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Biology of the smooth muscle cells in human atherosclerosis

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The aim of this study was to reconstruct dynamic biological steps of human atherosclerosis at different ages of life and, in particular, to clarify the role of the smooth muscle cells (SMCs) by means of evaluation of several markers implicated in proliferative diseases (c-fos, proliferating cell nuclear antigen: PCNA, apoptosis, chromosome 7). We examined the biological features of 67 atherosclerotic arterial lesions obtained from fetuses, infants, young people and adults. From each case serial sections were stained for histological examination, PCNA, c-fos and apoptosis detection by immunohistochemical methods and for chromosome 7 number evaluation by fluorescence *in situ* hybridization. In coronary specimens of fetuses we observed SMCs with c-fos positivity. In infant lesions the predominant result was positivity for PCNA. Similar results were obtained from the plaques from young subjects with a greater presence of PCNA-positive cells. In adult subjects numerous apoptotic cells were present in the stable plaques, whereas in the unstable plaques we frequently detected joint positivity for both PCNA and c-fos gene and supernumerary chromosomes 7. During the evolution of the atherosclerotic process we observed a biological modulation of SMC proliferation, which begins after activation of the c-fos gene, increases during progression of the lesion and declines in stable plaques, when apoptosis increases. In unstable plaques, the same early steps observed in fetus and infant arteries occur. The observation in some cases of chromosome 7 alterations, markers of tumorigenesis, suggests the possible transformation of an advanced atherosclerotic plaque into a neoplastic-like process.

Key words: Atherogenesis; smooth muscle cells; c-fos; apoptosis; chromosome 7.

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Atherosclerosis is a complex disease in which smooth muscle cells (SMCs) play a fundamental role. In particular, SMC proliferation is believed to be one of the fundamental mechanisms in the pathophysiology of the atherosclerotic process, acting as a defense mechanism in response to a variety of injuries (such as high levels of low-density lipoproteins, hypertension and cigarette smoking) (1–4). Ageing is also a well-known important risk factor for atherosclerosis (5, 6). However, atherosclerotic lesions can be detected already in the first months of life and even during fetal life. In fact, we have recently reported that cigarette smoking has an atherogenic effect on the coronary arteries of fetuses after the 35th gestational week in physiological pregnancies, even of mothers smoking only a few cigarettes a day (7). Of interest are also the artery wall alterations we observed in the first months of life, related to formula feeding and parental cigarette smoking (8, 9).

In the literature there is disagreement on the basic mechanisms and the sequence of changes that determine arterial atherogenic thickening and, in particular, on the role of the SMCs. Authors (10–13) maintain that within the first few weeks after injury, arterial SMCs proliferate in the tunica media and later migrate and proliferate in the intima, causing the intimal thick-

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ening. In accordance with the monoclonal theory, supported by various authors (1, 4, 14–16), only a certain subpopulation of SMCs, resulting from a somatic mutational event in a single cell that gives it and its progeny a proliferative advantage, is selectively expanded in intima. Other authors consider that SMC proliferation follows subendothelial edema and fragmentation of the internal elastica lamina. Subsequently, the lesions are complicated by lipid incursion (17–19).

It has also been reported that the first atherosclerotic reaction is lipoprotein transport across the endothelium into the artery wall and its retention in the intima, followed by the appearance of fatty streak lesions and successively by SMC proliferation (5, 20–22).

According to Ross (23), the earlier intimal lesions initiate instead with accumulation of macrophages, T-lymphocytes, intra- and extracellular lipids and SMCs. These latter turn from the contractile phenotype into the synthetic form, start to proliferate and to produce collagen, elastin and extracellular matrix material.

More recently, bone marrow has emerged as a new source of vascular SMCs that participate in intimal hyperplasia in important vascular disorders, such as lipid-induced atherosclerosis, transplantation arteriosclerosis and post-angioplasty restenosis (24–26).

The present study was undertaken to investigate the biological pattern of human atherosclerosis by means of evaluation of several markers implicated in proliferative diseases (c-fos, PCNA, apoptosis, chromosome 7) and, in particular, to clarify the role of the SMCs in the atherogenic process at different ages of life. This should make it possible to reconstruct a dynamic sequence of the biological changes of these cells during the evolution of atherosclerotic lesions from fetus to adult.

