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Spindle cell reaction to nontuberculous mycobacteriosis in AIDS mimicking a spindle cell neoplasm

Evidence for dual histiocytic and fibroblast-like characteristics of spindle cells*

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Summary. We report 5 patients with AIDS who had an unusual spindle cell proliferation in the lymph nodes and skin caused by nontuberculous mycobacteriosis. The spindle cell proliferation in these tissues may mimic a spindle cell neoplasm and pose a diagnostic problem if an infectious aetiology is not suspected. The fibroblast-like spindle cells contained numerous acid fast bacilli. They were strongly positive for antibody markers of monocyte/macrophage and leukocyte derivation: Leu M3, Mo-9, T-200, and HLA-DR, and variably positive for alpha-1 anti-chymotrypsin and lysozyme. Ultrastructurally these spindle cells were predominantly fibroblast-like with poorly developed features of macrophages. These results reveal the dual macrophage and fibroblastic character of the spindle cells and probably imply a functional differentiation rather than a histogenetic one.

Key words: Mycobacteria – Spindle cells – Facultative fibroblasts – AIDS

Introduction

Nontuberculous mycobacterial infections (NMI), are usually of limited virulence in the normal host, but may cause disseminated disease in immunocompromised patients (Greene et al. 1982; Klatt et al. 1987; Sohn et al. 1983). The classical histology of NMI tissue reactions in AIDS patients consists of sheets of foamy histiocytes packed with acid-fast bacilli (AFB) or poorly formed granulomata. Recently we had the opportunity to study lymph nodes and skin from 5 patients with AIDS who had NMI characterized by an unusual proliferation of fibroblast-like spindle cells. A spindle cell reaction such as this been previously described on occasion: the "histoid" variant of leprosy (Wade 1963) and a single case of cutaneous M. avium-intracellulare (MAI) infection after steroid immunosuppression (Wood et al. 1985). The uncharacteristic picture seen here might suggest a spindle cell neoplasm. The purpose of this report is to describe its unusual histological appearance and to study the nature of these fibroblast-like spindle cells which contain AFB.

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Materials and methods

Lymph nodes from 4 patients and skin biopsy from 1 patient were studied. Table 1 summarizes pertinent clinical findings of these patients. Formalin-fixed paraffin embedded tissue sections were stained with haematoxylin-eosin, and Ziel-Neelsen. For immunohistochemical study fresh tissue from 2 patients (cases 2 and 4) was snap frozen in isopentane and dry ice at −70° C, paraffin sections were available in cases 1, 2, 3, 5. Tissue from two cases (2, 5) were fixed in glutaraldehyde for electron microscopy. Table 2 summarizes specificity and source of the antibodies and results obtained by immunohistochemistry.

Immunohistochemical study of monocytes/histiocytes and leukocytes markers was done by biotin streptavidin-peroxidase method (Shi et al. 1988) as follows: Fresh frozen cryostat sections mounted on poly-L-lysine (Sigma, St. Louis, Mo.) coated slides were fixed in cold acetone (10 min) and air dried. Sections from formalin fixed paraffin embedded tissue were also mounted onto poly-L-lysine coated slides, then dewaxed, and rehydrated, and treated with 3% H_2O_2 for 5 min, at room temperature (RT) to quench endogenous peroxidase activity. All sections were washed with phosphate buffered saline (PBS) and incubated with normal goat serum (NGS) (Accurate Chemical and Scientific Corp Westbury NY) to block nonspecific

Table 1. Clinicopathological summary of spindle cell nontuberculous mycobacteriosis

Case	Patient	Immune status/ Risk factor	Tissue	Foam cells else where?	Culture confirmation
1	33M	Diagnosis AIDS homosexual	lymph nodes	yes, rectum	none
2	34M	HIV pos. IV drugs	skin	not known	M. kansasii
3	25M	Positive for CMV, herpes	lymph nodes node	yes, within same	none
4	27F	Diagnosis AIDS	lymph nodes	not known	M. avium-intracellulare
5	35M	HIV pos. IV drugs	lymph nodes	not known	M. avium-intracellulare

binding (30 min, RT). Cryostat sections were incubated with the primary antibodies for 1 h at RT. Paraffin sections were incubated with antibodies to alpha-1-antichymotrypsin (A-1-CT) and lysozyme overnight at 4° C. After 3 PBS washes, all sections were incubated with the appropriate biotinylated secondary antibody (Ab) (Bio Genex, San Ramon, CA) for 30–60 min RT, washed, and incubated with peroxidase conjugated streptavidin (Bio Genex) for 30 min RT. After 2 PBS washes, and 1 Tris buffer wash (0.05 M Tris, pH 7.6) the reaction was developed with freshly prepared diaminobenzidine tetrahydrochloride (50 mg) and hydrogen peroxide (33 microlitres) in Tris buffer (100 ml). Negative controls consisted of slides incubated with NGS, in lieu of primary antibody.

