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Asteroid bodies: Products of unusual microtubule dynamics in monocyte-derived giant cells. An immunohistochemical study

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Summary. We have studied asteroid bodies (ABs) of multinucleated giant cells (MGCs) in a series of sarcoid and foreign body granulomas with a standard streptavidin-biotin peroxidase technique, using commercial antibodies against collagen, vimentin and tubulin on routinely processed tissue as well as, in one case, on fresh frozen sections (FS). Our findings clearly indicate that ABs are products of the microtubule (MT) system and lack collagen. The tubulin in them stains in fresh FS but is «masked» in formalin-fixed tissue. It can be fully «unmasked» by dephosphorylation and partially by trypsinization. Compared to single microtubule organizing centers (MTOCs) in mononuclear cells serving as internal controls, ABs are obvious replicas of centrosome-nucleated MT assemblies from which they differ principally by the disproportionate size of their components and by the invariable vacuolation of the surrounding cytoplasm. Relying on bits of relevant information gleaned from the literature, our observations support the following preliminary conclusions: 1) spokes are massive bundles of MTs rich in tyrosinated α-tubulin coassembled in phosphorylated linkages with yet unidentified microtubule associated proteins (MAPs) and probably microfilament proteins; cores are masses of pericentriolar material including amorphous tubulins, MAPs, phosphoproteins and phospholipids; 2) their size, at least in some ABs, appears to indicate the presence of overlapping centrosome-nucleated MTOCs which in monocyte-derived MGCs are known to be multiple; 3) the cytoplasmic vacuolations around them reflect a collapse and retraction of intermediate filaments (IFs), indicating substantial ongoing MT depolymerization with disruption of MT-IF interactions; 4) ABs are products of unusual MTOC dynamics characterized by simultaneous MT assembly and depolymerization; such a phenomenon, termed «microtubule catastrophe«, has been recognized in vitro with centrosome-nucleated MT assemblies under conditions of low tubulin concentrations.

Key words: Asteroid body (AB), Cytoskeleton, Tubulin, Microtubule organizing center (MTOC), Centrosome

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Introduction

About five decades ago, the term «asteroid body» (Friedman, 1944) became standard nomenclature for peculiar star-shaped intracellular structures which, since the turn of the century, have been known to occur selectively in MGCs of various types of foreign body and, most notably, sarcoid granulomas. Measuring up to 30 μm in diameter and apparently floating in cytoplasmic vacuoles, they consist of a usually rounded core from which somewhat bent spokes radiate into the cytoplasm, evoking images of spiders or open umbrella frames. Earlier investigators referred to them under a variety of descriptive designations (fat needle stars, spider or spiculated bodies, astrospheres, stellate or radial inclusions, etc.) which, together with the diverse theories about their nature, have been enumerated in excellent reviews by Cunningham (1951) and by Cain and Kraus (1977).

Electron microscopic studies have resulted in discrepant interpretations of their composition: 1) bundles of criss-crossing collagen fibrils thought to be either trapped or in-situ elaborated by fused epithelioid cells (Azar and Lunardelli, 1969); 2) mainly collagen-free microfilaments and, to a lesser degree, microtubules proposed to arise from local aggregations of cytoskeletal components (Cain and Kraus, 1977, 1983), and 3) membrane lipid bilayers organized in lamellar and tubular forms (Papadimitriou et al., 1992).

Attempts at exploring the antigenic profile of ABs have, to the best of our knowledge, been reported only twice previously with similar discrepant results: positive immunofluorescence for vimentin (Cain and Kraus, 1983) and negative immunoperoxidase stains for

vimentin as well as for α- and ß-tubulin (Papadimitriou et al., 1992). Since of us originally supported the «collagen» concept (Azar et al., 1973), this study has been undertaken with the special incentive to sort out by immunohistochemical means, if possible, the conflicting electron microscopic interpretations, particularly with regard to the presence or absence of collagen.

Materials and methods

The paraffin blocks of formalin-fixed tissue from nine cases of sarcoid and four cases of foreign body granulomas were selected from the surgical pathology files at Harlem Hospital Center. The sarcoid series comprised lymph nodes in 4, lung in 2, breast in 1 and skin in 2 cases while the foreign body series consisted of 3 suture granulomas of skin and muscle and one post-traumatic dermal lesion containing exogenous material of vegetable origin. In addition, a fresh sarcoid spleen (1000 gr) was obtained in the course of the study and provided an abundant source for both fresh frozen sections and formalin-fixed tisuse.

Both cryostat and paraffin sections were cut at 5 micron thickness and were mounted on silane-coated slides. The labeled streptavidin-biotin (LSAB) method was used with commercial kits (DAKO, Carpinteria, CA, USA) and incubations with primary antibodies were carried out at 4-6 °C overnight. The following primary antibodies were used: polyclonal rabbit anti-human collagen type I (Biodesign Internat., Kennebunkport, ME, USA), monoclonal anti-human collagen type III (BioGenex, San Ramon, CA, USA), monoclonal anti-human collagen type IV (DAKO, Carpinteria, CA, USA), monoclonal anti-swine vimentin (DAKO), polyclonal rabbit anti-sea urchin tubulin, monoclonal anti-chick brain α-tubulin and anti-chick brain ß-tubulin (BioGenex, San Ramon, CA, USA).

The reagents employed in enzyme digestion procedures were all obtained from SIGMA, St. Louis, MO, USA. For collagen, a 0.8% solution of pepsin in 0.1M sodium acetate-HCl buffer, pH 2, was used at 37 °C for 15 minutes. Trypsinization was performed at room temperature for 20 minutes with a 0.1% solution of trypsin in 0.05M Tris-HCl buffer, pH 7.6. Dephosphorylation was carried out with a 1% solution of I-S alkaline phosphatase from bovine intestinal mucosa in 0.1M Tris-HCl buffer, pH 8.3, at 37 °C for 2 hours; to inhibit excessive proteolysis, this solution also contained 17.5 µg/ml PMSF (phenylmethylsulfonyl fluoride, dissolved in a small aliquot of dimethyl sulfoxide), 10 µm/ml leupeptin and 10 µm/ml pepstatin A. Sections exposed to enzymes were thoroughly rinsed in distilled water, immersed in a chilled 3% solution of hydrogen peroxide in methanol for 10 minutes and after repeated washes in 0.01M phosphate buffered saline, pH 7.2, were processed in the same way as controls. Positive immunostaining was visualized with diaminobenzidine. A light nuclear counterstain was obtained with 1% methyl green in citrate buffer, pH 5.3.