MATERIALS AND METHODS

We examined the biological features of a total of 67 atherosclerotic arterial lesions obtained from fetuses, infants, young people and adults. The study was performed on:

- Samples of coronary arteries obtained by autopsy from 16 fetuses, 10 males and 6 females, ranging in age from 35 to 40 weeks of gestation. Five of the stillborns had died of known causes (Potter's syndrome, dilated cardiomyopathy, septicemia, severe chorioamnionitis and umbilical cord torsion, respectively), whereas in 11 cases a precise death cause had not been determined. These cases we classified as SIUD (sudden intrauterine unexplained death) (Table 1).
- Samples of coronary arteries from autopsies performed on 21 infants, 14 males and 7 females, aged 1–34 months. Ten of these cases were SIDS (sudden infant death syndrome) victims, and 11 had died of other causes (Table 2).
- Autopsy samples of coronary and carotid arteries of 8 young subjects, 3 males and 5 females, aged 19–32 years, who died of various causes (Table 3).
- Carotid specimens containing obstructive atherosclerotic plaques obtained by surgery, performed for therapeutic purposes, from 22 patients, 13 males and 9 females, aged 60–90 years (Table 4).

Arterial fragments were fixed in 10% buffered formalin and embedded in paraffin, according to standard procedures. From each case serial sections were stained for histological examination with hematoxylin/eosin and trichromic Heidenhaim (Azan), for cell immunophenotyping, PCNA, c-fos and apoptosis detection by specific immunohistochemical methods and for chromosome 7 number evaluation by fluorescence *in situ* hybridization (FISH).

Cell immunocytochemistry

Immunophenotyping of cells present in the vascular lesions was performed with monoclonal antibodies, precisely against α-actine to identify the SMCs (clone 1A4; Sigma, St. Louis, MO, USA – dilution 1:200), CD68 for macrophages (clone PG-M1; Dako, Hamburg, Germany – dilution 1:100) and CD 45 for T-cells (clone OPD4; Dako – dilution 1:25). The primary antibodies were incubated for 1 hour at room temperature. After a PBS rinse for 10 min, the LSAB system (LSAB-2-kit, alkaline phosphatase, Dako) was performed following the manufacturer's instructions and, finally, a fuchsin chromogen (New Fuchsin Red, Dako) was applied for 20 min to obtain a red reaction product at the site of the target antigen in the cytoplasm. A counterstaining with hematoxylin was used to visualize the nuclei in the tissue sections.

PCNA immunohistochemistry

Sections were deparaffinized and transferred to a TRIS-HCl-buffered saline solution (TBS, pH=7.6). After blocking the endogenous peroxidase with 3% hydrogen peroxide for 5 min, sections were subsequently immunostained with the monoclonal PC10 antibody (Dako, Hamburg, Germany – dilution 1:200) using the avidin biotin complex (ABC complex) method with overnight incubation. Diaminobenzidine tetrahydrochloride (DAB, Sigma, St. Louis, MO, USA) was used as a chromogen, and a

light hematoxylin counterstain was used. Biotinylated rabbit anti-mouse IgM was used as a secondary antibody (Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA). All incubations were carried out in a humidified chamber at room temperature, and, after each incubation, the slides were extensively washed with three changes of TBS. Inflamed palatine tonsils were used as positive controls for PCNA. Sections incubated with normal mouse or rabbit IgG at the same dilution served as negative controls.

Only nuclei with intense immunostaining were considered to be PCNA positive.

c-fos immunohistochemistry

Sections were deparaffinized in xylene and rehydrated. Endogenous peroxidase was blocked by incubating with 3% hydrogen peroxide for 5 min. After washing in phosphate-buffered saline (PBS), the sections were incubated with 10% normal goat serum and then with 1:100 diluted polyclonal anti-c-fos antibody (SC-52P, Santa Cruz Biotechnology, CA, USA) at room temperature for 1 h. After washing in PBS for 5 min, the sections were incubated for 30 min with the biotinylated goat anti-rabbit IgG stained with diaminobenzidine tetrahydrochloride (DAB, Sigma) solution and counterstained with hematoxylin.

The cells with intense brown immunostaining were considered to be c-fos positive.

