

Cross-reactive and species specific *Mycobacterium tuberculosis* antigens in the immunoprofile of Schaumann bodies: a major clue to the etiology of sarcoidosis

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Summary. Sarcoidosis, once thought to be a variant of tuberculosis, is currently listed as a disease of unknown etiology. The present study was initiated by unpublished observations that Schaumann bodies - the laminated inclusions often encountered in sarcoid granulomas - cross-reacted with commercial polyclonal antibodies to *Mycobacterium bovis*, *Mycobacterium duvalii* and *Mycobacterium paratuberculosis*. Given the broad cross-reactivity of many mycobacterial antigens, those findings lacked specificity but warranted in depth probing of the immunoprofile of the bodies, particularly for specific mycobacterial antigens. Formalin-fixed tissue from eight patients with an established diagnosis of sarcoidosis was studied with panels of antibodies against both common cytoplasmic proteins and various mycobacterial antigens, using a labeled streptavidin-biotin-alkaline phosphatase technique. Our findings indicate that Schaumann bodies are indeed residual bodies of heterophagic mycobacterial derivation. They immunostained intensely for the lysosomal proteins muramidase and CD68, variably for some cytoskeletal proteins (tubulin, desmin, vimentin) and not at all for cytokeratin, muscle actin, alpha-1-antichymotrypsin and ferritin. Both cross-reactive and species specific antigenic determinants of *M. tuberculosis* complex were shown to be present. Affinity absorption with killed intact bacilli H37 Rv resulted in virtually equal loss of binding by all polyclonal antimycobacterial antibodies to cross-reactive ligands in Schaumann bodies. In addition, the bodies were clearly labeled with the monoclonal antibodies TB68 and TB71, known to recognize species specific epitopes of *Mycobacterium tuberculosis* complex. Although obtained on a small number of cases, our findings uphold Schaumann's original postulate that the laminated calcific inclusions represent remnants of «transformed tubercle bacilli».

Key words: Sarcoidosis, Mycobacterial antigenic determinants, Schaumann bodies, Heterophagic residual bodies

Introduction

Schaumann bodies (SBs) are complex, concentrically stratified concretions, up to 150 µm in diameter, that often enclose hematoxyphilic mineralized components or birefringent crystalline material. They may be virtually unstained in routine H&E stained sections and may display a brown autopigmentation in unstained sections. They occur in multinucleated giant cells (MGCs) but not in epithelioid cells. The small ones are usually spherical, compact and basophilic. The large ones are seldom preserved intact, appearing empty or fragmented, with laminated polycyclic contours. They sometimes appear to be extracellular and may be surrounded by hyaline fibrous tissue with or without recognizable remnants of MGCs.

SBs can be readily distinguished from comparable mineralized structures such as psammoma bodies, Gamna-Gandy corpuscles and round concretions or calcospherules in various other conditions by virtue of their unique morphology and location. However, structures duplicating their morphology and distinct location are known to occur also in conditions other than sarcoidosis and have, unfortunately, also been referred to as Schaumann bodies. As used in this report, the eponym Schaumann body has been restricted to sarcoidosis. Any look-alikes found in other conditions, even though their physical properties may be indistinguishable from those of genuine SBs, are being referred to as Schaumann body analogues (SBAs). Before the immunophenotype of the various SBAs can be established, we consider this distinction to be quite important.

The occurrence of SBAs has been documented in one instance in tuberculous salpingitis (Burne, 1953), more often in BCG granulomas (Lemming, 1940; Vortel,

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1962), in fungal infections (Nanta, 1930), in chronic berylliosis (Vorwald, 1948; Jones Williams and Williams, 1968; Freiman and Hardy, 1970) and, sporadically, in Crohn's disease (Warren and Sommers, 1948; Roge et al., 1991) and a few other conditions (Tayot et al., 1976; Young et al., 1978). The conchoid bodies of chronic berylliosis were claimed to be indistinguishable from true SBs (Jones Williams and Wallach, 1989), but most significantly, SBAs have been produced in experimental animals with injections of photochromogenic mycobacteria (Frenkel, 1958; Rasmussen and Caulfield, 1960), human tubercle bacilli (Dumont and Sheldon, 1965) and heterogeneous microbial agents (Okudaira et al., 1961), including leishmania (Binhazim et al., 1993).

In view of the compelling evidence that SBAs can be reproduced experimentally as a host-mediated response to a variety of infectious agents, we felt that a study aimed at exploring the possibility that sarcoid SBs were products of host-mycobacterial interactions, as originally envisioned by Schaumann (1941), was long overdue.

Materials and methods

Selection of cases

The test series comprised formalin-fixed paraffin-embedded surgical and autopsy material from a total of eight patients with an established diagnosis of

sarcoidosis. Well over 200 cases with sarcoid granulomas were pre-screened successively, for the period 1972-1981, from the surgical pathology files of the Medizinische Hochschule Hannover, Germany and, for the period 1966-1993, from cases available for review at Harlem Hospital Center. Sections with most numerous SBs in two cases from the former and six cases from the latter institution were selected for this study.

