Analysis of rat thymocytes differentiation, using ABCASS

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Several surface antigens are expressed on rat thymocytes, and their expression has been the subject of recent studies. We have attempted to use cell size and surface antigen, as markers for intrathymic differentiation, and tried to characterize thymic lymphocyte subpopulations, using a Automated Biological Cell Analyzing and Sorting System (ABCASS, Showa Denko). ABCASS, developed under the direction of Department Surgery, in collaboration with Section of Quantum Electronics (Prof. H. Inaba), Research Institute of Electrical Communication, can analyze cell sizes (forward light scatter) and fluorescenated surface antigens (fluorescence intensity), and sort them according to light scatter and/or fluorescence intensity using argon laser beam (wave length : 488 nm, power at 400 mW).

Cell suspensions from the rat thymus was obtained by mincing the tissues and filtering them through a stainless-steel screen. After the cells were washed with PBS, and suspended in 40% Percoll solution, the cells were fractionated by Percoll density gradient centrifugation. Namely, the Percoll density gradient was composed of the dilutions of 40,50,53,68 and 70%. After the centrifugation (1000 G for 30 min), the cells in the interface of each layer were carefully harvested. Cells in the interface between 50% and 58% Percoll concentration were called Fraction 1 (Fr.1) cells, between 58% and 63% Fraction 2 (Fr.2) and cells between 63% and 70% Fraction 3 (Fr.3). Samples of 5×10^6 cells in 50 μ l of serum free RPMI 1640 medium were incubated on ice for 30 min with 50 μ l of diluted OX-7 (120 μ g/ml). Then, the cells were washed twice in Ca Mg free PBS with 0.1% NaN_3 , resuspended in 100 μ l of fluorescein-conjugated F(ab')_2 fragment rabbit anti mouse IgG diluted to 1:10 in RPMI 1640 medium supplemented with 2% heat-inactivated rat serum. After another 30-min-incubation on ice, the cells were washed twice, resuspended in PBS with 0.1% NaN_3 and analyzed on ABCASS (Table 1). The data were expressed in linear arbitrary unit and, if necessary,

presented in dot plot.

In ABCASS analysis (Figure 1), most of the cells in Fr.1 appeared to be very large and to have low Thy 1 intensity. The peak of light scatter shifted toward the right (larger) and that of fluorescence intensity toward the left (lower), compared with those of unfractionated thymocytes. Also demonstrated were two distinct peaks, both of larger cells (light scatter), and a peak of cells with lower content of Thy 1 (fluorescence intensity) as well as peaks (in light scatter and in fluorescence intensity) of whole thymocytes. Fr.2 cells are composed of various sized cells having heterogeneous Thy 1 intensity. Fr.3 cells have character of typical thymocytes they have sharp peaks of both light scatter and fluorescence intensity, indicating that they are composed of homogenous population of cells with small sized and high Thy 1 intensity.

By Percoll discontinuous density gradient, rat thymocytes could be divided into three fractions of different characters ; cells of different cell size, different content of surface marker (Thy 1), when examined in dual parameter analysis (dot plot) of cell size (light scatter) and Thy 1 content (fluorescence intensity) by ABCASS.

Table

Figure