Results

Collagen

Immunostaining performed on formalin-fixed tissue with and without proteolysis produced completely negative results in ABs for collagen type I, collagen type III and collagen type IV. The distribution of collagen type I was superposable on that of collagen type III in sclerotic sarcoid lesions, outlining MGCs and whole tubercles as negative images in which ABs were only identifiable with phase contrast microscopy (Fig. 1a,b). Collagen type IV was seldom present as part of vascular basement membranes within granulomas.

Vimentin

In fixed tissue, ABs consistently failed to immunostain for vimentin in the presence of variable but often intense cytoplasmic immunostaining of MGCs. Not only cores and spokes but the entire vacuolated sphere around ABs appeared to be refractory to the antibody (Fig. 2). In fresh FS, large AB cores and the surrounding sphere were often weakly reactive but long spokes, whether slender or thick, remained clearly unstained. On the other hand, small ABs could not be distinguished against the background of intense cytoplasmic staining.

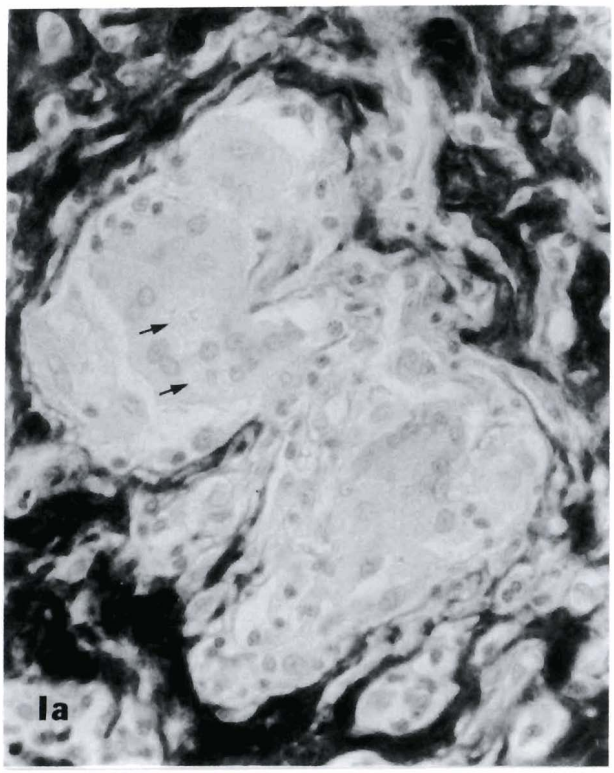
Tubulin

In routinely processed tissue without proteolysis, ABs did not immunostain with any of the three antibodies though there was weak to moderate cytoplasmic labeling of MGCs with the polyclonal antibody. In controls, the anti-α antibody acted much like an anti-muscle actin antibody, labeling smooth and striated muscle, myofibroblasts and myoepithelial cells. The anti-ß antibody crisply labeled cilia of tubal and bronchial mucosa, nerve trunks, cytoplasmic networks in stratified squamous epithelium and dot-like paranuclear bodies, presumed to be centrioles, in many types of cells.

In fresh FS briefly fixed in acetone (2 min) or 90% ethanol (15-20 min), ABs stained crisply for α-tubulin with the monoclonal antibody (Fig. 3), showing large ill-defined cores in the presence of diffuse cytoplasmic immunostaining. The polyclonal antibody produced identical results. By contrast, there was little or no cytoplasmic immunostaining for ß-tubulin, AB cores remaining largely unstained and AB spokes staining at best faintly. Dot-like putative centriolar bodies could not be recognized.

Pronase digestion of formalin-fixed sections completely destroyed tubulin antigenicity. Trypsinization produced negative staining for ß-tubulin but, at the same time, resulted in fairly uniform and strong immunolabeling of AB cores with the other two antibodies, including variable staining of spokes (Fig. 4a,b). AB cores were most often large, ill-defined and rounded but sometimes also elongated or U-shaped. The near perfect

radial symmetry of AB spokes known from classic illustrations was rare. Thick spokes, numbering from few to as many as two dozens or more, were usually seen alternating with fine short spokes in haphazard combinations, not necessarily radiating from the same point. In general, spokes were seldom straight and were



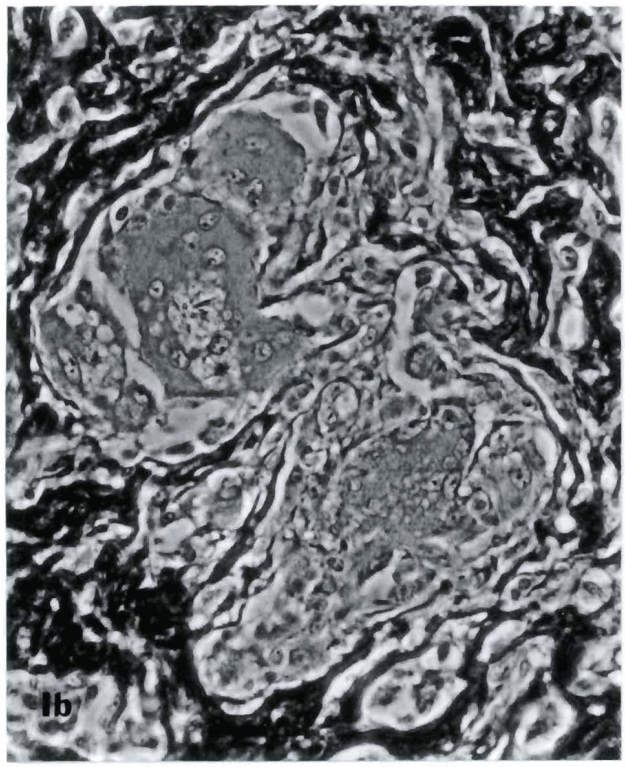


Fig. 1. Collagen type III immunostaining in sclerotic granuloma of sarcoid lymph node. **a.** Two non-reactive asteroid bodies (arrows), barely discernible in unstained cytoplasm of MGCs. **b.** Same field in dark phase contrast disclosing outline of asteroid bodies. x 400

