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RELATIVE ANTIBODY-FORMING CAPACITY OF SPLEEN CELLS AS A FUNCTION OF AGE

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Our past studies have shown that the *in vivo* culture model is quite suitable for assessing quantitatively the antibody-forming capacity of lymphoidal cells[1–4] That is, a linear $\log_2$ relation with a slope of 1.0 can be demonstrated between activity, as expressed in terms of a 6-day agglutinin titer, and cell number. Using this model, we found that there exists in the intact mouse an autoregulatory mechanism that permits only a fraction of the total population of competent cells to participate in a given immune response.[4] Thus, for example, the cells from one-tenth of a spleen from a preimmunized mouse transferred into the irradiated mouse can be shown to produce as much antibody in response to an optimum secondary dose of antigen as can an intact mouse. In view of this autoregulatory mechanism, it would seem that data obtained from studies on the antibody-forming capacity of individuals throughout their life span (e.g., see the comprehensive study of Wolfe and his coworkers[5]), although informative, could not be readily interpreted. Preliminary studies were therefore carried out using the *in vivo* culture model to determine the primary antibody-forming capacity of 76×10^6 spleen cells (anti-sheep RBC response) from (C3H/Anf Cum ♀ × 101/Cum ♂) donors varying in age from 1 week to 29 months.

Materials and Methods.—Approximately 175 (C3H/Anf Cum ♀ × 101/Cum ♂) donors, ranging in age from 1 week to 29 months, and 300 twelve-week-old isologous recipients were used in this study. The mice were caged in groups of five and allowed free access to food and water. Within a day after a single total-body exposure to 800 r, the recipients were used as *in vivo* cultures for spleen cells. A G.E. Maxitron X-ray machine was used. Irradiation conditions were as follows: 300 kvp at 20 ma; 170 r/min at 70 cm; inherent filtration, 4.75 mm of Be; added filtration, 3 mm of Al; and hvl, 0.470 mm of Cu. The 30-day LD_{50} and LD_{99} doses for these 12-week-old mice are ~750 and ~875 r, respectively.[6]

Cell suspensions were prepared by teasing spleens of donors in Tyrode's solution containing 1 per cent normal isologous mouse serum. After determining the viability of spleen cells by the Eosin dye uptake method of Schrek,[7] the concentration of cells was adjusted appropriately and a 1-ml aliquot was then injected intravenously into each irradiated mouse. Intraperitoneal injection of the test antigen, 1 ml of 1 per cent sheep RBC, immediately followed this cell-infusion procedure, and individual serum samples were collected 6 days later. Sera were frozen until completion of the experiment and then titrated by our standard twofold serial dilution agglutinin method.[8]

First, the "activity profile" was obtained by determining the primary antibody response of 6×10^6, 12×10^6, 24×10^6, 48×10^6, and 96×10^6 viable, nucleated spleen cells from 12-week-old donors. About 50 recipient mice were used. Then the relative antibody-forming capacities of 76×10^6 viable nucleated spleen cells from 1 week to 29-month-old donors were determined. About 200 recipient mice were used in this latter study. As negative controls, about 50 irradiated mice were given either the test antigen only or the spleen cells only. None of these control mice showed any detectable antibody titer.

Results.—As shown in Figure 1, a linear $\log_2$ relation was obtained between the primary 6-day antibody titer and cell number. The slope of the fitted regression line is 0.95, which is not significantly different from the slope of 1.0 obtained previously.[4] One is therefore justified in using this model to determine the relative primary antibody-forming capacity of a fixed number of cells from donors of varying ages, the cell dose being between 6×10^6 and 96×10^6. As already noted, a spleen cell dose of 76×10^6 was chosen.

The highest 6-day $\log_2$ titer which we detected, independent of the age of spleen cell donors, was 8.0. Therefore, for comparative purposes, an estimate of the relative primary antibody-forming capacity of 76×10^6 spleen cells from mice of varying age groups was made relative to this titer; i.e., $2^{-(titer_{max} - titer_i)}$, where $titer_{max}$ is 8.0 and $titer_i$ is the mean titer of spleen cells of any age group. The results are shown in Figure 2. The highest activity was obtained with cells from 8-month-old mice (~60%), and the lowest from 1-week-old mice (~1%). The antibody-forming capacity of spleen cells from the very old mice was only about one-fourth that of 8-month-old mice (~10%).

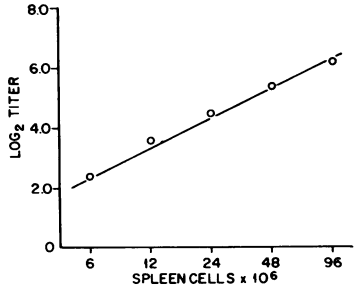

FIG. 1.—Primary anti-sheep RBC agglutinin response as a function of spleen cell dose (6-day titers).

