AGE DIFFERENCES IN PERIPHERAL BLOOD AND PHAGOCYTOSIS INDICES IN GERM-FREE AND CONVENTIONAL WISTAR RATS

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UDC 612.112.3:612.66]:574.24:578/.579

KEY WORDS: germ-free animals; phagocytic activity; erythrocytes; leukocytes; hemoglobin.

The phagocytic activity (PA) of leukocytes plays an important role in the afferent and efferent components of the immune system of the body. In old conventional animals the phagocytic activity of the leukocytes and macrophages is known to be depressed, and this is a regular feature of the immunobiological changes in animals with age [1, 12]. Meanwhile the role of the unmonitored microbial factor in the induction of these changes has been inadequately studied. In particular, age differences in the blood morphology of laboratory rats in the late stages of postnatal development have been inadequately investigated [2, 4, 5].

The object of this investigation was to compare the peripheral blood indices (number of leukocytes and erythrocytes, hemoglobin level) and also the PA of peritoneal macrophages in germ-free and conventional animals of different ages.

EXPERIMENTAL METHOD

Germ-free and conventional male Wistar rats aged 2.5, 9, and 35 months were used. The germ-free rats were obtained from France (IFFA-Credo, Lyon) and kept in isolators of Trexler type. Individual tests on laboratory mice showed absence of pathogenicity of the limited microflora of the germ-free rats of all three age groups. The animals were fed and looked after in accordance with specially compiled rules [8], and the microbiological control of nonpathogenicity satisfied all the demands of germ-free technology [7, 10]. Conventional Wistar laboratory rats were kept under standard conditions in the experimental biological clinic of the Institute of Gerontology, Academy of Medical Sciences of the USSR; the animals were given a diet in accordance with the nutritional standards adopted by the Ministry of Health of the USSR.

The leukocytes and erythrocytes were counted and the hemoglobin concentration determined by the usual laboratory methods [3, 9]. The PA of the leukocytes of the germ-free and conventional rats was determined by a modified method [11]. Peritoneal exudate cells obtained from animals by washing out with sterile Hanks' solution were used as the phagocyte. Determination of the cell composition of the washings showed that more than 90% of the total number of cells were macrophages (monocytes). The object for phagocytosis consisted of an 18-h culture of *Escherichia coli* 055, and the ratio between leukocytes and test object was 1:50. The mixture of leukocytes and bacteria, in a volume of 2 ml, was incubated in the presence of 0.25 ml of standard complement in a dilution of 1:10. To inactivate extracellular bacteria after the end of 30 min of incubation at 37°C a mixture of antibiotics (100 i.u. penicillin and 100 μg streptomycin) was added to the phagocytic system.

Samples of the phagocytic mixture were taken after 30 min and 1, 2, and 12 h. The residue of cells washed in physiological saline was disintegrated by pipeting in 1 ml of sterile distilled water. Tenfold dilutions were prepared from the resulting cell lysate and 0.5 ml of each dilution and seeded in petri dishes with Endo's agar. After incubation of the seedings for 12 h the number of growing colonies was counted and the number of living *E. coli* cells per 10^6 leukocytes determined. This index was used as a measure of PA in conventional and germ-free animals.

EXPERIMENTAL RESULTS

The results of investigation of age differences in morphology of the blood in conventional and germ-free laboratory rats showed significant differences between the two groups of animals. It will be clear from Table 1 that in the germ-free rats the number of erythrocytes remained unchanged during aging, whereas in conventional animals this parameter fell significantly with age by 27.4% compared with young animals.

Counting the leukocytes revealed greater age differences. In the germ-free animals, for instance, this parameter increased with age on average by 50%, whereas in ordinary rats during the same time interval it increased almost threefold, in agreement with the results of other investigations [4, 6, 13]. The age increase in the number of leukocytes in both groups

Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, Moscow. Laboratory of Physiology, Institute of Gerontology, Academy of Medical Sciences of the USSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR D. F. Chebotarev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 8, pp. 81-83, August, 1981. Original article submitted January 12, 1981.