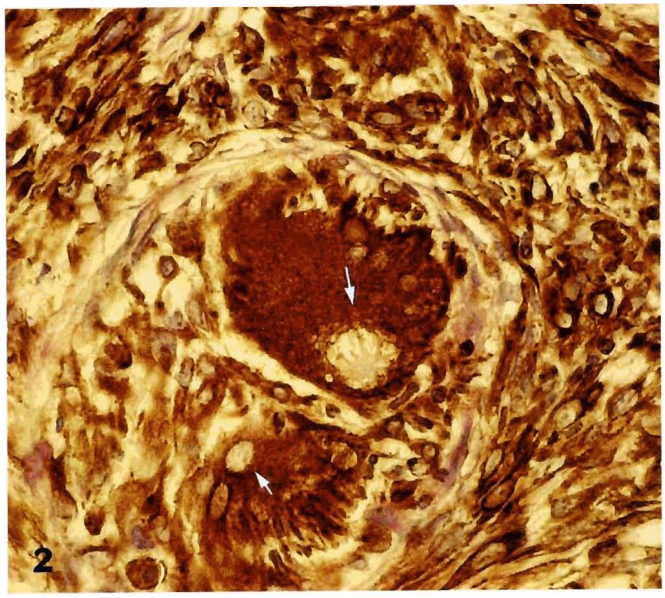


Fig. 2. High content of cytoplasmic vimentin in two MGCs, each with unstained asteroid body (white arrows) and equally unstained spheres around bodies. Sarcoid, skin. x 400

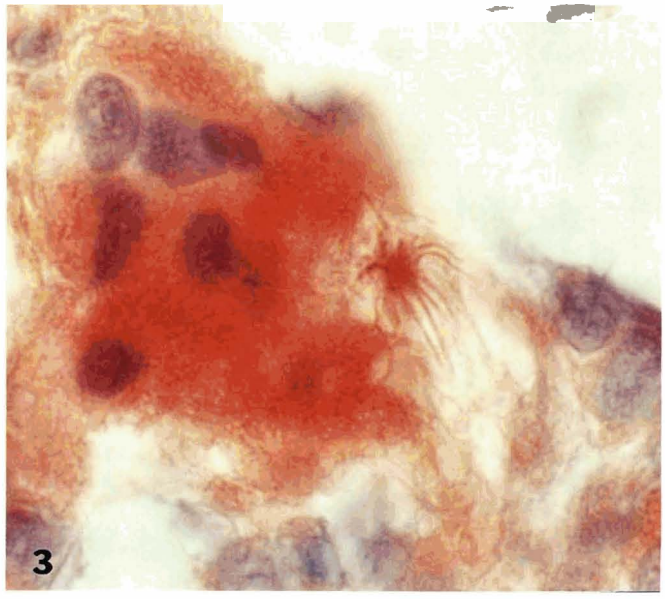
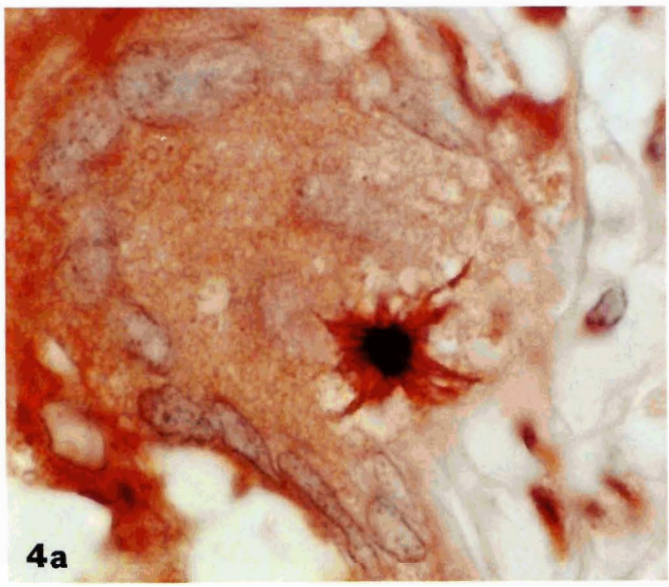


Fig. 3. Frozen 5-µm-thick section of sarcoid spleen immunostained for α-tubulin: crisply labeled asteroid body with ill-defined core. x 1,000

usually bent in parallel or in various directions.

A valuable by-product of tubulin stains following mild trypsinization was the demonstration of highly assorted zonal concentrations of cytoplasmic α-tubulin reactivity in many MGCs, most often with a perinuclear or bipolar distribution (Figs. 5, 6). These were also revealed with the polyclonal antibody and suggested the presence of dynamic MT arrays. In MGCs with such distinct MT arrays, ABs were seldom seen and, if present, they occurred in tubulin depleted cytoplasm (Fig. 5).

