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Lymphoid organ development in rabbits: Major lymphocyte subsets

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Abstract

Although rabbits represent an important animal model, little is known about the lymphoid organ development in this species. In the present study, lymphocyte subsets in peripheral blood, spleen, mesenteric and popliteal lymph nodes in newborn and 2-, 4-, 6- and 8-week old and adult were characterized. Lymphocyte subsets were detected using flow cytometry and monoclonal antibodies against rabbit CD4, CD8, T-cell-specific antigen and cross-reactive antibody against B-cell antigen CD79α. In neonates, lower numbers of T cells were detected in both peripheral blood and spleen than in mesenteric lymph nodes. In comparison with other compartments, $CD79\alpha^+$ cells prevailed in the spleen. Post-natal development was characterized by a decreased $CD4^+/CD8^+$ ratio due to increasing frequency of $CD8^+$ lymphocytes in all organs but mesenteric lymph nodes, where it was due to decreased numbers of $CD4^+$ lymphocytes. Another significant feature was the increase of B cells in peripheral blood and mesenteric lymph nodes.
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1. Introduction

Rabbit (*Oryctolagus cuniculus*) is one of the animal species often used as an experimental model in human and veterinary research. The rabbit model served for the study of infectious diseases such as syphilis [1], tuberculosis [2], human T lymphotropic virus-I [3] and human immunodeficiency virus [4]. Rabbit is also useful for studies of various non-infectious diseases such as atherosclerosis [5] or eye disorders [6] and is still the animal of choice for production of many polyclonal antibodies [7]. Despite that, at present, information related to prenatal and post-natal changes of lymphocyte subpopulations in peripheral blood (PB) and lymphoid organs is insufficient in this species.

The effect of age on lymphocyte subset distribution in different animal species was described in various papers. Joling et al. [8] studied the distribution of lymphocyte subpopulations in thymus, spleen and PB of specific pathogen-free pigs from 1 to 40 weeks of age. Sellon et al. [9] described age-related changes in lymphocyte subsets in perinatal cats, and Wilson et al. [10] described T-cell subsets

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in blood and lymphoid tissues obtained from fetal calves, maturing calves and adult cattle. Post-natal development of lymphocyte subpopulations in blood and lymphoid organs in dogs has also been characterized [11–13].

Due to the unique formation of the antibody repertoire in rabbit, most recent studies deal with the development of B lymphocytes [14] and generation of the antibody repertoire in gut-associated lymphoid tissue [15–17]. The influence of intestinal microflora on diversification of primary antibodies and selection of B cells has been intensively studied [18–20]. Age-related variations in the numbers of T and B lymphocytes in thymus, bone marrow, spleen, popliteal lymph nodes, appendix and PB of rabbits have been described [21]. Fluorescein-stained antiserum directed against rabbit thymus lymphocyte antigen (RTLA) and antiserum to rabbit gamma globulin were used for the determination of lymphocyte subsets in the present study. In later studies, only the status in different lymphoid organs or PB of adult rabbits is mentioned [22–25].

Therefore, the aim of the present study was to characterize post-natal development of lymphocyte subsets in PB, spleen, popliteal and mesenteric lymph nodes using well-defined monoclonal antibodies (mAbs).

2. Materials and methods

2.1. Animals

Outbred New Zealand White SPF rabbits, strain Crl:KBL, were obtained from Charles River Laboratories, Inc. (Wilmington, MA). Two females and one male were used as parents; they were housed in animal care facility with controlled conditions at the Veterinary Research Institute, Brno, Czech Republic. Twenty-eight rabbits of progeny generation aged 1 day, 2, 4, 6, 8 weeks (4 per group) and adult rabbits (eight 20-week-old animals) were sacrificed under general anesthesia by complete exsanguination using cardiac puncture. General anesthesia was induced by intramuscular administration of 5 mg/kg xylazine (Rometar 2% inj. ad us. vet., SPOFA a.s., Czech Republic) and 35 mg/kg ketamine (Narkamon 5% inj. ad us. vet. SPOFA a.s., Czech Republic). The animals were euthanized under the agreement of the Branch Commission for Animal Welfare of the Ministry of Agriculture of the Czech Republic.

2.2. Sample collection and cell isolation

Heparinized PB was obtained by cardiac puncture. Total leukocyte counts in blood samples were determined using the Digicell 500 cell counter (Contraves AG, Switzerland). Differential leukocyte counts were calculated from blood smears stained with May–Grünwald and Giemsa–Romanowski. Organ samples were collected into RPMI1640 medium and cell suspensions were prepared by careful teasing of lymphoid tissue using two forceps. All cell suspensions were filtered through a fine nylon mesh. After ammonium chloride-mediated lysis of erythrocytes in lymphoid organ suspensions, the cells were washed in washing and staining buffer (WSB) (PBS with 0.2% gelatin from cold water fish skin, 0.1% sodium azide and 0.05 mM EDTA, all reagents from Sigma) and resuspended in WSB and adjusted to the density of 5×10^6/ml.

2.3. Immunostaining

Commercially available mAbs were used for the detection of lymphocyte subsets.

For quantification of B-lymphocytes, R-PE conjugated mouse anti-human CD79α (clone HM57, DakoCytomation, Denmark, [26]) was used. Cell suspensions were fixed and permeabilized with IntraStain kit (DakoCytomation, Denmark) and labeled according to a protocol recommended by the producer.

The following anti-rabbit mAbs were used as primary immunoreagents for indirect immunofluorescence staining: anti-CD4 (RTH1A, IgG1), anti-CD8 (ISC27A, IgG2a), anti-panT2 (RTH21A, IgG1) and anti-CD45 (ISC18A, IgG2a) (VMRD Inc., USA). All mentioned primary mAbs were diluted 1:50 in WSB. The mAb panT2 (pT) is less characterized; however, in our preliminary study, we detected that this antibody marked 99% of thymocytes; all $CD4^+$ and $CD8^+$ leukocytes in thymus are pT^+ as well, and the population of pT^+ lymphocytes in PB is completely distinct from $CD79\alpha^+$ cells. To assess contamination of the lymphocyte gate by other cell types, the cross-reactive [27] mAb against human CD14 antigen (TÜK4, IgG2a, DakoCytomation, Denmark) diluted 1:5 was used. The antigen CD14 is expressed on the surface of monocytes and granulocytes, but not of lymphocytes.

As the secondary immunoreagent, FITC (dilution 1:100) or R-PE (dilution 1:500) labeled goat

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