0007-4888/81/9208-1095 $07.50

TABLE 1. Age Differences in General Hematologic Indices (in 1 mm^3 blood) in Germ-Free and Conventional Male Rats

Serial No.	Age of animals, months	Statistical index	Erythrocytes, millions		Leukocytes, thousands		Hemoglobin, mg, %	
			ordinary rats	germ-free	ordinary rats	germ-free	ordinary rats	germ-free
1	2½	n	10	4	10	4	10	4
		M	6,5	4,3	6,1	3,2	14,7	14,6
		$\pm m$	0,27	0,22	0,18	0,22	0,35	0,67
2	9	n	10	5	10	5	10	5
		M	5,2	4,4	8,9	2,8	13,2	15,1
		$\pm m$	0,28	0,15	0,55	0,11	0,25	0,44
		P_{1-2}	<0,05	>0,05	<0,05	>0,05	<0,05	>0,05
3	35	n	10	6	10	6	10	6
		M	5,1	4,4	17,6	4,0	12,1	15,3
		$\pm m$	0,52	0,21	1,52	0,06	0,79	0,60
		P_{1-3}	<0,05	>0,05	<0,05	<0,05	<0,05	>0,05
		P_{2-3}	>0,05	>0,05	<0,05	<0,05	>0,05	>0,05

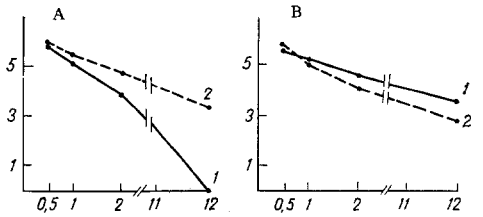

Fig. 1. Number of surviving *E. coli* cells at different times of incubation with mixture of leukocytes in conventional (A) and germ-free (B) rats. Abscissa, time of incubation (in h); ordinate, log of number of living microorganisms per 10^6 leukocytes. 1) Young, 2) old rats.

of rats, incidentally, took place on account of neutrophils, and there was a relative decrease in the number of lymphocytes.

A significant decrease in the hemoglobin concentration also was found in the old conventional rats compared with young, whereas in the germ-free rats there was no significant change in this parameter with age.

The results thus indicate that keeping rats under germ-free conditions helps to maintain constancy of the peripheral blood indices even during late ontogeny.

If the results showing age differences in PA of leukocytes are compared it will be seen (Fig. 1) that this parameter rises progressively in germ-free animals with age, whereas activity of the cells in conventional animals falls significantly with age.

Whereas the indices of phagocytosis of the young germ-free animals were lower than in conventional rats of the same age group, in old germ-free animals they were significantly higher than in old conventional rats. Consequently, PA of leukocytes is maintained for a longer period in germ-free animals than in conventional animals, and this fact correlates with data showing that rats with a restricted microflora have a longer maximal expectation of life [10].

There was, incidentally, a true increase in PA of the leukocytes, for the number of cells taking part in part in phagocytosis was identical in rats of all age groups. Meanwhile, when phagocytosis is studied *in vivo* these differences may be masked by the unequal leukocyte count in these animals. For instance, hematologic investigations (Table 1) showed that the leukocyte count was significantly lower in germ-free rats of all age groups that in conventional rats (on average by two-thirds).

Maintenance of a higher level of phagocytic activity of leukocytes in old germ-free rats is thus evidently connected with the absence of an uncontrolled microbial factor in the life of these individuals, so that the body is involved much less frequently in the phagocytic response of the immune system, which helps to maintain high phagocytic indices of the white blood cells even during the period of late ontogeny. Meanwhile, the low PA of leukocytes of old conventional rats is evidently the cause of the progressive rise in the number of leukocytes in the blood of these animals, necessary to maintain the body in homeostasis.

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