Choice of method

Certain characteristics of SBs recognized in preliminary studies had to be taken into account: 1) a powerful endogenous enzyme system difficult to consistently neutralize with common blocking measures for immunoperoxidase procedures; 2) a native yellow to brown pigmentation of some bodies which may lead to equivocal results with diaminobenzidine (DAB), and 3) our own observation that Hanker-Yates reagent (phenylenediamine/pyrocatechol), an alternative alcohol-resistant chromogen with a brown-black end-product, was more sensitive than DAB to endogenous reactive agents within SBs and was cumbersome to use with the Tubbs-Sheibani technique (Tubbs and Sheibani, 1981).

Regardless of the method to be adopted, the strong hematoxyphilia and affinity for basic dyes exhibited by parts or totality of SBs precludes the satisfactory application of nuclear counterstains. In addition, the high content of muramidase (lysozyme) in the bodies requires

Table 1. Antibodies against cytoskeletal, lysosomal and other endogenous proteins.

ANTIGEN	CLONE	DILUTION/ENZYME	SOURCE
Cytokeratin	AE1	1:100/T	BioGenex
Tubulin	polyclonal	PD/T	BioGenex
Ferritin	polyclonal	1:200	BioGenex
Muscle actin	HHF35	1:8000	Enzo Diagnostics, Inc.
Vimentin	V9	1:20	DAKO Corporation
Desmin	D33	1:100	DAKO Corporation
CD68	KP1	1:60/T	DAKO Corporation
Muramidase (lysozyme)	polyclonal	PD/T	DAKO Corporation
Alpha-1-antichymotripsin	polyclonal	PD/T	DAKO Corporation

PD: prediluted; T: trypsin.

Table 2. Antimycobacterial polyclonal and monoclonal antibodies.

ANTIGEN	CLONE/DESIGNATION	DILUTION	SOURCE
<i>M. bovis</i> *, BCG	polyclonal	1:800	DAKO Corporation
<i>M. paratuberculosis</i> *	polyclonal	1:800	DAKO Corporation
<i>M. duvalii</i> *	polyclonal	1:800	DAKO Corporation
<i>M. tuberculosis</i> *, 16 kD	F24-2-3/(IT-4)	1:1000	CDC/WHO, Atlanta, GA
<i>M. tuberculosis</i> , 16 kD	TB68/(IT-20)	1:30	CDC/WHO
<i>M. tuberculosis</i> , 38 kD	TB71/(IT-23)	1:5	CDC/WHO
<i>M. tuberculosis</i> *, SOD 23 kD	F116-5/(IT-61)	1:5000	CDC/WHO
<i>M. leprae</i> , 35 kD	MMP-1-3C3/(mc9247)	1:10000	CDC/WHO

*: cross-reactive with other species; SOD: superoxide dismutase; IT or mc in brackets: CDC/WHO reference numbers.

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the use of negative control sera that are free of antimuramidase antibodies.

Immunohistochemistry

Re-hydrated five micron tissue sections mounted on silane coated slides were first treated with 2.28% periodic acid in distilled water for 10 min, followed by 0.02% fresh sodium borohydride for 2 min in order to neutralize endogenous alkaline phosphatase activity (Bulman and Heyderman, 1981). Slides were then washed in three changes of PBS. Sections requiring proteolysis were pre-digested for 15 min at room temperature with 0.1% trypsin in tris-hydrochloride buffer, pH 7.6, containing 0.1% CaCl₂, and again thoroughly rinsed in buffer. Incubations with the primary antibodies were carried out at 4 °C overnight, using subsequently the labeled streptavidin-biotin-alkaline phosphatase method with commercial kits (DAKO Corporation, Carpinteria, CA). The primary antibodies against endogenous proteins are listed in Table 1, those used to localize mycobacterial antigens in Table 2. Two drops of a 30 mg/ml solution of levamisole were added to each 5 ml substrate with dissolved Fast Red TR and, after filtering the solution, color development was allowed to proceed for 15 min at room temperature. Counterstaining was omitted except for selected sections which were stained with 1% methyl green in citrate buffer, pH 5.3. Sections were mounted with Glycergel (DAKO Corporation), a glycerol-gelatin water-soluble mounting medium.

Known controls were used for cytokeratin (lung), ferritin (fetal liver) and for three mycobacterial species: *M. tuberculosis* (lung and epicardium from two cases of miliary tuberculosis), *M. leprae* (skin from a case of lepromatous leprosy) and *M. avium/intracellulare* (MAI) (lymph nodes from two AIDS patients). Internal controls for the remaining antibodies were adequate in most tissues. In negative controls, primary antibodies were substituted with diluent buffer or, for polyclonal antibodies, with commercial non-immune sera (L1802 or

L1828, DAKO Corporation).

Affinity absorption of antimycobacterial polyclonal antibodies (P-ABs) was performed as follows: loopfuls of heat-killed bacilli *M. tuberculosis*, strain H37 Rv, were suspended in 5 ml aliquots of each 1:800 working dilutions of antibodies to *M. duvalii*, to the BCG strain of *M. bovis* and to the 2E strain of *M. paratuberculosis* and placed on a tube rocker at room temperature for one and six hours, respectively, followed by centrifugation at 2500 rpm for 10 min and careful separation and filtration of supernate.