Apoptosis immunohistochemistry

The sections were deparaffinized and incubated with 20 μg/ml proteinase K (Sigma. St. Louis, MO, USA). After blocking of the endogenous peroxidase with 3% hydrogen peroxide for 5 min, deoxynucleotidyl transferase (TdT 0.3 U/ml) was used to incorporate digoxigenin-conjugated deoxyuridine (dUTP 0.01 mM/ml) into the ends of DNA fragments. The signal of TdT-mediated dUTP Nick End Labeling (TUNEL) was then detected by an anti-digoxigenin antibody conjugated with peroxidase (Apoptag Peroxidase *in situ* Apoptosis Detection kit, Oncor, Gaithersburg, MD, USA). Apoptotic nuclei were identified by the presence of dark brown staining. Counterstaining was performed by immersing the slides in methyl green for 10 minutes.

Immunohistochemistry evaluation

All the immunostained sections were examined under a light microscope using a ×40 lens.

The positivity of the immunoreactive SMCs for each immunohistochemical technique was classified as: −=no positive cells; +=a few dark positive cells (<10%); ++=a number of positive cells ranging from 10 to 50%; and +++=a high number of positive cells (>50%).

Fluorescence in situ hybridization (FISH)

We used an α-satellite DNA probe specific for the centromeric region of chromosome 7, labeled with biotin (Oncor Inc., Gaithersburg, MD, USA). The centromeric probe was prepared by mixing 1.5 μl of the probe with 30 μl of Hybrisol VI (Oncor). The probe was applied to the prepared air-dried slides (15 μl) and coverslipped. Both probes and target DNAs underwent denaturation on the slides on a 67°C+2 hot plate for 5 min and then incubation overnight in a pre-warmed humidified chamber at 37°C. The hybridized signals were detected using a commercial kit (FITC avidin detection kit, Oncor). Propidium iodide 2.5 μg/ml in anti-fade was used for counterstaining. For scoring, a Leitz Orthoplan with a Ploemopak incident-light fluorescence microscope was used, equipped with ultraviolet excitation filter sets. Only interphase cell nuclei with intact morphology were scored. The number of hybridization spots in each cell was considered.

Statistical analysis

The statistical value of the results was determined using the analysis of the variance test (F-test). The selected level of significance was $p<0.05$.

DEFINITION OF TERMS

Preatherosclerotic lesions

The tunica media appears fragmented with intense infiltration by SMCs, often arranged in columns perpendicularly to the axis of the tunica itself. These cells infiltrate the intima, which is thickened. This thickening is also due to deposits of acid mucopolysaccharides, mainly consisting of type A and C chondroitin sulfates and of hyaluronic acid. Monocytes and/or foam cells are present in low or moderate numbers. Rare B –

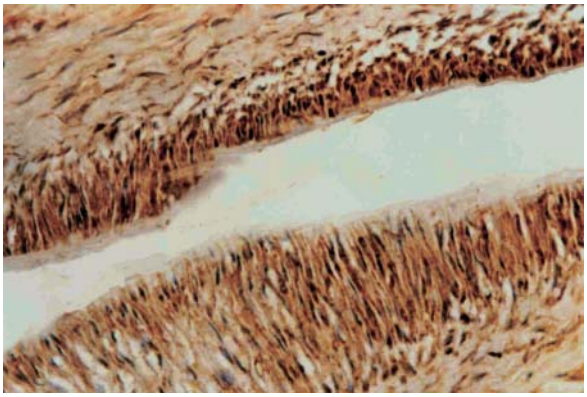

Fig. 1. Epicardial coronary artery (left anterior descending branch) of a fetus of 36 gestational weeks (case 10, Table 1). The coronary lesion shows high positivity for the c-fos protein. c-fos immunohistochemistry. Magnification 400×.

