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SPARC: A Protein that Modulates Cell Cycle and Cell-Matrix Interactions

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ABSTRACT

SPARC (secreted protein, acidic and rich in cysteine) belongs to a group of proteins that have been shown, by virtue of their anti-adhesive properties, to mediate interactions between cells and the extracellular matrix. In this report we show that SPARC adsorbed to nitrocellulose does not support the attachment of bovine endothelial cells and fibroblasts. In contrast, the glycoprotein laminin mediated the attachment of endothelial cells, and native type I collagen promoted the adhesion of fibroblasts. When presented to endothelial cells as a soluble protein, SPARC inhibited the uptake of [^{3}H]-thymidine in a dose-dependent manner; this effect was enhanced in the absence of serum and was mimicked by a 20-amino acid synthetic SPARC peptide. The appearance of SPARC in odontoblasts, in myoblasts, and in restricted populations of cells in the perichondrium or periosteum of embryonic mouse bone provides additional support for its function as a modulator of cell growth and differentiation in distinct tissues.

INTRODUCTION

Initial descriptions of SPARC (also termed osteonectin and BM-40) in bone and mineralized tissues were followed by numerous reports that confirmed a more extensive distribution of this glycoprotein (1-4). Although the collection of cells, tissues, and organs that were subsequently shown to transcribe SPARC mRNA might at first appear prodigious (3, 5), it nevertheless was apparent that expression was coincident in most cases with *a)* tissues undergoing developmental or injury-related remodeling (e.g., osteogenesis, somitogenesis, angiogenesis), *b)* proliferating cells (embryonic or tumor cells), *c)* non-cycling cells (parietal endoderm, steroidogenic cells), or *d)* invasive, highly migratory cells (trophoblastic giant cells, parietal endoderm, and cells of metastatic tumors) (reviewed in 6). A feature common to all these cellular populations, however, was changes in cell shape ostensibly brought about by concomitant changes in cell-extracellular matrix interactions. It was subsequently shown that SPARC inhibited the spreading of fibroblasts, endothelial cells, and smooth muscle cells in vitro (7), and that it diminished the number of focal contacts produced by cultured endothelial cells (8). Moreover, teratocarcinoma cells stably transfected with sense or antisense SPARC cDNA exhibited altered morphologies in vitro: cells which expressed high levels of SPARC protein were rounded and aggregated, whereas underexpressing lines (anti-sense transfectants) appeared as individual, spread cells (9). Collectively,

these experiments indicate that SPARC affects cell shape, and they afford a provisional explanation for the pattern of expression that has been observed for SPARC in vivo.

Molecular cloning and structural characterization of SPARC have delineated regions of the protein that could be specific functional domains (10-12). Studies with synthetic peptides have identified the two Ca^{+2}-binding regions of SPARC as active inhibitors of cell spreading (13). Another synthetic peptide from a cationic, cysteine-rich region of SPARC was recently shown to inhibit proliferation of endothelial cells (14). Temporary withdrawal from the cell cycle could conceivably facilitate cell migration and would be compatible with a release of cells from the surrounding extracellular matrix. Proteins that disrupt cell-matrix interactions, as opposed to those that generally promote cell spreading, include tenascin, thrombospondin, and SPARC, and have been the subject of a recent review (6).

In this report we show the expression of SPARC at cellular interfaces in tissues from embryonic mice and examine the ability of native SPARC to support the direct attachment or adhesion of both endothelial cells and fibroblasts. We also assess the growth-inhibitory properties of SPARC and a cationic SPARC peptide on endothelial cells cultured in diminishing amounts of fetal bovine serum. Due possibly to the interaction between SPARC and bovine serum albumin (BSA) (15), we found that SPARC inhibited DNA synthesis less effectively in the presence of serum. These experiments establish that SPARC can act independently as an anti-attachment factor for endothelial cells and fibroblasts, and that it can function as an inhibitor of endothelial cell proliferation in the absence of albumin, a protein that is generally not found in the mammalian embryo prior to parturition.