Results

Histological sections of all 5 cases showed similar features characterized by a marked spindle cell proliferation. The lymph node architecture was replaced by whorls of fibroblast-like cells intermixed with dense collagen bands and sparse chronic inflammatory infiltrate (Figs. 1a, b). The skin in case 2 revealed a uniform subepidermal dense infiltrate of plump spindle cells in fascicles resembling fibrohistiocytic lesion (Figs. 2a, b). The lymph node of one case (case 4) was replaced by an atypical lymphohistiocytic proliferation admixed with spindle cells and occasional Reed-Sternberg-like cells. Areas of hyalinization were present (Figs. 3a, b). In only one case (case 3) could the diagnosis of mycobacterial infection be suspected prior to Ziel-Neelsen stain because the lesions showed spindle cells admixed with more typical foamy histiocytes and poorly formed granulomata in other areas. Ziel-Neelsen stain revealed abundant intracellular AFB in parallel arrangement within these spindle cells in all cases (Fig. 4).

Table 2. Summary or antibodies, specificities, sources and results

Antibody to (dilution)	Specificity	Source	Results	Number of cases
T-200 1/100 (CD45)	All haemopoietic cells including lymphocytes macrophages monocytes	A	+++	1/1
HLA-DR 1/100	Ia-like antigen on lymphocytes Langerhans cells macrophages endothelial cells some epithelial cells occasionally fibroblasts	B	+++	2/2
Leu M3 1/100	(CD14) monocyte/ macrophage	C	+++	1/1
Mo-9 1/100	(CD14) monocyte/ macrophage	D	+++	2/2
Alpha-1-Anti-chymotrypsin (A-1-ACT) :1/500	histiocytes/ macrophages	B	+/++	4/4
Lysozyme 1/300	histiocytes/	B	+/++	4/4

Legend:
A: Hybritech Inc., San Diego, Ca.
B: Dako, Santa Barbara, Ca.
C: Becton and Dickinson, Mountainview, Ca.
D: Dr. A. Dimitriu-Bona, Mount Sinai School of Medicine, New York, NY
Scale:
0: no staining
+: minimal staining
++: moderate staining
+++: strong staining

Immunohistochemical findings in cryostat sections (Table 2) give optimal results when reacted with mouse monoclonal antibodies (MoAb) for monocytes/macrophage and leukocyte markers: (anti-T-200, anti-HLA-DR, anti-Leu M3, anti-Mo-9). The spindle cells of the skin lesion (case 2) showed diffuse +3 cytoplasmic staining with Mo Ab to Leu M3, Mo-9 (Fig. 4 inset), T-200, HLA-DR. In one case (4), only anti-HLA-DR and anti-Mo-9 were used, both with diffuse +3 cytoplasmic staining (Fig. 2). Antibodies to A-1-CT and lysozyme stained all cases, diffusely, albeit weakly (+1

