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Inflammatory events in a vascular remodeling model induced by surgical injury to the rat carotid artery

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1 The aim of our study was to gain insight into the molecular and cellular mechanisms of the inflammatory response to arterial injury in a rat experimental model.

2 Rats (five for each experimental time) were subjected to brief clamping and longitudinal incision of a carotid artery and monitored for 30 days. Subsequently, Nuclear Factor-kappaB (NF-κB) expression was measured by electrophoretic mobility shift assay. Heat shock protein (HSP) 27, HSP47 and HSP70 were evaluated by Western blot. Morphological changes of the vessel wall were investigated by light and electron microscopy.

3 In injured rat carotid artery NF-κB activity started immediately upon injury, and peaked between 2 and 3 weeks later. Western blot showed a significant increase of HSP47 and HSP70 7 days after injury. At 2 weeks postinjury, HSP27 expression peaked. Ligth microscopy showed a neointima formation, discontinuity of the media layer and a rich infiltrate. Among infiltrating cells electron microscopy identified dendritic-like cells in contact with lymphocytes.

4 Our model of surgical injury induces a significant inflammatory process characterized by enhanced NF-κB activity and HSPs hyperexpression. Dendritic-like cells were for the first time identified as a novel component of tissue repair consequent to acute arterial injury.
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Abbreviations: cpm, counts per minute; EMSA, electrophoretic mobility shift assay; HSP, heat shock protein; NF-κB, nuclear factor-kappa B; NT, uninjured carotid; SDS, sodium dodecyl sulphate; SMCs, smooth-muscle cells; T, injured carotid; TBE, Tris borate EDTA

Introduction

The endothelial dysfunction that results from mechanical artery injury is associated with restenosis after revascularization procedures such as angioplasty, stenting, and bypass grafting (Drachman & Simon, 2005; Liapis & Paraskevas, 2005). Evidence that the inflammatory response activated after vessel injury induces restenosis has been obtained at cellular, molecular, and genetic level (Toutouzas *et al*., 2004). However, the relationship between inflammation and restenosis is complex and only in part understood (Bhatt, 2004). Inflammation stimulates the migration and proliferation of smooth-muscle cells (SMCs), which intermix with infiltrating leukocytes to form an intermediate lesion. If these responses continue unabated, they can thicken the artery wall, which compensates by gradual dilation, so that the lumen remains unaltered, a phenomenon termed 'remodeling' (Ross, 1999; Steffens & Mach, 2004). Mitogenic stimuli promote SMCs proliferation through multiple intracellular signaling pathways. Many of these stimuli activate the nuclear factor-kappa B (NF-κB), on the other hand the expression of several genes involved in the inflammatory and proliferative response of cells is regulated at transcriptional level by NF-κB (Gerondakis & Strasser, 2003). Heat shock proteins (HSPs) are induced by several stresses as heat, toxic substances, injury, surgery, and even behavioral and psychological stresses (Pockely, 2002). NF-κB activation has been identified in human atherosclerotic lesions and in a rat model of arterial injury (Yoshimura *et al*., 2001) whereas all cell types in the blood vessel wall respond to environmental stress with the synthesis of HSPs (Snoeckx *et al*., 2001). Although these observations suggest that both NF-κB and HSPs play a critical role in vasculopathy induced by arterial injury, it is not well known the relationship between inflammatory cell infiltration and these molecular factors in restenosis progression. Circulating bone marrow-derived progenitor cells have been implicated in vascular repair: they could be a source of neointimal cells, which contribute to restenosis, graft vasculopathy, and hyperlipidemia-induced atherosclerosis, and could differentiate to SMCs (Hibbert *et al*., 2003; Kong *et al*., 2004). Among cells derived from blood-borne precursors, dendritic cells are known to be involved in early neointima formation (Takahashi & Lee, 2003). Dendritic cells induce a primary immune response, and their number is greatly increased in various inflammatory events (Cravens & Lipsky, 2002; Gogolak *et al*.,

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2003). We have used a rat experimental model of carotid surgical injury to identify molecular and cellular modifications occurring after acute arterial injury. Specifically, we investigated the time course of NF-κB activation, and the expression of HSPs (HSP70, HSP27, HSP47). We also examined the morphological changes occurred in the vascular remodeling process.

Methods

Animals

Studies were performed on adult male Wistar rats (200–250 g). All surgery and experimental procedures were approved by the Animal Ethics Committee of the Second University of Naples. The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Animal care was in compliance with the Italian (D.L. 116/92) and European Community (E.C. L358/1 18/12/86) guidelines on the use and protection of animals in experimental research. All rats were acclimated in individual cages for 1 week before experimentation. They were maintained on a 12-h light/dark cycle at 24°C, at a relative humidity of 60%, and they received food and water *ad libitum*.