Results

Mycobacterial antigens

The results of immunostaining for cross-reactive and species-specific determinants are shown in Table 3. There were significant differences between P-ABs and murine monoclonal antibodies (MoABs) both as regards binding to known controls and to SBs.

In known controls, the three P-ABs produced staining of remarkably similar intensity in all targets regardless of the species represented either by the antigen or by the antibody. Dense bacillary clusters in miliary tuberculosis, lepromatous leprosy and MAI infections were distinctly labeled. However, delineation of individual bacilli was unsatisfactory and, in general, quite inferior to that achieved in routine acid-fast stains. Immunoreactivity was concentrated in granules and connecting segments stained too faintly to allow localization of scattered single organisms. With the exception of intracellular MAI (Fig. 1), diffusion of immunoreactive substances around bacilli obscured their outlines, particularly in tuberculosis.

All SBs, irrespective of size or shape, were selectively labeled with the P-ABs in each case of sarcoidosis. The intensity of immunostaining was moderate independent of the antibody used (Fig. 2) but was usually less distinctive than that observed in preliminary studies with the Harker-Yates detection

Table 3. Immunostaining of cross-reactive and species specific mycobacterial antigens in Schaumann bodies as compared to known tissue controls.

ANTIBODY	SCHAUMANN BODIES	KNOWN TISSUE CONTROLS		
		TB	MAI	Leprosy
<i>M. bovis</i> , BCG (P)	++	++	++	++
idem, affinity absorbed	±	++s	++	++
<i>M. duvalii</i> (P)	++	++	++	++
idem, affinity absorbed	±	++s	++	++
<i>M. paratuberculosis</i> (P)	++	++	++	++
idem, affinity absorbed	±	++s	++	++
F24-2-3 (IT-4) (M)	++	++	±	±
TB68 (IT-20) (M)	+++	++	-	-
TB71 (IT-23) (M)	+	+	-	-
F116-5 (IT-61) (M)	±	++	++	++
MMP-1-3C3 (mc9247) (M)	-		±	++

TB: *M. tuberculosis*; MAI: *M. avium/intracellulare*; Leprosy: *M. leprae*; affinity absorbed: with killed intact bacilli *M. tuberculosis*, strain H37 Rv; (P): polyclonal; (M): monoclonal; -: negative; ±: negative or equivocal; +: weak; ++: moderate; ++s: spotty moderate, decreased reaction sites; +++: intense immunostaining.

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system in immunoperoxidase procedures (Fig. 3). Occasional MGCs displayed spotty granular staining on a case specific basis unrelated to the presence or absence of SBs.

Affinity absorption of the P-ABs, as outlined in Materials and methods, produced unexpected results. While immunostaining of MAI and Hansen's bacilli was not perceptibly affected, immunostaining of tubercle bacilli was characterized by apparently fewer reaction sites rather than decreased intensity or absence of staining. On the other hand, absorbed antibodies in each instance failed to label SBs with an unequivocal signal, most bodies remaining blank or, at best, showing faint pink hues. Results were identical with antibodies absorbed for one hour and those absorbed for six hours.

The reported specificity of the MoABs, based on RIA, ELISA, immunoblot and related techniques of serologic analysis (Engers et al., 1986; Khanolkar et al.,

1992; Young et al., 1992), was tested on our three mycobacterial species in formalin-fixed tissue controls. We observed two minor discrepancies. First, the MoAB MMP-I-3C3 (mc9247), reported to recognize a specific 35 kD membrane-associated *M. leprae* antigen (Young et al., 1992), was found to show limited cross-reactivity with MAI. Second, the MoAB F24-2-3 (IT-4) directed towards a heat-shock protein of *M. tuberculosis*, cross-reacted weakly with both leprosy and MAI bacilli as well as with nuclear DNA in many cells of MAI infected lymph nodes. The two MoABs which reacted only with *M. tuberculosis* were TB68 (IT-20), another member of the 16 kD heat-shock protein binding family, and TB71 (IT0-23) which is known to recognize a 38 kD phosphate transport «binding protein». Immunostaining with TB71 was weak even at high concentrations of antibody (1:3 to 1:5). The remaining MoAB of the series, F116-5 (IT-61), which recognizes the 23 kD

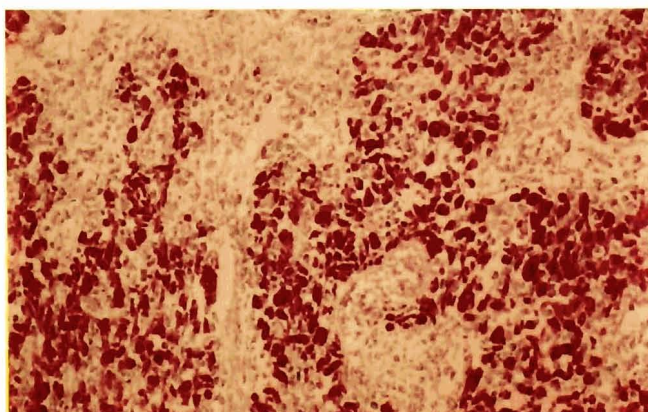


Fig. 1. Macrophages with densely packed MAI in control lymph node labeled with polyclonal antibody against *M. paratuberculosis* show virtually no diffusion of red staining antigens. Streptavidin-biotin-alkaline phosphatase method, visualized with Fast Red TR and counterstained with methyl green. x 200

