PLAQUE-FORMING AND ROSETTE-FORMING CELLS IN THE SPLEEN OF NZB/BLN MICE DURING ONTOGENY

V. A. Trufakin, V. G. Dudin, S. I. Il'nitskaya, V. A. Kozlov, and T. P. Noppe

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The number of plaque-forming cells (PFCs) and rosette-forming cells (RFCs) in response to sheep's red cells on the fourth day after immunization was determined in the spleen of NZB/BLN and C57BL mice aged from 30 to 360-370 days. The number of PFCs increased with age in the NZB/BLN mice by 35-40 times and in the C57BL mice by 5 times. It was 3 times greater in NZB/BLN mice at the age of 30 days than in C57BL mice, and 20 times greater at the age of 360-370 days. At all stages of ontogeny the number of RFCs was 1.5-2.5 times greater in the NZB/BLN mice than in the C57BL mice. The number of RFCs in the NZB/BLN mice fell with age. The number of lymphocytes, especially medium-sized, increased with age in the spleen and thymus of the NZB/BLN mice. Depression of the function of the population of T-lymphocytes is postulated.

KEY WORDS: antibody-forming cells; thymus; immune system in ontogeny.

In NZB mice with genetically determined autoimmune hemolytic anemia the thymus-dependent immunologic functions are depressed with age: the ability to induce a graft versus host reaction is diminished [4, 12], the response to phytohemagglutinin and foreign lymphocytes is depressed [7, 9], the number of θ-positive lymphocytes decreases, and autoimmune thymus-dependent lymphocytopenia develops [11]. However, an earlier immunologic maturation and intensification of bone marrow-dependent immunologic functions at an early age against certain antigens – sheep's red cells and bovine serum albumin [5, 8, 13] – were observed in these animals.

It was accordingly decided to study the response of NZB mice at an older age to heterologous red cells.

The dynamics of the number of plaque-forming cells (PFCs) and rosette-forming cells (RFCs) in the spleen of NZB mice in ontogeny was studied and the cell composition of the thymus and spleen was investigated at the same time.

EXPERIMENTAL METHOD

Mice of the subline NZB/BLN were used in the experiments at different ages: from newborn to age 360-370 days (the mice of subline NZB/BLN were obtained in the 68th generation in 1969 from the National Institute of Health, Bethesda, USA). C57BL mice were used as the control group. The animals were immunized with sheep's red cells in a dose of $5 \cdot 10^8$. On the fourth day after immunization the number of PFCs [6] and of RFCs [14] was determined. The number of PFCs was expressed per 10^6 nucleated viable spleen cells and the number of RFCs was expressed per 10^3 cells. The significance of the results was estimated by Student's method.

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TABLE 1. Number of PFCs and RFCs in the Spleen of NZB/BLN and C57BL Mice during Ontogeny (M ± m)

Age of mice (in days)	Number of PCFs/10^6 spleen cells		Number of RFCs/ 10^3 spleen cells	
	NZB/BLN	C57BL	NZB/BLN	C57BL
30	39,4±2,7	14,2±1,2	3,3±0,4	1,6±0,2
90—100	52,6±4,4	37,4±2,3	6,6±1,0	1,8±0,4
180—190	154,9±12,9	87,8±7,9	1,8±0,2	2,0±0,3
270—280	243,1±19,1	80,4±10,5	3,1±0,4	2,5±0,4
360—370	1410,7±62,7	76,6±12,6	4,9±0,8	1,9±0,3

TABLE 2. Composition of Spleen Cells of NZB/BLN Mice in Ontogeny (in %)

Cells	Age of mice (in days)							
	newborn	10	30	60—70	90—100	180—190	210—220	270—280
Lymphocytes:								
large	1,4	2,3	7,9	8,9	9,9	11,6	10,5	11,1
medium-sized	15,9	17,3	20,2	24,8	29,4	34,8	30,5	31,9
small	19,0	20,6	34,1	45,7	48,3	36,9	30,5	33,2
Plasma cells	0,4	1,4	2,7	3,9	5,1	5,6	7,2	8,3
Other cells	63,3	58,4	35,1	16,7	7,3	11,1	21,3	15,5

Legend. Other cells include reticulum, mast, and erythroid cells, monocytes, etc.

