*table 1*).

Diet-induced up-regulation of activated p65, VCAM-1 and ICAM-1

We found a clear increase in immunoreactivity for activated NF-κB in the endothelial cells of the aortic sinus after 10 days on the atherogenic diet (*figure 2A*). The increase in endothelial NF-κB activity was associated with a substantial up-regulation of endothelial VCAM-1 and ICAM-1 (*figure 2B* and *2C*, *table 1*). Quantitative analysis of the percentage of positive cells showed a highly significant difference between the mice on the atherogenic diet and those on the standard diet (*table 1*).

Prevention of diet-induced endothelial activation by in vivo *electrotransfer of IL-10-encoding plasmid*

The *in vivo* electrotransfer technique ensured the production of high levels of circulating IL-10 in the mice transfected with pCor-IL-10 plasmid (2015.25 ± 121.16 pg/mL at day 2, $n = 3$, and 319.75 ± 35.40 pg/mL at day 10, $n = 3$). Circulating levels of IL-10 following injection with the empty plasmid were below the level of detection. *In vivo* electrotransfer of pCor-IL-10 plasmid completely prevented diet-induced expression of activated p65 and inhibited VCAM-1 and ICAM-1 up-regulation (*figure 2D-2F*, *table 1*). Overall, there was a very significant positive correlation between the presence of activated p65 and the expression of both adhesion molecules on the endothelial lining ($r = 0.82$, $p < 0.0001$ for VCAM-1; $r = 0.68$, $p < 0.001$ for ICAM-1).

DISCUSSION

Endothelial expression of VCAM-1 and ICAM-1 is a critical event in the initiation of the atherosclerotic process [1, 2]. These adhesion molecules are up-regulated in atherosclerotic-prone areas and their expression precedes the recruitment of inflammatory cells within the arterial wall [32, 33]. Moreover, the use of neutralizing antibodies that block the ligation of these molecules was shown to inhibit leukocyte adhesion on the endothelial cell surface and to reduce the development of atherosclerotic lesions [35-37]. ICAM-1 is constitutively expressed on the endothelial cell surface. In this study, we also observed a constitutive expression of VCAM-1 on the endothelial lining of lesion-prone areas, a finding consistent with the results of Iiyama *et al*. [33]. We also showed constitutive expression of the activated form of p65 in occasional endothelial cells exclusively located in lesion-prone areas, as shown by Hajra *et al*. [38]. It is likely that this represents the *in vivo* effects of local changes in shear stress on NF-κB activation, a phenomenon that has been established *in vitro* [4, 5]. We also found a significant increase in endothelial expression of activated NF-κB shortly after the administration of the atherogenic diet and showed a close relationship between this activation and the up-regulation of VCAM-1 and ICAM-1. Our *in vivo* findings are in agreement with *in vitro* studies showing that upregulation of VCAM-1 and ICAM-1 on endothelial cells is under inflammatory control and is highly dependent on NF-κB activation [39, 40]. We and others have shown that both the development and the stability of atherosclerotic lesions depend on the balance between pro- and anti-inflammatory cytokines and that IL-10 plays a major role in this balance [17, 19]. Pinderski *et al*. reported an *in vitro* decrease in monocyte adhesion to endothelial cells transfected with an adenovirus encoding for IL-10 [19], suggesting a direct effect of IL-10 on endothelial adhesiveness. Therefore, we designed this study to examine the role of IL-10 in diet-induced endothelial activation *in vivo*. In order to achieve high levels of circulating IL-10, we used a previously described strategy of electrotransfer of an expression plasmid DNA encoding for murine IL-10 [18, 30]. A single injection of the encoding plasmid resulted in very high levels of circulating IL-10 during the 10-day period of the experiment [41]. Interestingly, this efficient *in vivo* delivery of IL-10 clearly prevented both diet-induced NF-κB activation and induction of cell adhesion molecules. Further studies should determine whether these effects occur through deactivation of circulating leukocytes or whether they are directly related to a direct effect of IL-10 on endothelial cells. In conclusion, our study provides, for the first time, *in vivo* evidence for a strong inhibitory role of IL-10 on endothelial NF-κB activation and expression of VCAM-1 and ICAM-1 in a mouse model of atherosclerosis. *In vivo* delivery of IL-10 cDNA by means of intramuscular electrotransfer appears to be an efficient strategy for downregulating atherogenic diet-induced endothelial activation.

