Arch Histol Cytol, **66 (2)**: 145-153 (2003)

Morphological and immunocytochemical characterization of cultured fibroblast-like cells derived from adult human synovial membrane

F. Vandenabeele[1], C. De Bari[2], M. Moreels[1], I. Lambrichts[1], F. Dell'Accio[2], P. L. Lippens[1] and F. P. Luyten[2]

[1]*Laboratory of Histology, Biomedical Research Institute-DWI, Limburgs Universitair Centrum, Diepenbeek; and* [2]*Laboratory for Skeletal Development and Joint Disorders, Onderwijs & Navorsing, Katholieke Universiteit Leuven, Leuven, Belgium*

Summary. The synovial membrane (SM) is a source of multipotent mesenchymal stem cells (MSCs), which appeared microscopically to be a relatively homogeneous population of fibroblast-like cells (FCs) in culture (De Bari *et al.*, 2001). The aim of this study was to investigate phenotypic characteristics of the SM-derived FCs (SD-FCs) that could elucidate their origin inside the synovial tissue. Morphological characterization of SD-FCs was assessed by electron microscopy and by expression of surfactant protein A (SP-A). This study, yielded substantial evidence that SD-FCs show ultrastructural and immunocytochemical features of type B synoviocytes; they contained characteristic lamellar bodies (LBs) that are secreted by exocytosis. LB secretion ability was maintained upon passaging (P3-P10). Immunocytochemistry showed that SD-FCs express surfactant protein A (SP-A). Taken together, these results indicate that multipotent SD-MSCs may originate from the synovial lining, having a phenotype highly similar to that of type B synoviocytes. We believe our data highlight the potent ability of type B synoviocytes to have a multilineage differentiation potential.

Received July 15, 2002; revised January 23, 2003

Address for correspondence: Frank Vandenabeele, MD, PhD, Laboratory of Histology, Biomedical Research Institute-DWI, Limburgs Universitair Centrum, 3590 Diepenbeek, Belgium
Tel: + 32 11 268111, Fax: + 32 11 268199
E-mail: frank.vandenabeele@luc.ac.be

Introduction

The human synovial membrane (SM) is composed of a cellular lining layer adjacent to the joint cavity (*'lamina synovialis intima'*) and a supportive layer (*'lamina synovialis subintima'*) that merges with the fibrous layer of the joint capsule. The cellular lining layer comprises two cell populations loosely set in a specialized extracellular matrix: bone marrow-derived macrophage-like (type A) synoviocytes, and locally derived fibroblast-like (type B) synoviocytes. Type B synoviocytes show the ultrastructural features of active, protein-producing cells (Kurz and Schünke, 1997; Shikichi *et al.*, 1999; Iwanaga *et al.*, 2000) and are principally involved in the secretion of collagen, fibronectin, lubricating glycoproteins, and hyaluronic acid into the interstitium and joint cavity (for review, see Ghadially, 1982, 1983).

Human fibroblast-like type B synoviocytes are characterized by the presence of lamellar bodies (LBs) that are secreted by exocytosis into the synovial fluid (Dobbie *et al.*, 1994, 1995; Dobbie, 1996; Riemann *et al.*, 2001, Vandenabeele et al., 2001). In this respect they differ significantly from other classes of fibroblasts, *e.g.* subintimal fibroblasts.

LBs were first described in the type II pneumocytes of the alveolar septa (Stratton, 1976a, b, 1977; Kalina and Young, 1980; Stratton *et al.*, 1988). Their presence is a cardinal clue in the identification of surfactant-producing cells, in which they represent newly synthesized surfactant (Telford and Bridgman, 1990). LBs consist of very regularly arranged concentric lamellae (lipid bilayers) around an electron-dense matrix core (the surfactant matrix) and have

an average bilayer periodicity of 5 nm (Stratton, 1976a, b, 1977; Kalina and Young, 1980; Stratton *et al.*, 1988). Surfactant is a lubricant secreted by the exocytotic extrusion of LBs (Stratton, 1978). It is composed of approximately 10% protein and 90% lipid, of which the largest portion is saturated phospholipid (Hills, 1989; Rooney *et al.*, 1994; Schwarz and Hills, 1996). The phospholipids are synthesized in the rough endoplasmatic reticulum (RER) and transported to the Golgi apparatus, and then transferred to the LBs, for their storage (Schmitz and Müller, 1991; Risco *et al.*, 1994). Surfactant contains unique apoproteins designated as surfactant proteins (SP)-A, SP-B, SP-C, and SP-D (Rooney *et al.*, 1994). SP-A is the most abundant and the best characterized apoprotein of the LB, and is a reliable marker for surfactant-producing cells.