TABLE 1. *Artery lesions in fetuses*

Case no.	Age (gw)/ sex	Diagnosis of death	Atheroscl. lesion	Biological markers			
				Immunohistochemistry			FISH
				c-fos	PCNA	Apoptosis	Chrom. 7 alterations
1	35/M	SIUD	A	++	−	−	−
2	35/F	Potter's syndrome	A	++	−	++	−
3	35/M	SIUD	A	−	−	−	−
4	36/M	Dilated cardiomyopathy	A	−	−	−	−
5	36/F	Septicemia	A	−	−	+	−
6	36/F	SIUD	A	++	−	+	−
7	36/M	SIUD	A	+++	−	+++	−
8	36/M	SIUD	A	++	−	−	−
9	36/F	SIUD	A	+++	−	+++	−
10	36/M	SIUD	A	+++	−	−	−
11	37/F	SIUD	A	++	−	−	−
12	38/M	Chorioamnionitis	A	++	−	++	−
13	38/M	SIUD	A	−	−	−	−
14	38/M	SIUD	A	+++	−	+++	−
15	40/M	Umbilical cord torsion	A	++	−	−	−
16	40/F	SIUD	A	+++	−	+	−

gw=gestational week; SIUD=sudden intrauterine unexplained death; A=preatherosclerotic lesion; −=negative immunohistochemistry; +=low positivity; ++=moderate positivity; +++=high positivity.

lymphocytes are also seen. The endothelium is morphologically intact. The internal elastic membrane appears fragmented (7).

Juvenile soft atherosclerotic plaques

These are plaques with a rich cellular content due to extensive infiltration by SMCs, associated with moderate infiltration by monocytes/foam cells and rare lymphocytes. Amorphous deposits of acid mucopolysaccharides mainly composed of B chondroitin sulfates and lipids are present, occasionally also in the internal portion of the tunica media. There is evident fragmentation of the elastic fiber system and of the tunica media, which appears to be focally thinner, especially in the areas of greater proliferation. The endothelium is morphologically intact. Reduction of the lumen varies from approximately 10–15% to 30–40% (8, 9).

Stable atherosclerotic plaques

Stable advanced lesions usually have uniformly dense fibrous connective caps, a small amount of lipids, irregular masses of calcified material, cell debris and scarce cellular components (27).

Unstable atherosclerotic plaques

These are lipid-rich plaques with a lipid core and thin fibrous caps highly infiltrated by macrophages, T-and B-lymphocytes and SMCs with widespread vascularization (27).

Both stable and unstable plaques can determine a lumen obstruction of >70%.

RESULTS

Immunohistochemical studies of the biological markers applied to the first group of arterial specimens, all diagnosed as preatherosclerotic lesions (Table 1, fetuses), showed in 12 cases

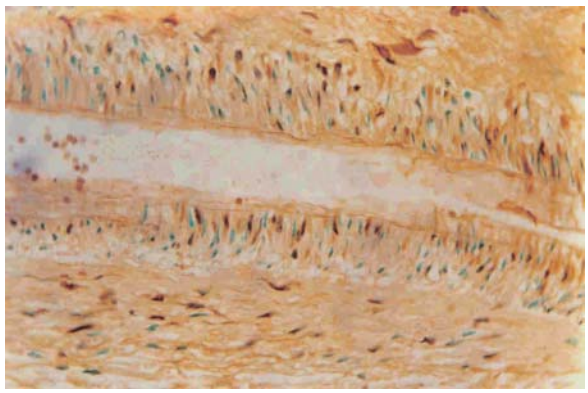

Fig. 2. Epicardial coronary artery (left anterior descending branch) of a fetus of 38 gestational weeks (case 14, Table 1). The coronary lesion shows high positivity for the apoptosis. apoptosis immunohistochemistry. Magnification 400×.

TABLE 2. *Artery lesions in infants*

Case no.	Age (months)/ sex	Diagnosis of death	Atheroscl. lesion	Biological markers			
				Immunohistochemistry			FISH
				c-fos	PCNA	Apoptosis	Chrom. 7 alterations
1	1/M	SIDS	A	+	−	+	−
2	1/F	Peritonitis	B	−	+	−	−
3	1/M	Hypertrophic cardiomyopathy	A	++	−	−	−
4	2/F	Pericarditis	A	++	−	−	−
5	2/M	SIDS	B	−	+	−	−
6	4/M	SIDS	B	−	+	−	−
7	5/M	SIDS	B	−	−	+	−
8	6/M	SIDS	B	−	+	−	−
9	6/F	Cardiac fibroma	B	−	−	−	−
10	7/M	SIDS	B	−	+	−	−
11	8/F	SIDS	B	−	−	−	−
12	8/M	SIDS	A	++	−	+	−
13	9/M	Micronodular cirrhosis	A	+	−	−	−
14	10/M	Congenital toxoplasmosis	A	+	−	+	−
15	10/F	SIDS	B	+	+	−	−
16	10/M	SIDS	B	−	++	+	−
17	11/M	Broncopneumonia	A	−	+	−	−
18	12/M	Extrahepatic biliary atresia	B	−	+	−	−
19	18/F	Broncopneumonia	A	−	+	−	−
20	21/M	Acute glomerulonephritis	B	−	++	−	−
21	34/F	Mucopolysaccharidosis	B	−	++	−	−