Carotid injury

The surgical procedure is described in detail elsewhere (Forte *et al*., 2001). Briefly, male Wistar were anesthetized with an intraperitoneal (i.p.) injection of ketamine hydrochloride (80 mg kg^{-1} i.p.). They were then given a dose of the antibiotic ticarcillin (50 mg kg^{-1} i.v.). Sterile techniques were used throughout the procedure. An incision (median length: 4 cm) was made in the animal's anterior neck region. The carotid was identified and a plastic scanlom clamp for coronary artery bypass grafting was applied on the carotid for 10 s to effect a crushing lesion. At the same point a 0.5-mm longitudinal incision was made through the full thickness of the carotid wall. Hemostasis was obtained with a single adventitial 8.0-gauge polypropylene stick. Five rats were killed at different time points (0, 2, 4, 6, 24 and 48 h; 7, 14, 21, and 30 days) after vascular injury and the carotid arteries were removed and processed as described below.

Electrophoretic mobility shift assay (EMSA)

The carotid segments (injured and uninjured; injured carotid (T) and uninjured carotid (NT), respectively) were excised from five rats at each indicated time (0, 2, 6, and 24 h; 7, 14, and 30 days) after injury. They were frozen in liquid nitrogen, immediately suspended in 150 μl of ice-cold hypotonic lysis buffer (10 mM HEPES, 10 mM KCl, 0.5 mM phenylmethylsulphonyfluoride, 1.5 μg ml^{-1} soybean trypsin inhibitor, 7 μg ml^{-1} pepstatin A, 5 μg ml^{-1} leupeptin, 0.1 mM benzamidine, 0.5 mM dithiothreitol) and homogenized at the highest setting for 1 min in a Polytron PT 3000 (Kinematica, Cincinnati, OH, U.S.A.) tissue homogenizer. The homogenates were chilled on ice for 15 min, supplemented with 20 μl of 10% Nonidet P-40, and then vigorously shaken for 15 min. The nuclear fraction was precipitated by centrifugation at 1500 g for 5 min, the supernatant containing the cytosolic fraction was removed and stored at −80°C. The nuclear pellet was resuspended in 100 μl of high salt extraction buffer (20 mM HEPES pH 7.9, 10 mM NaCl, 0.2 mM EDTA, 25% v v^{-1} glycerol, 0.5 mM phenylmethylsulphonyfluoride, 1.5 μg ml^{-1} soybean trypsin inhibitor, 7 μg ml^{-1} pepstatin A, 5 μg ml^{-1} leupeptin, 0.1 mM benzamidine, 0.5 mM dithiothreitol) and incubated with shaking at 4°C for 30 min. The nuclear extract was then centrifuged for 15 min at 13,000 × g and the supernatant was aliquoted and stored at −80°C. Protein concentration was determined with the Bio-Rad protein assay kit. Double-stranded oligonucleotides containing the NF-κB recognition sequence (5′CAACGGCA GGGGAATCTCCCTCTCCTT-3′) were end-labeled with ^{32}P-γ-ATP. Nuclear extracts containing 5 μg protein were incubated for 15 min with radiolabeled oligonucleotides (2.5–5.0 × 10^4 cpm) in 20 μl reaction buffer containing 2 μg poly dI-dC, 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 10% (v v^{-1}) glycerol. The specificity of the DNA/protein binding was determined by competition reaction in which a 50-fold molar excess of unlabeled wild-type, mutant or Sp-1 oligonucleotide was added to the binding reaction 15 min before addition of radiolabeled probe. In supershift assay, antibodies reactive to p50 or p65 proteins were added to the reaction mixture 15 min before the addition of radiolabeled NF-κB probe. Nuclear protein–oligonucleotide complexes were resolved by electrophoresis on a 6% nondenaturing polyacrylamide gel in 1 × TBE buffer (Tris-borate-EDTA) at 150 V for 2 h at 4°C. The gel was dried and autoradiographed with an intensifying screen at −80°C for 20 h. Subsequently, the relative bands were quantified by densitometric scanning of the X-ray films with a GS-700 Imaging Densitometer (Bio-Rad) and a computer program (Molecular Analyst; IBM).