MATERIALS and METHODS

SPARC was purified from culture medium conditioned by PYS-2 cells (4), and SPARC peptide 2.1 was synthesized as previously described (13). Affinity-purified rabbit anti-murine SPARC IgG was used on murine embryonic tissues in conjunction with an avidin-biotin-peroxidase technique (4). Measurement of [^{3}H]-thymidine incorporation was performed as described by Funk and Sage (14). Other methods have been detailed in the figure legends.

RESULTS and DISCUSSION

The anti-adhesive glycoprotein tenascin, which shares several functional properties with SPARC although the proteins are structurally dissimilar (6, 8), is produced during embryogenesis at sites of interaction between epithelial and mesenchymal cells (6). We have therefore reexamined the expression of SPARC at several cellular interfaces at late stages of murine embryogenesis, as these areas of extensive cellular interaction are generally characterized by rather extreme changes in cell shape and by abrupt alterations in the synthesis and deposition of extracellular matrix. As shown in Figure 1, SPARC was abundant in the alveolar bone surrounding an early bell-staged, unerupted tooth but was absent from both the enamel-producing ameloblasts (epithelium) and the dentin-producing odontoblasts (mesenchyme) at d 16 of murine embryogenesis (A and C). By d 18,

however, many of the odontoblasts in a later bell-staged tooth from a similar section of the jaw stained positively with affinity-purified anti-SPARC IgG, whereas ameloblasts and the enamel layer (between the ameloblasts and odontoblasts) were unreactive (B and D).

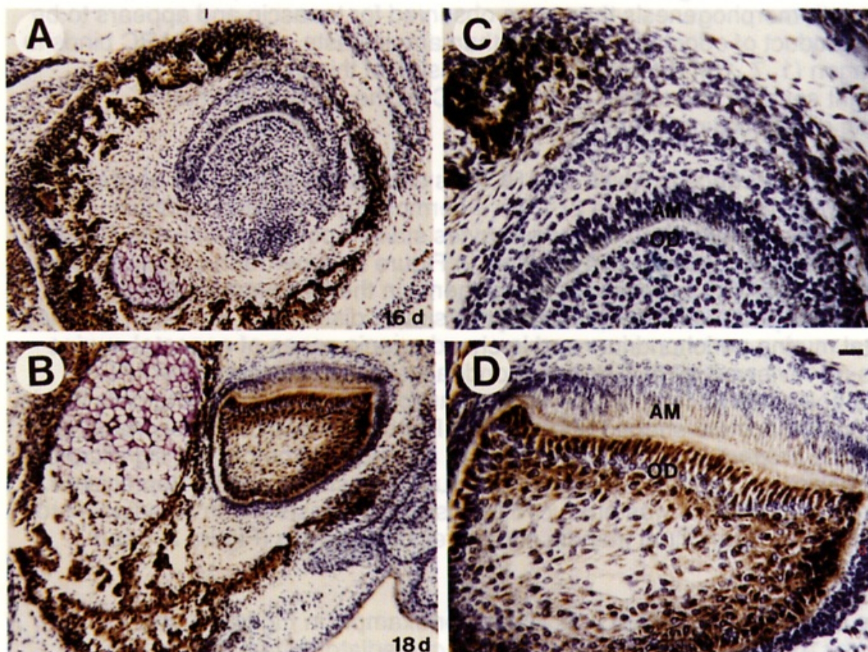

Figure 1. *Expression of SPARC by Odontoblasts in the 18 d Mouse Embryo.*

Sections of jaw from 16 d and 18 d mouse embryos were exposed to anti-SPARC IgG and immune complexes were visualized by an avidin-biotin-peroxidase technique. Toluidine blue was used as a counterstain.

A, 16 d unerupted tooth (early bell stage) was unstained, although abundant reaction product (brown stain) was apparent in the surrounding alveolar bone.

B, 18 d unerupted tooth (late bell stage) exhibited reactivity with anti-SPARC IgG. Alveolar bone and cartilage structure (left of tooth) also show positive staining.

C, higher magnification of A. AM, ameloblasts; OD, odontoblasts.

D, higher magnification of B. Bar = 40 µm. Note that peripheral odontoblasts expressed SPARC, whereas ameloblasts and the enamel layer between the ameloblasts and odontoblasts appeared negative.

