DENSITY GRADIENT SEPARATION
OF LOA LOA AND DIPETALONEMA PERSTANS MICROFILARIAE
FROM INFECTED PATIENTS

by
J.-Y. CESBRON¹, J. CHANDENIER², H. TAELEMAN³, D. HENRY¹ & A. CAPRON¹
¹Centre d'Immunologie et de Biologie Parasitaire, Unité Mixte INSERM 167-CNRS 624,
Institut Pasteur, 1 rue du Pr A. Calmette, F-59019 Lille Cédex, France
²Département des Maladies Tropicales et Parasitaires du Groupe Hospitalier
Pitié-Salpêtrière, Paris, France
³Prince Leopold Institute of Tropical Medicine, Antwerpen, Belgium

Immunological studies in human filariases require parasite material in relatively large quantities. However, the lack of suitable laboratory cycles of Loa loa and Dipetalonema perstans make the extraction of larvae from patients with high levels of microfilaraemia indispensable. Moreover, the parasites obtained have to be viable and free of extraneous material.

We initially tried using established methods for obtaining parasites from patients blood. First, microfilariae were collected from serum obtained after clotting whole blood in an Erlenmeyer flask stored at a slight angle overnight (5). This classic procedure was simple but had the disadvantage that many (70%) of the larvae were lost in the blood clot. We next tried to isolate parasites by filtration after lysis of blood cells by saponin or distilled water. However, this required considerable effort, time and a large number of filters, since the plug of fragmented ghost cell membranes and the microfilariae rapidly blocked the filter (5). Only 2 ml of whole blood was sufficient to obstruct one membrane.

Since the classical procedures for isolating microfilariae did not give satisfaction, we developed a technique from the observation reported by Muscoplat (3). Our best results were obtained with a two step procedure:

1) Human blood samples (10 ml) were collected in lithium heparin and carefully layered over 20 ml of iso-osmotic Percoll [90 ML Percoll (Pharmacia, Uppsala, Sweden)/10 ml 0.15 M NaCl/66 ml RPMI]. This preparation was then put into a 30 × 110 mm conical plastic tube (Falcon-Becton Dickinson, Grenoble, France) and was centrifuged for 20 minutes at 400 × g at 15°C. Three layers were observed after centrifugation: the upper one consisted of mononuclear cells and platelets, the lower was composed of polynuclear cells and erythrocytes, while the middle layer contained microfilariae, and was drawn off (10 ml approx.) with a syringe.

2) As the microfilarial layer was often contaminated by a few blood elements, these were best removed by filtration of the content of the syringe on a cellulose nitrate membrane of 8 µm and 5 µm porosity for isolating L. loa or D. perstans microfilariae respectively (SM1B, Sartorius, Göttingen, West-Germany). Gentle steady pressure was exerted on the syringe plunger until the resistance became firm. The microfilariae were washed by passing
5 ml of sterile distilled water through the filter which lysed some blood cells blocking the pores of the membranes. Then the parasites were eluted by washing the filter in a Petri dish with RPMI using a Pasteur pipette. Finally, after 2 extra washes in RPMI, the parasites were concentrated by gentle centrifugation (250 × g, 5 minutes at room temperature). When not used immediately for in vitro studies, the microfilariae were cryopreserved (1). We were able to process 200 ml of blood a day using this technique and to obtain a yield of 60-80% of the microfilariae free of blood cells.

In order to retain their viability, when larvae were incubated at 37°C in 5% CO₂ in air in flat bottomed microplates (NUNC, Roskilde, Denmark) with 20μl of fresh serum and monocytes at feeder cells, more than 90% were routinely viable for more than 2 weeks. However, the quantity of microfilariae collected by this procedure, some tens of thousands, were not sufficient to provide material for immunological studies.

In order to collect large quantities of microfilariae we used apheresis which procedure was applied for the first time to dog blood by Greenough and Buckner (2) and to human blood by Muylle et al. (4) and allows the processing of 3 liters of blood in 2 hours. These authors have shown that thebuffy coat obtained by this discontinuous centrifugation was ten or more times concentrated compared with original blood. The buffy coat sample obtained in this manner was then easily treated by the procedure described above and, routinely more than 8 × 10⁶ viable L. loa microfilariae could be isolated after a single purification cycle.

Acknowledgements — We thank M.F. Massard and C. Colson for secretarv assistance and Dr R.J. Pierce for critical reading of this manuscript.

Received for publication on October 31, 1985.

REFERENCES