TWO POPULATIONS OF SMALL LYMPHOCYTES

G. RUHENSTROTH-BAUER and CHRISTINE LÜCKE-HUHLE. From the Max-Planck-Institut für Biochemie, Munich

It is well known that the lymphocytes of the rat thoracic duct consist of two populations, namely about 5% large lymphocytes and 95% small ones. The two populations differ not only in morphological but also in biological respects (1, 2). Furthermore, we have evidence that the small lymphocytes themselves also consist of two populations. This is particularly so in rodents, but it is also found in other animals with respect to circulating life span, origin, and perhaps function (3–7). This paper provides further strong support for this concept on morphological and possibly functional grounds. This conclusion stems from observations of lymphocyte electrophoretic mobilities and cell volume and from parallel changes in both these parameters associated with changes in function of the whole population.

The electrophoretic mobilities of 308 rat thoracic duct lymphocytes from normal, adult Sprague-Dawley rats were measured with a Zeiss cytopherometer (8). These results are displayed in the cytopherogram (Fig. 1). That this distribution represents two populations was demonstrated in the following manner (9). The left-hand, ascending limb of the cytopherogram was reflected about the maximum value, and a symmetrical curve was obtained. A second histogram was then constructed on the right-hand side of the cytopherogram by plotting the differences between this symmetrical right-hand limb and the experimentally observed frequency of occurrence of cells of the indicated higher mobilities. If the two resultant overlapping distributions are plotted as integrated curves on probability paper, then each population gives rise to a straight line (Fig. 2). Thus the original cytopherogram is the sum of two populations of cells each showing a Gaussian distribution of mobilities. Population I, comprising 77% of the total, has a mean electrophoretic mobility μ = $-1.060 \pm 0.066 \times 10^{-4}$ [$cm^2sec^{-1}v^{-1}$]; and

population II, 23% of the total, has a mobility of $-0.930 + 0.050 \times 10^{-4}$ [$cm^2sec^{-1}v^{-1}$]. Hereafter, the electrophoretically slower lymphocyte population will be referred to as type A and the faster as type B.

Secondly, the volume distribution of rat thoracic duct-lymphocytes was measure (Fig. 3) with the apparatus described by Gutmann (10), and the distribution was analyzed in the same manner as for the electrophoretic mobilities (Fig. 4). Here, also, the total distribution could be broken down into two populations, each having a Gaussian distribution. 79% of the cells had a mean volume of 141 $\pm$ 28 μ^3, and 21% a mean of 196 $\pm$ 24 μ^3. If we treat the lymphocytes as spherical cells, these
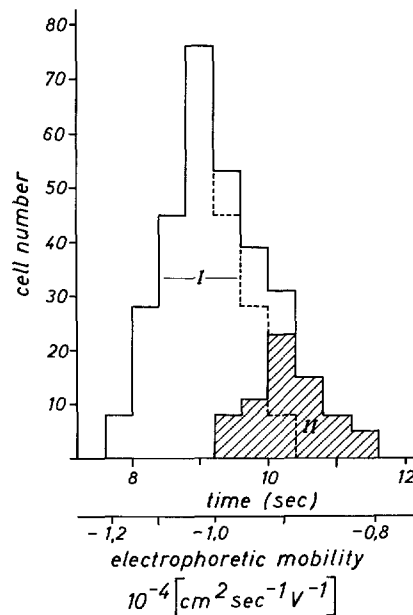

FIGURE 1 The distribution of the electrophoretic mobility of small rat thoracic duct lymphocytes. I, A type lymphocytes; II, B type lymphocytes (see text).

values correspond to cell diameters of 6.5 and 7.2 μ, respectively.

The close correspondence of the proportions into which the total small lymphocyte population may be split by these two unconnected physical meas-
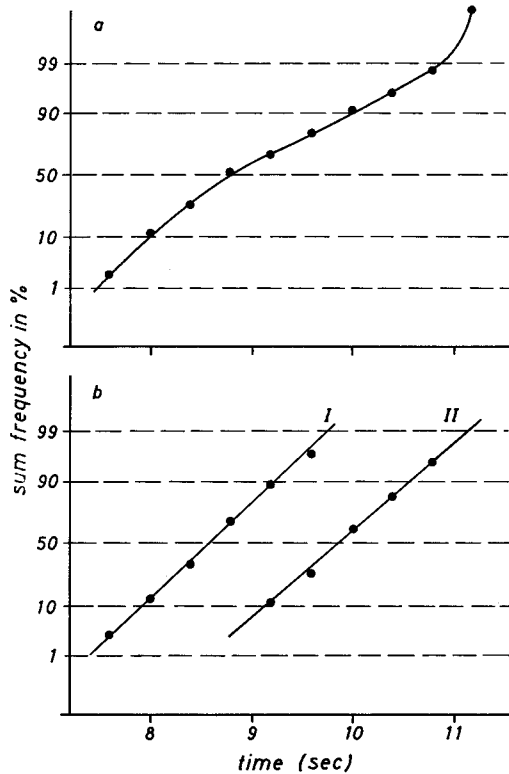