High levels of SPARC (osteonectin) have been reported as characteristic of the emerging odontoblast phenotype, coincident with the production of type I collagen (16, 17) and subsequent to the expression of tenascin and the proteoglycan syndecan by the condensing mesenchyme (17). Tung et al (16) additionally described the presence of osteonectin in ameloblasts, stratum intermedium, and stellate reticulum of unerupted porcine molars, whereas we noted low to undetectable levels of this protein in 18 d murine teeth. These discrepancies could result from the use of different reagents and of species that exhibit alterations in dentinogenesis. From Figure 1 we can conclude that SPARC is produced at later stages of tooth morphogenesis than were observed for tenascin and appears to be primarily a product of odontoblasts rather than ameloblasts. Since SPARC binds to type I collagen (1, 7, 13), the coincident expression of this extracellular fibrillar protein might serve in part to sequester SPARC in the provisional matrix surrounding differentiating odontoblasts.

A second interface in which SPARC was detected is shown in a section of a 16 d fetal murine hindlimb (Figure 2). The innermost layer of perichondrium adjacent to a nest of hypertrophic chondrocytes stained positively with anti-SPARC IgG. At the stage of osteogenesis depicted in Figure 2, hypertrophic chondrocytes were negative, whereas extracellular components in the degenerating/calcifying zone exhibited a positive immunoreactivity. A similar distribution has been reported in 18 d fetal murine perichondrium and in chondrogenic zones of neonatal murine phalanges (4, reviewed in 6). The perichondrial cells that expressed SPARC exhibited a polygonal to ovoid shape, in comparison with nonexpressing cells in this layer which were spindle-shaped (Figure 2B). Further studies on the secretory and osteoinductive properties of both morphotypes are required before the significance of the expression of SPARC in the perichondrium/periosteum can be appreciated. We also note that SPARC was produced by fusing myoblasts which lie adjacent to the cells of the perichondrium that did not express the protein (Figure 2, open arrow).

The distribution of SPARC, as shown for example in Figures 1 and 2, prompted our examination of SPARC as a direct mediator of cell attachment. Although soluble SPARC and certain SPARC peptides have been shown to diminish the spreading of endothelial cells and fibroblasts (7, 13), the activity of SPARC adsorbed onto a substrate, in the absence of other proteins, had not been tested.

As described in Figure 3, native SPARC, which adsorbs poorly to plastic, was bound to dishes that were precoated with nitrocellulose. When presented to either bovine aortic endothelial (BAE) cells (C) or to fetal calf ligament fibroblasts (F), SPARC did not support attachment. In contrast, both laminin (B) and type I collagen (E) adsorbed to nitrocellulose mediated the respective attachment of endothelial cells and fibroblasts. In addition to its activity as a factor that inhibits spreading and diminishes focal contacts in vitro (7, 8), SPARC appears to be nonpermissive for the attachment of endothelial cells and fibroblasts. As Chiquet-Ehrismann and her colleagues reported for tenascin, SPARC also disrupted

interactions between cells and purified extracellular matrix components (7, reviewed in 6), an activity likely to be biologically relevant during tissue remodeling.

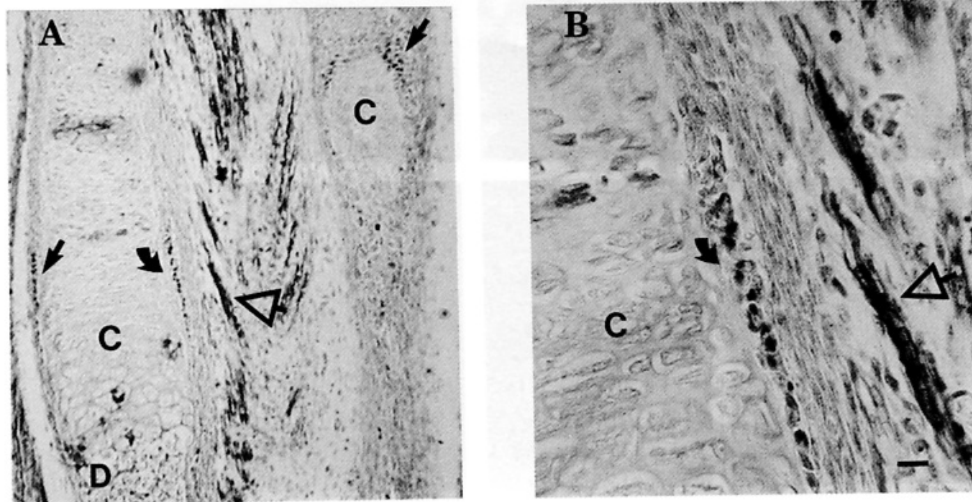

Figure 2. *Expression of SPARC in Myoblasts and Perichondrium of the 16 d Mouse Embryo.*

Sections of hindlimb were treated as described in Figure 1.

