PROPERTIES AND KINETICS OF THE B LYMPHOCYTE POPULATION OF RATS DURING DEVELOPMENT

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Cells carrying surface immunoglobulins (Ig^+ cells) detected by the indirect immunofluorescence method, and cells forming rosettes with sheep's red blood cells (SRBC) with antibodies adsorbed on their surface and with complement (RFC), were found in the liver and spleen of rat fetuses at the 15th and 20th days of development. The relative percentage of Ig^+ cells and RFC in the liver remained low and about the same level in rats on different days of postnatal development. In the spleen and bone marrow the number of Ig^+ lymphocytes and RFC increased during the first month of the rat's life, to reach a maximum in animals aged 30 days, and fell sharply in old rats. No Ig^+ cells or RFC were present in the thymus or they were found in very small numbers at certain times of investigation. Ig^+ lymphocytes with "caps" of fluorescence on their surface appeared in the spleen and bone marrow on the fifth and 10th days of life of the rat and their number rose considerably by the age of 30 days and in adult rats. No such cells were present in the lymphoid organs of old (40 months) animals.

KEY WORDS: *B lymphocyte*; *ontogeny*; *surface immunoglobulins*; *rosette-formation test*.

The study of the conditions and successive stages of differentiation of B lymphocytes in mammalian ontogeny has attracted the attention of research workers as an approach to the discovery of the mechanisms of immunogenesis and to the solution of the problem of the existence of an analog of Fabricius' bursa, creating a specialized environment for the differentiation of B-cells responsible for the humoral immune response. Several workers have described a population of B lymphocytes with definite surface markers (immunoglobulins — Ig, receptors for the third component of complement and for the Fc fragment of IgG) at different stages of individual development of mice, rabbits, pigs, and man [3, 5-7, 12, 13].

The object of this investigation was to study some properties and the kinetics of the population of B lymphocytes in the lymphoid organs of rats during pre- and postnatal development of the animals.

EXPERIMENTAL METHOD

Experiments were carried out on 15- and 20-day Wistar rat fetuses and on Wistar rats of different ages from newborn to 40 months. Suspensions of lymphocytes were obtained from the liver, spleen, thymus, and bone marrow by the method described earlier [1]. To detect lymphocytes carrying surface immunoglobulins (Ig^+ cells), lymphocytes washed 3 times with cold buffered physiological saline (pH 7.4), in a volume of 0.25 ml (concentration 4×10^6 cells in 1 ml), were incubated at 4°C for 20 min with an equal volume of hyperimmune rabbit serum against rat IgG in a dilution of 1:10 (the serum contained antibodies against heavy and light chains). After washing 3 times, the cell suspensions were incubated again with donkey serum against rabbit globulins, labeled with fluorescein isothiocyanate. The lymphocytes were washed 3 times and examined under the ML-3 luminescence microscope. The lymphocytes were provisionally identified in transmitted light from a tungsten source. Altogether 200 cells were counted and the percentage of IgG lymphocytes determined. Differences in the character of fluorescence on the surface of the lymphocytes were noted and cells belonging to one of the following four types were counted: 1) lymphocytes with one or two points of weak fluores-

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TABLE 1. Age Changes in Number of Cells with Characteristic Receptors for B Lymphocytes in Lymphoid Organs of Rats, %

Organ	Type of B lymphocytes	Prenatal period		Postnatal period							
		days						months			
		15	20	0	1	5	10	30	6	24	30
Spleen	Ig+	—	2,5 (2—3) n=2	11,9 (3—21) n=4	11,1 (4—19) n=5	18 (5—31) n=7	18,5 (11—23) n=9	37,4 (24—45) n=16	33 (29—38) n=7	12,5 (8—18) n=2	6,6 (6—8) n=3
	RFC	—	7,1 (3—10) n=2	4 (3—5) n=4	9,5 (3—16) n=6	12,7 (12—13) n=2	15,8 (9—21) n=3	22,3 (19—31) n=5	19,6 (16—22) n=5	8,5 (3—13) n=4	—
Thymus	Ig+	—	0 (0—0) n=2	0,5 (0—2) n=4	0 (0—0) n=4	1,2 (0—6) n=7	0,6 (0—2) n=10	0,6 (0—3) n=20	2,3 (0—6) n=0,6	—	1 (0—3) n=3
	RFC	—	0,5 (0—1) n=3	0 (0—0) n=4	0 (0—0) n=3	0,5 (0—1) n=2	0,4 (0—1) n=4	0,3 (0—0,5) n=6	0,2 (0—0,5) n=3	—	—
Liver	Ig+	2,5 (3—2) n=2	2,5 (1—4) n=2	3,7 (0—12) n=4	13 (6—20) n=3	7,2 (5—10) n=4	8,2 (5—13) n=8	6,7 (3—11) n=19	—	—	11 (6—20) n=3
	RFC	—	2,6 (0—6) n=7	2 (1—3) n=4	4,1 (3—7) n=8	4,5 (2—6) n=3	1,7 (0—4) n=6	3,3 (2—5) n=3	2,6 (1—4) n=5	2,5 n=1	—
Bone marrow	Ig+	—	—	—	—	7,6 (3—13) n=5	7,1 (3—12) n=9	16 (8—20) n=10	16 (13—23) n=5	—	5 (3—6) n=3
	RFC	—	—	—	—	2,5 (1—4) n=2	4 (1—7) n=4	10,4 (3—17) n=6	10 (4—15) n=6	4,2 (6—12) n=4	—