There is substantial evidence that pulmonary surfactant phospholipids and SP-A are present in a number of tissues, including gastric and intestinal mucosae, mesothelial tissues (mesentery, peritoneum, and pleura), the Eustachian tube, and synoviocytes (Dobbie *et al.*, 1994; Karchev *et al.*, 1994; Dobbie, 1996; Bourbon and Chailley-Heu, 2001; Khubchandani and Snyder, 2001). *In vivo*, surfactant phospholipids play an important role in boundary lubrication, facilitating tissues to slide over each other (for review, see Hills, 2000). This lubrication system is particularly important in synovial joints. The lubricating ability of synovial fluid is attributed to its relatively high levels of the saturated surface active phospholipid that is deposited as LBs and oligolamellar layers at the boundary of the articular surface (Rabinowitz *et al.*, 1983; Hills *et al.*, 1998; Hills, 1989; Dobbie *et al.*, 1994, 1995). The expression of SP-A is a general feature of organs exposed to pathogens; it plays a role in the host defence at the interface with the external milieu (Bourbon and Chailley-Heu, 2001). In organs that are not exposed to external pathogens, its role is likely to exert anti-inflammatory and immunomodulatory functions, as suggested by humoral autoreactivity directed against SP-A in rheumatoid arthritis synovial fluids (Dobbie *et al.*, 1994; Trinder *et al.*, 2000).

Recently, cultured mesenchymal cells isolated from the human SM were shown to have the ability to proliferate extensively in culture and to contain chondrogenic, osteogenic, myogenic and adipogenic potentials, establishing their progenitor nature (De Bari *et al.*, 2001). These SM-derived multipotent mesenchymal stem cells (SD-MSCs) appeared microscopically to be a relatively homogeneous population of fibroblast-like cells (FCs) and may be promising candidates for joint surface defect repair; however, their exact nature was not fully elucidated. Adult MSCs reside in different organs and tissues and are unique cells with a self-renewal capacity and multilineage differentiation potential. They play a crucial role in tissue repair and homeostasis.

Given the promising possibility of SD-MSCs as candidates for tissue regeneration, we set out to study the precise morphology of the FCs in the SD-MSC cultures. The present study aims to demonstrate whether the SD-FCs show the phenotypic characteristics of type B synoviocytes (presence/secretion of LBs; expression of SP-A immunoreactivity) which could elucidate their origin in the synovial lining.

Materials and Methods

Isolation and culture of human adult SD-MSCs

Clones of SD-MSCs were isolated from SM-derived cells established in a primary culture from adult human SM (n = 2 different, unrelated donors). Biopsies of SM were obtained post-mortem within 12 h of death. Both the harvest of synovial tissue and isolation of SD-MSCs were performed as described previously (De Bari *et al.*, 2001). Experiments were performed using confluent cell populations of the third to tenth passages. Cells were cultured in a growth medium consisting of Dulbecco's modified Eagle's medium (DMEM; Life Technologies) supplemented with 10% fetal calf serum (FCS; Hy Clone Per Bio) and an antibiotic-antimycotic solution (100 units/m*l* penicillin, 100 μg/m*l* streptomycin, and 0.25 μg/m*l* amphotericin B; Life Technologies) at 37℃ in a humidified atmosphere of 5% CO_2 in T25 culture flasks. The growth medium was replaced every three days. Non-adhering cells were removed by changing the growth medium. The attached cells were harvested with trypsin-EDTA (0.25% trypsin, 1mM EDTA; Life Technologies), and replated at a 1 : 3 dilution in the growth medium. At passage 3, SD-MSCs formed a homogeneous-appearing population of fibroblast-like cells, negative for the expression of the macrophage-specific CD14 gene, as determined by a reverse transcription-polymerase chain reaction (De Bari *et al.*, 2001). Human dermal fibroblasts (HDFs) were cultured in parallel as control cells and kept under identical conditions. All experiments performed in this study were done between P3 and P10. At each passage, trypsin-released cells were seeded at a density of 2 x 10^4/m*l* on glass slides for immunocytochemistry, on plastic coverslips (Thermanox®; Electron Microscopy Sciences) for transmission electron microscopy (TEM) and on 0.2 μm anopore membranes (Nunc® 25 mm Tissue culture inserts) for scanning electron microscopy (SEM). Seeded cells were cultured as described above in a 24-well plastic culture plate. As controls, human dermal fibroblasts (HDFs) were seeded in parallel and kept under identical conditions.