A, Chondrocytes (*C*) indicate the developing tibia and fibula. Staining with anti-SPARC IgG was apparent in perichondrium and/or periosteum (denoted by straight black arrows), in degenerating or calcifying chondrocytes (*D*), and in striated muscle (open arrow).

B, higher magnification of *A*. Curved black arrow points to same area in *A* and *B*, which shows cells in the perichondrial layer, immediately adjacent to the hypertrophic chondrocytes (*C*), that exhibited a positive, intracellular staining reaction in the presence of anti-SPARC IgG. Bar = 50 µm.

The experiments shown in Figure 3 were performed in Dulbecco's modified Eagle's medium (DMEM) containing 1% BSA to avoid possible complications resulting from proteins in serum that would adsorb to the nitrocellulose and/or compete directly with SPARC (e.g., vitronectin, fibronectin, or thrombospondin). Since SPARC is known to bind serum albumin in vitro (15), it could be argued that BSA inactivated SPARC in this attachment assay. In earlier studies, however, we had shown that soluble SPARC and SPARC peptides functioned as anti-spreading factors when presented to cells in the presence of fetal bovine serum (FBS) (7, 13), although cellular responses were blunted at higher concentration of FBS (7). Given that the concentration range of albumin in tissues is considerably lower than those in plasma or serum, and that SPARC inhibits endothelial cell proliferation (14), it was important to assess the effect of SPARC on the cell cycle in the absence of FBS. These experiments are summarized in Figure 4.

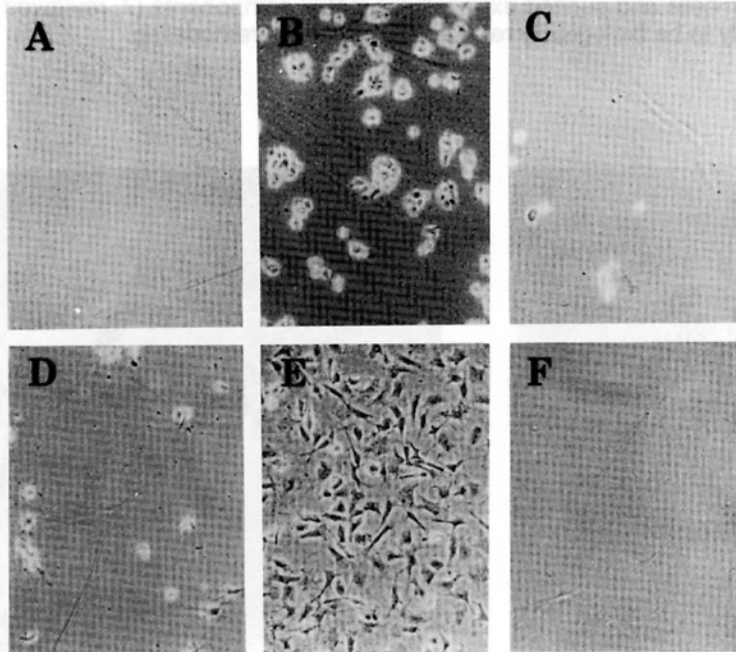

Figure 3. *SPARC does not Support Attachment of Endothelial Cells and Fibroblasts.*

Nitrocellulose was dissolved in methanol, and aliquots of the solution were placed in tissue culture wells and dried overnight (18). Native SPARC, BSA, laminin, and type I collagen were bound overnight to the respective wells, and the nitrocellulose-coated surfaces were subsequently blocked with BSA.

BAE cells and fibroblasts were released with trypsin, resuspended in DMEM containing 1% BSA for 1h, and plated in the same medium onto the treated substrates. After 5h, the wells were washed such that unattached or loosely adherent cells were removed. The remaining cells were fixed and subsequently photographed.