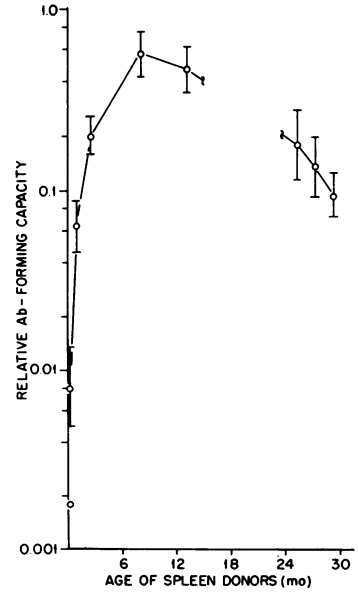

FIG. 2.—Relative antibody-forming capacity of 76×10^6 spleen cells as a function of age $[2^{-(titer_{max} - titer_i)}]$.

Supplementary data of interest were the following: (*a*) The body weight increased almost linearly from 1 week to 13 months of age (~5 to 45 gm), and decreased from

13 to 29 months of age (~45 to 30 gm) (see Fig. 3). (*b*) The linear increase in spleen weight from 1 week to 3 months of age was very rapid (~20 to 80 mg), whereas the increase from 3 to 20 months of age, although linear, was relatively slow (~80 to 120 mg; see Fig. 4). (*c*) The number of nucleated cells per milligram of wet weight of spleen remained relatively constant throughout the life span (2.03×10^6; 1.89×10^6 to 2.16×10^6, 95% confidence interval).

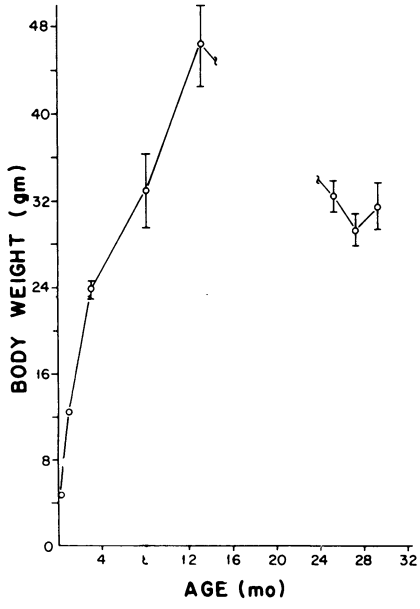

FIG. 3.—Change in body weight as a function of age, $x \pm (t)$ (s.d./$\sqrt{n}$).

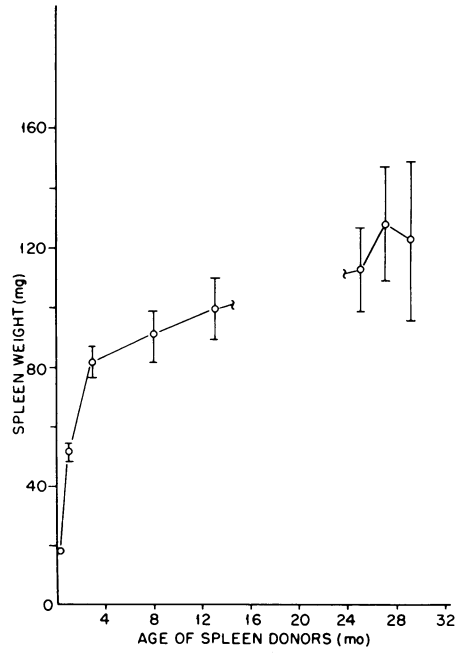

FIG. 4.—Change in spleen weight as a function of age, $x \pm (t)$ (s.d./$\sqrt{n}$).

Discussion.—As we would expect, there is an accelerated rise in the antibody-forming capacity of spleen cells during the first 3 months of life. The increase in spleen and body weight was also rapid during this period. However, the number of nucleated cells per wet weight of spleen remained relatively constant (~2×10^6 per mg) throughout the life span. An unexpected observation of interest is that the activity of spleen cells from the so-called "immunologically mature" 3-month-old mice[8, 9] is only about a third of that of the 8-month-old mice. From 3 to 8 months of age, the spleen and body weights increased only about 10 and 15%, respectively. These results would suggest that, during this period of growth up to about 8 months of age, the immunologically competent cells in the spleen could be (*a*) proliferating at a rate faster than that of the incompetent cells, (*b*) "maturing" at a fast rate from an inactive stage, (*c*) becoming more efficient in synthesizing antibody, or (*d*) colonizing from other organ areas. Capalbo's diffusion chamber model[10] should be most suitable to determine which one, or any combination, of these factors is mainly responsible for this increase in the antibody-forming capacity of spleen cells.