Western blot analysis

Carotid arteries (injured and uninjured; T and NT, respectively) were rapidly excised from five rats at the indicated times (0, 4, and 48 h, and 7, 14, and 21 days after injury), snap-frozen in liquid nitrogen and stored at −80°C until ready for processing. Tissues were pulverized with a mortar and pestle, and suspended in a cold cell lysis buffer (0.125 mol l^{-1} Tris-HCl pH 6.8, 20% glycerol, 4% SDS and 10% β-mercaptoethanol added with a protease inhibitor cocktail). After centrifugation, a portion of supernatant was used for protein concentration determination using a Bio-Rad protein assay. Equal amounts of protein (20 μg) were loaded onto a 10% polyacrylamide gel, electrophoresed and transferred to nitrocellulose membranes. The blots were blocked for 1 h with 5% nonfat milk reagent dissolved in TTBS (1 × Tris-buffered saline with 0.05 % Tween-20). Membranes were incubated with an anti-HSP70 monoclonal antibody (1 : 500), or an anti-HSP27 polyclonal antibody (1 : 500) or an anti-HSP47 monoclonal antibody (1 : 1000). Anti-β-actin monoclonal antibody was used as an internal standard. After further washing in TTBS 0.05%, horseradish peroxidase conjugated polyclonal goat anti-mouse and anti-rabbit IgG were used as secondary antibodies. The blots were visualized with Supersignal West Femto Maximum Sensitivity Substrate and autoradiographed. Protein levels were quantified by scanning densitometry (Gel Doc-2000, Bio-Rad).

Light and electron microscopy and immunohistochemistry

Light and electron microscopic analyses were performed in injured and uninjured carotids (from three rats) 7 days after surgery. Samples for microscopy were fixed in 2% formaldehyde and 2.5% glutaraldehyde in 0.1 mol l^{-1} cacodylate buffer, pH 7.4, osmicated and embedded in epoxy resin. Sections 1–2 μm thick were stained with alkaline toluidine blue and examined by light microscopy to select areas for electron microscopy. Sections about 70 nm thick were stained with lead acetate and uranyl acetate and observed in a Jeol JEM 1010 electron microscope (Tokyo, Japan), at 80 kV.

The injured and uninjured from rats 7 days after surgery (five animals) were used for immunohistochemical analyses. Samples were fixed with periodate-lysine-paraformaldehyde mixture in 0.1 mol l^{-1} phosphate buffer, pH 7.4 (McLean & Nakane, 1974; Pieri *et al*., 2002), for 1 h, washed in the same buffer for 24 h at room temperature, cryoprotected with 30% sucrose and snap frozen. Cryosections were laid on poly-L lysine-coated slides. After blocking nonspecific binding sites with 10 mg ml^{-1} bovine serum albumin in PBS, a mouse monoclonal antibody against rat MHC-II-polymorphic (clone F17-23-2, reacting with RT1l and RT1n) was applied overnight at 4°C, followed by fluorescein isothiocyanate-labeled, goat antimouse polyclonal antibodies (1 : 50, 60 min at 37°C). Omission of the primary antibody and substitution with an irrelevant one were used as controls for the immunohistochemical reaction. The slides were mounted with Gel/Mount (Biomeda, Foster City, CA, U.S.A.) and observed in an Axioskop microscope equipped for epifluorescence (Zeiss, Oberkochen, Germany).

Materials

Animals were obtained from Harlan Italy (San Piero al Natisone, Italy). All buffer reagents for EMSA and Western blot analysis, ketamine hydrochloride, ticarcillin disodium salt, anti-β-actin monoclonal antibody and all reagents for light and electronic microscopy were from Sigma (Milan, Italy). ^{32}P-γ-ATP was from Amersham (Milan, Italy). Double-stranded oligonucleotides were from Tib Molbiol, Boehringer-Mannheim (Genoa, Italy). Antibodies for supershift assay and secondary antibodies anti-mouse and anti-rabbit for western blot were obtained from Santa Cruz (Milan, Italy). Protein assay kit was from Bio-Rad (Milan, Italy). All antibodies for HSPs were purchased from StressGen Biotechnologies Corp. (Victoria BC, Canada). Secondary antibodies for immunohistochemical analysis were from Sigma (Milan, Italy). Mouse monoclonal antibody against rat MHC-II-polymorphic was from Serotec, Kidlington, Oxford, U.K. Supersignal West Femto Maximum Sensitivity Substrate for signal detection was obtained from Pierce Biotechnologies (Rockford, IL, U.S.A.).

Statistical analysis

All data were reported as means $\pm$ s.e.m. of n experiments. A two-tailed paired Student's t-test was used to compare individual left and right carotid data. All other data were analyzed by an unpaired Student's t-test or by one way analysis of variance (ANOVA) and Bonferroni-corrected P-value for multiple comparisons when indicated. P-values <0.05 were considered statistically significant.