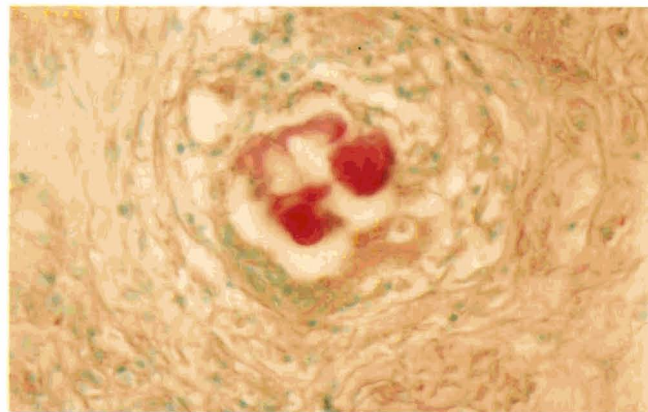


Fig. 2. Schaumann body in sclerotic sarcoid granuloma of lung labeled with anti-*M. bovis* (BCG) exemplifies usual degree of binding by polyclonal antibodies to cross-reactive ligands in the bodies. Same procedure as for preceding control. x 400

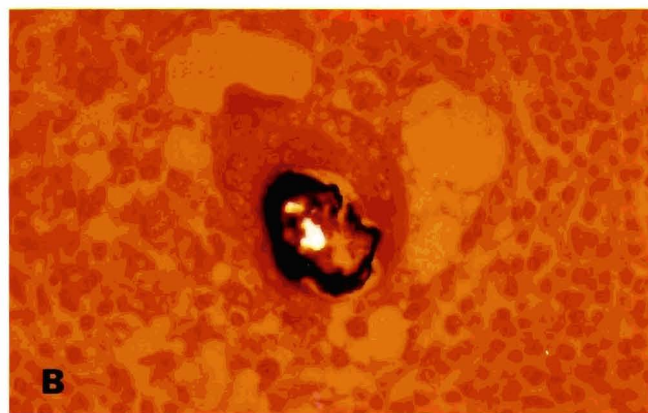
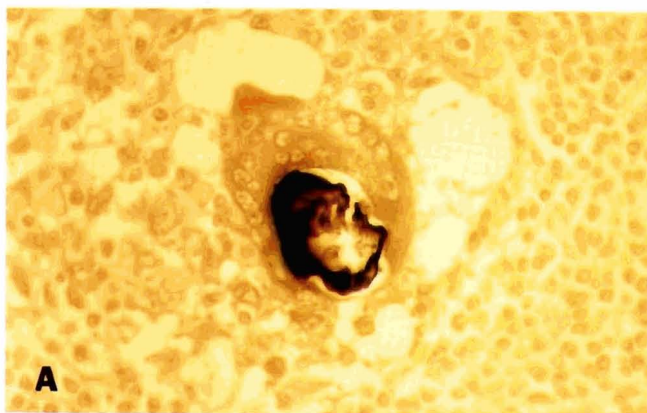


Fig. 3. Example of coloration obtained with Hancer-Yates reagent (p-phenylene-diamine/pyrocatechol) in immunoperoxidase procedures. A. Brown to black end-product delineates periphery of Schaumann body in sharp contrast to surrounding background in sarcoid lymph node immunostained with polyclonal anti-*M. duvalii*, using PAP procedure without counterstain. Note faint diffuse cross reactivity with nuclear DNA. B. Same body seen in polarized light reveals presence of birefringent crystals within central mineralized portion which is largely devoid of demonstrable antigens. x 400