REFERENCES

1. Ross R. Atherosclerosis – an inflammatory disease. *N Engl J Med* 1999; 340: 115.
2. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005; 352: 1685.
3. Walpola PL, Gotlieb AI, Cybulsky MI, Langille BL. Expression of ICAM-1 and VCAM-1 and monocyte adherence in arteries exposed to altered shear stress. *Arterioscler Thromb Vasc Biol* 1995; 15: 2.
4. Chappell DC, Varner SE, Nerem RM, Medford RM, Alexander RW. Oscillatory shear stress stimulates adhesion molecule expression in cultured human endothelium. *Circ Res* 1998; 82: 532.
5. Gonzales RS, Wick TM. Hemodynamic modulation of monocytic cell adherence to vascular endothelium. *Ann Biomed Eng* 1996; 24: 382.
6. Nagel T, Resnick N, Atkinson WJ, Dewey Jr. CF, Gimbrone Jr. MA. Shear stress selectively upregulatesintercellular adhesion molecule-1 expression in cultured human vascular endothelial cells. *J Clin Invest* 1994; 94: 885.
7. Jeng JR, Chang CH, Shieh SM, Chiu HC. Oxidized low-density lipoprotein enhances monocyte-endothelial cell binding against shear-stress-induced detachment. *Biochim Biophys Acta* 1993; 1178: 221.
8. Frostegard J, Wu R, Haegerstrand A, Patarroyo M, Lefvert AK, Nilsson J. Mononuclear leukocytes exposed to oxidized low density lipoprotein secrete a factor that stimulates endothelial cells to express adhesion molecules. *Atherosclerosis* 1993; 103: 213.
9. Khan BV, Parthasarathy SS, Alexander RW, Medford RM. Modified low density lipoprotein and its constituents augment cytokine-activated vascular cell adhesion molecule-1 gene expression in human vascular endothelial cells. *J Clin Invest* 1995; 95: 1262.
10. Li H, Cybulsky MI, Gimbrone Jr. MA, Libby P. An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. *Arterioscler Thromb* 1993; 13: 197.
11. De Caterina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone Jr. MA, Shin WS, Liao JK. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest* 1995; 96: 60.
12. Iademarco MF, McQuillan JJ, Rosen GD, Dean DC. Characterization of the promoter for vascular cell adhesion molecule-1 (VCAM-1). *J Biol Chem* 1992; 267: 16323.
13. Neish AS, Williams AJ, Palmer HJ, Whitley MZ, Collins T. Functional analysis of the human vascular cell adhesion molecule 1 promoter. *J Exp Med* 1992; 176: 1583.
14. Marui N, Offermann MK, Swerlick R, Kunsch C, Rosen CA, Ahmad M, Alexander RW, Medford RM. Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. *J Clin Invest* 1993; 92: 1866.
15. Voraberger G, Schafer R, Stratowa C. Cloning of the human gene for intercellular adhesion molecule 1 and analysis of its 5'-regulatory region. Induction by cytokines and phorbol ester. *J Immunol* 1991; 147: 2777.
16. Uyemura K, Demer LL, Castle SC, Jullien D, Berliner JA, Gately MK, Warrier RR, Pham N, Fogelman AM, Modlin RL. Cross-regulatory roles of interleukin (IL)-12 and IL-10 in atherosclerosis. *J Clin Invest* 1996; 97: 2130.
17. Mallat Z, Heymes C, Ohan J, Faggin E, Leseche G, Tedgui A. Expression of interleukin-10 in advanced human atherosclerotic plaques: relation to inducible nitric oxide synthase expression and cell death. *Arterioscler Thromb Vasc Biol* 1999; 19: 611.
18. Mallat Z, Besnard S, Duriez M, Deleuze V, Emmanuel F, Bureau MF, Soubrier F, Esposito B, Duez H, Fievet C, Staels B, Duverger N, Scherman D, Tedgui A. Protective role of interleukin-10 in atherosclerosis. *Circ Res* 1999; 85: 17.
19. Pinderski Oslund LJ, Hedrick CC, Olvera T, Hagenbaugh A, Territo M, Berliner JA, Fyfe AI. Interleukin-10 blocks atherosclerotic events in vitro and in vivo. *Arterioscler Thromb Vasc Biol* 1999; 19: 2847.
20. Namiki M, Kawashima S, Yamashita T, Ozaki M, Sakoda T, Inoue N, Hirata K, Morishita R, Kaneda Y, Yokoyama M. Intramuscular gene transfer of interleukin-10 cDNA reduces atherosclerosis in apolipoprotein E-knockout mice. *Atherosclerosis* 2004; 172: 21.
21. Caligiuri G, Rudling M, Ollivier V, Jacob MP, Michel JB, Hansson GK, Nicoletti A. Interleukin-10 deficiency increases atherosclerosis, thrombosis, and low-density lipoproteins in apolipoprotein E knockout mice. *Mol Med* 2003; 9(1-2): 10.
22. Potteaux S, Esposito B, van Oostrom O, Brun V, Ardouin P, Groux H, Tedgui A, Mallat Z. Leukocyte-derived interleukin 10 is required for protection against atherosclerosis in low-density lipoprotein receptor knockout mice. *Arterioscler Thromb Vasc Biol* 2004; 24(8): 1474.
23. Pinderski LJ, Fischbein MP, Subbanagounder G, Fishbein MC, Kubo N, Cheroutre H, Curtiss LK, Berliner JA, Boisvert WA. Overexpression of interleukin-10 by activated T lymphocytes inhibits atherosclerosis in LDL receptor-deficient mice by altering lymphocyte and macrophage phenotypes. *Circ Res* 2002; 90: 1064.
24. Wang P, Wu P, Siegel MI, Egan RW, Billah MM. Interleukin (IL)-10 inhibits nuclear factor kappa B (NFkappa B) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. *J Biol Chem* 1995; 270: 9558.
25. Lentsch AB, Shanley TP, Sarma V, Ward PA. In vivo suppression of NF-kappa B and preservation of Ikappa B alpha by interleukin-10 and interleukin-13. *J Clin Invest* 1997; 100: 2443.
26. Krakauer T. IL-10 inhibits the adhesion of leukocytic cells to IL-1-activated human endothelial cells. *Immunol Lett* 1995; 45: 61.
27. Noble KE, Harkness D, Yong KL. Interleukin 10 regulates cellular responses in monocyte/endothelial cell co-cultures. *Br J Haematol* 2000; 108: 497.
28. Kawachi S, Jennings S, Panes J, Cockrell A, Laroux FS, Gray L, Perry M, van der Heyde H, Balish E, Granger DN, Specian RA, Grisham MB. Cytokine and endothelial cell adhesion molecule expression in interleukin-10-deficient mice. *Am J Physiol Gastrointest Liver Physiol* 2000; 278: G734.
29. Aihara H, Miyazaki J. Gene transfer into muscle by electroporation in vivo. *Nat Biotechnol* 1998; 16: 867.
30. Mir LM, Bureau MF, Gehl J, Rangara R, Rouy D, Caillaud JM, Delaere P, Branellec D, Schwartz B, Scherman D. High-efficiency gene transfer into skeletal muscle mediated by electric pulses. *Proc Natl Acad Sci USA* 1999; 96: 4262.
31. Nakano A, Matsumori A, Kawamoto S, Tahara H, Yamato E, Sasayama S, Miyazaki JI. Cytokine gene therapy for myocarditis by in vivo electroporation. *Hum Gene Ther* 2001; 12: 1289.
32. Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. *Arterioscler Thromb Vasc Biol* 1998; 18: 842.
33. Iiyama K, Hajra L, Iiyama M, Li H, DiChiara M, Medoff BD, Cybulsky MI. Patterns of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression in rabbit and mouse atherosclerotic lesions and at sites predisposed to lesion formation. *Circ Res* 1999; 85: 199.
34. Brand K, Page S, Rogler G, Bartsch A, Brandl R, Knuechel R, Page M, Kaltschmidt C, Baeuerle PA, Neumeier D. Activated transcription factor nuclear factor-kappa B is present in the atherosclerotic lesion. *J Clin Invest* 1996; 97: 1715.