SIDS=sudden infant death syndrome; A=preatherosclerotic lesion; B=juvenile plaque; −=negative immunohistochemistry; +=low positivity; + +=moderate positivity; +++=high positivity.

(75%) moderate or intense (++/+++) c-fos positivity of the SMCs both in the tunica media, also in zones adjacent to structural alterations, and in the perpendicular columns infiltrating the intima (Fig. 1). In these cases, apoptotic SMCs were frequently present (Fig. 2). In particular, in cases 7, 9 and 14 we observed that both c-fos and apoptosis were intensely positive (+++). PCNA was negative in all specimens.

In the second group of lesions (Table 2, infants), there were 8 preatherosclerotic lesions and 13 juvenile plaques. The predominant result was positivity for PCNA, although in a low number of SMCs, observed in 12 cases (57%). The number of PCNA-positive SMCs was increased in cases 16, 20 and 21 from subjects aged 10, 21 and 34 months, respectively. Rare/moderate numbers of cells with activation of the c-fos gene were observed in 7 cases (33%) and with positivity for apoptosis in 5 cases (24%).

Similar results emerged from the immunohistochemical study of juvenile plaques from young subjects (Table 3), with a greater presence of PCNA-positive cells.

In all these groups of atherosclerotic lesions (Tables 1–3), FISH demonstrated two fluorescent spots relative to chromosome 7 per nucleus.

Table 4 shows the biological features of the fourth group in this study (adult subjects). The lesions consist of 9 "stable" and 13 "unstable" plaques. The stable plaques were prevalently characterized by the presence of apoptotic cells and by negativity of the other markers.

In 62% of the unstable plaques (8 cases), a high proliferative index featuring numerous PCNA-positive SMCs was detected. C-fos gene activation was demonstrated in 6 cases (46%). Specific joint positivity for both PCNA and c-fos was present in 38% (5 cases). The FISH technique demonstrated supernumerary chromosomes 7 (trisomy and/or tetrasomy) (Fig. 2) in a fair number of SMCs in 6 cases, 4 of which were also characterized by c-fos and PCNA positivity (cases 2, 6, 8 and 14).

For all the applied techniques, immunophenotyping in consecutive sections allowed a confirmation of the cells characterized by c-fos, apoptosis, PCNA positivity and numerical alterations of chromosome 7 as SMCs.

TABLE 3. *Artery lesions in young subjects*

Case no.	Age (years)/ sex	Diagnosis of death	Atheroscl. lesion	Biological markers			
				Immunohistochemistry			FISH
				c-fos	PCNA	Apoptosis	Chrom. 7 alterations
1	19/M	Chronic myeloid leukemia	B	−	+++	−	−
2	20/M	Cerebral hemorrhage	B	−	++	−	−
3	20/M	Neuroblastoma	B	+	−	−	−
4	22/F	Dilated cardiomyopathy	B	−	++	+	−
5	24/F	Multiple myeloma	B	−	+++	−	−
6	25/F	Cerebral hemorrhage	B	−	++	−	−
7	25/F	Hypetrophic cardiomyopathy	B	+	−	+	−
8	32/F	Myocardial infarction	B	−	++	−	−

B=juvenile plaque; −=negative immunohistochemistry; +=low positivity; ++=moderate positivity; +++= high positivity.