The maximum activity was obtained with cells from 8-month-old mice. This is

not too surprising if we assume that immunological maturity is closely related to chronological maturity. According to G. E. Cosgrove, Jr. (personal communication), the mean life span of this hybrid strain of mice is about 26 months. This would suggest that at about 8 months of age, these mice have lived a third of their mean life span.

The gradual decrease in the antibody-forming capacity of spleen cells from 8 months to the terminal stage of life seems typical of a "biological decay" pattern. During this time there is a continuous, slow increase in spleen weight, whereas the body weight reaches a maximum at 12 months of age and decreases thereafter. The number of cells per unit weight of spleen remains essentially constant throughout life and it is therefore possible to estimate the relative antibody-forming capacity of the spleen as a function of age; i.e., relative antibody-forming capacity of 76×10^6 spleen cells $\times$ spleen weight. In doing so, we found that the slight increase in the spleen weight does not compensate for the two- to threefold decrease in the activity per unit cell number, i.e., antibody-forming capacity per spleen weight clearly decreased during this period. One could define the latter two-thirds of the life span operationally as the phase during which the spleen "ages." The immune status of the individual would reflect this aging. The decrease in antibody-forming capacity beyond 8 months of age could be due to (*a*) "emigration" of the competent cells from the spleen to other organs, (*b*) a decrease in the efficiency of the competent cells to synthesize antibodies, or (*c*) the possibility that more cells are being "tied up" by naturally invading antigens.

Although there is still much to be learned, this quantitative approach has permitted us to have a better insight into the problem of maturation and aging of the antibody-synthesizing machinery.[11]

Summary.—Using the *in vivo* culture model, we have been able to demonstrate that the relative antibody-forming capacity of spleen cells of donor mice varies with age from 1 week to 29 months. A very rapid increase in activity was noted from 1 week to 1 month of age, and less rapid increase from 1 to 8 months. The maximum antibody-forming capacity was obtained with cells from 8-month-old donors. A gradual decrease seemed to take place over the subsequent 21 months of life. These data, together with those on body and spleen weights, broaden our perspective on the problem of maturation and aging of the antibody-protein synthesizing machinery.

We wish to express our gratitude to M. A. Kastenbaum and D. G. Gosslee of the Mathematics Panel of this Laboratory for the statistical treatment of the data.

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AN APPARENT LUNAR PERIODICITY IN THE SEXUAL CYCLE OF CERTAIN PROSIMIANS

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During the course of other work by two of us, on a considerable group of lemurs and lorisoids in captivity in New Haven, Connecticut, certain types of cyclical behavior have become apparent. Since it has been necessary to reconstruct the living quarters of some of these animals, the disturbance of which precludes further observations in the immediate future, the more remarkable aspects of this cyclical behavior are reported at the present time, largely in the hope of stimulating other workers who may have access to prosimians to watch for comparable phenomena.

The animals observed consisted of a group of four (2♂♂, 2♀♀) *Lemur fulvus* E. Geoffroy, a pair of *Lemur albifrons* E. Geoffroy, 3♀♀ and 1♂ *Galago senigalensis* E. Geoffroy, one ♂ and one immature ♀ *G. crassicaudatus* E. Geoffroy, and three pairs *Perodicticus potto* (P. L. S. Müller). These animals lived from three to twelve months, up to December 1, 1961, in a room at the top (fifth floor) of a low tower with windows on all walls. The most important animals, 2♂♂ of *Lemur fulvus rufus* Audebert, one ♀ of *L. f. rufus* ("Calo"), and one of *L. f. fulvus* ("Sal") occupied a large section of the room, could reach windows looking north and east, could easily see out from windows looking south and less easily from one small window facing west. A pair of pottos and both *G. crassicaudatus* occupied a similar section; the *L. albifrons* and the *G. senegalensis* lived in smaller but naturally illuminated cages, the other two pairs of pottos in darker cages. These animals are referred to collectively as group 1.

A pair of *Lemur fulvus rufus* kept in the kitchen of a dwelling house with a window facing west-north-west and with little view of the night sky has served as a control. These animals are designated as group 2.

All these animals are reported to breed seasonally. The lorisoids breed biannually, producing young about April and October.[1–3] Gestation lasts between 3 and 4 months for *G. senegalensis*[2–4] to 6 months for *Loris*.[1] Lemurs have a single annual breeding period. Mating occurs in the southern hemisphere during April, May, and June and parturition from September through January.[5] In the northern hemisphere the seasons become reversed with birth occurring about April and May.[1, 5] Pregnancy lasts about 4 months.[1, 5]

Oestrous cycles of 4 to 6 weeks are well documented in *G. senegalensis*,[4, 6] in *Loris*,[1] and in the lemuriforms *Microcebus* and *Cheirogaleus*.[5, 8] However, there is no record of a 4- to 6-week oestral cycle in *Lemur*, although periods of swelling and