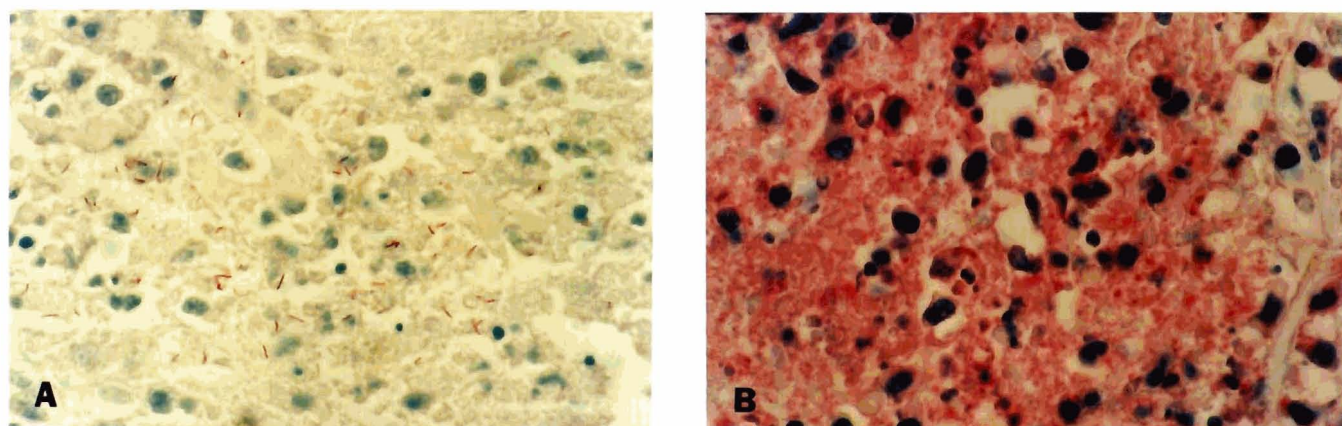


Fig. 4. Routine acid-fast stain compared with result of immunostaining in miliary tuberculosis. **A.** Acid-fast bacilli neatly delineated in Ziehl-Nelsen stain, extrabacillary antigens remaining unstained and undetectable. **B.** Comparable area from same case immunostained with MoAB TB68 shows multiple ill-defined points of reactivity without bacillary outlines against background of weak diffuse immunostaining (counterstained with Mayer's hematoxylin). x 1,000

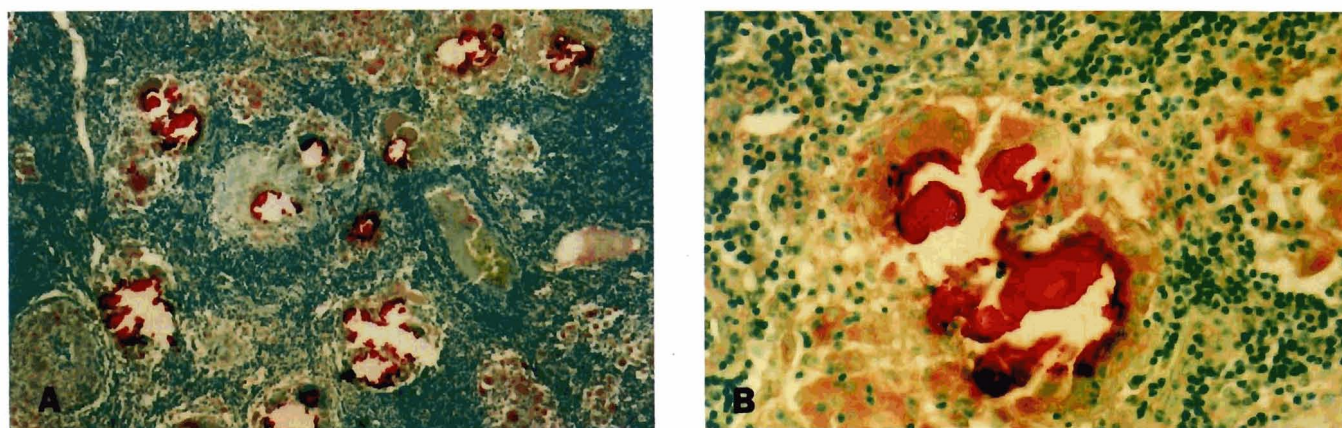


Fig. 5. **A.** Area of sarcoid lymph node with multiple Schaumann bodies, selectively immunolabeled with the MoAB F24-2-3 which recognizes LMW heat-shock proteins of *M. tuberculosis* complex but has limited cross-reactivity with other species. **B.** Close-up view of one of the bodies reveals fairly homogenous distribution of target antigen with little superposed peripheral staining by methyl green counterstain. Cytoplasm of giant cell and epithelioid cells is weakly immunoreactive. **A.** x 100; **B.** x 400

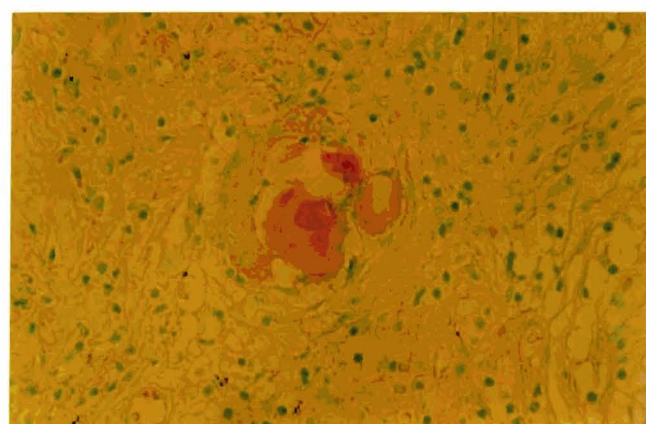


Fig. 6. Example of generally weak immunostaining of Schaumann bodies with the MoAB TB71 which recognizes a phosphate transport «binding protein» specific of *M. tuberculosis* complex. Methyl green counterstain. x 400

