

## RETICULOCYTE INTERFERENCE WITH LYMPHOCYTE SEPARATION IN BLOOD OF PATIENTS WITH KALA-AZAR

During separation of lymphocytes in an investigation of subpopulations of T-cells in kala-azar patients,<sup>1</sup> contamination of the lymphocyte layer with erythrocytes was observed. Studies were performed to determine the nature of the erythrocytes and also the method with which these cells could be removed.

For the separation of the lymphocytes, 5 ml of venous blood was drawn from each patient and controls (children < 5 years old), mixed gently with 10% ethylenediaminetetraacetic acid (EDTA), and layered on 3 ml of Ficoll-Hypaque (Behring, Germany) with a specific gravity of 1.077. The tubes were centrifuged at 300 × G for 40 minutes. The above procedure was originally described by Boyum.<sup>2</sup>

Heavy contamination of the lymphocyte layer with erythrocytes was observed which made it extremely difficult to determine the surface markers of the lymphocytes. Smears were prepared from the lymphocyte layer and stained with Giemsa and also two drops from the lymphocyte suspensions were mixed with two drops of New Methylene blue (C.I. 52030 0.5 g dye in 100 ml distilled water containing 1.6 g potassium oxalate) in a capillary tube and left for 10 minutes.<sup>3</sup> Thin films were prepared and air dried; observation of these smears under oil immersion demonstrated a large number of cells with various amounts of reticulum. For removal of reticulocytes 0.83% ammonium chloride was added to Tris buffer (BDH Chemicals Ltd, Poole, England) and adjusted to pH 7.4. Two ml of this buffer was added to the lymphocyte suspensions containing the reticulocytes and incubated at 37°C for 20 minutes; the tubes were then centrifuged for 10 minutes at 150 × G. The lymphocyte-free erythrocytes

were used for further study. A reticulocyte-free lymphocyte layer was obtained when blood from age matched control children (n = 15) or adults (n = 21) was used for separation of lymphocytes, in contrast to 28 patients with kala-azar studied.

Anemia is a common feature in patients with visceral leishmaniasis.<sup>4</sup> Erythropoietic activity and reticulocytosis is greatly increased in these patients; reticulocytes sediment at a lower rate than granulocytes in Ficoll-Hypaque gradient solution, and therefore contamination of the lymphocyte layer with reticulocytes occurred.

S. Ardehali

A. Moin Rezakhanlou

Department of Microbiology, Medical School, Shiraz  
University of Medical Sciences,  
Shiraz, Islamic Republic of Iran.

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