Results

Kinetic analysis of NF-κB activation

The NF-κB/DNA binding activity in nuclear protein extracts from NT and T carotid arteries was evaluated by EMSA. As shown in Figure 1a and b, NF-κB/DNA binding activity occurred at basal level in nuclear extracts from NT carotids at time 0. Upon injury, a retarded band appeared at time 0, significantly increased until 14 days and decreased thereafter. Conversely, in nuclear extracts from NT carotids, NF-κB/DNA binding activity started 2 h after injury, progressively and significantly increased until 24 h and decreased thereafter. The composition of the NF-κB complex activated by carotid injury was determined by competition and supershift experiments (Figure 1c). The specificity of the NF-κB/DNA binding complex was demonstrated by the complete displacement of NF-κB/DNA binding in the presence of a 50-fold molar excess of unlabelled NF-κB probe in the competition reaction. In contrast a 50-fold molar excess of unlabeled mutated NF-κB probe or Sp-1 oligonucleotide did not affect DNA-binding activity. The anti-p50 and anti-p65 antibodies gave rise to a characteristic supershift of the retarded complex. Addition of anti-p50 + anti-p65 to the binding reaction caused a marked reduction of the intensity of the NF-κB-specific bands suggesting that the NF-κB complex contained p50 and p65 dimers.

Time course of HSPs protein expression after carotid injury

Western blot analysis revealed a significant increase of HSP70 protein expression at 7th day after injury as compared to NT carotids, whereas at 14 and 21 days after the injury the values were similar to NT (Figure 2). HSP27 expression gradually increased and became statistically significant at 7 and 14 days after injury; the signal returned to basal levels 21 days after injury (Figure 3). HSP47 expression appeared only at day 7 in T carotids, and undetectable in NT carotids throughout the study. The signal decreased to basal level already 14 days from injury (Figure 4).

Light and electron microscopy and immunohistochemistry

On the basis of the results obtained in our previous study (Forte *et al*., 2001), and EMSA and Western blot analyses, we have chosen the 7th day after the injury, in order to investigate morphological changes in injured carotids (T). Light microscopy (Figure 5) showed a neointima formation and a discontinuity of the media layer leading to cell transmigration from the vascular lumen to media and adventitia. At electron microscopy (Figure 6) immature, organelle-rich cells were detected within the vessel lumen and the neointima, some also within the media and adventitia. These cells contain a rough or smooth endoplasmic reticulum, a well-developed Golgi apparatus and one or more microfilament peripheral bundles. Lysosomes were absent or a few. Some infiltrating cells in the deeper part of neointima, the media and the adventitia were dendritic in shape and organized in a mesh containing lymphocytes. By contrast, no alteration was observed in the NT arteries (Figure 7). At immunofluorescence, cells of dendritic shape and expressing MHC-II antigens were found