Figure 2 The curves from Fig. 1 transposed in Gaussian sum frequency paper. a, all cells; b, cells after distribution.
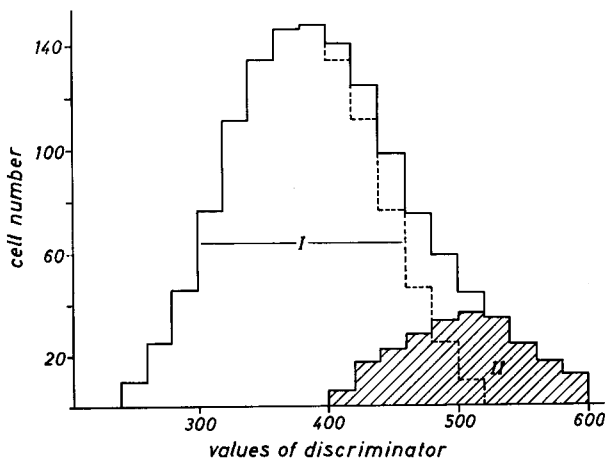
urements suggests strongly that the A lymphocytes are those with the larger cell volume and that the B lymphocytes are those with the smaller. This hypothesis is confirmed by the following observation. 1–2 hr after a whole-body irradiation with 800 R, the lymphocytes of the rat thoracic duct are in most cases almost only B type when examined electrophoretically. Volume measurements in such cases similarly show that the population is composed almost entirely of lymphocytes of smaller mean volume. Radiation of thoracic duct lymphocytes in vitro alters neither their electrophoretic nor their volume distribution. Our finding is also in harmony with the results of Gowans and Knight (2) who stated that about 80% of the small lymphocytes recirculate quickly so that these cells disappear in a relatively short time during a prolonged duct drainage. On the other hand, the remaining 20% recirculate relatively slowly and give, even after 5 days drainage, a constant low lymphocyte count.

The small lymphocytes of the peripheral blood of the normal healthy rat are almost all of electrophoretic type A. At the maximum, 5% show a higher electrophoretic mobility. An exact determination of cell-volume distribution on peripheral lymphocytes is not possible with present methods since, for this determination, blood is pretreated with saponin to destroy the erythrocytes. Saponin also, however, damages the lymphocyte membrane, so that electrical methods of volume measurement can give no reliable values. However, information can be obtained from changes in the peripheral lymphocyte population after two types of stimuli.
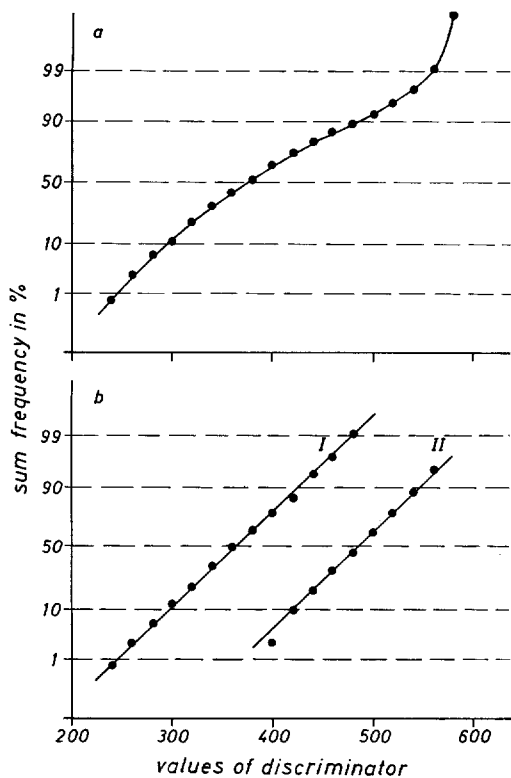
A few hours after whole-body irradiation with

Figure 3 The volume distribution of small thoracic duct lymphocytes.