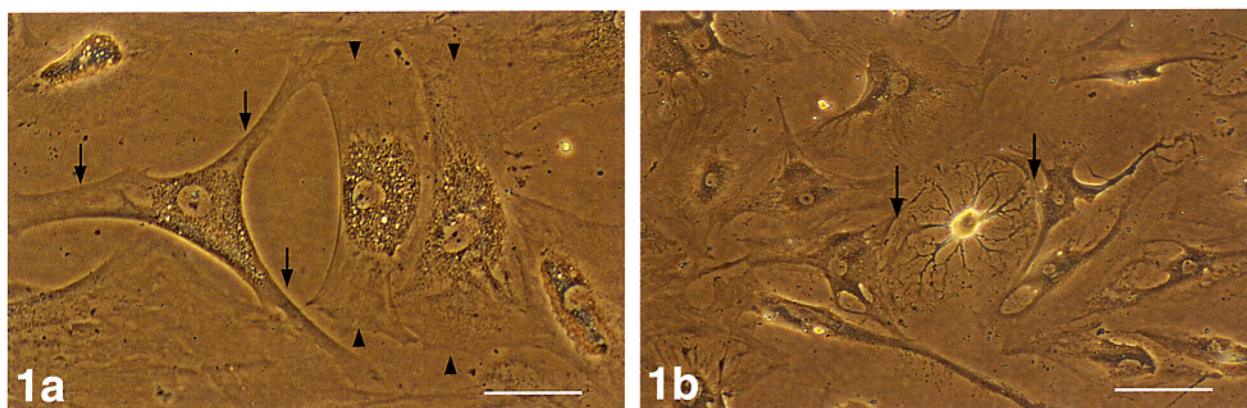

Fig. 1. Phase-contrast microscopic findings of SD-FCs. **a:** An oligodendritic cell with three elongated cell processes (arrows) and two fibroblast-like cells with broad spread cytoplasm (arrowheads). **b:** A polydendritic cell with multiple stellate cell processes anchored to neighbouring cells (arrows). Bars = 50 μm (a) and 100 μm (b).

Immunohistochemistry

Unless otherwise stated, all procedures were performed at room temperature. SD-MSCs and HDFs seeded on glass slides allocated for immunohistochemistry were stained using the peroxidase-based EnVision System®(Dako Diagnostics N.V./S.A.; Heverlee, Belgium). After washing in 0.01 M phosphate-buffered saline (PBS), the cells were fixed in 0.5% glutaraldehyde and 2% paraformaldehyde in a 0.01 M PBS solution (pH 7.2) for 1 h. The cells were permeabilized with 0.05% Triton X-100 (Boehringer Mannheim, Germany) in 0.01 M PBS solution (pH 7.2) for 30 min prior to labelling. After washing three times with 0.01 M PBS solution, the cells were incubated with rabbit polyclonal anti-human surfactant protein A (SP-A) antibody (Chemicon International, Inc; Biognost, Belgium) at a dilution of 1:4000 in 0.01 M PBS overnight at 4℃. After washing with PBS, cells were incubated with anti-rabbit peroxidase-conjugated secondary antibodies (EnVision®; Dako Diagnostics N.V./S.A.) for 30 min. A high sensitivity diaminobenzidine (DAB) chromogenic substrate system (Dako Diagnostics N.V./S.A.) and Mayers' hematoxylin were used to visualize the immunoperoxidase and to counterstain, respectively. Cells were coverslipped in an aqueous mounting medium. Control SD-MSCs and HDFs were subjected to the same immunoperoxidase staining, with the primary antibody omitted.

Electron microscopy

For examination by TEM, SD-MSCs and HDFs from parallel cultures were seeded on plastic coverslips. In addition, SD-MSCs and HDFs grown in culture flasks were carefully released from the flash wall with a cell scraper and the suspended cells were collected by centrifugation. The pellets and the cell-seeded coverslips were fixed overnight in a solution containing 2% glutaraldehyde in a 0.05 M cacodylate buffer (pH 7.3) at 4℃. The fixative was gently aspirated with a glass pipette and the cells were postfixed in 2% osmium tetroxide for 1 h, stained with 2% uranyl acetate in 10% acetone for 20 min, put through dehydrating series of graded concentrations of acetone, and embedded in araldite according to the conventional method. Semithin sections (0.5 μm) were stained with a solution of thionin and methylene blue (0.1% aqueous solution) for light microscopy. Ultrathin sections were mounted on 0.7% formvar-coated grids, stained with uranyl acetate and lead citrate, and examined in a Philips EM 208 transmission electron microscope operated at 80 kV. For SEM, SD-MSCs were grown on 0.2 μm anopore membranes, fixed overnight in 2% glutaraldehyde in a 0.05 M cacodylate buffer (pH 7.3) at 4℃, and dehydrated in graded concentrations of acetone. MSCs were then put through critical point drying, followed by gold-palladium coating. Cells were viewed in a Philips XL30 FEG scanning electron microscope.