Pretreatment of formalin-fixed sections with alkaline phosphatase resulted in distinct tubulin immunolocalization within spokes while tubulin staining of cores



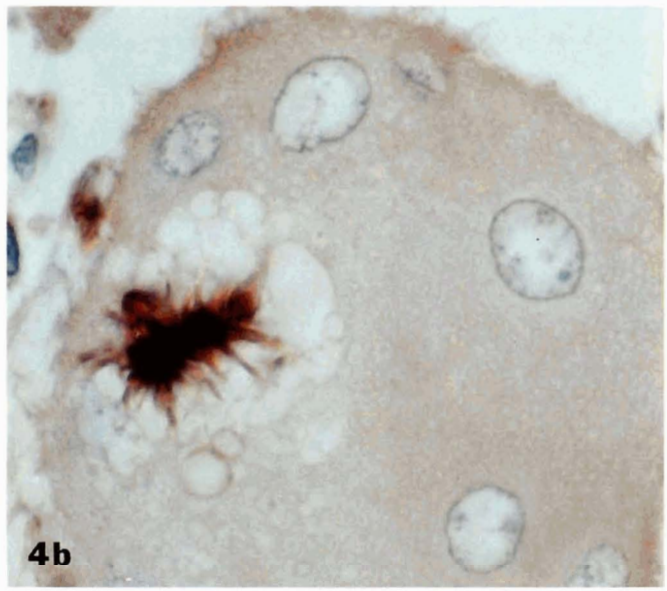
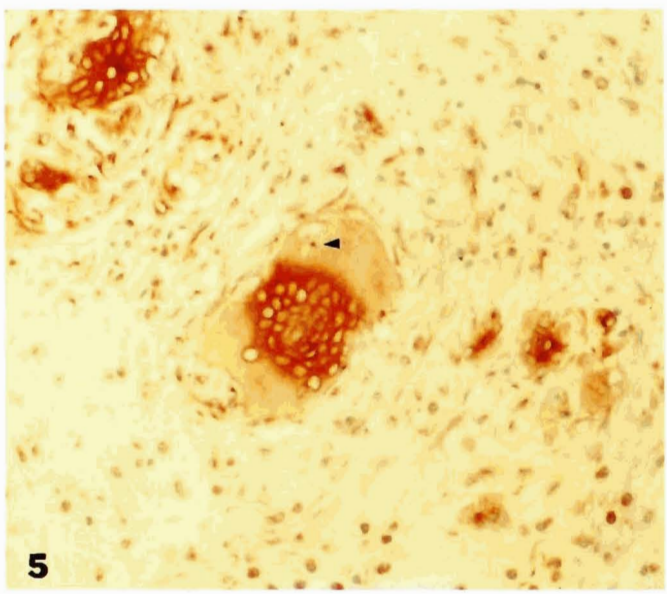


Fig. 4. Tubulin immunoreactivity of asteroid bodies in trypsinized sections. **a.** Intensely reactive rounded core and less pronounced staining of spokes in MGC of sarcoid lymph node. **b.** Caterpillar-like core with short spokes radiating from multiple points in tubulin depleted MGC of sarcoid spleen. x 1,000



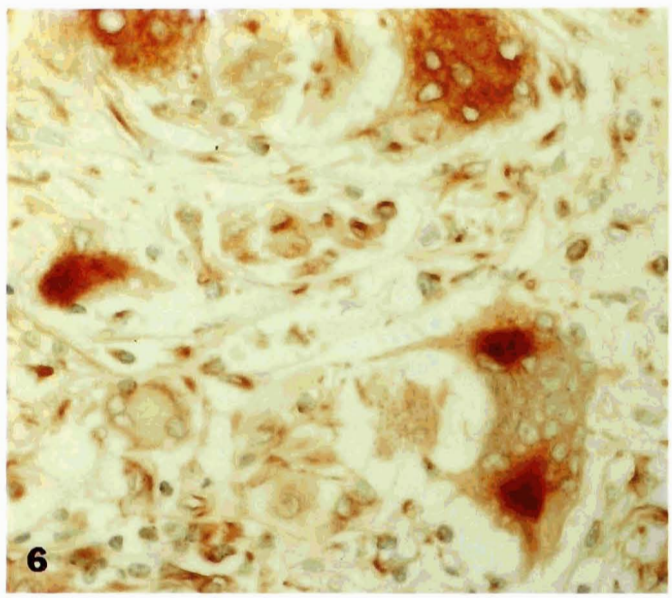


Fig. 5. Example of perinuclear microtubule arrays in MGC with centrally clustered nuclei immunostained for tubulin (polyclonal) after trypsinization. Note small reactive core (arrowhead) in peripheral cytoplasm. Sarcoid spleen. x 100

Fig. 6. Dynamic bipolar microtubule arrays intensely labeled for α-tubulin in one of MGCs apparently engaged in outflanking maneuver (lower right). Trypsinized section of sarcoid spleen. x 200

and MGC cytoplasm was noticeably reduced (Figs. 7, 8). Despite the presence of inhibitors of proteolysis in the alkaline phosphatase solution, prolonged dephosphorylation (in excess of 3 hours) reduced overall tubulin immunoreactivity significantly.

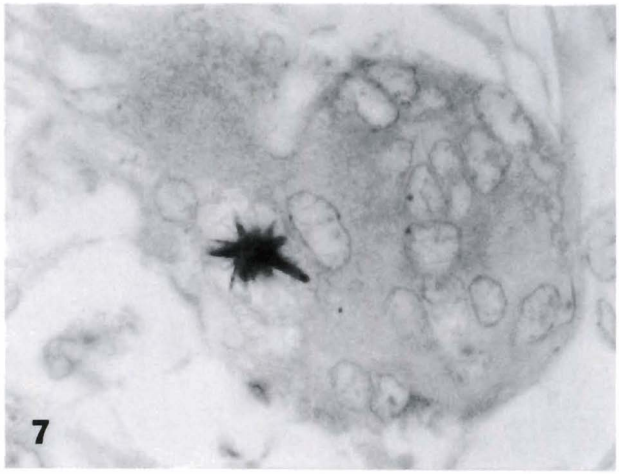


Fig. 7. Selective tubulin immunolabeling of solitary asteroid body «unmasked» by dephosphorylation in section of pulmonary sarcoid granuloma. x 1,000

