SPHEROIDAL BODIES AND GLOBI OF HUMAN LEPROSY

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Summary: From the plasma and/or buffy coats of 80% of 38 cases of (tuberculoid and lepromatous) leprosy have been isolated in pure culture a group of spheroidal organisms (spheroidal bodies of leprosy, SPBL) showing on various media a versatility of differention ranging from naked protoplasts to globi containing acid-fast rods. The acid-fastness of the latter, like the unique acid-fastness of leprosy bacilli from lepromatous leprosy, can be extracted with C_5H_5N. Inoculation of chick embryos with SPBL elicits the nodular response evoked by homogenates of lepromatous tissue. From these nodules SPBL can be recovered in pure culture. SPBL appears to be the long sought etiologic agent of leprosy.

Introduction: One hundred years ago G. H. Armauer Hansen established an association between the clinical condition now known as lepromatous leprosy and the presence of innumerable rods seen in OsO_4-fixed leprous tissue (16). These bacilli of Hansen (HB) were said to be noncultivable and in 1931 were given the Latin name of Mycobacterium leprae (20, 3). Recently, Fisher (11, 12) and Convit and Pinardi (7) have independently shown that basic Fuchsin ($C_{20}H_{19}N_3$) bound to HB can be extracted with C_5H_5N whereas that bound to various mycobacteria cannot. Thus acid-fastness of leprosy bacilli is pyridine-extractable; that of mycobacteria is not. Further, Convit, Avila, Goihman and Pinardi have shown that the response of a class of leprosy patients to injected HB is markedly different

from their response to mycobacteria (6). Thus there are sound reasons for questioning the mycobacterial nature of HB. A number of microbiologists have isolated either acid-fast or nonacid-fast members of the Mycobacteriaceae directly from leprosy patients or from human leprous matter passaged through experimental animals. These reported cultivable organisms have been assessed as contaminants (5). Etemadi, working with pools of leprosy bacilli from lepromatous patients, has demonstrated the presence in the pools of particular classes of mycolic acids (10). Thus the microbiological, chemical and immunological data from leprosy patients are in conflict with regard to a mycobacterial etiology.

We describe here a spheroidal body of leprosy (SPBL) which is capable, under specific conditions, of differentiating into dividing protoplast or into filterable genomes or into large sporangia-like bodies or globi* containing either acid-fast cocco-bacilli or typical acid-fast leprosy bacilli. Our results place the cultivation efforts of earlier microbiologists in a favorable light and offer a useful key to the overall biology, the etiology and immunopathology leprosy.

Materials and Methods: Inoculum (Sources of SPBL)

Venous blood was taken aseptically in syringes previously rinsed with heparin (as "liquaemin" = 100 mg heparin/ml preserved 1.0% $C_6H_5CH_2OH$), left 2 hours at RT and spun down at 3,500 rpm. Resulting buffy coats were 2 x rinsed in 20 ml sterile Na_2HPO_4-KH_2PO_4 buffered saline (pH 7.2) in standard petri dishes, agitated to free them of adhering RBC,

*Although the globus is a hallmark of lepromatous leprosy few agree on its nature (8). From Denny (9) we take the following definition: " . . . . characteristic colonies growing within an as yet unidentified restraining membrane." (Italics are ours.) And we suggest that production of globi from SPBL in vitro is an essential criterion for the identification of the SPBL of leprosy.

separated with sterile wooden sticks into pieces measuring ± 5 x 5 mm and inoculated into culture tubes. The remaining plasma was spun at 20,000 x g for 2 hours; and the pellet distributed into culture tubes.

Media: Isolation and Holding Medium (IHM)

To 65 ml sterile, molten (56° C) beef heart infusion - neopeptone broth [infusion from 1 lb fresh, ground beef heart filtered through Reeve-Angel No. 230 filter paper; Difco Neopeptone, 10 g; NaCl, 5 g; Difco Bacto Agar, 13.8; distilled water to make 1 l; pH adjusted to 7.6 with KOH] the following items, warmed to 46° C, were aseptically added: tissue culture select horse serum (BBL), 27.5 ml; 0.1% (w/v) cholesterol in horse serum, 2.5 ml; sugar supplements previously described (13) 20% glucose, 2.5 ml; 40% galactose, 0.25 ml; 20% glycerol, 0.5 ml; and 10^6 units/ml (w/v) penicillin V, 0.1 ml.

Butter Agar (BA) has been described elsewhere (13).

Warning: As for all work with soft bacteria, the sera used in media must be tested for contamination with soft bacteria, even certified sera.

Chick Embryo Inoculations. The standard procedures for the inoculation of chorioallontoic membranes (1) were employed for testing the capacity of SPBL to form the nodules described by Gay-Prieto and associates (14, 15).