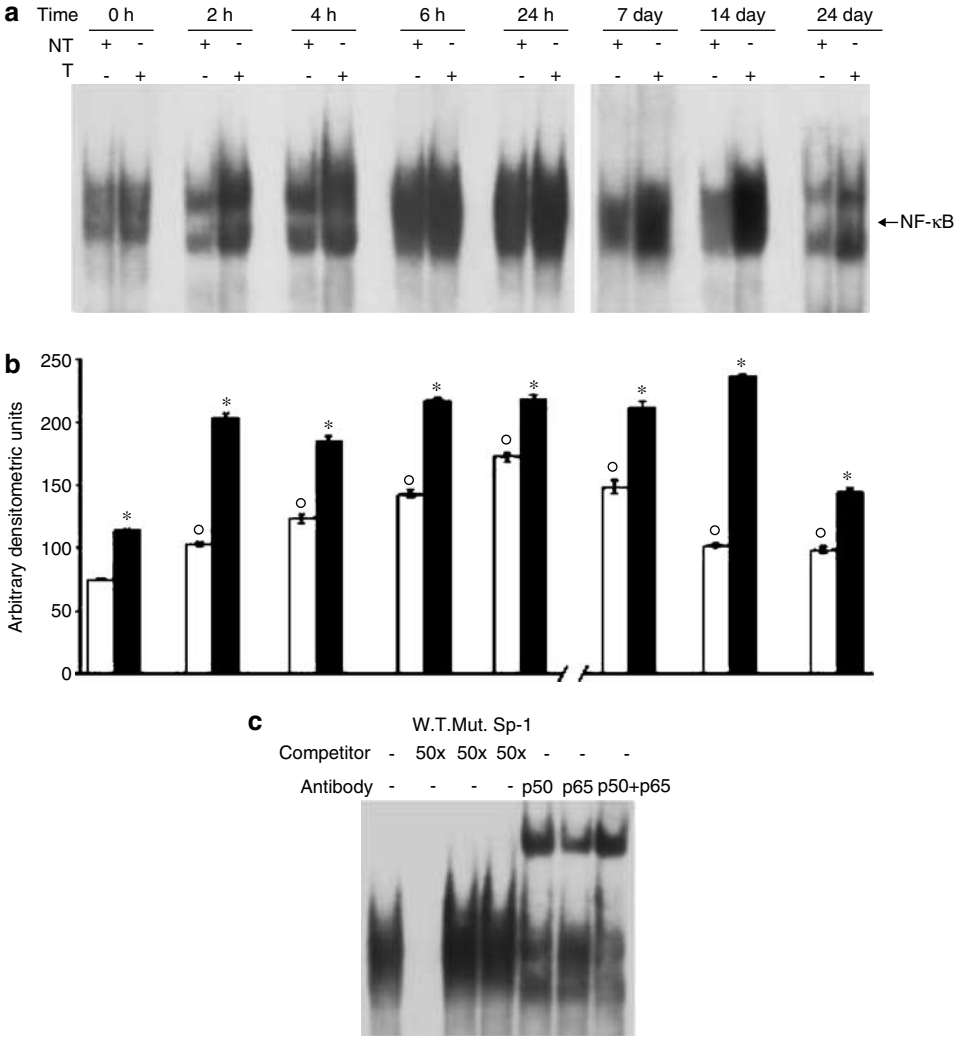

Figure 1 Kinetic analysis of NF-κB activation and characterization of NF-κB complex. Representative EMSA (a) as well as densitometric analysis (b) show NF-κB/DNA binding activity in nuclear extracts from uninjured (NT) and injured (T) carotid arteries. Nuclear extracts from NT and T carotid arteries were prepared as described in the text and incubated with ^{32}P-labeled NF-κB probe. Data in (a) are from a single experiment (from three to five carotids) and representative of three separate experiments. Data in (b) are expressed as mean $\pm$ s.e.m. of three experiments. $^{\circ}P<0.01$ vs NT carotids at time zero; $^{*}P<0.01$ vs NT carotid for each time point. Characterization of NF-κB/DNA complex in nuclear extracts of injured rat carotid arteries at 24 h (c). In competition reaction nuclear extracts were incubated with radiolabeled NF-κB probe in the absence or presence of: identical but unlabeled oligonucleotides (W.T., 50 $\times$), mutated non-functional NF-κB probe (Mut., 50 $\times$) or unlabeled oligonucleotide containing the consensus sequence for Sp-1 (Sp-1, 50 $\times$). In supershift experiments nuclear extracts were incubated with antibodies against p50, p65, p50 + p65, 15 min before incubation with radiolabeled NF-κB probe. Data illustrated are from a single experiment and are representative of three separate experiments.

in the same location and distribution as the dendritic cells shown by electron microscopy (Figure 8). Control sections appeared unstained (not shown).

Discussion

Using our rat carotid model, we monitored the inflammatory events consequent to surgical injury by examining NF-κB activation, HSPs expression, and the occurred morphological changes. NF-κB is a dimeric transcription factor that can be rapidly activated by the inflammatory cytokines TNF-α and IL-1, T-cell activation signals, growth factors and stress inducers (Gerondakis & Strasser, 2003). In our model NF-κB was activated within minutes of injury; it increased remarkably and remained high until 14 days after injury, after which it decreased. NF-κB activation occurred in NTs at time 0; it increased until 24 h postinjury and then started to decline. Thus, inflammation was much more severe and persistent in injured than in uninjured vessels. However, NF-κB activation in uninjured arteries indicates that injury induces systemic inflammation. This result is in agreement with another report of NF-κB activation in organs distal from the inflammation site (Liu *et al*., 2003). However, we detected inflammatory cell infiltration and tissue remodeling only at the injury site. It is conceivable that a slight increase in tissue NF-κB levels is not necessarily linked to processes leading to tissue damage and structural subversion. In addition, we detected both p50 and

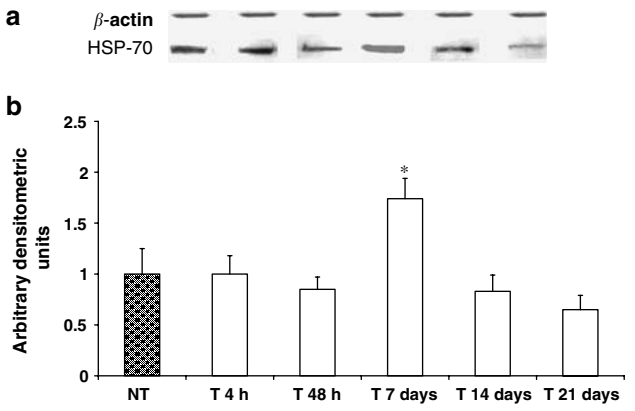

Figure 2 Kinetic analysis of HSP70 expression in injured (T) and uninjured (NT) carotids. The samples obtained from carotids at different times after injury or from NTs were analyzed by Western blot assay using specific monoclonal antibody as indicated in Material and methods. Results are expressed in arbitrary densitometric units as compared to the protein expression level in NT. (a) Representative Western blot for HSP 70 expression. (b) The relative amount of HSP70 was quantified by scanning densitometry and normalized to that of β-actin. Each column represents the mean $\pm$ s.e.m. of five independent experiments for each time point. $^{*}P<0.05$ vs NT.

