SIZE OF POPULATION OF ANTIBODY-FORMING CELLS IN ANIMALS OF DIFFERENT SPECIES BEFORE AND AFTER IMMUNIZATION

L. A. Pevnitskii, L. N. Fontalin, and V. V. Solov'ev

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An important problem in current immunology is the quantitative estimation of changes taking place in lymphoid tissue after immunization. Another aspect of this problem is the presence of cells in lymphoid tissue predetermined in relation to a particular antigen, the number of these cells, the causes of this predetermination, and so on.

Until recently, the study of these problems has been extremely difficult, because it has been impossible to investigate individual antibody-forming cells either by the Coons' method or by other immunomorphological methods, because of their small number in a particular organ.

In the present investigation the method of local hemloysis in a gel was used to determine the number of antibody-forming cells in the spleen of animals of different species before immunization and in the early periods after injection of antigen.

EXPERIMENTAL METHOD

Adult animals of various species—inbred (mice of line CC57BR, August rats) and noninbred (rabbits, guinea pigs, hamsters, albino rats)—were used in the experiments. The antigen consisted of sheep's erythrocytes injected intravenously. The number of antibody-forming cells was determined by the method of Jerne and Nordin [13] with certain modifications [2]. In certain experiments the titer of hemolysins and hemagglutinins in the animals' blood was determined by the usual method. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Antibody-forming cells were found before immunization in all the animals of the species investigated (Fig. 1). The number of these "normal" antibody-forming producing cells was greatest in the rabbits and rats, and smallest in the guinea pigs and mice. Hamsters occupied an intermediate position. This distribution of the animals corresponds to the frequency with which normal antibodies are found in their blood. The experiments carried out to titrate hemagglutins and hemolysins in the unimmunized animals showed that in rabbits and rats antibodies are found more frequently and in higher titers than in mice.

Only isolated reports of changes in the number of "normal" antibody-forming cells in the lymphoid tissue of animals exposed to various procedures unconnected with immunization with specific antigen may be found in the literature [8]. The authors have previously shown [2] that 6-thioguanine, if injected into animals during immunization with sheep's erythrocytes, sharply reduces the number of antibody-forming cells. In this connection it was interesting to study the action of this analogue of one of the bases of the nucleic acids on the population of antibody-forming cells in unimmunized animals.

The results of experiments conducted on mice in which 6-thioguanine was injected intraperitoneally into the animals as a single dose of 6-12 mg/kg body weight, showed that 2-3 weeks after its injection the number of "normal" antibody-forming cells is significantly increased above the control level (Table 1).

Laboratory of Immunologic Tolerance, Division of General and Radiation Immunology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR G. V. Vygodchikov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 64, No. 10, pp. 60-63, October, 1967. Original article submitted February 13, 1967.

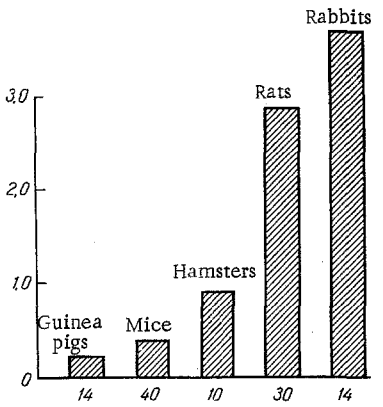

Fig. 1. Number of antibody-producing cells in spleen of intact animals (per 10^6 spleen cells). Ordinate) number of antibody-forming cells; abscissa) number of animals investigated.

TABLE 1. Effect of 6-Thioguanine on Production of "Normal" Antibody Forming Cells (M±m)

Group of mice	No. of mice	No. of antibody-forming cells	
		in the spleen	per 10^8 cells
Receiving 6-thioguanine	72	134±15	45±4
Intact	49	85±10	32±4

The results of the study of changes in the number of antibody-producing cells in mice and rats after immunization with 0.5-1 billion sheep's erythrocytes are given in Fig. 2. The number of antibody-producing cells in the spleen rises rapidly, so that the increase, which after 12 h is not significant, becomes significant after 24 h. The number of antibody-producing cells is maximum on the 4th day after immunization, after which it falls. In the stage of logarithmic growth the number of cells is doubled approximately after 10 h, in agreement with data for the rate of division of plasmablasts and immature plasma cells [3, 17-19].