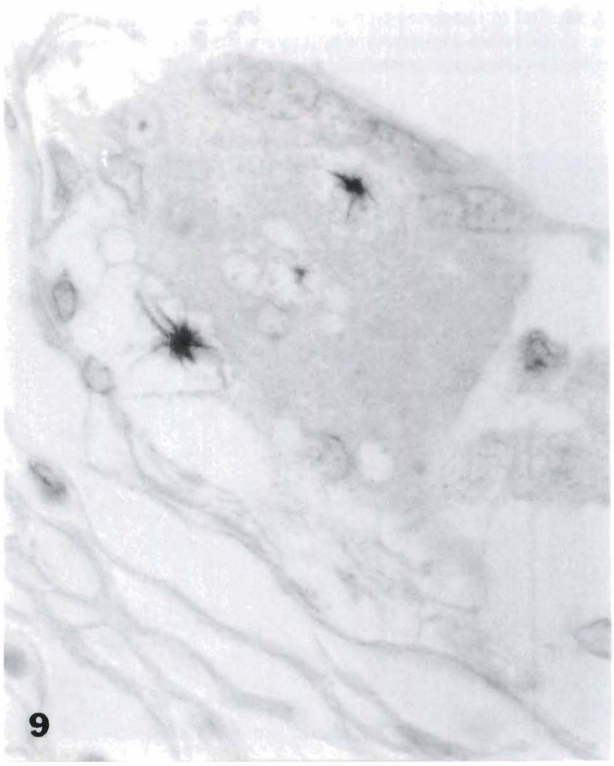


Fig. 9. Multiple asteroid bodies, one quite tiny in center, immunostained for α-tubulin after trypsinization in sarcoid granuloma of lymph node. Note lack of cytoplasmic staining in MGC. x 1,000

Asteroid body morphology and correlations

In foreign body granulomas, ABs tended to be large, solitary and centrally located, with bulky cores and few thick tapering spokes (Fig. 8). In sarcoid granulomas, the

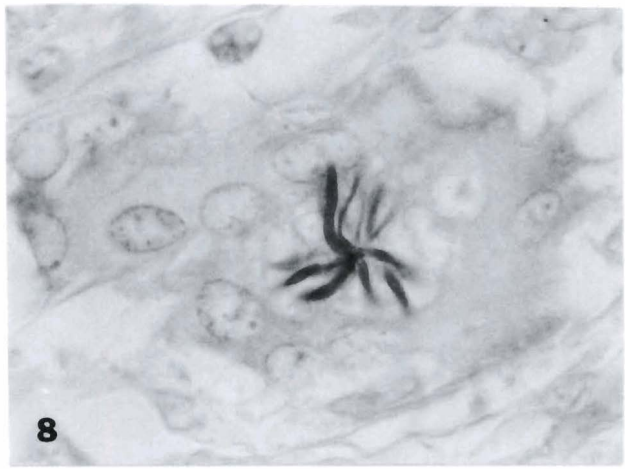


Fig. 8. Large asteroid body with thick tapering spokes and no obvious core immunostained for tubulin in dephosphorylated section of cutaneous foreign body granuloma. x 1,000

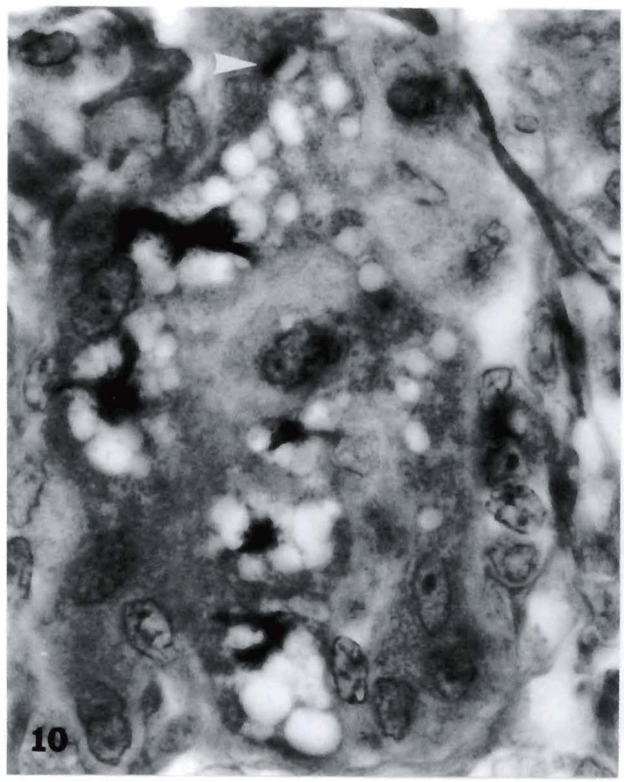


Fig. 10. Tubulin (polyclonal) immunostaining in trypsinized section of sarcoid granuloma: single MGC with 5 somewhat disfigured asteroid bodies, the lowermost consisting only of U-shaped core. Part of 6th asteroid body (white arrowhead) at top of field. Lymph node. x 1,000

presence of 3 to 6 ABs in a single plane of section of a giant cell, generally not recognized in H&E stained sections, was not uncommon (Figs. 9, 10).

In trypsinized and dephosphorylated sections, tubulin stains facilitated the visualization of single paranuclear microtubule organizing centers (MTOCs) in mononuclear cells. These usually displayed the morphology of minute asters in plasma cells or appeared

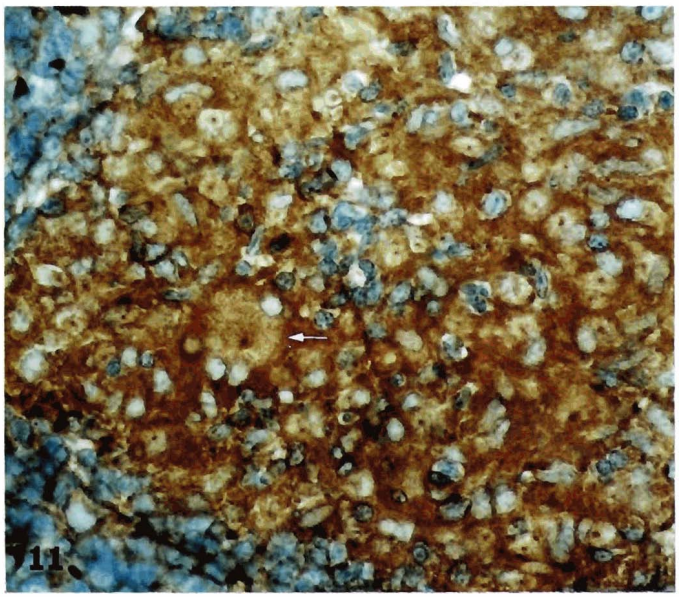


Fig. 11. Example of single MTOCs in epithelioid cells seen as spherical clearings with central converging profiles of tubulin reactivity. Small MGC with few peripheral nuclei (white arrow) likewise shows just single MTOC. Trypsinized section of sarcoid lymph node, polyclonal tubulin. x 400