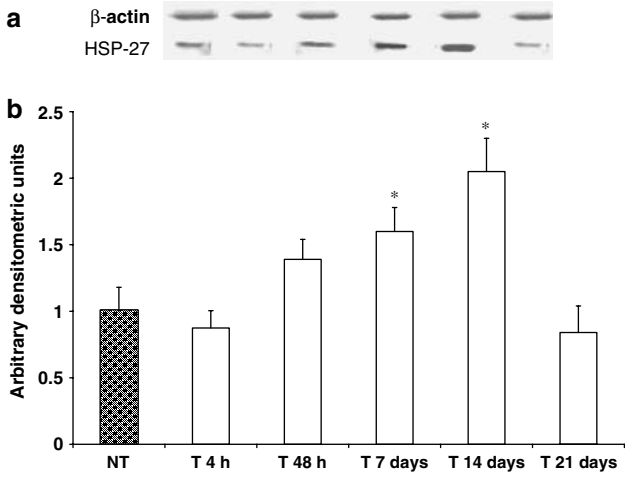

Figure 3 Kinetic analysis of HSP27 expression in injured (T) and uninjured (NT) carotids. The experiments were performed as described in Figure 2. (a) Representative Western blot for HSP 27 expression. (b) Each column represents the mean $\pm$ s.e.m. of five independent experiments for each time point. $^{*}P<0.05$ vs NT.

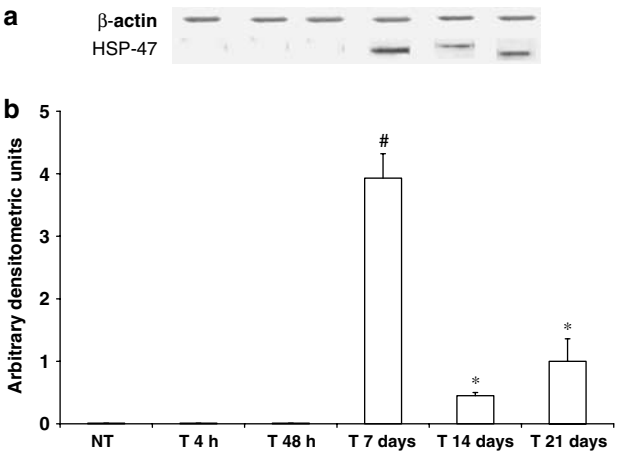

Figure 4 Kinetic analysis of HSP47 expression in injured (T) and uninjured (NT) carotids. The experiments were performed as described in Figure 2. (a) Representative Western blot for HSP 47 expression. (b) Each column represents the mean $\pm$ s.e.m. of five independent experiments for each time point. $^{*}P<0.05$ vs NT; $^{\#}P<0.01$ vs NT.

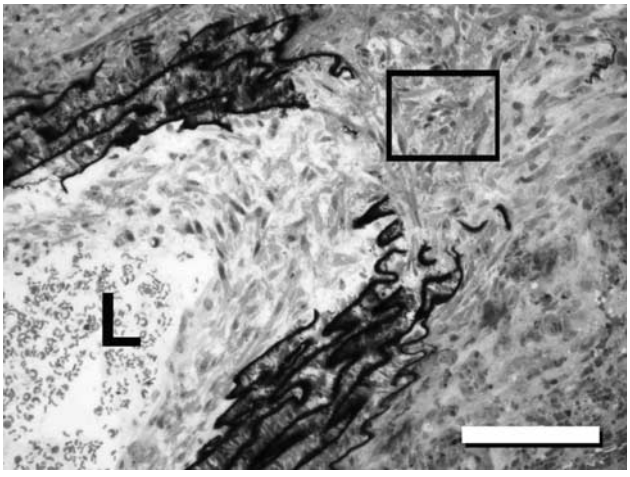

Figure 5 Light microscopy of a vessel wall 7 days after injury. Epoxy resin section, stained with alkaline toluidine blue, shows media discontinuity, neointima formation and cell infiltration which starting from the neointima goes through media and adventitia layers. The cells within the squared inset has been observed at the electron microscopy (see Figure 6). Field is representative of three independent experiments. L = vessel lumen, Bar = 100 μm.

p65 subunits in Ts indicating that the complex NF-κB in rat arteries results from the association of these heterodimeric forms as already described (Cercek *et al*., 1997).