A-C, BAE cells plated onto surfaces treated with BSA (*A*), laminin (*B*), and SPARC (*C*).

D-F, fibroblasts plated onto surfaces treated with BSA (*D*), type I collagen (*E*), and SPARC (*F*). Neither SPARC nor BSA supported cell attachment, whereas laminin and type I collagen respectively mediated the attachment of endothelial cells and the adhesion (indicated by spread cells) of fibroblasts.

As previously reported (14), SPARC decreased the incorporation of [^{3}H]-thymidine into BAE cell DNA in a dose-dependent manner; however, this effect was enhanced over control levels when the cells were treated with SPARC in the absence of FBS or BSA (Figure 4A). A similar result was obtained with SPARC peptide 2.1, a synthetic 20-residue peptide enriched in cysteine and histidine that exhibits anti-proliferative activity (14). Figure 4B depicts an inhibition of

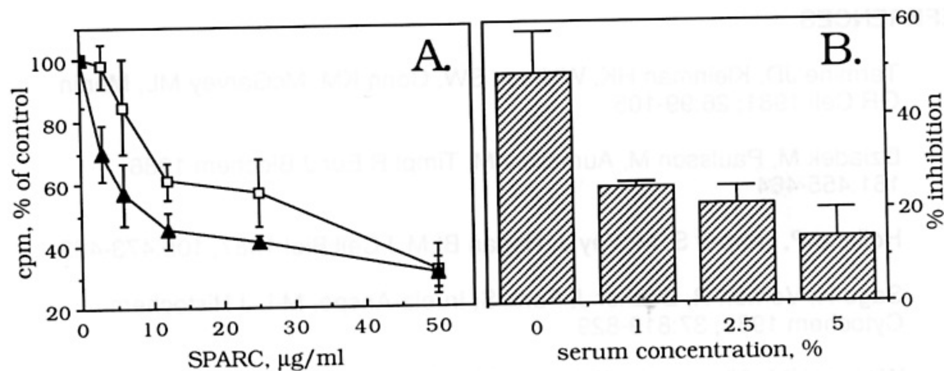

Figure 4. *Inhibition of Thymidine Incorporation by SPARC and SPARC Peptide 2.1 is More Effective in the Absence of Serum*

A, Growth-arrested BAE cells were dissociated in trypsin and were replated at subconfluent density (approximately 10^4 cells/cm^2) in DMEM, with (□) or without 2.5% FBS (▲), containing 1 μCi/ml [^{3}H]-thymidine and increasing amounts of SPARC. After 24 h, duplicate wells were washed twice in cold phosphate-buffered saline (PBS) and fixed in 10% trichloroacetic acid (TCA). TCA-insoluble material was hydrolyzed in 0.4 N NaOH and quantitated in a liquid scintillation counter. Points represent the mean of triplicate wells ±SD.

B, Synchronized BAE cells were dissociated with trypsin. The cell pellets were washed with serum-free DMEM and resuspended in serum-free DMEM containing 1 μCi/ml [^{3}H]-thymidine and 0.4 mM peptide 2.1 or an equal volume of PBS. Cells were then seeded into 24-well plates at subconfluent density, and FBS was added to duplicate wells to final concentrations of 0-5%. After 24 h, cells were washed and fixed as described in A. Values shown are the percent inhibition, relative to controls, of thymidine incorporation expressed as the mean ±SD. Results are representative of three independent experiments.

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We have recently shown that native SPARC binds to platelet-derived growth factor (PDGF) (BB and, to a lesser extent, AB isoforms) and inhibits the binding of PDGF to its cognate receptors on fibroblasts (19). One mechanism by which SPARC modulates the cell cycle could therefore result from the sequestration of PDGF in, for example, remodeling tissues. The ability of SPARC to titrate the activity of growth and morphogenetic factors on selected cellular populations provides further support for the hypothesis that this protein subserves a significant role at specific times during embryogenesis or tissue repair.