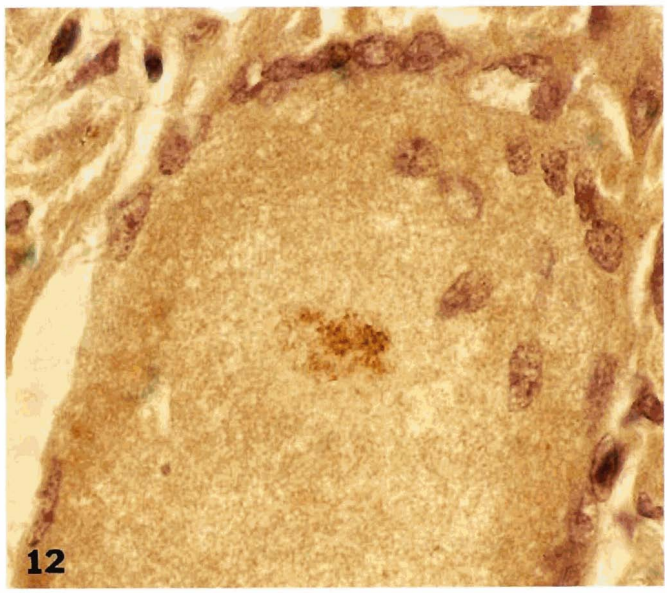


Fig. 12. Part of Langhans type giant cell with central clusters of tubulin-reactive granules, consistent with aggregated centrosomes. Polyclonal tubulin after alkaline phosphatase digestion. x 1,000

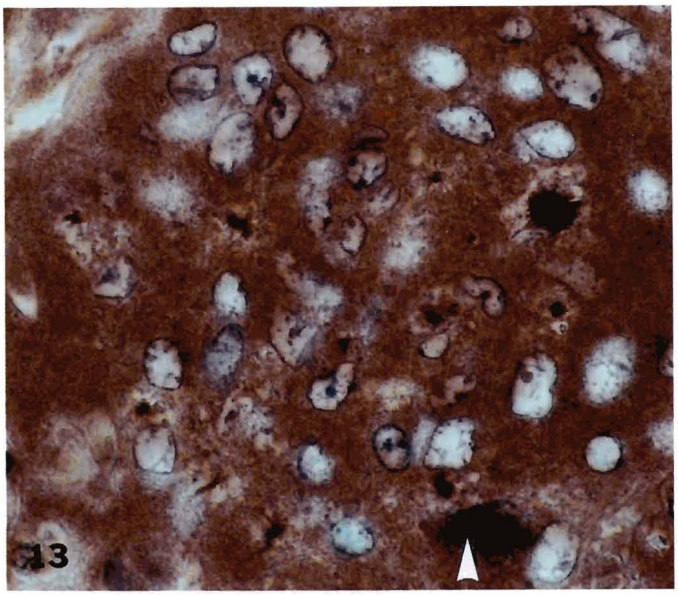


Fig. 13. Part of large MGC with dispersed nuclei showing two asteroid bodies with bulky cores (larger one out of focus, marked by white arrowhead) as well as at least 10 structures resembling MTOCs or small asteroid bodies. Trypsinized section of sarcoid spleen immunostained for tubulin. x 1,000

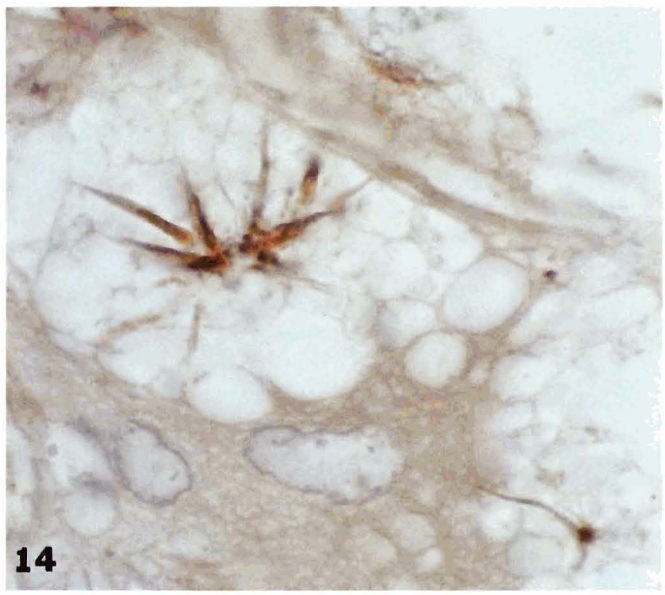


Fig. 14. Granular condensations of tubulin reactivity at root of spokes sectioned beyond center of core. Note small asteroid body in right lower corner with only two clearly stained spokes. Dephosphorylated sarcoid spleen. x 1,000

as clear spheres with central converging profiles of tubulin reactivity in epithelioid cells (Fig. 11). Occasional small MGCs with few peripheral nuclei revealed similar single MTOCs. Centriole-like bodies were not recognized in such structures. On the other hand, central cytoplasmic pooling of numerous dot-like bodies, presumably centrosomes, was sporadically evident in appropriate planes of section of large MGCs of the Langhans type with more than 30 nuclei. These had the appearance of solid granules without rays and were arranged in tight circles or irregular clusters (Fig. 12). In general, such large Langhans type MGCs appeared to be stabilized cells lacking both active MT arrays and ABs. In some MGCs with randomly clustered or dispersed nuclei, multiple structures resembling MTOCs were sometimes conspicuously demonstrated throughout the cytoplasm (Fig. 13). The distinction between these tiny tubulin containing asters and small ABs could not always be made reliably since the tell-tale vacuolations around the smallest ABs were often equivocal.

