Analysis of leucocytes from *Leucoraja erinacea* by flow cytometry

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Flow cytometric analysis has been valuable for research on immune cells, mainly of mammals, and recently other organisms like teleosts as well. But this method has been rarely applied for elasmobranchs. Among elasmobranchs the little skate is becoming one of the most important model species, because NIH recently has approved a project for the whole genome sequencing of this species[1]. So we aimed to use flow cytometry for immune cells of the little skate, particularly to characterize lymphocytes in more detail.

In elasmobranchs lymphocytes exist in the blood and several types of immune organs like the spleen. But direct analyses of blood or spleen by flow cytometry were difficult because their major population is red blood cells, preventing the detailed analysis of other cell populations like lymphocytes. Thus, the first thing to be done was to remove red blood cells from the skate peripheral blood cells or single cell suspensions prepared from the spleen, for enrichment of lymphocytes. For this purpose, density gradient centrifugation was tested with RediGrad reagent (Amersham Biosciences). We expected that after centrifugation the population represented primarily by lymphocytes would be trapped on surface of the gradient and be separated from both red blood cells and granulocytes in the pellets. Observation under the microscope and May-Grunwald Giemsa staining[2], suggested that cells collected near the surface of the gradient (1.095 g/ml) were predominately lymphocytes. The cells were then analyzed by flow cytometry (Becton Dickinson) together with control samples taken before centrifugation (Figure 1). The putative lymphocyte population in the spleen was greatly enriched after the centrifugation (93%) compared with before centrifugation (62%). Similar results were obtained from the peripheral blood cells (data not shown).

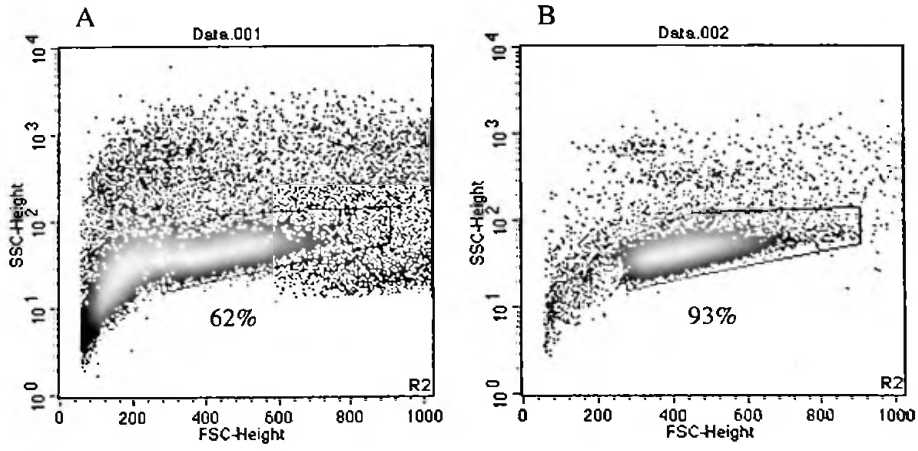

Fig. 1. FACS analysis of skate spleen leucocytes. Samples were obtained before (A) and after (B) density gradient centrifugation. Putative lymphocytes were gated by FSC and SSC properties.

We conclude that flow cytometric analysis is applicable for the leucocytes of elasmobranchs and will be extremely useful for characterization of various types of cell populations from these species when combined with monoclonal antibodies raised against them. This is the first work applying density gradient centrifugation and flow cytometric analysis to any species of skate or ray.

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2. **Walsh, C. J. and C. A. Luer.** Elasmobranch Husbandry Manual. In: *Elasmobranch Hematology: Identification of Cell Types and Practical Applications.* edited by M. Smith, D. Warmolts, D. Thoney and R. Hueter.Columbus, Ohio, USA: Ohio Biological Survey, Inc., 2004. p307-323.