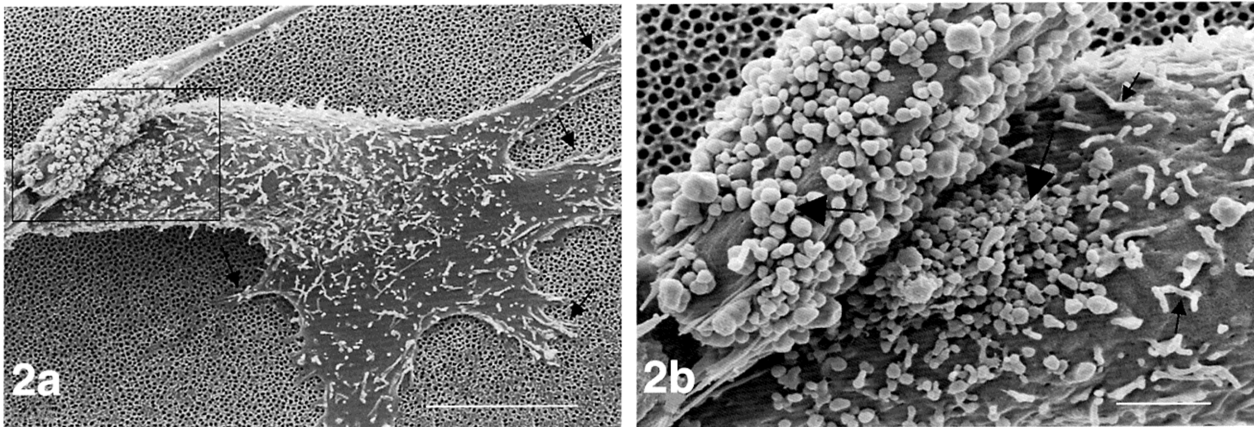

Fig. 2. Scanning electron microscopy of SD-FCs. **a:** A typical example of a SD-FC with a broadly spreading cytoplasm and many cytoplasmic projections (arrows) spreading from the front and back ends. Note the varicose tip of a cell process in direct contact with the cell (boxed area). **b:** Detail of the boxed area in Figure 2a, showing filopodia (small arrows) and numerous surface-related globular structures (large arrows). Bars = 10 μm (a) and 2 μm (b)

Results

A variety of cell morphologies were observed during culture. Initially, after enzymatic dispersion, the seeded SD-MSCs were round. Cells attached within the first few hours of culture. From day 2 onwards, SD-MSCs proliferated and began to change their morphology by extending cell processes developing towards FCs with an abundant and irregularly branched cytoplasm. A majority of SD-FCs evolved an 'oligodendritic' phenotype, with 2–3 elongated cell processes (Fig. 1a). A minority of SD-FCs transformed into a 'polydendritic' phenotype, with 4 or more stellate cell processes, which divided into small terminal branches and anchored to neighbouring cells (Fig. 1b). Typical fibroblasts, with broad sheet-like protrusions of cytoplasm (lamellipodia), front-to-back-end polarity, and a large distinct nucleus possessing one or two prominent nucleoli (Fig 1a), appeared by day 4 and eventually became the predominant subtype on day 10. The rounded and 'polydendritic' phenotypes persisted with time in culture.

SEM showed SD-FCs to be endowed with filopodia on their cell surface (Fig. 2b). SD-FCs anchored to the substratum with many cytoplasmic projections at the front and back ends of the lamellipodia (Fig. 2a). At their tips, the filopodia and cytoplasmic projections eventually displayed varicose end bulbs, often in direct contact with neighbouring cells (Fig. 2a, b). Globular structures, averaging 500–1000 nm ϕ, were associated with the lamellipodia and varicose end bulbs (Fig. 2b).

TEM demonstrated that all SD-FCs displayed characteristic features of active fibroblasts, with the presence of a well-developed RER, a prominent Golgi apparatus, abundant membrane caveolae, and a high concentration of actin-like microfilaments. No significant TEM differences between the subtypes of SD-FCs could be observed. Cytoplasmic lipid inclusions and straight microtubules were frequently seen. Quite frequently a single cilium was seen in a sectioned SD-FC, arising from one member of a centriolar pair and extending into the extracellular space (Fig. 3c). Adjacent SD-FCs were interconnected by gap junctions and intermediate junctions at the level of the cell extensions (Fig. 3b). The nuclei were euchromatic. The heterochromatin was limited to a thin band near the nuclear envelope. The nuclei contained one or several nucleoli and displayed a nuclear fibrous lamina (Fig. 3a).

TEM demonstrated that the SD-FCs contained LBs (Fig. 3a, c, 4a). LBs showed a variable morphology, with spherical or ovoid LBs predominating (Fig. 4a). They averaged about 500 nm in diameter. In the ovoid LBs the long axis varied between 400 and 1000 nm. The LBs contained 2 to 8 more or less equally spaced bilayers concentrically surrounding a 'matrix core' (*sensu* Stratton, 1978). The periodicity of the lamellae was ca 4 nm. Usually the matrix core contained electronlucent vesicles and variable numbers of electron-dense granular material, resembling glycogen deposits (Fig. 4b, c). The lamellae in the LBs formed closed or open circles. In SD-FCs, spherical LBs containing concentric closed lamellae were predominant. Different stages of LB maturation could be observed in the same cell