Although the effect of HSPs on atherosclerosis is well understood (Mehta *et al*., 2005), their role in inflammatory events consequent to surgical injury are poorly investigated. In our model, HSP70 was significantly increased 7 days after injury. Moreover, its expression was correlated with a high density of infiltrating cells through the vessel wall suggesting that HSP70 limits the damage after vascular injury (Greenberg *et al*., 2001). A previous finding that HSP70 is induced in response to acute hypertension suggested that HSP70 protects the vasculature from damage during hemodynamic stress (Xu *et al*., 1995). In our model, HSP27 expression increased in Ts to reach a maximum 14 days from injury, after which it declined at returned to basal level 21 days after injury. This result is relevant in the light of the finding that pharmacological induction of HSP27 may prevent restenosis postvascular intervention (Connolly *et al*., 2003). In addition, HSP70 and HSP27 expression was unchanged in NTs, which suggests that the increased expression of two HSPs in the injured vessels may be correlated with the protection against the stressful conditions. HSP47 was found to be expressed only at 7 days postinjury, and never in NTs. This time course strongly suggests that HSP47 was specifically induced by injury. HSP47 is a 47-kDa heat-shock-inducible glycoprotein that has been found associated with procollagen in the endoplasmic reticu-

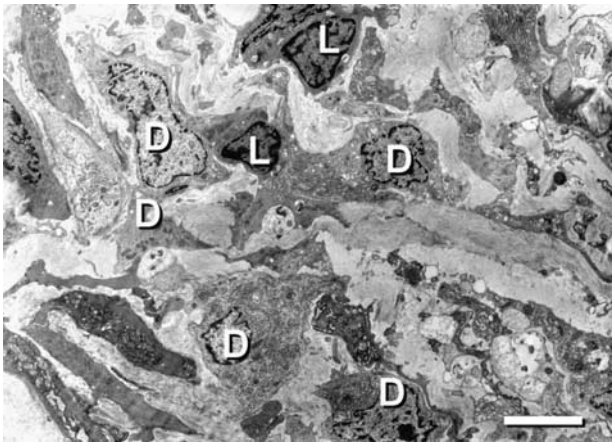

Figure 6 The picture shows the ultrastructure of the border between media and adventitia layers (squared insert of Figure 5) of a carotid artery 7 days after injury: dendritic-like cells (D), organized in a mesh and in contact with lymphocytes (L), are observed in the inner part of the neointima, within the media and adventitia. Field is representative of three independent experiments. Bar = 5 μm.

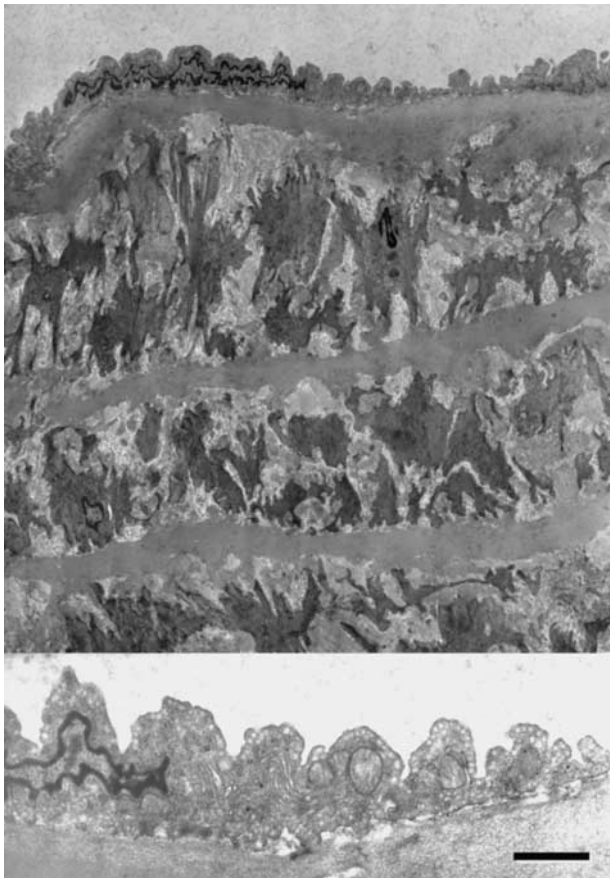

Figure 7 Untreated carotid artery of a rat 7 days after injury, with normal appearance at electron microscopy. The mature endothelium with its thin basement membrane attached to the inner elastic lamina is enlarged in the bottom panel. Field is representative of three independent experiments. Bar = 7 μm for the top panel and 2 μm for the bottom panel.

