OVAL CELLS AND CYSTIC STRUCTURES IN MULTIPLICATION OF *M LEPRAE*

V N Bhatia, Gopi Thawani

Ten preserved biopsy suspensions from leprosy patients were inoculated in biphasic medium and incubated alternatively in refrigerator and at 37°C. The cultures were observed every week for 3 months. The typical actinomycetoid growth appeared within 3-5 days. Microscopically, two types of cysts (unstained and dark) were seen along with oval cells. Dark cysts showed development of irregular septae or cracks breaking the mass into irregular quadrangular pieces. Granular acid-fast material could be made out inside the cracks and around the cysts. Oval cells were seen either independently or organised around pink homogeneous material. The oval cells were stained blue or pink. Some of them showed both blue and pink shades.

Key words : Oval cells, Cystic structures, *M leprae*

Introduction

The presence of cystic structures and oval cells in leprosy material and cultures is now well documented.[1-7] Oval cells described by us[6] have been noted by Chakraborty et al (1987)[8] as well who claimed them to be arthrospores. Chatterjee (1976)[9] reported on non-acid fast coccoid precursors which become acid fast later. The present study, deals with morphology of cystic structures and oval cells and their relationship with acid fast forms of *M leprae*.

Materials and Methods

Ten preserved biopsy suspensions from multibacillary leprosy patients were inoculated in a biphasic medium. The methods followed were same as described in our earlier paper.[7]

The biphasic medium had slope made of CMY-PD agar (Corn meal 20 gm, yeast 4 gm, peptone 10 gm, dextrose 10 gm and agar 20 gm in one litre distilled water) and an overlay consisting of Earle's balanced salt solution without $NaHCO_3$.

The tubes were incubated alternatively at refrigeration temperature and at 37°C. The changes from cold to hot were made at weekly intervals. The control cultures were incubated at refrigeration temperature and at 37°C. All cultures were observed for 3 months.

The smears were prepared every week and examined end to end for the presence of biological structures. Their morphology and staining character were noted in detail. Cover slip preparations were examined along with smears in each case. Silver staining and staining for capsular material were carried out on selected smears.

Results

The smears showed cystic structures and oval cells right from the first week. The cystic structures were of two types (a) unstained and (b) dark (Fig.1). The unstained cysts appeared to enlarge, burst and release its contents to repeat the cycle. The material contained coarse granules, oval/round cells and spherules of varying size along with fine mycelial twigs. The dark cysts increased in size in later weeks. Some of these became club shaped. The bigger cysts developed septae or cracks breaking the mass into irregular quadrangular piecess (Fig.2). The acid fast material was visible clearly around the cystic

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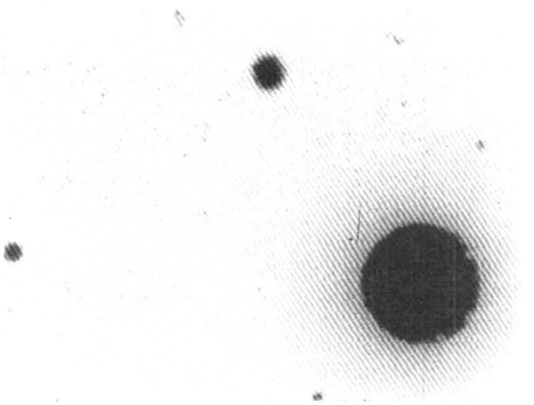

Fig. 1. Showing dark cysts of different sizes (X 200).

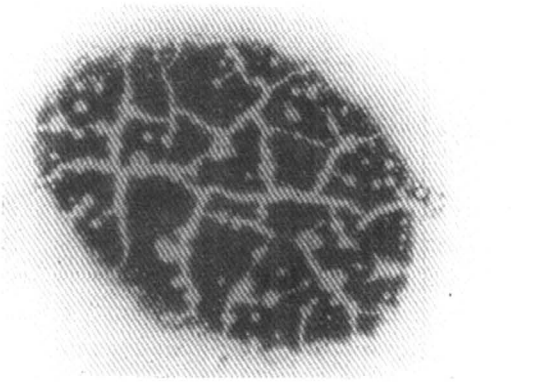

Fig. 2. Big dark cyst breaking into quadrangular pieces (X 200).

mass and in spaces within the dark ball. The leaking material showed presence of oval cells, AFB, coccoid granules and empty vacuoles.

Some interesting features were observed about oval/round cells. Two types of oval cells were seen (a) non-acid fast and (b) acid fast. Few cells with both non-acid fast and acid fast shades were also encountered. Some oval cells were arranged in organized manner as discontinuous whirls or big clusters against homogeneous pink background. Sometimes this pink material was seen independent and oval cells could be made out very clearly in it. These cells in fact could be seen in different shapes viz (i) oval, (ii) ring shaped, (iii) bipolar, (iv) U-shaped or, (v) D-shaped open at one or both ends, (iv) compressed into bacillary form, (vii) having granules at one or two poles, (viii) coccoid forms where lumen has not developed and (ix) solid ovoids.

Discussion

Approach to cultivation of *M leprae* has changed since demonstration of certain biological structures in the leprosy material. The most important and one of earliest recognized structures in leprosy is "empty looking" cyst. This empty looking cyst is actually not empty. On breaking, the cyst releases granular contents which are believed to contain precursors of *M leprae*.[6,10]

We recently got success in propogatting cystic structures in good number in the artificial medium by adding urea to the biphasic medium and xylol as overlay. During prolonged work on cystic structures extending to a decade, we have made following observations :

i) The cystic structures can bulge, throw pseudopodia-like elongations which lengthen, intermingle and fuse with each other enclosing circular spaces.

ii) The cystic structures burst to release granular material. This material contains coccoids, oval cells, spherules along with fine mycelial twigs. This material gets dried with time.

iii) With ageing cystic structures become brownish and their walls become thickened and hard. They enclose the contents which are otherwise discharged on bursting. These contents also become dried and turn brown.

iv) Some cysts get transformed into dark-stained round or club-shaped masses, some times with an outer membrane giving it glomerular appearence.

With above features seen in culture, the

cystic structures should no more be called as L forms. L forms are bacteria which become soft, delicate and pleomorphic by losing their cell walls. Compared to this, cystic structures of leprosy are more robust, organized and are not devoid of cell wall. The elongations, pseudopodia, projections and bulgings have a strong cell wall. Thus it appears that these cysts are an important component in life cycle of *M leprae* which may be the link connecting bacillary phase with the mycelial phase.

It is reasonable to believe that Chatterjee, Chakraborty and Bhatia groups have been dealing with one and the same organism. Each worker describes it sincerely according to his own understanding. The need is to combine the descriptions made by these groups to get the complete picture.

Chatterjee (1976, 1980)[9,10] claims that non-acid fast coccoids turn acid fast with time. Chakraborty et al (1987)[8] appear to believe that whole nocardial hypha in toto is acid fast and breaks to form acid fast bacilli. Our work supports views of Chatterjee.[9,10] We described oval cells which like coccoid precursors are non-acid fast to begin with and become acid fast with time. Considering the magnification and resolution we get in today's microscope, it is possible that coccoids of Chatterjee are same as oval cells or the two may be related to each other in some way. Like Chatterjee[9,10] we have observed conversion of non-acid fast to acid fast forms both individually and in form of islands. In addition, we found forms which divide by vertical split, each resulting into a pair of acid-fast bacilli. We also observed other forms of these cells as listed under results. All these become acid fast and result in various morphological forms characteristic of *M leprae*. We consider this phenomenon as an important feature in multiplication of *M leprae*.

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