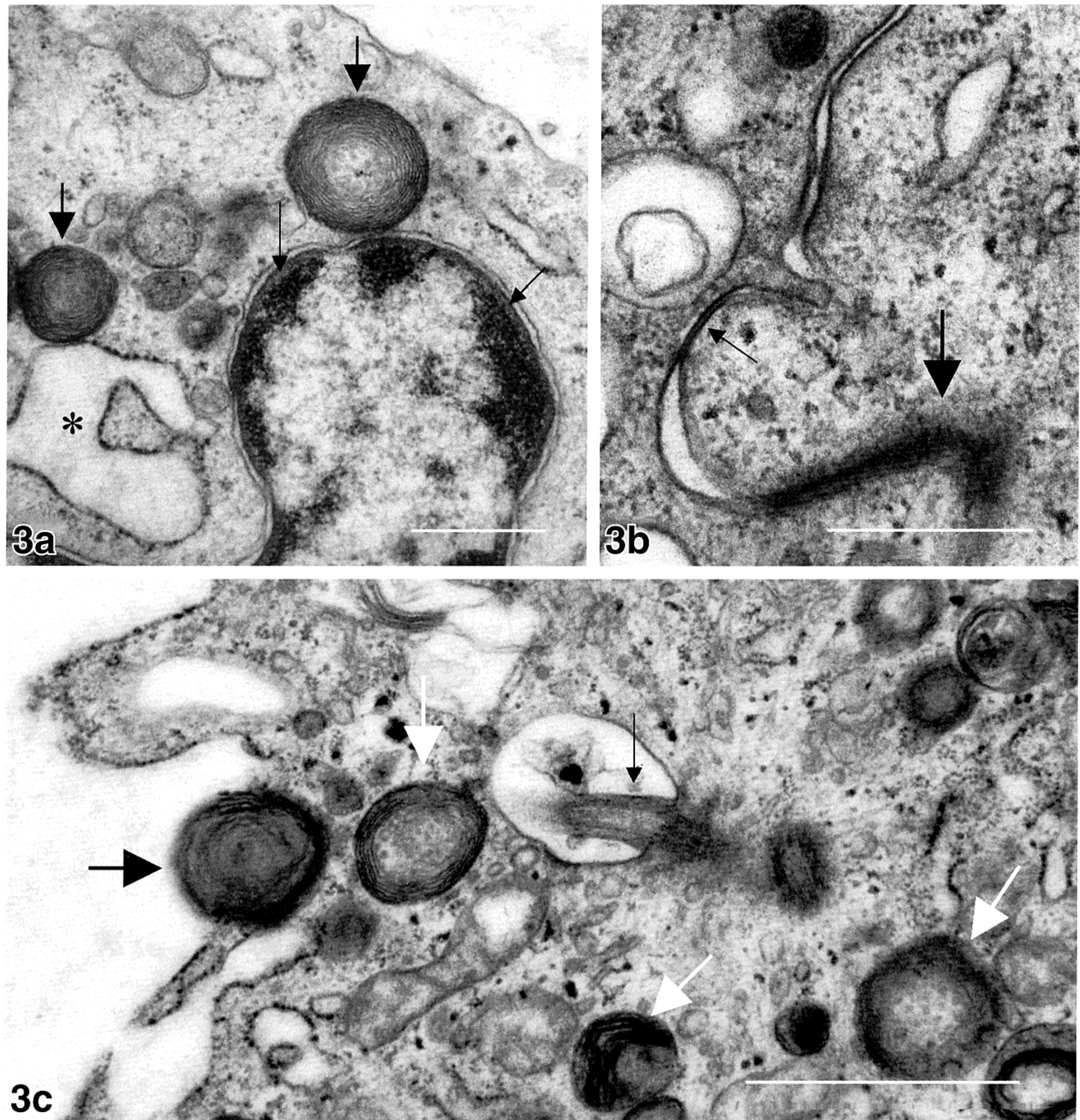

Fig. 3. Transmission electron micrographs of SD-FCs. **a:** A nuclear fibrous lamina (small arrows), distended cisternae of the RER (*) and LBs (large arrows). **b:** Gap junction (small arrow) and intermediate junction (large arrow). **c:** LB in the process of extrusion into the extracellular space (large black arrow). Note the cilium arising from one member of a centriolar pair projecting into the extracellular space (small arrow) and the variable morphology of LBs (large white arrows).
Bars = 500 nm (a, b) and 1000 nm (c)