Results and Discussion: Gram-negative SPBL showed up as increasing areas of whiteness under the liquids of synuresis in the IHM and in the BA media. Visible growth took up to two months to appear. Inoculum from fresh, untreated cases often yielded detectable turbidity within 24 to 48 hours. Wet mounts, prepared from turbid areas and examined with phase contrast microscopy, revealed actively growing, phase-dense, tear-drop

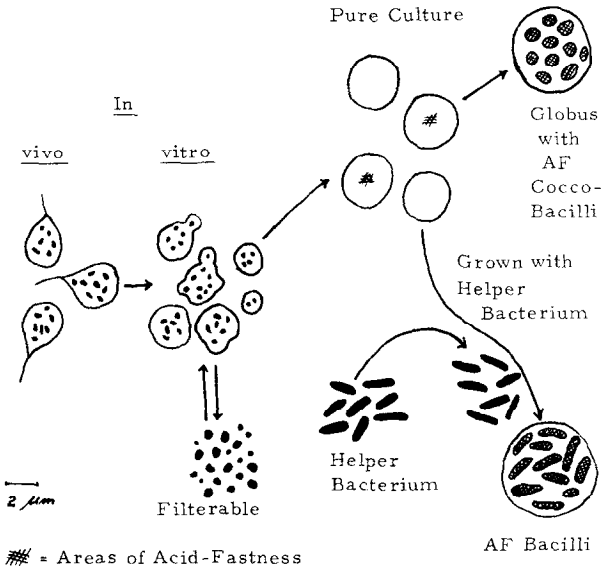

Fig. 1.

shaped bodies 1 or 2 µ long. Organisms which appeared early seemed to have a hyaline tail-like appendage. With increased numbers this configuration gave way to tear-shaped blobs (see Figure 1). After several million organisms had accumulated on BA, a few large, refractile sporangia-like bodies (globi) containing acid-fast cocco-bacilli were found. From a case of lepromatous leprosy a gram-positive, nonacid-fast bacillus was isolated along with the SPBL. When the SPBL and the bacillus (helper bacterium) grew together, the globi that developed contained, instead of cocco-bacillary forms, large acid-fast rods indistinguishable from leprosy bacilli. Sterile extracts of the helper bacilli improved the development of globi but never gave globi equal to those developing in mixed culture. The acid-fastness of the bacilli like that of leprosy bacilli was extractable with pyridine.

Viable SPBL could be derived from old SPBL cultures and leprous plasma which had been passed through Millipore filters that retain bacteria (see also 4, 14).

Gay-Prieto, Huertos and Puertas described the formation of a characteristic nodule on the chorioallantoic membrane of the 12 day chick embryos initiated by homogenates of leprous tissue (14). Nodulation was transmissible and only after it had been passaged several times did globus-like bodies, analagous to those shown in the upper part of Figure 1, appear. Our results, coupled with those of Gay-Prieto et al and with those of Shepard (18), indicate that the eclipse period observed in experimental infection in the chick embryo and in the mouse is not due to the slow generation time of leprosy bacilli as assumed by Shepard (18, 19) but, is as suggested by Gay-Prieto, the length of time it takes the inoculum (SPBL) to reach the critical numbers required for globus-formation. To date neither we nor any other investigators have evidence to suggest that bacilli formed in globi are capable of division. The role of helper bacteria in deciding the kind of acid-fast morphotypes to be found in globi remains to be determined. Its investigation should reveal hitherto unsuspected bacterial interactions important in effecting morphogenesis.

When SPBL were inoculated onto the chorioallantoic membrane of 12 day old chick embryos, within 4 days there developed the characteristic nodules described by Gay-Prieto (14) from which SPBL grew in culture within 15 days. If such lesions are accepted as characteristic and diagnostic for the behavior of SPBL in the chick embryo, then Koch's postulates have been fulfilled with regard to SPBL.

In the past decade the inoculation of the footpads of normal (18) or thymectomized and total-body irradiated mice [given post-irradiation transfusions (17) or having one femur shielded with lead during the irradiation (2)] has been an accepted procedure for obtaining increases in leprosy bacilli derived from human lepromatous tissue and associated pathology.

We intend to obtain additional proof of the identity of in vitro grown SPBL by mouse footpad inoculation and have in process both mice inoculated in footpads and armadillos inoculated intracutaneously and intravenously with SPBL. Results from these inoculations cannot be known for six or more months.

The evidence presented here serves to equate SPBL with the leprosy bacillus generating system found in human leprosy because of:

1) the uniqueness of the sporangium-like globus which derives in vitro from the SPBL, 2) the characteristic pyridine-extractable acid-fastness which bacilli in SPBL-derived globi share with bacilli in globi from cases of lepromatous leprosy, 3) the characteristic nodular lesions which SPBL elicit in the embryo of the chick and 4) the fact that SPBL have so far been recovered from a large number of leprosy patients and from no other place in nature.

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