TABLE 4. *Artery lesions in adults. All the plaques are obtained by surgery for therapeutic purposes*

Case no.	Age (years)/ sex	Atheroscl. lesion	Biological markers			
			c-fos	PCNA	Apoptosis	FISH
						Chrom. 7 alterations
1	60/M	C	−	−	+	−
2	61/F	D	++	++	−	Trisomy
3	65/F	C	−	−	++	−
4	66/M	D	−	+++	−	−
5	66/F	C	−	−	++	−
6	67/M	D	+	++	−	Trisomy/tetrasomy
7	68/F	D	−	−	−	−
8	70/M	D	++	++	−	Tetrasomy
9	71/M	C	−	−	+++	−
10	71/F	D	+	−	+	−
11	72/M	D	−	+++	+	Trisomy
12	73/M	C	−	−	+	−
13	75/M	C	−	−	−	−
14	75/M	D	++	++	−	Trisomy
15	75/F	D	++	++	−	−
16	77/F	D	−	+++	−	−
17	79/M	D	−	−	−	−
18	82/F	D	−	−	+	Trisomy
19	82/F	D	−	−	−	−
20	84/M	C	−	−	+	−
21	88/F	C	−	+	−	−
22	90/M	C	−	−	−	−

C=stable plaque; D=unstable plaque; −=negative immunohistochemistry; +=low positivity; ++=moderate positivity; +++=high positivity.

The statistical analysis disclosed a significant prevalence of the c-fos positivity in fetuses compared to the other age groups ($p<0.05$). The PCNA positivity was higher in the infants group ($p<0.05$). The chromosome 7 alterations, detected only in the adults group with unstable plaques, were significantly higher compared to the other groups ($p<0.001$).

DISCUSSION

The dynamic sequence in the evolution of atherosclerotic plaques has been widely studied on the basis of the morphology and immunohistochemical composition, yielding controversial results (10–26).

In this study, the analysis of several bio-

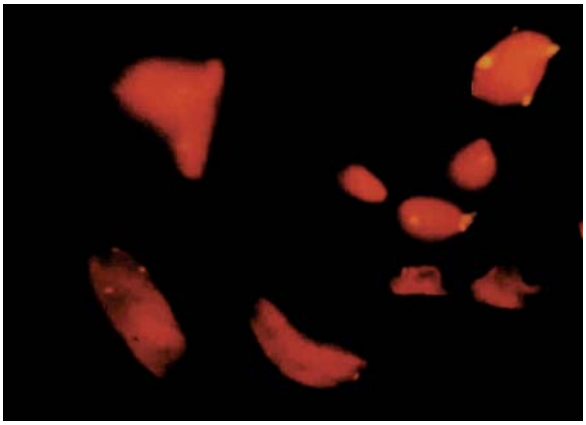

Fig. 3. Unstable plaque of carotid artery in a 75 year-old man (case 14, Table 4). The FISH method with the probe for chromosome 7 shows nuclei with three hybridization signals (=trisomy 7). Magnification 1,000×.

markers implicated in proliferative diseases (in particular c-fos, PCNA, chromosome 7 trisomy) provided a greater insight into the pathogenic mechanism of atherosclerosis, by identifying the biological events occurring at the onset of the lesions as well as during their progression.

The preatherosclerotic lesions that we observed in the coronary walls of fetuses, beginning from the 35th gestational week, represent the first structural modifications induced by atherogenic factors. The histological examination shows loss of polarity of the SMCs of the tunica media, forming columns of cells located perpendicularly to the axis of the media itself and infiltrating the subendothelial connective tissue. Based on the biological data observed in such lesions, we can state that the start of human atherosclerosis is represented by c-fos gene activation in the SMCs of the tunica media. In fact, we observed numerous c-fos positive cells in such lesions and in the media adjacent to the structural alterations. The activation of the c-fos gene seems to promote a process of SMC transformation with loss of the differentiated state and acquisition of the ability of ameboid movement, then migration toward the intima.

Several authors (6, 10, 28, 29) have reported that in atherosclerotic plaques there are two types of SMCs, the contractile or quiescent type, characterized by the presence of typical SMC contractile proteins, such as α-actin and SM myosin, and inability to undergo cytokinesis, and the synthetic or activated type, characterized by loss of contractile function, a decrease of contractile proteins with a typical switch in actin expression from α- to β form, an increase in rough endoplasmic reticulum, as well as increased synthetic and proliferative capacity. These dedifferentiated cells are the cells involved in intimal thickening and correspond to the cells defined by Gabbiani et al. as "myofibroblasts", having features in common with both SMCs and fibroblasts (30, 31).

The proto-oncogene c-fos belongs to the family of Immediate Early Genes, so defined for their ability to be activated quickly in the presence of noxious signals in a variety of vascular and non-vascular cells, since they do not require *de novo* protein synthesis (32–35).