Although dephosphorylation removed most tubulin reactivity from AB cores the presence of centrosomes, respectively centrioles, could not be conclusively demonstrated with these stains. While the spokes of many small ABs appeared to radiate from a single point, suggesting a single MTOC, dephosphorylated cores of large ABs often revealed multiple granular condensations at the roots of spokes (Fig. 14) that were occasionally arranged in tight circles (Fig. 15) with a tiny central non-reactive window (Fig. 16).

Discussion

The shape, movement and internal organization of eukaryotic cells is determined by three major cytoskeletal systems, the microtubules (MTs), the intermediate filaments (IFs) and the microfilaments (MFs). The complex functions and interactions of these systems as well as the role they play in pathologic processes have only begun to be effectively probed with modern methods during the last two decades (Rungger-Brändle and Gabbiani, 1983).

Our findings clearly indicate that ABs are products of the MT system. Because ABs are found exclusively in certain types of MGCs it is important to understand how MT networks are organized in these cells as compared to cells that do not display them.

Microtubules (MTs)

The major component of MTs is the 110 kD heterodimer tubulin which is composed of α and ß subunits, each a 55kD acidic protein. MTs are hollow non-contractile fibrils with a diameter in the neighborhood of 25 nm which are co-assembled through polymerization of tubulin with several as yet incompletely characterized proteins, known as microtubule associated proteins or MAPs. According to available data (Olmsted, 1986), some MAPs have phosphorylated binding domains on the MT lattice and provide a most important link for MT-IF and MT-MF interactions. MTs often appear in bundles of 30 or more and may be periodically connected by fine cross-bridges, formed by one of the

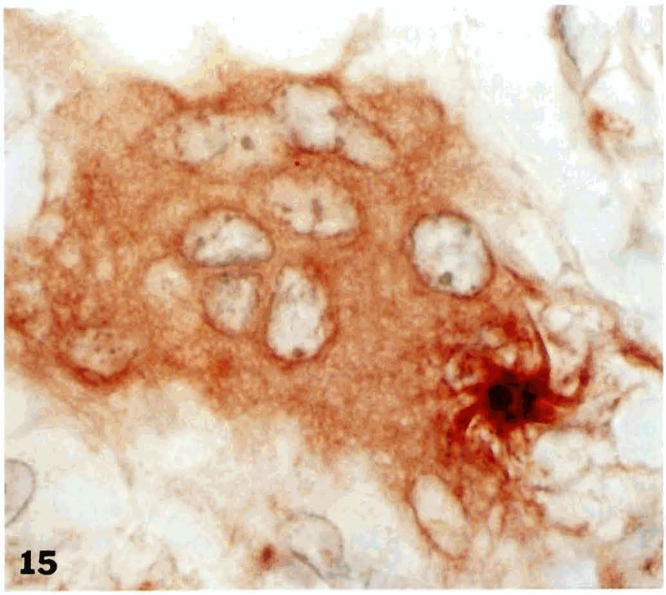


Fig. 15. Multiple granular masses intensely immunostaining for tubulin in dephosphorylated core of asteroid body. Sarcoid spleen. x 1,000

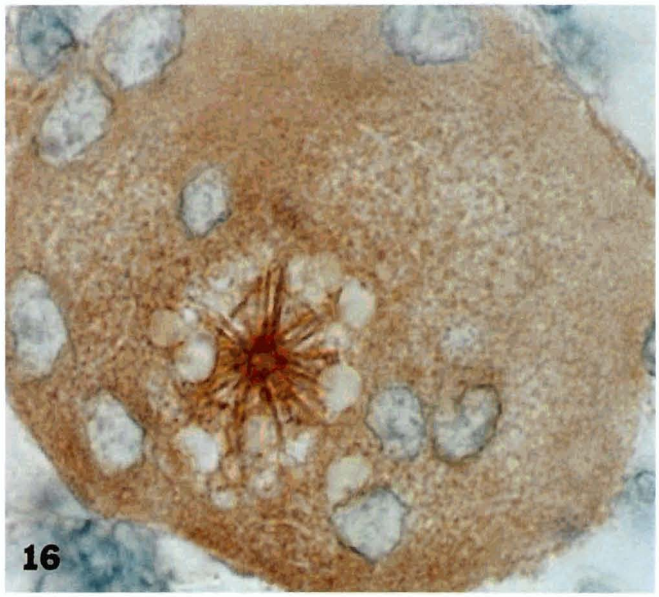


Fig. 16. Example of tubulin immunostaining in dephosphorylated core of multiple granules arranged in circle around tiny central non-reactive window. Sarcoid spleen. x 1,000

MAPs, dynamin (Scholey, 1990), that drives active sliding between MTs.

The centrosome as a major microtubule organizing center (MTOC)

MTs usually originate from a nucleating or initiating site in the cell for which the term MTOC has become widely accepted. According to the literature (Brinkley, 1985), MTOCs may have many different forms (centrioles, basal bodies, polar bodies, kinetochores, etc.) but the best documented MTOC in interphase cells is the centrosome. The centrosome is a dynamic, replicating structure that is usually located near the nucleus and is recognized ultrastructurally by converging MT profiles and one or more pairs of centrioles surrounded by a variable amount of electron dense amorphous pericentriolar material (PCM). The PCM, which contains MAPs, phosphoproteins and soluble tubulins (Olmsted, 1986), may function as a MTOC independent of centrioles. On the other hand, MTs may assemble at dispersed sites in the cytoplasm independent of centrosomes. A recently recognized promotor of MT assembly, γ-tubulin, has been reported to be a ubiquitous component of MTOCs (Oakley et al., 1990). Although the centrosome appears to exert a more widespread influence on the organization of cytoplasm than just the arrangement and distribution of MTs, it is recognized as a major MTOC and is a preferred site for MT assembly.