A number of authors have studied the morphology of antibody-producing cells as revealed by Jerne's method [5-7, 21]. The results of these investigations are contradictory and, in addition, the microscopic methods (especially electron microscopy [5]) used by these authors enabled them to study only a limited number of cells in the population. In our own laboratory M. S. Svirskii used a method of fractionating a suspension of spleen cells by centrifugation in a density gradient of sucrose solution. It has previously been shown [1, 9, 10] that spleen cells may be subdivided in this way into a "top" fraction, of which more than 99% are small lymphocytes, and a "bottom" fraction rich in plasma cells and plasmablasts.

Experiments carried out on mice immunized with sheep erythrocytes (Table 2) showed that the number of antibody-forming cells detectable by Jerne's method in the lymphocyte fraction is 1-50th of that in the fraction containing plasma cells and plasmablasts.

Turning now to the discussion of the results obtained, the interspecies differences in numbers of antibody-forming cells in the animals before immunization must first be examined. It is reported in the literature that antibody-producing cells are present in the spleen of unimmunized animals [6, 8, 12, 20], but no comparative data on this question are available. The results of the present experiments show (see Fig. 1) that in animals whose tissues contain Forssman antigen (guinea pigs, hamsters) or an antigen related to it (mice), the number of antibody-producing cells is much smaller than in animals without Forssman antigen (rabbits, rats). This suggests that the relatively large number of antibody-forming cells in rabbits and rats may be due to the spontaneous immunization of these animals with Forssman antigen, which is widely distributed in the surrounding microflora and in food products [11, 21].

By analogy with the hypothesis expressed above it may be supposed that the presence of cells forming antibodies to sheep's erythrocytes in unimmunized animals is always connected with spontaneous immunization with antigens in the surrounding environment related to the antigens of sheep erythrocytes. However, another view is held which may explain the appearance of normal antibodies (corresponding to the "normal" antibody-forming cells) by somatic mutations [4]. The results of the authors' experiments (see Table 1) with administration of 6-thioguanine, which may be incorporated into the DNA of certain tissues [14], and which evidently has a mutagenic action, are in agreement with this hypothesis although they cannot be regarded as strict proof. It is possible that the observed increase in the number of antibody-forming cells may be connected, not with the mutagenic action of 6-thioguanine, but with its other properties. This problem requires special investigation.

Analysis of the phase of increase in the number of antibody-forming cells after immunization reveals a correlation between the number of these cells in intact and vaccinated rats and mice (Fig. 2). In rats, which had more antibody-producing cells before immunization, correspondingly more of these cells are formed after immunization. It may, therefore, be assumed that the cells of "normal" and "immune" antibodies have common precursors. The number of antibody-forming cells in the spleen of intact mice corresponds fairly accurately to the number of precursor cells determined by other methods [15, 16].

TABLE 2. Antibody-Forming Activity of Fractionated Population of Spleen Cells (M±m)

	No. of antibody-forming cells per 10^6
Top fraction	11±2
Bottom	592±28
Initial suspension	461±21

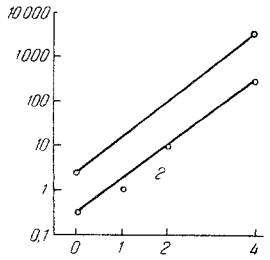

Fig. 2. Number of antibody-forming cells in the spleen of rats (1) and mice (2) before and after immunization (per 10^6 spleen cells). Ordinate) Number of antibody-producing cells (logarithmic scale); abscissa) days after immunization.

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