cence; 2) lymphocytes with many points of fluorescence on 1/4 of the cell surface; 3) lymphocytes with many points of fluorescence on 1/2 or more of the cell surface; 4) lymphocytes with a "cap" of fluorescence on one pole of their surface. The specificity of fluorescence was determined in accordance with the criteria suggested by Coons and Kaplan [4]. In rats at the same stages of development the relative percentage of lymphocytes forming rosettes with sheep's red blood cells (SRBC) on which antibodies and complement were adsorbed (RFC) was determined in the lymphoid organs. For this purpose, thrice washed SRBC were incubated for 30 min at 37°C, first with anti-SRBC serum taken in a subagglutinating dilution, after which 2 ml of a suspension of thrice washed SRBC was incubated under the same conditions with 0.1 ml of mouse serum as the source of complement. The concentration of thrice washed SRBC was adjusted to 8×10^7 ml and the suspension was used for the rosette-formation test by the method described previously [11].

EXPERIMENTAL RESULTS

The results of counting the number of Ig^+ lymphocytes and RFC in the lymphoid organs of rats of different ages are summarized in Table 1. They show that the liver of 15-day rat fetuses contains 2.5% of Ig^+ cells. Their number increases considerably in day-old rats and remains at the same level in the rat liver during the first month of life, and in adult and old animals. A gradual increase in the number of Ig^+ cells was found in the spleen starting from the 20th day of intrauterine life and until the age of 30 days, when it reached its maximum (37%). In rats aged 24 and 40 months there was a sharp decrease in the number of Ig^+ lymphocytes (12.5 and 6.6% respectively). Lymphocytes were found in the bone marrow of rats aged 5 days in sufficient numbers for preparing cell suspensions and determining the number of Ig^+ cells (7.6%). In the later stages of the investigation the age dynamics of the Ig^+ cells in the bone marrow corresponded on the whole to that in the spleen. In the thymus, starting from the neonatal period and at all subsequent times of the investigation there were either very few Ig^+ cells or none at all.

Assessment of the types of fluorescence showed that lymphocytes of the thymus at all times of the investigation gave the first type of fluorescence (Fig. 1). The same type of fluorescence was found in lymphocytes of the liver and spleen of the rat fetuses. As the animals developed, cells with types 2 and 3 of fluorescence appeared in the liver and spleen. In rats aged 5 days lymphocytes with typical "caps" of fluorescence appeared in the spleen. Later the number of lymphocytes with "caps" of fluorescence on their surface increased in the spleen and reached 18 or 18.5% of the total number of lymphocytes in the spleen of rats