More recently, experimental studies (36–38) have shown that in vascular SMCs, proto-oncogene expression and in particular c-fos m-RNA levels can be stimulated by exogenous and endogenous oxidants. Also oxidants present in the gas phase of cigarette smoke, as well as nicotine, have been implicated in atherogenesis (39, 40). We have recently reported (7) atherosclerotic lesions in the coronary walls of fetuses, attributable to the mothers' smoking during pregnancy.

In some of the primary lesions observed in the present study in fetuses, immunopositivity of the SMCs for apoptosis was observed, while the other biological markers (PCNA and chromosome 7 trisomy) had negative results. These findings can be interpreted, as suggested by several authors (41, 42), as physiological attempts to prevent the evolution of the atherogenic process and to preserve the wall structure in normal condition.

Atherosclerotic progression is testified by the biological events in the coronary wall alterations in infants of a few months. These consist in a moderate number of PCNA-positive SMCs and rare cells with both activation of the c-fos gene and apoptosis. This suggests that c-fos gene overexpression could promote a proliferative process, as demonstrated by the PCNA positivity. In fact, PCNA, principally expressed during the S phase of the cell cycle (43, 44), has been suggested to be a specific marker of cellular replication.

The evolution of the lesions, detected in the arteries of infants and young subjects (from 1

to 32 years), involves increased PCNA positivity testifying to an increase of the proliferative activity of SMCs, while the other parameters (c-fos, apoptosis and chromosome 7 trisomy) are generally negative. Instead, SMC apoptosis characterizes the advanced stable atherosclerotic lesions found in adults. Our findings are in agreement with reports by other authors (45, 46) stating that programmed cell death may play a role in the conversion of a hypercellular lesion to a more fibrotic atheroma.

Of particular interest is the biological pattern of the reactive atherosclerotic lesions whose well-known anatomo-clinical picture is represented by unstable plaques. In fact, in high percentages of unstable atherosclerotic plaques we found marked immunohistochemical PCNA (75% of the cases) and c-fos (40%) positivity of the SMCs. Specific joint positivity for both markers was present in 33% of the cases.

These data confirm our previous observations in studies on the biological aspects of stable and unstable atherosclerotic carotid plaques (27, 47).

Therefore, we believe that PCNA expression in the SMCs of unstable atherosclerotic plaques may be preceded by c-fos proto-oncogene activation, which could successively stimulate cell division. The joint presence of both c-fos and PCNA positive SMCs could be a signal of considerable reactivation of the atherogenic process. Using the FISH technique, we observed supernumerary chromosomes 7 in the SMCs, particularly in areas of intense reactivation of the atherosclerotic process. In our previous investigations we demonstrated that this genetic event frequently occurs in tumors of various types (48–50). It is interesting to note that genes for growth factors, EGF at band p12–13 and PDGF at the pter 7q22 region, are mapped on chromosome 7 (51, 52). This suggests that in atherosclerosis an excess number of chromosomes 7 could be related to overexpression of these genes, corresponding to an increase in SMC proliferative activity, which could even occur irregularly as in tumor cells. Therefore, the presence of trisomy and tetrasomy 7 in areas of atherosclerotic reactivation would make unstable plaques similar to a neoplastic process.

In conclusion, our findings suggest important roles of the c-fos gene, PCNA, apoptosis and chromosome 7 in the biology of vascular SMCs. In particular, during the evolution of the atherosclerotic process, we observed a biological modulation of SMC proliferation, that begins after a brief activation of the c-fos gene, increases during progression of the lesion and declines in stable plaques, when apoptosis increases. In the reactivation of the atherosclerotic process, typical of unstable plaques (21), the same early steps as those observed in fetus and infant coronary arteries occur. Moreover, the observation in some cases of markers of tumorigenesis, such as chromosome 7 alterations, suggests the possible transformation of an advanced atherosclerotic plaque into a neoplastic-like process, thus supporting the monoclonal theory of atherosclerosis (1, 4, 14–16, 51–53). This hypothesis proposes that atherosclerotic lesions begin in response to pathological stimuli as a somatic mutational event transforming a single SMC into the progenitor of a clone provided with a proliferative advantage, as seen in carcinogenesis.

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