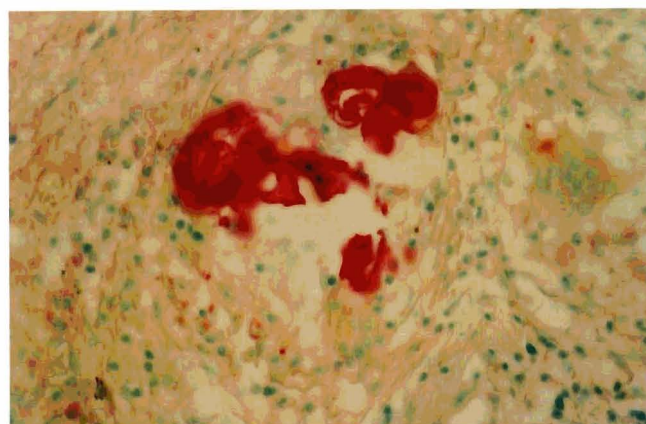


Fig. 7. Schaumann bodies in pulmonary sarcoid granuloma immunostained with the MoAB TB68 convey the impression of being saturated with the target antigen, known to be LMW heat-shock proteins specific of *M. tuberculosis* complex. Methyl green counterstain. x 400

superoxide dismutase protein of *M. tuberculosis*, cross-reacted not only with all three control species but also with unrelated coliform and coccoid organisms of the intestinal flora.

In general, the MoABs failed to delineate individual bacilli in controls and accentuated the punctate or globular immunostaining of granular complexes previously observed with the P-ABs (Fig. 4). Diffusion of antigen was least apparent among leprosy bacilli stained with MMP-I-3C3 and was most pronounced around bacilli in miliary tuberculosis stained with F116-5 and TB68.

SBs were immunolabeled with variable intensity by all four MoABs of the IT group. Staining of the bodies was moderate with F24-2-3 (Fig. 5), weak with TB71 (Fig. 6) and ranged from negative to weak with F116-5. The most intense and uniform immunostaining was achieved with TB68 (Fig. 7). In addition to complete lack of color, reactions interpreted as negative included faint pink hues which were occasionally observed in bodies stained with F116-5 and MMP-I-3C3. Whether seen as homogeneous structures when small or as concentrically laminated structures when large, SBs showed a clear tendency for the immunoreactive substance to be concentrated peripherally, which resulted in stretches of thin but uninterrupted outer rim staining. Crystalline material, whenever present, did not immunostain but was often obscured and showed loss of birefringence in polarized light. In several cases, both F24-2-3 and TB68 yielded granular reactivity in the cytoplasm of scattered MGCs and epithelioid cells. Asteroid bodies did not react with antimycobacterial antibodies.

Cytoskeletal antigens

Neither SBs nor the cytoplasm of MGCs were immunoreactive for cytokeratin and muscle actin. Vimentin was the only intermediate filament protein expressed by MGCs consistently with variable intensity. SBs were seldom found to contain vimentin positive parts in the form of isolated streaks or patches. Diffuse labeling of whole bodies was exceptionally seen in small compact bodies. Most SBs, however, immunostained for both desmin and tubulin. The cytoplasm of MGCs was desmin negative but showed variable reactivity for tubulin in trypsinized sections. Asteroid bodies also expressed tubulin in trypsinized sections but did not stain with the remaining antibodies.

Lysosomal and other cellular antigens

Some of the strongest and more consistent immunostaining of SBs was obtained with the two macrophage markers, the P-AB to muramidase (lysozyme) and the MoAB KP1 (CD68). The cytoplasm of MGCs and, to a lesser degree, that of epithelioid cells and other mononuclear cells was also strongly immunoreactive. By contrast, SBs were usually negative for alpha-1-anti-

chymotrypsin in the presence of strong reactivity in MGCs. Neither SBs nor MGCs immunostained for ferritin. If present, ferritin reactivity was weak and was limited to few cells in the periphery of epithelioid cell tubercles. Asteroid bodies did not react with any antibodies of this group.

Discussion

The structures we now call Schaumann bodies had been described long before sarcoidosis was recognized as a disease entity. A detailed review of the earliest records on the subject, dating back to 1850, has been provided by Potts (1951). In this century, it was Schaumann who first called attention to their common occurrence in «lymphogranulomatosis benigna», the term he had introduced for sarcoidosis (Schaumann, 1941). Schaumann referred to them as «L.B. bodies» and found them arising within, rather than being engulfed by, the giant cells. After years of exhaustive studies, he considered the presence of L.B. bodies as clues of pivotal importance to the etiology of the disease. Relating their occurrence to «pleomorphic non-acid-fast microorganisms» he had once successfully cultured from involved lymph nodes, Schaumann invoked numerous supportive details observed over the years by himself and by others to postulate that the laminated intracellular inclusions represented remnants of transformed tubercle bacilli.

