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Autore	Maddaluno, Marcella
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Sommario	Percutaneous coronary intervention (PCI) is the current procedure that allows the endovascular treatment of occlusive artery disease, without the need of bypass surgery. The most problematic complication of PCI, with or without stent implantation, is the restenosis defined as the re-narrowing of the enlarged artery and characterized by an immune/inflammatory response going with a hyperplastic reaction, involving smooth muscle cell (SMC) migration/proliferation, and remodelling of the arterial wall. In an effort to improve on current therapy for restenosis we are prompted to consider new strategies for prevention and treatment, focusing on understanding of molecular mechanisms and identifying possible therapeutic targets. This thesis aims at four issues described as follows: 1. Effect of NBD peptide on injury-induced neointimal formation The activation of nuclear factor-?B (NF-?B) is a crucial step in the arterial wall's response to injury. NF-?B essential modulator-binding domain (NBD) peptide blocks the activation of the I?B kinase complex, selectively abrogating the inflammation-induced activation of NF-?B. In this study, we investigated the effect of NBD peptide on neointimal formation using two animal models of arterial injury: rat carotid artery balloon angioplasty and wire-induced carotid injury in

apolipoproteinE-deficient (apoE-/-) mice. Local treatment with the NBD peptide (300 ?g/site) significantly reduced the number of proliferating cells in rat carotid arteries 7 days after angioplasty (by 40%; P<0.01) and reduced injury-induced neointimal formation (by 50%; P<0.01) at day 14. These effects were associated with a significant reduction of NF-?B activation and monocyte chemotactic protein-1 (MCP-1) expression in the carotid arteries of rats treated with the peptide. In addition, the NBD peptide (0.01 to 1 ?M) reduced rat SMC proliferation, migration, and invasion in vitro, processes contributing to the injury-induced neointimal formation in vivo. Similar results were observed in apoE-/- mice in which the NBD peptide (150 ?g/site) reduced wire-induced neointimal formation at day 28 (by 47%; P<0.01). Our results demonstrate that the NBD peptide reduces neointimal formation and SMC proliferation/migration, both effects associated with the inhibition of NF-?B activation. 2. Use of the anti-inflammatory agent bindarit to control neointimal hyperplasia Chemokines are a family of proteins that regulate the migration of circulating leukocytes to sites of arterial injury as well as the activation of SMCs. Many chemokine genes are under the control of NF-?B. Bindarit is an original compound with peculiar anti-inflammatory activity due to a selective inhibition of the chemokines MCP-1, MCP-3, and MCP-2. In the present study we evaluated the effect of bindarit on neointimal formation using both animal models described above. Treatment of rats with bindarit (200 mg/kg/day) significantly reduced balloon injury-induced neointimal formation by 39% at day 14 without affecting re-endothelialisation and reduced the number of medial and neointimal proliferating cells at day 7 by 54% and 30%, respectively. These effects were associated with a significant reduction of MCP-1 levels both in sera and in injured carotid arteries of rats treated with bindarit. In addition, in vitro data showed that bindarit (10-300 µM) reduced rat SMC proliferation, migration, and invasion. Similar results were observed in apoE-/- mice in which bindarit administration resulted in a 42% reduction of the number of proliferating cells at day 7 after carotid injury and in a 47% inhibition of neointimal formation at day 28. Analysis of the cellular composition in neointimal lesions of apoE-/- mice treated with bindarit showed that the relative content of macrophages and the number of SMCs were reduced by 66% and 30%, respectively, compared with the control group. This study demonstrates that bindarit is effective in reducing neointimal formation in both non-hyperlipidaemic and hyperlipidaemic animal models of vascular injury by a direct effect on SMC proliferation and migration and by reducing neointimal macrophage content. All of these data were associated with the inhibition of MCP-1 production. 3. Role of Monocyte Chemotactic Protein-3 in human coronary smooth muscle cell proliferation Few studies have examined the role of MCP-3 in vascular pathologies such as atherosclerosis and restenosis in which SMC proliferation plays an important role. In this study, we investigated the effect of MCP-3 on human coronary artery SMC (CASMC) proliferation. MCP-3 induced concentration-dependent CASMC proliferation with the maximum stimulatory effect at 0.3 ng/mL (about 50% vs unstimulated cells) assessed by bromodeoxyuridine (BrdU) uptake and direct cell counting. Anti-MCP-3 Ab (20 ng/mL) completely inhibited cell proliferation, demonstrating the specificity of the proliferative effect of MCP-3. Moreover, the MCP-3-induced CASMC proliferation was blocked by RS 102895 (0.06-6 ?M), a specific antagonist of chemokine receptor 2

	(CCR2). The mitogenic effect of MCP-3 appeared to be dependent on ERK1/2 MAPK and PI3K signalling pathway activation, as demonstrated by the reduction of MCP-3-induced CASMC proliferation observed after the treatment of cells with U0126 (1 ?M) and LY-294002 (5?M), selective inhibitors of ERK 1/2 and PI3K activation, respectively. We found no relationship between MCP-3-induced CASMC proliferation and NF-?B activation. Moreover, we found that tumor necrosis factor-? (TNF-?, 30 ng/mL) and interleukin-1? (IL-1?, 1 ng/mL) both induced time-dependent increase of MCP-3 production by CASMCs, which was reduced by the anti-MCP-3 Ab (20 ng/mL), suggesting that the mitogenic effect of these stimuli is due, at least in part, to MCP-3. Our results demonstrate that MCP-3 is produced by human CASMCs and directly induces CASMC proliferation in vitro, suggesting a potential role for this chemokine in vascular pathology. 4. Antigen presentation and costimulatory molecules expression by murine smooth muscle cells The findings that SMCs express MHC II molecules during arterial response to injury suggested their active role in cellular immunity. Since it is not known if vascular SMCs can function as antigen presenting cells, in the present study we investigated the contribution of SMC in antigen presentation. Firstly, we examined the MHC II and some costimulatory molecules expression in SMCs. The percentage of MHC II, CD54 (ICAM-1), CD44 and OX40L positive unstimulated SMCs was about 2%, 30%, 87% and 5%, respectively. The stimulation with IFN-? (100 ng/mL)) significantly caused a 7 to 8 fold increase in the percentage of MHC II positive cells (P<0.01), a 2 fold increase in the percentage of ICAM-1 positive cells (P<0.01), while it did not affect the expression of CD44 and OX40L. To assess the antigen presentation by SMCs we employed the Ealpha (E?)-GFP/Y-Ae system that allows visualisation of antigen uptake, as the E? is GFP labelled, and tracking of antigen presentation using the Y-Ae Ab to detects E? when bound to MHC II. Treatment of SMCs with E? for 24 h induced an increase in the percentage of GFP positive cells, both in presence or absence of IFN-?-stimulation, without affecting the percentage of Y-Ae positive cells. Treatment with E? of dendritic cells, used as positive control, significantly caused a 50 to 60 fold increase in the percentage of both GFP and Y-Ae positive cells. Our results show that cultured murine SMCs express MHC II molecules after stimulation with IFN-? but are not able to present the antigen in the context of MHC II.
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