Letters to the Editor

Internal birefringence and the recognition of Leishmania parasites

The parasites of visceral leishmaniasis may be found in stained smears of bone marrow or splenic aspirates, predominantly in macrophages or monocytes but frequently scattered loosely about the intercellular spaces. When they are numerous, there may be little difficulty in their detection and recognition; they appear with Romanowsky staining as oval bodies, about 2–5 μm by 1–3 μm, and the leishmanial or amastigote forms seen in direct smears have a dark staining round nucleus and a smaller, more rod shaped, kinetoplast (Fig. 1). When parasites are infrequent, as is usually the case in bone marrow smears, they may not be easy to recognise; in size and shape they closely resemble platelets and their internal structure is not always discernible or clearly distinguishable from the granular content of platelets.

We have recently noted, in the bone marrow of a patient admitted to Addenbrooke’s Hospital under the care of Dr D Rubenstein and found to have kala azar, that the kinetoplast of the amastigote form of Leishmania donovani is sharply birefringent under polarised light (Fig. 2). Study of several specimens of different Leishmania species in Romanowsky-stained smears (one of L donovani (man) kindly provided by Professor W Peters of the London School of Hygiene and Tropical Medicine and others of hamster tissues infected respectively with L tropica major (USSR, rodent), L braziliensis braziliensis (Brazil, man), L donovani infantum (Ethiopia, man) and L mexicana amazonensis (Brazil, rodent) kindly provided by Dr PJ Gardener of the Molteno Institute, Cambridge) has confirmed this observation as valid for all parasites studied. We report it here in the hope that others may find this a helpful feature, as we have, in confirming the suspected presence of the parasites. Platelets do not show birefringence. No doubt the same feature would be visible in the parasites seen in skin biopsies from cutaneous leishmaniasis, but such material has not been available for us to study.

As far as we are aware the birefringence of the kinetoplast in Leishmania has not previously been reported. Gardener1 drew attention to the presence of refractile granules in one isolate of L braziliensis braziliensis (LV63) though they were not present in other isolates from the same species (LV20 and LV64). Scorza et al3 described similar refractile inclusions, separate from the kinetoplast, in a new species of Leishmania (L garnhami) found in cases of cutaneous leishmaniasis in the Venezuelan Andes region. These bodies, which appear so far to be restricted to the above isolates, may perhaps correspond to liposomes visible by electron microscopy, at least in L garnhami.3 In the LV63 isolate of L braziliensis braziliensis the position, staining and refractile characteristics of these granules can be clearly distinguished from the birefringent kinetoplasts, as shown in Figs. 3 and 4.

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References
1 Handman E, Remington JS. Serological and immunochemical characterization of monoclonal antibodies to Toxoplasma gondii. Immunology 1980; 40:579.

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polarised
rotation—that is, rounder than (b), but 4 of a showing inclusions—for clearly distinguishable larger much tissue example, kinetoplasts—for (LV63). Fig. 3 A Leishman stained preparation showing a binucleated macrophage, from a hamster tissue smear, containing numerous amastigote forms of L braziliensis braziliensis (LV63). The rod-shaped kinetoplast—for example, b—and the much larger nuclei of the parasites are clearly distinguishable from the small rounded inclusions—for example, a—some of which appear brown under normal light but which are shown in Fig. 4 to be sharply refractile.

A selective agent for anaerobic cocci

Anaerobic cocci often occur in combination with other organisms, notably Bacteroides spp, in clinical samples, yet the separation of anaerobic cocci from mixed cultures can be difficult and time-consuming, especially as there are no satisfactory selective media for these organisms. Most of the selective media that have been described for anaerobes select either Gram-positive and Gram-negative anaerobes together or Gram-negative anaerobes alone. Wren, in his assessment of selective media for anaerobes, found that a nalidixic acid-Tween medium gave the best recovery of anaerobic cocci from clinical samples—yet this medium also allowed good growth of most of the isolates of Gram-negative anaerobes. There is therefore a need for a selective medium that can select out Gram-positive anaerobes, especially anaerobic cocci, from mixed cultures that include Bacteroides spp. This letter describes in brief the use of bicozamycin (bicyclomycin; CGP 354E; FR1881), an anti-diarrhoeal agent with specific activity against Gram-negative enteric pathogens, as a selective agent for anaerobic cocci. The results will be reported in detail elsewhere.

In the course of studies on the invivo activity of bicozamycin (supplied by Ciba-Geigy PLC) against anaerobes of clinical interest (Watt and Brown, to be published), we found that whereas all of the test anaerobic cocci were resistant to 256mg/l of bicozamycin, almost all of the Bacteroides spp were inhibited at this concentration. We decided to assess the use of 10% horse blood agar containing 500mg/l bicozamycin as a selective medium. The bicozamycin was easily dissolved in distilled water to give a stock solution of 50g/l bicozamycin; 10 ml of this solution was added to one litre Columbia blood agar medium without the agar was still molten to give a final concentration of 500 mg/l. The final surface pH of this medium was 7.1. We compared the surface growth, as assessed visually, of a range of clinical isolates and reference strains on blood agar with and without bicozamycin. The results (Table) show that whereas all of the 87 anaerobic cocci strains (including three Veillonella spp) and 18 of 21 strains of clostridia grew well on the medium, only 3 of 58 strains of Gram-negative anaerobic bacilli grew on this medium and then only with difficulty. Further studies have confirmed the ability of the medium to select out anaerobic cocci or clostridia from mixed culture—although a few strains of clostridia have failed to grow on the medium we have not encountered any strains of anaerobic cocci that failed to grow. Indeed, quantitative tests have shown that the medium supports the growth of inocula of anaerobic cocci of ≤ 20 CFU/ml.

The medium as described does allow

Growth of test anaerobes on media with and without bicozamycin

<table>
<thead>
<tr>
<th>Test organism</th>
<th>No of strains</th>
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<tr>
<td></td>
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<tr>
<td>Pc acascharolyticus</td>
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<tr>
<td>Other anaerobic cocci</td>
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<td>20</td>
</tr>
</tbody>
</table>

BA = Blood agar; BBA = bicozamycin blood agar.
* Very poor surface growth as assessed visually.

References

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