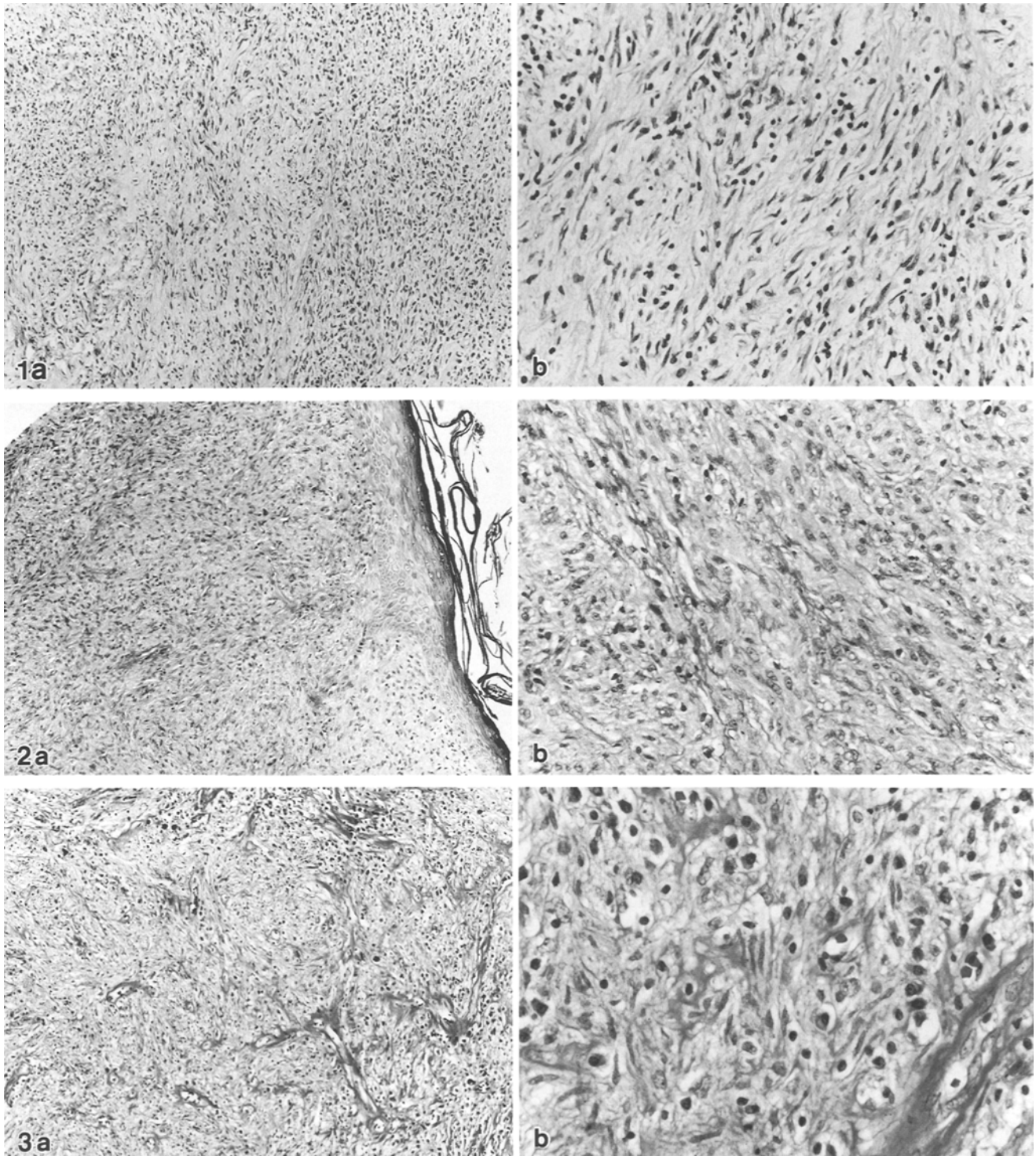

Fig. 1. Lymph node with fascicles of spindle cells and sparse inflammatory infiltrate (case 1). Haematoxylin and eosin, (**a** 100× and **b** 250× final magnification)

Fig. 2. Skin with subepidermal infiltrate of spindle cells mimicking a fibrohistiocytic tumor (case 2). **a** 100× and **b** 250× final magnification)

Fig. 3. Lymph node showing hyalinization and spindle cells with few plasma cells (case 5. **a** 100× and **b** 400× final magnification)

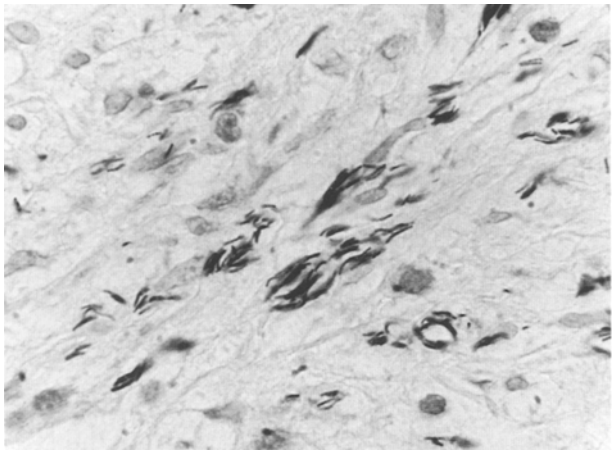

Fig. 4. Numerous acid fast bacilli are present in spindle cells (Ziel Neelsen stain, 1000× final magnification). *Inset:* Cytoplasmic staining of spindle cells with Mo-9 antibody (1000× final magnification)

to +2 intensity). Antibody to alpha-1-antitrypsin gave negative results.