The physical properties and chemical composition of SBs have been explored more or less in depth by several investigators (Teilum, 1949; Uehlinger, 1964; Zak, 1964; Jones Williams and Williams, 1967), with variable results. A comprehensive study illustrated with excellent color prints of relevant histochemical reactions appeared in an article by Frizzera and Mancini (1966). Most studies agreed on two points: first, that the fundamental constituents of the bodies were acid mucopolysaccharides, glycoproteins and lipoproteins as well as mineral salts of calcium and iron; and second, that the structures formed around a pre-existing nidus which could be either endogenous or exogenous. As a result, SBs were concluded to be a form of residual bodies (Jones Williams and Williams, 1967). Their ultrastructure has been likened to that of Michaelis-Gutman bodies (Pluot et al., 1974). Noteworthy among dissenting theories was the suggestion that SBs evolved from asteroid bodies (Uehlinger, 1964; Kirkpatrick et al., 1988).

The exact nature and histogenesis of the birefringent crystals occurring both within an outside SBs or SBAs remain unsettled (Engle, 1951; Vortel, 1962; Zak, 1964). Jones Williams and Williams (1960, 1967) regarded the crystals to be composed mainly of calcite and maintained that they were endogenous, invariably constituting the nidus around which the bodies evolved. More recently, Reid and Andersen (1988) found that the crystals were mainly composed of calcium oxalate.

As for the results obtained with antimycobacterial

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antibodies, their interpretation must be contingent on an understanding of the variety and nature of mycobacterial antigens. From the outset, studies with immunodiffusion (Stanford, 1973), immunoelectrophoresis (Daniel and Janicki, 1978; Harboe et al., 1979; Closs et al., 1980) and radioimmunoassay (Harboe et al., 1978) revealed a highly complex antigenic make-up of the genus *Mycobacterium*. Because at least some of the distinct soluble antigenic constituents detected in each species are shared by all species of mycobacteria (Stanford, 1973) polyclonal antibodies raised against one species would be expected to cross-react with all other mycobacterial species. On the other hand, single antigenic determinants recognized by MoABs may also be widely shared among different mycobacterial species, particularly in the 65 kD heat-shock protein range. Some of these antibodies have also shown broad inter-genus cross-reactivity, binding to antigens in gram-negative bacteria, gram-positive bacteria, spirochetes and rickettsiae (Shinnick et al., 1988).

So far, few of the epitopes recognized by MoABs have proved to be species specific (Khanolkar et al., 1992). For instance, out of 31 MoABs submitted for evaluation in 1985 to the WHO Immunology of Tuberculosis (IMMTUB) Workshop, none was found to be specific for a given mycobacterial species or strain when results of all tests were taken into account (Engers et al., 1986). Only 6 showed restricted specificity to *M. tuberculosis* complex (i.e., reacting with *M. tuberculosis*, *M. bovis* BCG and *M. africanum*). Three of these, F24-2-3, TB68 and TB71, have been used by us in the present study although some of their characteristics have in the meantime been updated. As of May 1994, the WHO MoAB bank at CDC lists F24-2-3 (IT-4) as cross-reactive with other species and both F24-2-3 and TB 68 (IT-20) as subclass IgG₁ recognizing 16 kD epitopes rather than 14 kD epitopes, as previously reported (Engers et al., 1986; Young et al., 1992). The MoAB TB71 (IT-23), once thought to be strictly specific for *M. tuberculosis*, has been characterized as binding to both *M. tuberculosis* and *M. africanum* but not to *M. bovis* or to other species (Morris and Ivanyi, 1985).

Although our control series was limited to three species, the results obtained by us seem to confirm the reported specificity of the MoABs in the IT group (Table 3). The focal punctate, granular or globular immunostaining of tubercle bacilli would seem to indicate that most target epitopes are concentrated in unevenly distributed reaction centers. This antigenic heterogeneity is the main reason why some MoABs fail to delineate individual organisms. The diffusion of antigenic products we observed is an additional impediment. Earlier immunohistochemical studies have shown that both polyclonal and monoclonal antimycobacterial antibodies can be helpful in localizing precisely such dispersed antigens in tuberculous lesions whenever routine acid-fast stains are negative (Humphrey and Weiner, 1987; Barbolini et al., 1989).

The MoABs we are dealing with have not, to the

best of our knowledge, been investigated for non-specific binding or cross-reactions on formalin-fixed tissue previously. Our observation that F24-2-3 may stain the nuclei of some cells was not surprising since we had noted similar cross-reactions with antimycobacterial P-ABs during our preliminary studies. Other kinds of shared antigens have been documented between *M. bovis*, BCG and neoplastic cells (Minden et al., 1974), including malignant melanoma cells (Minden et al., 1976). Recently, shared antigenicity between antimycobacterial MoABs and human DNA has been demonstrated by ELISA and suggested to play a role in the genesis of autoimmunity (Shoenfeld et al., 1986).

Our own limited testing of the antibodies on irrelevant tissue controls showed that three of them (F24-2-3, TB68 and MMP-I-3C3) cross-reacted to a variable extent with cardiac lipochrome, a reaction not previously observed with P-ABs. In addition, following pronase digestion, both TB68 and F116-5 (but not F24-2-3) bound strongly to nuclear DNA of most cells, regardless of cell type.