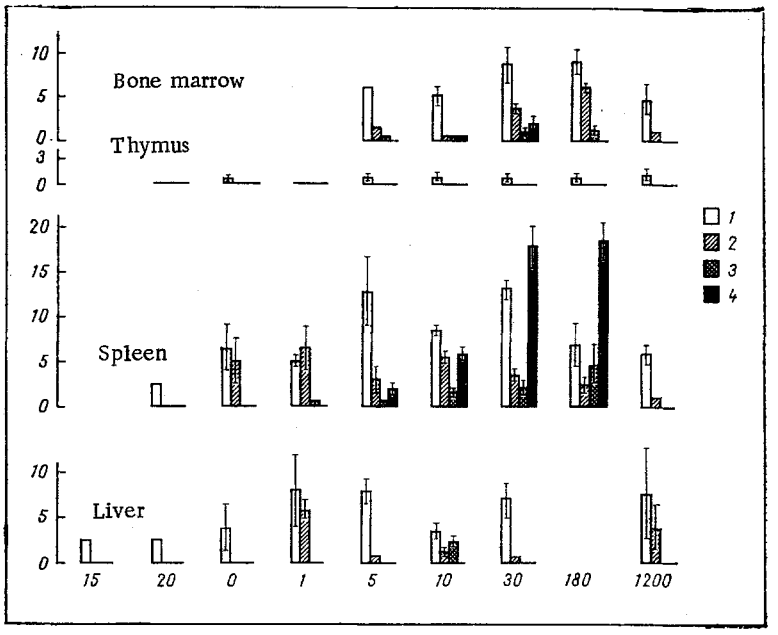

Fig. 1. Changes in number of cells with different types of fluorescence in lymphoid organs of rats at different stages of ontogeny. Abscissa, age of rats in days (to left of 0, prenatal period); ordinate, number of cells with different types of fluorescence (in %). 1) Type 1 of fluorescence, 2) type 2, 3) type 3, 4) type 4.

aged 30 days and 6 months respectively. A considerable number of cells with "caps" of fluorescence also was found in the bone marrow of the 30-day-old-rats. In the spleen and bone marrow of the old rats no lymphocytes with type 3 fluorescence or with "caps" of fluorescence were present (Fig. 1).

The results of the rosette-formation test showed that changes in the number of RFC in the lymphoid organs of rats of different ages (Table 1) correspond to changes in the number of Ig^+ cells. The fact that the number of RFC at nearly all times of the investigation was smaller than the number of Ig^+ cells is in harmony with observations that in the rosette-formation test with SRBC with antibodies and complement adsorbed on their surface, not all but only some of the population of B lymphocytes is revealed [2].

At the same time, there are grounds for considering that changes in the whole population of B lymphocytes can be judged from changes in the number of Ig^+ cells in the lymphoid organs of rats of different ages. Goldschneider and McGregor [8] showed that approximately equal numbers of positively reacting cells can be detected by the indirect immunofluorescence method in the lymphoid organs of adult rats both when a strictly specific serum against B lymphocytes and a serum against IgG are used.

The times of appearance of the Ig^+ cells and their relative percentages in the lymphoid organs of rats of different ages were found to agree almost exactly with the results obtained in analogous experiments on mice [6, 10, 11].

The results of the present investigation show that B lymphocytes appear in the liver and spleen of rats during prenatal development of the animals. Meanwhile, Wistar rats are known to begin to respond by antibody synthesis to T independent antigen (paratyphoid vaccine) at the age of 9 days [14], i.e., in rats there is a hiatus between the time of appearance of the Ig^+ cells and development of the ability to give a humoral immune response. After analyzing this situation in mice, Spear et al. [12] suggested that certain qualitative changes must precede the formation of immunocompetence of the B lymphocytes. In this connection the regular changes in the type of fluorescence in the Ig^+ cells of rats of different ages, discovered in the present investigation, are interesting. Since cells with multiple points and with "caps" of fluorescence on their surface appeared in the spleen and bone marrow of rats aged 5 and 10 days, the increase in the number of immunoglobulin receptors on the surface of the B lymphocytes and the formation of "caps" of fluorescence in the presence of antigen are probably characteristic features of more mature cells. This hypothesis is supported by

results obtained by Osmond and Nossal [11], who investigated the density of immunoglobulin receptors on the surface of lymphocytes from the bone marrow and spleen of adult and newborn mice by autoradiography, using sera containing different concentrations of antiglobulin, and concluded that immature B lymphocytes have a lower density of immunoglobulin receptors than mature cells.

It can accordingly be concluded from data in the literature and from the results of the present investigation that in rats, as the B lymphocytes mature the density of their immunoglobulin receptors increases and correlation is found between the appearance of "caps" of fluorescence (the 5th- to 10th day of life of the rat) in the presence of antiglobulin serum and formation of the immunocompetence of the B cells (the ninth day of life of the rat) [14].

In old rats the number of lymphocytes with receptors characteristic of B cells in the spleen and bone marrow was reduced by 75-84% and cells with type 3 fluorescence and with "caps" of fluorescence on the surface disappeared. Assuming that in old rats, just as in old mice [9, 10], ability to give a humoral immune response is sharply reduced, it can be concluded that when maturation and differentiation of the B lymphocytes are disturbed by aging correlation exists between the ability of the body to give reactions of humoral immunity and the ability of the B lymphocytes to form "caps" in the presence of antigen. This hypothesis is completely amenable to experimental verification.

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