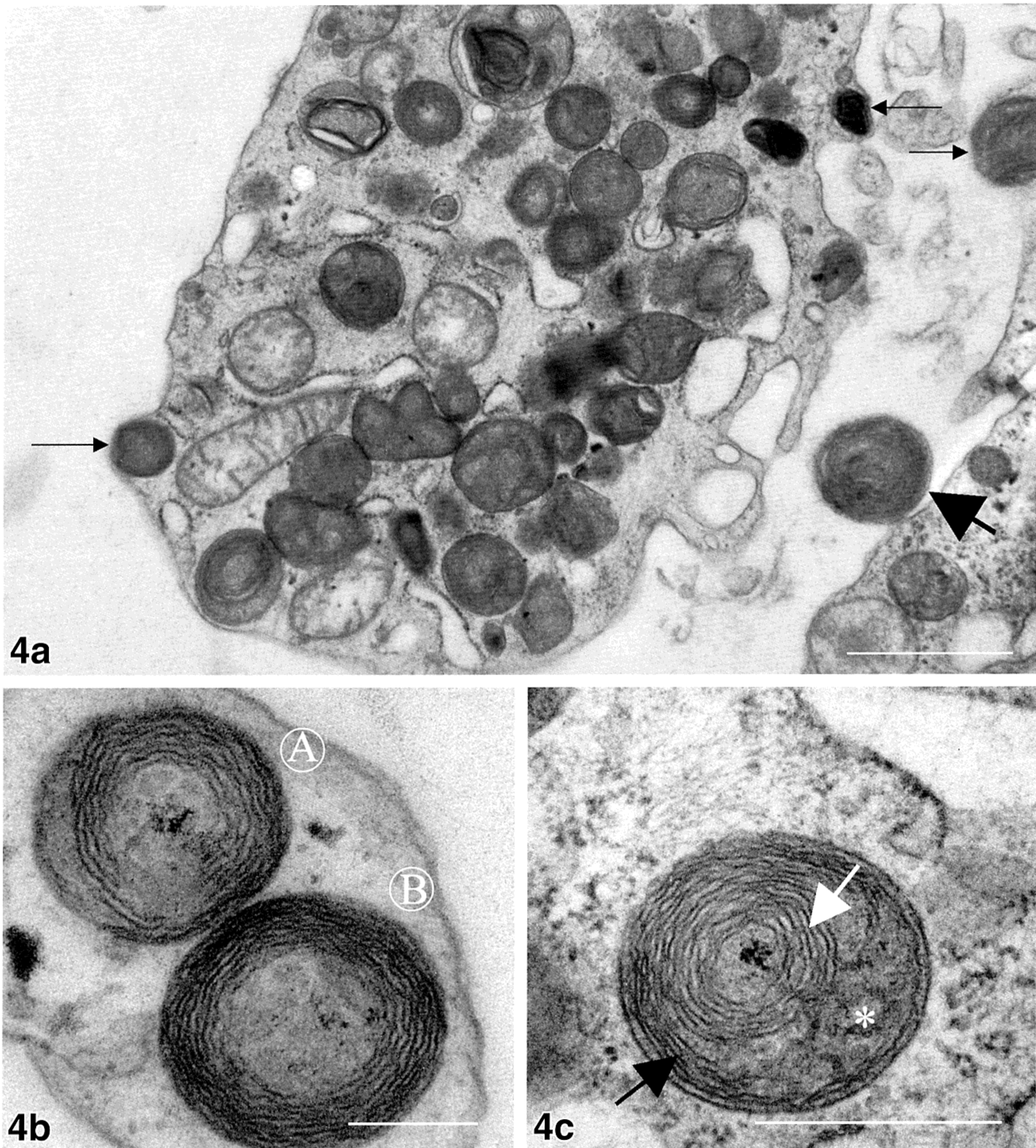

Fig. 4. Transmission electron micrographs of LBs. **a:** LBs are extruded by exocytosis (small arrows) into the growth medium and eventually appear on the cell surface (large arrow). **b** and **c:** LBs at different stages of maturation. Mature (B) and immature (A) LB present in the same cell are shown in **b**. A typical example of an immature LB with a projection core (asterisk), a band of peripherally located compact lamellae (black arrow), and a second band of concentric lamellae (white arrow) surrounding the matrix core is shown in **c**. Note the glycogen-like deposits in the matrix core of LBs.
Bars = 1000 nm (a), 200 nm (b) and 500 nm (c)

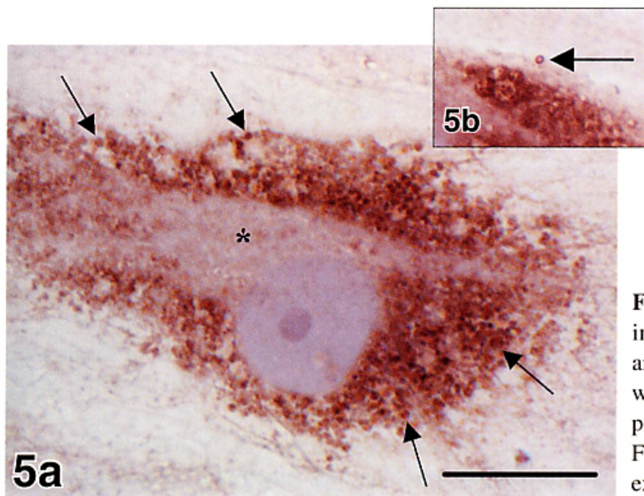

Fig. 5. Immunocytochemical staining for SP-A. **a:** The immunoreactivity is granular in appearance (arrows) and correlates well with the presence of LBs. Note the weak SP-A expression in the juxtanuclear region occupied by the Golgi complex (asterisk). **b:** Detail of a SD-FC showing a SP-A positive surface-related LB (arrow) extruded into the extracellular space. Bar = 20 μm

(Fig. 4b). Immature LBs contained a membrane-bound array of electron-dense material (Fig. 4c), which corresponded to the 'projection core' (*sensu* Stratton, 1978). The developing lamellae appeared to elaborate from the projection core, which was absent as such in mature LBs (Fig. 4b). Mature LBs appeared to be discharged into the growth medium by exocytotic extrusion (Fig. 3c, 4a). Comparable structures, averaging 500 nm in diameter, could be readily identified by SEM (Fig. 2b). Upon passaging, the relative number of both mature and immature LBs gradually decreased, with immature LBs predominating in late passages (P8–P10). LBs were absent in parallel cultures of HDFs.