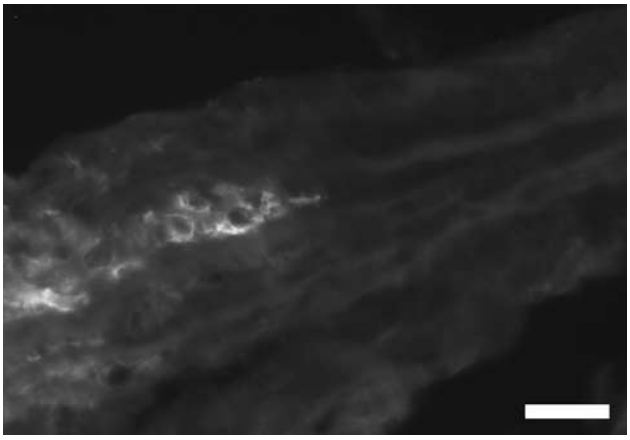

Figure 8 A cluster of dendritic cells expressing MHC-II membrane antigens is shown deep in the neointima of a carotid artery 7 days upon injury. Field is representative of three independent experiments. Immunofluorescence, Bar = 20 μm.

lum, and evidence suggests that it acts as a collagen-specific chaperone (Nagata, 2003; Sauk *et al*., 2005). Our data are in agreement with HSP47 overexpression in rat carotid SMCs associated with collagen overproduction and intimal thickening consequent to balloon injury (Murakami *et al*., 2001; Sluijter *et al*., 2004). The increase in HSP47 expression in our model was transient; in fact, HSP47 expression started to decline 14 days after injury. Therefore, this protein was expressed mostly at the onset of the fibrosing process, around 7 days after treatment, a process that was well advanced 14 days after injury. Several studies report that the heat stress response protects against tissue injury by increasing the expression of HSPs and suppressing the NF-κB activation (Chen *et al*., 2004; Schell *et al*., 2005). In this study, we show that the NF-κB activation is occurring at early time points and is sustained until 14 days after vascular injury whereas a greater and significant level of HSPs expression is mainly detectable at late time points (7 and 14 days) suggesting for these HSPs a protective role in vascular injury and remodeling. Further studies are necessary to examine the roles played by HSPs (HSP70, HSP47 and HSP27) as well as the mechanism of the interaction of HSPs and NF-κB pathway.

Our light and electron microscopy data confirm the recruitment of inflammatory cells in response to acute vascular injury. These cells could be blood-borne and differentiated into endothelial, macrophage and dendritic cells. We cannot exclude that some infiltrating cells differentiate into SMCs, however, the expression of filamentous actin, as shown by microfilament bundles at electron microscopy, is typical of several cells that enter the arterial wall during response to injury. It has been suggested that circulating precursor-derived endothelial cells are involved in the repair of blood vessels after ischemic injury, tumor angiogenesis, and atherosclerosis (Sata *et al*., 2002). Among the blood-borne cells in the arterial wall, dendritic cells deserve special attention given their pivotal role in immune responses. Dendritic cells are present early and throughout the immune response, and are in contact with lymphocytes (Bailey & Fleming, 2003). We found dendritic cells in the deeper part of the intima, in the media and the adventitia, which suggests that most if not all these precursor-derived cells reached the luminal surface of the vessel wall and

proceeded to differentiate as they moved into the tissue. Thus, dendritic cells may play a regulatory role in remodeling the arterial wall. Dendritic cells in contact with lymphocytes in nonlymphoid tissues, as found here, are hallmarks of secondary immune responses (Gallè *et al*., 2001; Cirrincione *et al*., 2002) and indicate that dendritic cells are mature and capable of stimulating lymphocytes and other cells as well (Foti *et al*., 1999; Sallusto *et al*., 1999; Zhu *et al*., 2000; Scimone *et al*., 2005).

In conclusion, our model of surgical injury induces a significant inflammatory process characterized by enhanced NF-κB activity also in distant sites. The activation of HSPs suggests they exert a protective function in vascular injury and remodeling. In particular, the time course-expression of the collagen-specific chaperonin, HSP47, suggests that this molecule is a sensitive and specific marker of the early phases of the fibrosing process. Moreover, the presence of many dendritic cells, their localization throughout the vessel wall and their contacts with lymphocytes is new and intriguing, and adds another facet to traditional, as well as novel concepts concerning the origin and nature of neointimal cells. Our study is in progress to better characterize the specific role of dendritic cells and their correlation with NF-κB and HSPs activity.

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