EVALUATION OF THE STATISTICAL DISTRIBUTION OF THE NUMBER OF ANTIBODY-FORMING CELLS IN NONIMMUNE AND IMMUNIZED MICE

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It is shown that the number of 19S-antibody-forming cells in the mouse spleen obeys a lognormal distribution. This applies to both nonimmune animals and to animals immunized with sheep's red cells, whether intact or treated with cyclophosphamide. It is concluded from the results that in statistical analysis of the numbers of antibody-forming cells detected by the local hemolysis in gel method it is correct to use the geometric mean.

The local hemolysis in gel method [10] has become widely used in immunological research for the detection and counting of antibody-forming cells (AF cells). If sheep's red cells are used as the antigen, the usual criterion of immunologic reactivity is the number of AF cells in the spleen formed on the 4th-5th day after primary intravenous or intraperitoneal immunization (the time of accumulation of the maximal number of "direct" or 19S-AF-cells [2, 7-9, 12, 16]). The number of AF cells is counted in 10^6 nucleated spleen cells and in the whole organ, the man (x) and standard error of the mean ($S_{\bar{x}}$) are calculated, and Student's t-test is used to determine the significance of the difference between the means. It must be emphasized that in most investigations conducted by the local hemolysis in gel method, absolute values for the number of AF cells are used to calculate the means, i.e., the arithmetic mean is determined.

At the outset of a study of the effect of immunodepressants on the immunologic reactivity of mice to sheep's red cells by the method of Jerne and Nordin [10] the writer used the number of AF cells in 10^6 nucleated spleen cells as the chief criterion, calculating the arithmetic mean [1]. However, it was discovered during subsequent investigations that the immune response of mice to sheep's red cells is characterized by high variability of the number of AF cells forms, and this is true both of intact animals and of animals receiving immunodepressive treatment. The error of the arithmetic mean was frequently so great that the difference between the control and experimental values was not significant, even if the difference between the mean values itself was considerable. If, however, the statistical analysis was carried out, not on the number of AF cells, but on logarithms of its individual values in the animals of the comparable groups (i.e., if the geometric mean, $\bar{x}_g$ was calculated), in some cases this allowed the significance of differences between the means to be established, which was not possible when the arithmetic means were used. This state of affairs is illustrated by the data taken from Table 1 for the number of AF cells in the spleen of immunized CC57BR mice and of noninbred albino mice. Even if the number of observations was relatively large, the arithmetic means (177,409 ± 7448 and 159,223 ± 6161, respectively), did not differ significantly ($t = 1.9$; $P = 0.057$), whereas the differences between the geometric means ($\log \bar{x}_g$ 5.149 ± 0.021 and 5.080 ± 0.016, respectively) is significant ($t = 2.6$; $P = 0.016$).

The problem thus arises of the extent to which it is permissible to use the geometric mean for statistical analysis of data regarding the production of AF cells in experiments in vivo. To solve this problem it had to be shown which of the known theoretical distributions corresponds to the variance series actually

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TABLE 1. Number of AF Cells in Mice and Its Correlation with Normal and Lognormal Distribution

Calc. of number of 19S-AF cells	Mice tested	Statistical index				
		n	mean and mean error	σ	χ^2	P
Per 10^6 nucleated spleen cells	Nonimmune CC57Br	153	$\bar{x}=0,38\pm0,03$	0,42	97,414	<0,01
			$\lg \bar{x}_g=\bar{1},400\pm0,031$	0,387	5,129	>0,25
	Immunized CC57Br	245	$\bar{x}=469\pm19$	295	39,403	<0,01
			$\lg \bar{x}_g=2,575\pm0,020$	0,317	6,589	>0,10
	Immunized noninbred	428	$\bar{x}=416\pm14$	290	65,617	<0,01
			$\lg \bar{x}_g=2,519\pm0,015$	0,314	13,624	≥0,05
	Immunized noninbred receiving cyclophosphamide	119	$\bar{x}=134\pm10$	113	34,858	<0,01
			$\lg \bar{x}_g=1,941\pm0,043$	0,471	2,901	>0,25
Per whole spleen	Nonimmune CC57Br	153	$\bar{x}=101\pm9$	117	87,831	<0,01
			$\lg \bar{x}_g=1,820\pm0,032$	0,394	4,816	>0,25
	Immunized CC57Br	245	$\bar{x}=177\,409\pm7\,448$	116 573	40,280	<0,01
			$\lg \bar{x}_g=5,149\pm0,021$	0,328	10,266	>0,10
	Immunized noninbred	428	$\bar{x}=159\,223\pm6\,161$	127 411	132,101	<0,01
			$\lg \bar{x}_g=5,080\pm0,016$	0,338	11,014	>0,10
	Immunized noninbred receiving cyclophosphamide	119	$\bar{x}=64\,057\pm5\,538$	58 451	40,856	<0,01
			$\lg \bar{x}_g=4,621\pm0,042$	0,460	4,484	>0,25

obtained from the numbers of AF cells discovered in the animals. It was assumed that differences would be found in the degree of correlation if the numerical values of the variants or their logarithms were used, and ultimately the choice would have to be made between x and $\overline{x_g}$ for the analysis of the results of experiments of this type.

This paper is based on the results of more than 150 experiments to determine the number of 19S-AF cells in the spleen of nonimmune animals and animals immunized with sheep's red cells (noninbred albino mice and CC57BR mice), which were either intact or had received preliminary treatment with the immunodepressant cyclophosphamide. The experiments were carried out at different times, but in all cases the conditions were standard. The weight of the mice (males) was 20-26 g. The dose of red cells for immunization was 5×10^8 cells, injected intravenously. Mice receiving cyclophosphamide (200 mg/kg, intraperitoneally) were immunized 7 days after treatment. The number of AF cells in the spleen was determined on the 4th day after immunization, using the method of local hemolysis in agar [10] with some modifications described by the writer previously [1]. The degree of correlation with the normal or lognormal distribution was determined by the χ^2 criterion after formation of a variance series by breaking down the values obtained for the number of AF cells into classes and converting them into values of sigma (σ) [3]. The calculations were carried out on an "Iskra-12" keyboard computer. The results are given in Table 1.

This table shows that the number of AF cells in the animals under the different experimental conditions obeys a lognormal distribution. This is valid both for nonimmune and for immunized mice, whether intact or receiving immunodepressive treatment. The rule thus discovered is evidently attributable to the characteristics of accumulation of AF cells in the lymphoid tissue of the animals, and it is in harmony with the view that this process is based on proliferation of AF cells and of their precursors [4-6, 14, 15]. In other words, the lognormal distribution is determined by the logarithmic growth in the population of AF

cells. In this connection it is interesting to note that Burnet and Fenner [5] in 1949 concluded that proliferative processes played a role in antibody formation on the basis of, besides other data, the fact that the distribution of antibody titers in the blood of immunized animals obey a lognormal distribution.

It can be concluded from the facts in this paper that for statistical analysis of numbers of AF cells detected by the local hemolysis in gel method it is correct to use the geometric mean. Other workers have reached the same conclusion when determining the number of hemolysin-producing cells in the mouse spleen [13, 17].

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