SP-A immunostaining revealed granular immunoreactivities in the SD-FCs, correlating well with the presence of LBs (Fig. 5a). Expression of SP-A persisted with time in culture. Parallel cultures of HDFs were SP-A negative.

Discussion

The present study investigated the nature of cultured mesenchymal cells isolated from the human SM, and found that these cells show the phenotypic characteristics of fibroblast-like (type B) synoviocytes.

The most important finding was that the SM-derived cells, unlike fibroblasts of other origins, contained LBs. The ultrastuctural features of the LBs, consisting of very regularly arranged concentric membranes around an electron-dense matrix core, and the specific periodicity of the bilayers were strikingly similar to those found in human synoviocytes (Dobbie *et al.*, 1994, 1995; Dobbie, 1996; Riemann *et al.*, 2001; Vandenabeele *et al.*, 2001) and type II pneumocytes (Stratton, 1976a, b, 1977; Kalina and Young, 1980; Stratton *et al.*, 1988). The surface morphology of SD-FCs as revealed by our SEM observations is comparable to that of LB-secreting epithelial cells in the Eustachian tube (Karchev *et al.*, 1994). In both TEM and SEM images of SD-FCs, LBs appeared protruding from the cell surface. The presence of globular elements in association with the lamellipodia (SEM) is interpreted as the exocytosis of LBs; the morphology and the dimensions of the discharged elements are consistent with this hypothesis. Furthermore, it is known that mature human LBs are released by a merocrine type of secretion *via* exocytosis (Stratton, 1978). Our observations concerning the maturation processes of LBs support the findings from previous reports, *i.e.* the projection core is the site from which the successive lamellae elaborate (Sud, 1977; Stratton, 1978; Dobbie et al., 1994).

A second finding in this study was that cultured mesenchymal cells were positive for SP-A, a hydrophilic protein found in the phospholipid-rich material designated surfactant. This is consistent with previous studies in which synoviocytes have been shown to secrete a surfactant-like material (Dobbie, 1996; Bourbon and Chailley-Heu, 2001). Surfactant is additionally known to be secreted by the exocytotic extrusion of the LBs, the storage-sites for the surfactant-phospholipids and ultrastructural hallmark of surfactant-producing cells.

Together with ultrastructural features, the immunohistochemical findings confirm that the SD-FCs have a phenotype highly similar to type B synoviocytes, different from subintimal and other classes of fibroblasts. From our studies it seems likely that SD-MSCs topographically address the synovial lining. Cultures of SD-MSC isolated by De

Bari *et al.* (2001) might have been initiated as primary cultures of synoviocytes, likely type B synoviocytes. The finding of three subtypes of SD-FCs based on morphology and cell surface phenotype, comparable to those described by Hendler *et al.* (1985) in primary cultures of human SM, supports this hypothesis. The present findings also highlight the potential of type B synoviocytes to have a multilineage differentiation potential. The chondrogenic potential of SM — previously reported in rodents — gives credence to this hypothesis (Iwata *et al.*, 1993; Nishimura *et al.*, 1999).

Acknowledgments

The authors thank Marc Jans, Jeanine Santermans and Marie Josee Sleypen (Laboratory of Histology, Biomedical Research Institute-DWI, Limburgs Universitair Centrum) for their excellent technical assistance and Cosimo De Bari for kindly providing the SM-derived mesenchymal stem cells. We are especially grateful to Dr. Jan D'Haen (Institute for Materials Research, Limburgs Universitair Centrum) for help with the scanning electron microscopy, to Marc Withofs for help with the photographic work, and to Professor Em. Dr. Julia Creemers for valuable discussions about the manuscript.

References

Bourbon JR, Chailley-Heu B: Surfactant proteins in the digestive tract, mesentery, and other organs: evolutionary significance. *Comp Biochem Physiol A Mol Integr Physiol* 129: 151-161 (2001).

De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP: Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthr Rheum* 44: 1928-1942 (2001).

Dobbie JW: Surfactant protein A and lamellar bodies: a homologous secretory function of peritoneum, synovium, and lung. *Perit Dial Int* 16: 574-581 (1996).

Dobbie JW, Tasiaux N, Meijers P, Derson JD, Bodart C, Hind C, Bourguet C, Perret J: Lamellar bodies in synoviocytes, mesothelium and specific epithelia as possible site of auto-antigen in rheumatoid disease. *Br J Rheum* 33: 508-519 (1994).

Dobbie JW, Hind C, Meijers P, Bodart C, Tasiaux N, Perret J, Anderson JD: Lamellar body secretion: ultrastructural analysis of an unexplored function of synoviocytes. *Br J Rheum* 34: 13-23 (1995).

Ghadially FN: *Ultrastructural pathology of the cell and matrix. A text and atlas of physiological and pathological alterations on the fine structure of cellular and extracellular components.* Butterworths, London, 1982.

Ghadially FN: Synovial membrane. In: *Joints. Fine structure of synovial joints* (Ghadially FN, ed). Butterworths, London 1983 (p. 1 - 41)

Hendler PL, Lavoie PE, Werb Z, Chan J, Seaman WE: Human synovial dendritic cells. Direct observation of transition to fibroblasts. *J Rheumatol* 12: 660-664 (1985).