Based on our results, the immunoprofile of SBs is mainly characterized by a high content of lysosomal proteins, as anticipated for residual bodies of lysosomal digestion, and by significant levels of both cross-reacting and species specific antigenic determinants of the *M. tuberculosis* complex. The cross-reacting ligands detected by P-ABs may be inferred to occur in low concentrations and, when the observed loss of binding by affinity absorbed reagents is taken into account, to indicate a common variety of surface antigens that are equally shared between *M. tuberculosis* and the other species involved (i.e. *M. bovis*, *M. paratuberculosis* and *M. duvalii*). Of the mycobacterial target epitopes detected by the MoABs, the LMW heat-shock proteins specific of *M. tuberculosis* complex appear to permeate the bodies in highest concentrations, followed by the equally species specific 38 kD phosphate transport «binding protein» in low concentration and by the presence, often in trace amounts, of the broadly cross-reactive 23 kD superoxide dismutase protein.

The participation of the cytoskeletal proteins vimentin and tubulin in the composition of SBs must be viewed as evidence for the intracellular evolution of the bodies, including those which are eventually encountered in extracellular location. The paradoxical staining of SBs for desmin appears to be a false reaction which, we have reason to suspect, involves a cross-reaction with degraded alpha-tubulin.

A plausible explanation for the concentric stratification of SBs may have been provided by ultrastructural studies concerning the evolution of SBAs in experimental mycobacterial infections of hamsters (Rasmussen and Caulfield, 1960; Dumont and Sheldon, 1965). Diffusion of bacterial products from viable organisms in the center of developing corpuscles was found to be the primary triggering event for the subsequent concentric precipitation rings of dense granular material and mineral microcrystals, analogous

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to the rings produced in gels by Liesegang (Liesegang, 1926). Further enlargement of the bodies by additional precipitation layers was described to continue until the central bacteria disintegrated or became mineralized (Rasmussen and Caulfield, 1960).

The tendency to enhanced peripheral immunostaining observed in SBs supports this mechanism and proves that mycobacterial products indeed do diffuse into the surrounding cytoplasm during the evolution of the laminated bodies. Moreover, in order to result in such large structures as many SBs are, this diffusion must keep going on through a series of successive precipitation lines and microcrystal barriers set up by the cell's defense mechanisms. This requires a viable organism with a remarkable capability for intracellular survival.

Mycobacteria fully meet these requirements. Past experimental studies on parasitic species have established the fact that mycobacteria can survive and multiply in phagolysosomes indefinitely (D'Arcy Hart et al., 1972). It has even been suggested that lysosomal enzymes not only fail to inhibit mycobacterial growth but might actually provide nutrients of low molecular weight beneficial for the growth of these chronic intracellular pathogens (Brown et al., 1969).

However, the preceding considerations apply to conventional forms of mycobacteria and these, as a rule, are not found in uncomplicated sarcoidosis. We believe that cell wall-deficient (CWD) forms of a stabilized mycobacterial L-phase are involved. If minute filterable, non-acid-fast CWD forms indeed occur in the granulomas, the failure to detect them microscopically is not surprising. In fact, CWD mycobacterial forms have been isolated by Mattman (1993) from the blood of 53 out of 55 sarcoid patients tested. Although these organisms closely resembled CWD *M. tuberculosis* they showed several different aspects and could not be fully characterized because reversion, even with a year's incubation, was not achieved (Judge and Mattman, 1982). Of the agents capable of inducing the CWD state in mycobacteria, mycobacteriophages must be considered the most likely candidates to induce a stabilized L-phase. Although lysogenic strains of some mycobacterial species have been isolated from clinical material (Grange and Bird, 1978), naturally occurring lysogeny in *M. tuberculosis* has not been convincingly demonstrated (Grange and Redmond, 1978). Despite evidence of extrachromosomal DNA in *M. tuberculosis*, no plasmids could be identified in this species (Zainuddin and Dale, 1990). Nevertheless, the possibility that mycobacteriophages play a role in the etiology of sarcoidosis is supported by valid arguments (Mankiewicz and Beland, 1964) and remains to be further investigated.

In conclusion, SBs are distinctive residual bodies of heterophagic mycobacterial derivation. A necessary prerequisite for their evolution and concentrically laminated structure is the initial presence of viable organisms. Antigenically, these appear to be part of the *M.*

tuberculosis complex but, for the time being, involvement by a hitherto unrecognized strain, or by more than one strain or species, cannot be ruled out. The high content of specific mycobacterial heat-shock proteins indicated an organism in distress. Barring the unlikely event that the occurrence of identical immunophenotypes will be demonstrated for some SBAs in other conditions, this feature alone may have important implications for the diagnosis of sarcoidosis by immunohistochemical means.

We further conclude that the birefringent crystals frequently associated with SBs are not the nidus around which the bodies evolve but are by-products of intracellular immune processes. Asteroid bodies do not partake in the evolution of SBs but, according to a recent study (Gadde and Moscovic, 1994), are products of unusual dynamics of microtubule organizing centers in monocyte-derived giant cells.

It is our hope that the results of this limited series will provide the incentive for further efforts to verify the present findings on a larger scale as well as for attempts to establish the antigenic profile of SBAs in such conditions as Crohn's disease, malignancy-associated pseudo-sarcoid reactions and chronic berylliosis.

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