FIGURE 4 The curves from Fig. 3 transposed in Gaussian sum frequency paper. *a*, all cells; *b*, cells after distribution.

also seen in the spleen and lymph nodes, the thymus and bone marrow again remaining unlabeled.

Despite the fact that four times as many B as A lymphocytes drain from the thoracic duct into the blood, the total lymphocyte population of the blood contains 20 times as many A cells as B cells. This implies that B lymphocytes leave the blood stream much faster than A lymphocytes. An approximate calculation from our data gives a mean circulation time in the blood of about 19 hr for the A lymphocytes and for B lymphocytes about 9 min. The total number of A lymphocytes in the body seems to be greater than that of B lymphocytes.

The question arises whether the two morphological types of small lymphocytes described above have different functions. For this purpose, according to the method of Jerne and Nordin (11), rats were immunized with sheep erythrocytes, and 5 days later the blood or thoracic duct lymphocytes were assayed for antibody production against sheep red cells. Relating the number of hemolytic plaques to the number of tested leukocytes, in five animals, one in 320 ± 187 of the circulating leukocytes produced antibody, and from seven animals, one in 9,200 ± 4,200 of the thoracic duct cells produced plaques. In five animals irradiated with 800 R of X-rays 2–3 hr before the duct drainage, only one cell in 61,000 ± 45,000 thoracic duct cells was producing antibody. Despite the large experimental error of the Jerne technique, especially in the measurement of the peripheral blood leukocytes, these results suggest that there is a correlation between the number of A lymphocytes in the cell populations examined and the number of antibody-producing cells. This is shown particularly by the experiments with irradiated animals where practically all the cells plated were B lymphocytes and only about 0.02‰ were Jerne positive. It is possible, therefore, that in the A lymphocytes we are dealing with an immunologically competent lymphocyte in the original sense of Medawar (12) and that the immunologically committed cells, following an antigenic stimulus, appear within this group. However, we must not exclude the possibility that immunological activity, as measured by the Jerne technique, is a property of a different cell type present in very small numbers in the cell populations we have examined and, therefore, are not detected on morphological grounds.

In summary, it may be said that our experiments

800 R, the peripheral lymphocytes are exclusively of electrophoretic type B. Secondly, 15 min after an intravenous or intraperitoneal injection of 0.2 ml of 96% ethanol, there appear up to 30% type B lymphocytes in the blood. 1 hr after the injection, these cells have disappeared from the circulation, and the electrophoretic distribution is returned to normal.

In order to clarify the distribution of A and B lymphocytes in the body, populations of A lymphocytes from the blood and A and B lymphocytes from the thoracic duct were labeled in vitro with tritiated adenosine and reinjected intravenously into rats (2). 24 hr after the injection of labeled A lymphocytes, the peripheral lymphocytes contained 4% labeled cells. Also, labeled cells were found in the spleen and lymph nodes, while the bone marrow and thymus remained free of labeled cells. After injection of the labeled 20:80 mixture of A and B lymphocytes from the thoracic duct, at 24 hr about 2% of the circulating lymphocytes carried a label. In this case labeled cells were

are in agreement with the hypothesis that the so-called small lymphocytes of the rat in fact comprise two cell populations, the A and B lymphocytes. The mean electrophoretic mobility of the A lymphocytes is $-0.930 \pm 0.050 \times 10^{-4}$ [cm² sec^{-1}v^{-1}], and their mean volume is 196 ± 24 μ^3. The mean electrophoretic mobility of the B lymphocytes is $-1.060 \pm 0.066 \times 10^{-4}$ [cm² sec^{-1}v^{-1}]. and their mean volume is 141 ± 28 μ^3. In the thoracic duct these types normally occur in an approximately 20:80 mixture, but after 800 R the duct population contains almost only B lymphocytes. Splenectomized animals show no alteration in the normal distribution of A and B cells in the thoracic duct after irradiation. The blood of normal rats contains almost exclusively A type lymphocytes. After irradiation, the blood stream shows a dose-dependent increased number of B lymphocytes, while the A lymphocytes are very greatly diminished in number. An injection of ethanol likewise causes a rapid appearance of B lymphocytes in the peripheral blood. With the aid of Jerne technique, it could be shown that there is a correlation between the proportion of A lymphocytes in the total leukocyte population and the number of cells responding immunologically to sheep erythrocytes.

The experimental details of this work will be published elsewhere (13).

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