Hills BA: Oligolamellar lubrication of joints by surface active phospholipid. *J Rheumatol* 16: 82-91 (1989).

Hills BA: Boundary lubrication in vivo. *Proc Inst Mech Eng* 214: 83-94 (2000).

Hills BA, Ethell MT, Hodgson DR: Release of lubricating synovial surfactant by intra-articular steroid. *Br J Rheumatol* 37: 649-652 (1998).

Iwanaga T, Shikichi M, Kitamura H, Yanase H, Nozawa-Inoue K: Morphology and functional roles of synoviocytes in the joint. *Arch Histol Cytol* 63: 17-31 (2000).

Iwata H, Ono S, Sato K, Kawamura M: Bone morphogenetic protein-induced muscle- and synovium-derived cartilage differentiation in vitro. *Clin Orthop* 296: 295-300 (1993).

Kalina M, Young SL: Multilamellar bodies in alveolar septal cells. *Exp Lung Res* 1: 201-209 (1980).

Karchev T, Watanabe N, Fujiyoshi T, Mogi G, Kato S: Surfactant-producing epithelium in the dorsal part of the cartilaginous Eustachian tube of mice. Light, transmission, and scanning electron microscopic observations. *Acta Otolaryngol* 114: 64-69 (1994).

Khubchandani KR, Snyder JM: Surfactant protein A (SP-A): the alveolus and beyond. *FASEB J* 15: 59-69 (2001).

Kurz B, Schünke M: Articular chondrocytes and synoviocytes in culture: influence of antioxidants on lipid peroxidation and proliferation. *Ann Anat* 179: 439-446 (1997).

Nishimura K, Solchaga LA, Caplan AI, Yoo JU, Goldberg VM, Johnstone B: Chondroprogenitor cells of synovial tissue. *Arthr Rheum* 42: 2631-2637 (1999).

Rabinowitz JL, Gregg JR, Nixon JE: Lipid composition of the tissues of human knee joints. II. Synovial fluid in trauma. *Clin Orthop* 190: 292-298 (1983).

Riemann D, Hansen GH, Niels-Christiansen L-L, Thorsen E, Immerdal L, Santos AN, Kehlen A, Langner J, Danielsen EM: Caveolae/lipid rafts in fibroblast-like synoviocytes: ectopeptidase-rich membrane microdomains. *Biochem J* 354: 47-55 (2001).

Risco C, Romero C, Bosch MA, Pinto da Silva P: Type II pneumocytes revisited: intracellular membranous systems, surface characteristics, and lamellar body secretion. *Lab Invest* 70: 407-417 (1994).

Rooney SA, Young SL, Mendelson CR: Molecular and cellular processing of lung surfactant. *FASEB* 8: 957-967 (1994).

Schmitz G, Müller G: Structure and function of lamellar bodies, lipid-protein complexes involved in storage and secretion and secretion of cellular lipids. *J Lipid Res* 32: 1539-1570 (1991).

Schwarz IM, Hills BA: Synovial surfactant: lamellar bodies in type B synoviocytes and proteolipid in synovial fluid and the articular lining. *Br J Rheum* 35:821-827 (1996).

Shikichi M, Kitamura HP, Yanase H, Konno A, Takahashi-Iwanaga H: Three-dimensional ultrastructure of synoviocytes in the horse joint as revealed by the scanning electron microscope. *Arch Histol Cytol* 62: 219-229 (1999).

Stratton CJ: The three-dimensional aspect of mammalian lung multilamellar bodies. *Tissue Cell* 8: 693-712 (1976a).

Stratton CJ: The high resolution ultrastructure of the periodicity and architecture of lipid-retained and extracted lung multilamellar body laminations. *Tissue Cell* 8: 713-728 (1976b).

Stratton CJ: The periodicity and architecture of lipid retained and extracted lung surfactant and its origin from multilamellar bodies. *Tissue Cell* 9: 301-316 (1977).

Stratton CJ: The ultrastructure of multilamellar bodies and surfactant in the human lung. *Cell Tissue Res* 193: 219-229 (1978).

Stratton CJ, Zasadzinski JAN, Elkins D: Lung lamellar body amphiphilic topography: a morphological evaluation using the continuum theory of liquid crystals. *Anat Rec* 221: 503-519 (1988).

Sud BN: Electron microscopic study of the human type II pneumocyte and surfactant synthesis. *Anat Rec* 187: 724-725 (1977).

Telford IR, Bridgman CHF: *Introduction to functional histology.* Harper & Row, New York, 1990

Trinder PK, Hickling TP, Sim RB, Brackertz D, Loos M, Maeurer MJ: Humoral autoreactivity directed against surfactant protein-A (SP-A) in rheumatoid arthritis synovial joints. *Clin Exp Immunol* 120: 183-187.

Vandenabeele F, Lambrichts I, Lippens PL, Creemers J: *In vitro* loading of human synovial membrane with 5-hydroxydopamine: evidence for dense core secretory granules in type B cells. *Arch Histol Cytol* 64: 1-16 (2001).