Multiple centrosomes in monocyte-derived MGCs

MGCs form by fusion of cell types of the monocyte line (epithelioid cells in sarcoidosis). Using antibodies against centrosomes and tubulin, Moudjou et al. (1989) convincingly demonstrated that both avian and human MGCs of the osteoclast type conserved the centrosomes of all cells partaking in the fusion process and consequently possessed as many centrosomes as there were nuclei in the cell. They further found that whether the centrosomes were regularly spaced in the peripheral cytoplasm or were relocated in the vicinity of nuclei depended on MT dynamics. A diametrically different mode of centrosome distribution has been described in Langhans type MGCs in which centrosomes are aggregated in the midportion of the cytoplasm equidistant from the peripherally disposed nuclei (Rhee et al., 1979). We have found ample evidence that these two extremes of centrosomal distribution in MGCs indeed exist but neither in osteoclasts nor in classical Langhans cells are associated with the occurrence of ABs. Most MGCs displaying ABs, particularly in sarcoidosis, are transitional forms with fewer than 30 randomly clustered or dispersed nuclei in which the multiple centrosomes may be presumed to have a similar unsettled distribution. These cells most often show signs of active internal reorganization reflected by prominent zonal displays of dense MT arrays.

Pointers in the histogenesis of ABs to aberrant MTOC dynamics

Centrosomes have been successfully isolated together with their PCMs from cultured neuroblastoma cells known to have multiple centrosomes (Mitchison and Kirschner, 1984). When such isolates dispersed in small clusters were incubated with appropriate concentrations of tubulin they produced radial MT assemblies strikingly similar to ABs, including thick tapering bundles. These experiments proved that single asters of MT assemblies could originate from several aggregated centrosomes. We have made numerous observations that suggest a similar multicentric origin for at least some large ABs from overlapping MTOCs.

The tendency of ABs to be localized in tubulin depleted cytoplasm of MGCs strongly suggests that a phenomenon described in vitro as «microtubule catastrophe» (McIntosh, 1984) may be operative in vivo. It is based on evidence that centrosomes can remain the centers of dynamic asters at tubulin concentrations well below those that cause rapid depolymerization of free MTs but that, paradoxically under such conditions, there is simultaneous growth of some populations of MTs while others catastrophically disassemble and disappear (Mitchison and Kirschner, 1984). We view the cytoplasmic vacuolations around ABs as indirect evidence for this phenomenon, since rapid MT depolymerization in vivo experimentally induced by drugs or antibodies is known to result in collapse of vimentin filaments through disruption of MT-IF interactions (Blose et al., 1984).

Correlations with previous findings

There is growing evidence that MT populations with different tubulin compositions and distinct properties may coexist in the same cell (Gundersen et al., 1984; Prescott et al., 1989). Interphase MTs rich in tyrosinated α-tubulin have been found to bind a higher percentage of MAP2 than MTs made with detyrosinated tubulin (Kumar and Flavin, 1985) and to be functionally distinct (Kreis, 1987). In this context, an earlier demonstration of tyrosine in ABs by the diazotization method (Lucas and Davies, 1973) is of great interest because it indicates that the high α-tubulin content observed by us in both ABs and cytoplasmic MT arrays is consistent with the presence of tyrosinated α-tubulin. MTs rich in tyrosinated tubulin have been found to be more dynamic and less stable than the detyrosinated group, which may be an important modification in cells undergoing changes in shape, differentiation state or disease condition.

The reasons why previous electron microscopic studies of ABs almost invariably failed to identify MTs are not clear. Only Cain and Kraus (1977) recognized MTs as a minor component of the stars. Kirkpatrick et al.

(1988) mentioned tubule-like structures unrelated to true MTs and Papadimitriou and Drachenberg (1992) found MT-like structures with an average diameter of 26 nm but, rather than true MTs, interpreted them as accumulations of phospholipids arranged in membrane bilayers.

The ultrastructural periodicity of the fibrils shown in the first electron micrographs of ABs and interpreted as collagen (Azar and Lunardelli, 1969) has not been recognized in subsequent studies. The main reason, we believe, may have been the degree of preservation of the examined tissues. Thanks to first hand knowledge by one of us (EAM) of how meticulously those early specimens were processed, we have no doubt than the periodicity in question was no artifact. However, re-evaluation of some of the original electron micrographs reveals that neither the diameter of the fibrils (about 25 nm) nor the spacing of the bandings (25-30 nm) justified the conclusion that they were collagen since the corresponding parameters for collagen fibrils are: diameter less than 60 nm for type I, 70-100 nm for type III, and average periodicity of 67 nm (Fleischmajer et al., 1990). Although little information exists on the physical arrangement of MAPs on the MT lattice, periodic spacings have been demonstrated at least for MAP2 (36 nm, Voter and Erickson, 1982) and for MAP1 (21±5 nm, Vallee and Davis, 1983). Apart from the apparently unusual MT dynamics taking place in ABs, it is important to realize that MTs in general are highly labile structures that are readily depolymerized and may disappear by fixation with various fixatives, especially on fixation in the cold.

Our negative results for collagen conclusively disprove the suggestion that MGCs might function as facultative fibroblasts or be involved in trapping exogenous collagen during the fusion process of their formation.

The frequent glove-like, close juxtaposition of unit membranes to AB spokes, mentioned in several electron microscopic descriptions (Azar and Lunardelli, 1969; Kirkpatrick et al., 1988; Papadimitriou and Drachenberg, 1992) is probably the result of rapidly expanding structures as they impinge on some of the many membrane bounded vesicles of the smooth endoplasmic reticulum or, less likely, on residual membranes of partly fused cells. Both the strategic location of centrosomes in the Golgi area and the finding that fusing cells have their Golgi zones facing each other (Rhee et al., 1979) may be important contributing factors.

Our conclusion that asteroid bodies constitute products of unusual centrosome-MTOC dynamics in MGCs undergoing active internal reorganization remains to be confirmed by further studies which, we hope, this communication will promote.

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