For the morphological investigation of the thymus and spleen, intact nonimmunized NZB/BLN mice were fixed in Carnoy's fluid. The cell composition was counted in preparations stained with hematoxylin–eosin and azure II-eosin.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the relative number (per 10^6 spleen cells) of PFCs increased with age in both the NZB/BLN and the C57BL mice; by the age of 360-370 days the number of PFCs in the NZB/BLN mice had increased by 35-40 times, but in the C57BL mice by only 5 times. In the NZB/BLN mice at all stages of ontogeny more PFCs were discovered than in the C57BL mice; they were 2-3 times more numerous at the age of 30 days, and 20 times more numerous at the age of 360-370 days. It must also be mentioned that the increase in the number of PFCs in the NZB/BLN mice took place throughout the period of ontogeny, whereas in the C57BL mice it occurred only until the age of 180-190 days.

The dynamics of the number of RFCs in the spleen of the NZB/BLN and C57BL mice (Table 1) did not repeat that of the PFCs. The number of RFCs in the spleen of the NZB/BLN mice was 1.5-2.5 times greater than in the C57BL mice at all stages on ontogeny except at the age of 180-190 days, when the number of RFCs was reduced in the NZB/BLN mice.

The number of large, medium-sized, and small lymphocytes and of plasma cells increased with age in the spleen of the NZB/BLN mice (Table 2). However, starting from the age of 200 days, a relative decrease in the number of lymphocytes was observed, mainly on account of the small type.

A unique reaction of the medium-sized lymphocytes appeared in the thymus of the NZB/BLN mice (Table 3), both in the cortex and in the medulla. Their number rose considerably at the age of 90-210 days, whereas the number of large lymphocytes remained unchanged and the number of small lymphocytes fell sharply.

The observed increase in the number of PFCs in response to injection of heterologous red cells and the increase in the number of lymphocytes and plasma cells in the spleen thus indicate a possible increase in the intensity of bone marrow-dependent immune reactions with age in NZB/BLN mice. Presumably this increase is due to depression of the function of a certain population of T-lymphocytes responsible for reciprocal control over antibody formation in the body [3, 11]. Probably as a result of hypofunction of the

TABLE 3. Composition of Thymus Cells of NZB/BLN Mice in Ontogeny (in %)

Cells	Age of mice (in days)							
	newborn	10	60—70	90—100	180—190	210—220	270—280	360—370
Cortex:								
large lymphocytes	8,1	13,9	9,8	12,1	10,6	12,7	10,2	7,3
medium-sized	34,3	35,4	49,7	48,8	63,8	59,9	43,2	44,0
small	52,7	45,2	34,9	34,1	19,5	24,6	44,6	47,2
other cells	4,9	5,5	5,6	5,0	6,1	2,8	2,0	1,5
Medulla:								
large lymphocytes	26,7	29,2	32,5	26,9	30,1	26,5	20,5	37,3
medium-sized	39,3	36,0	43,8	50,5	40,1	49,8	38,9	38,8
small	15,7	17,4	9,6	13,8	9,7	9,1	9,8	6,8
other cells	18,3	17,4	14,1	8,8	20,1	14,6	30,8	17,1

Legend. Other cells include reticulum and mast cells, pycnotic cells, etc.

T-lymphocytes in NZB/BLN mice, with increasing age there is a decrease in the number of RFCs, a heterogeneous population consisting of cells of both thymus- and bone marrow-dependent origin [1, 2, 10]. Intensive proliferative processes and the marked increase in the number of medium-sized lymphocytes in the thymus cannot evidently make good the low level of function of the thymus-dependent lymphocytes population.

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