35. Shih PT, Brennan ML, Vora DK, Territo MC, Strahl D, Elices MJ, Lusis AJ, Berliner JA. Blocking very late antigen-4 integrin decreases leukocyte entry and fatty streak formation in mice fed an atherogenic diet. *Circ Res* 1999; 84: 345.

36. Patel SS, Thiagarajan R, Willerson JT, Yeh ET. Inhibition of alpha4 integrin and ICAM-1 markedly attenuate macrophage homing to atherosclerotic plaques in ApoE-deficient mice. *Circulation* 1998; 97: 75.

37. Schapira K, Lutgens E, de Fougerolles A, Sprague A, Roemen A, Gardner H, Koteliansky V, Daemen M, Heeneman S. Genetic deletion or antibody blockade of alpha1beta1 integrin induces a stable plaque phenotype in ApoE-/- mice. *Arterioscler Thromb Vasc Biol* 2005; 25: 1917.

38. Hajra L, Evans AI, Chen M, Hyduk SJ, Collins T, Cybulsky MI. The NF-kappa B signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to atherosclerotic lesion formation. *Proc Natl Acad Sci USA* 2000; 97: 9052.

39. Weber KS, Draude G, Erl W, de Martin R, Weber C. Monocyte arrest and transmigration on inflamed endothelium in shear flow is inhibited by adenovirus-mediated gene transfer of IkappaB-alpha. *Blood* 1999; 93: 3685.

40. Lockyer JM, Colladay JS, Alperin-Lea WL, Hammond T, Buda AJ. Inhibition of nuclear factor-kappaBmediated adhesion molecule expression in human endothelial cells. *Circ Res* 1998; 82: 314.

41. Deleuze V, Scherman D, Bureau MF. Interleukin-10 expression after intramuscular DNA electrotransfer: kinetic studies. *Biochem Biophys Res Commun* 2002; 299: 29.