Ultrastructurally the lesion contained mostly fusiform fibroblast-like cells with elongated crenulated nuclei. The cytoplasm contained abundant dilated rough endoplasmic reticulum (RER). These cells were often surrounded by abundant collagen. These fibroblast-like cells contained numerous ingested bacilli consistent with mycobacteria and occasional membrane bound lysosome-like granules (Fig. 5a). Occasional pinocytotic vesicles were seen along the cell membrane. Cytoplasmic tubuloreticular structures were occasionally observed (Fig. 5b). Small, polygonal cells with round to oval nuclei were frequent, containing bacilli. These cells were of undetermined origin. Poorly developed macrophages with few lysosomal granules and lacking filopods and ruffled membrane, were seen. Cytoplasm was not abundant as in epithelioid cells.

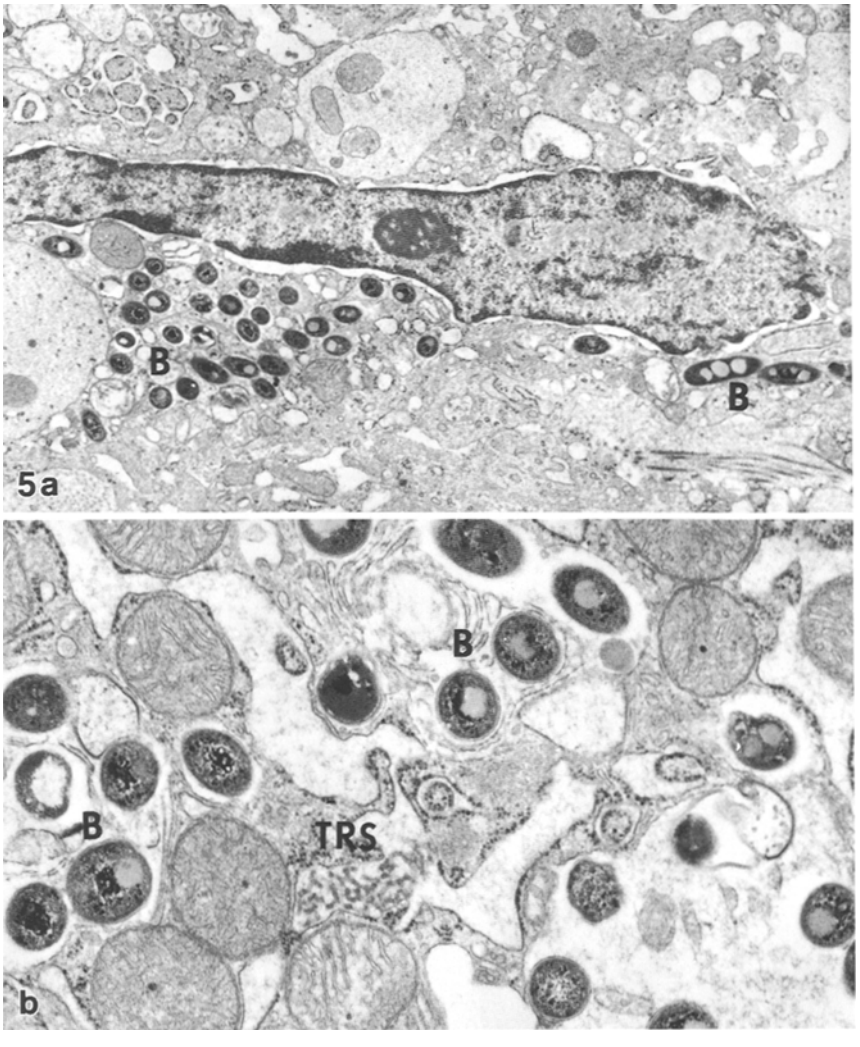

Fig. 5a. Fibroblast-like cells with abundant dilated endoplasmic reticulum, intracellular bacilli (*B*) and abundant collagen (*arrows*). (17000× final magnification). **b** Tubuloreticular structures (*TRS*) in a cell containing bacilli (*B*). (28300× final magnification)