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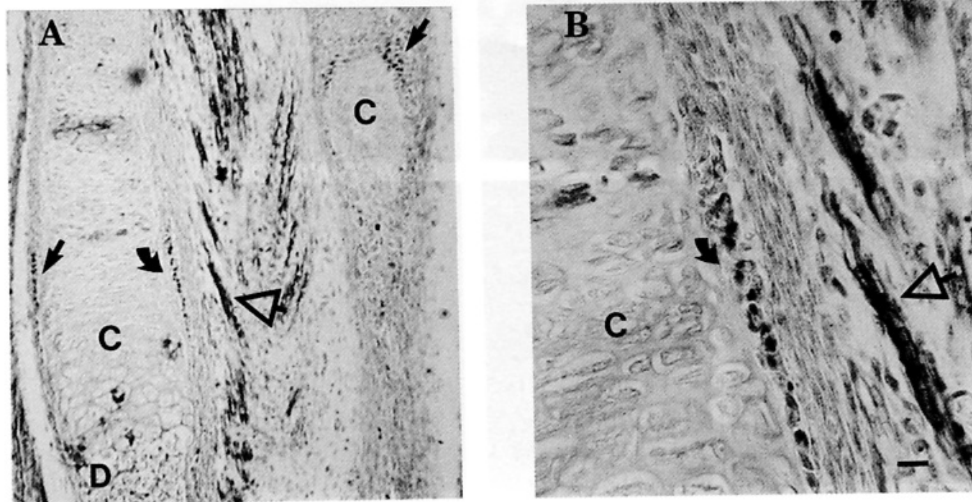

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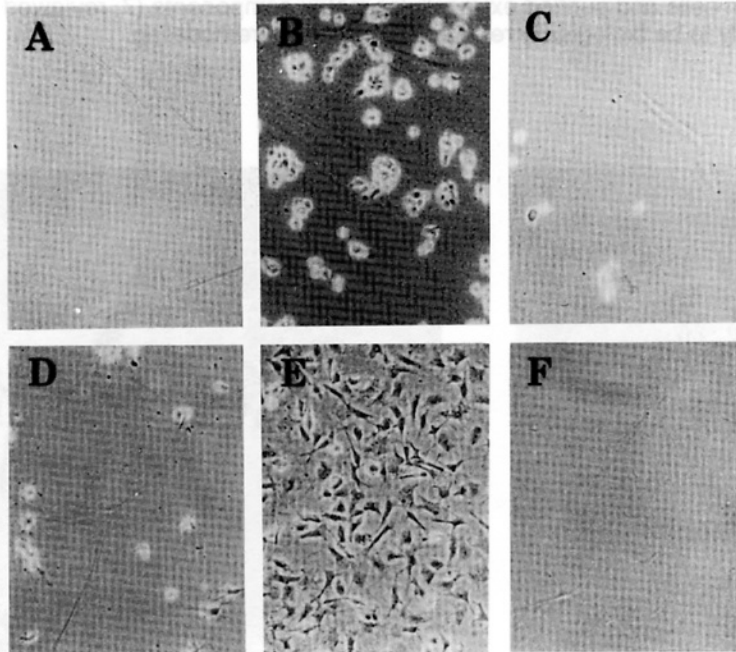

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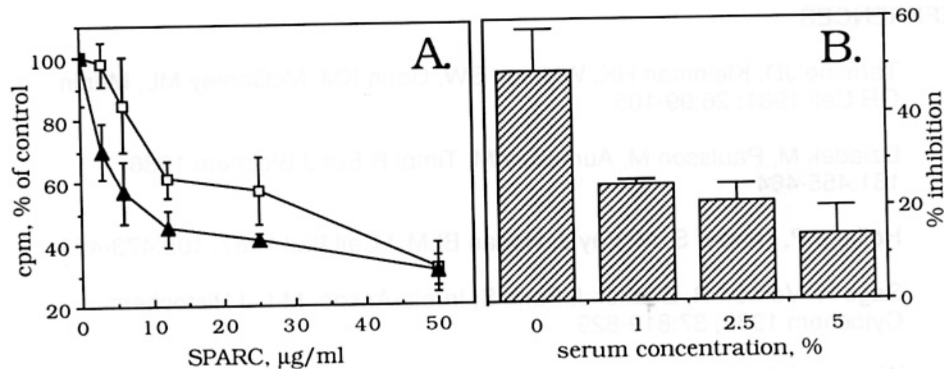

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