Discussion

The marked spindle cell reaction in lymph nodes and skin tissue in these five cases of nontuberculous mycobacteriosis is an unusual manifestation in AIDS patients. A similar reaction to MAI has been previously reported involving the skin of a man on steroid immunosuppression after cardiac surgery who was presumably HIV negative (Wood et al. 1985). The authors did not discuss possible mechanisms of this type of tissue reaction. Spindle reactions to other mycobacteria in particular M leprae occur. Wade in 1963 described the "histoid" variant of leprosy. He compared this apparently monocellular process to fibroma or fibrosarcoma, hence the name "histoid" meaning composed of a single tissue type. The term "histoid" may be confused with "histioid", so we prefer the descriptive term "spindle cell reaction".

We emphasize the potential diagnostic problem in distinguishing this entity from spindle cell neoplasms such as Kaposi's sarcoma, malignant fibrous histiocytoma (MFH), or nodular sclerosing Hodgkin's disease. The clinical setting of AIDS offers a clue that an infectious aetiology should be considered regardless of the histological picture. Rarely NMI may occur together with a spindle cell neoplasm, but this could be ruled out here since most of these spindle cells diffusely expressed monocyte/macrophage antigenic markers. Microscopic features in these lesions suggests a spectrum of cellular reaction: spindle cells at one end, at the other end spindle cells admixed with histiocytes. In histoid leprosy, admixture with more typical histiocytes has also been described (Owens et al. 1969; Porichha and Bhatia 1987).

Results of immunohistochemical studies in Table 2 support monocytic/macrophage differentiation of these spindle cells. The MoAb panel studied in the 2 available cryostat cases suggests that these spindle cells are of monocyte/macrophage and leucocytic origin. Anti-Leu M3 (Clone Mo-9), specifically detects determinants of monocyte/macrophages. Both cases studied were diffusely positive for this marker. Fibroblasts do not express Leu M3, Mo-9, T-200, and usually would not express HLA-DR unless the fibroblasts are altered (Pober 1983). Four of 5 cases studied with antibodies to conventional histiocytic markers (Alpha-1-ACT, antilysozyme) were weakly positive. The weak intensity of these markers correspond to paucity of lysozyme seen by EM. These markers are not lineage specific and could be present on many cell types due to absorption from tissue fluids (Soini and Miettinen 1988). However in the context of distinguishing histiocytic/macrophage origin from a fibroblastic one these markers are useful as alpha-1-antitrypsin, lysozyme, alpha-1-antichymotrypsin are generally negative in fibrosarcomas (DuBoulay 1982).

The ultrastructure of these lesions reveals that these spindle cells are fibroblast-like as well as phagocytic. The fibroblastic features seen include a fusiform shape, abundant cytoplasmic dilated RER, and investment with collagen. Poorly developed macrophages were observed which may represent an intermediate form between macrophage and fibroblast (Fu et al. 1975) but typical macrophages or epithelioid histiocytes were rarely observed. The ultrastructural features reported in histoid leprosy had more "histiocytic" features. Job et al. (1977) described elongated cells with increase RER, numerous small finger-like villi, large lysosomal granules in leprosy. Jihe et al. (1982) described "fusiform" macrophages also embedded in collagen.

It is uncertain how often a spindle reaction such as we describe here occurs. It is speculated that given the right circumstance such as immunosuppression any cell can acquire a histiocytic/phagocytic phenotype. The present lesion and that of histoid leprosy are associated with immunosuppression. Interestingly, we found occasional tubuloreticular structures in cells with bacilli by EM. While they are not specific, they have been associated with HIV infection. HIV may infect macrophages although it has 10,000 fold affinity for CD4 receptors for T cells. It is postulated (Gartner et al. 1986) that some HIV subtypes may have a preferentially increased affinity for macrophages. The role of HIV as a cofactor in the present lesion is undetermined, and may not be necessary for a spindle cell reaction, as HIV is not a cofactor in histoid leprosy. Mycobacteria may contribute to this peculiar phenomena as has been suggested in lepromatous leprosy (Ridley and Ridley 1980).

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