GENETIC CONTROL OF THE NUMBER AND AVIDITY OF ANTIGEN-BINDING B LYMPHOCYTES IN THE MOUSE SPLEEN

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The number and avidity curves of IgM-positive B lymphocytes forming rosettes with trinitrophenyl-treated sheep's erythrocytes (TNP-RFC) were compared in BALB/c, C57BL/6, (C57BL/6 × BALB/c)F_1, F_1 × BALB/c, and F_1 × C57BL/6 mice. Trinitrophenyl-bovine serum albumin (TNP_{24}-BSA), dinitrophenyl-BSA (DNP_{23}-BSA), and sulfanyl-BSA ($Sulf_{17}$-BSA), in various dilutions, were used as inhibitors. The number of TNP-RFC was 60% greater in the spleen of the BALB/c mice than in that of the C57BL/6 mice. The F_1 hybrids occupied an intermediate position, whereas the number of TNP-RFC was 35% greater in F_1 × BALB/c hybrids than in F_1 × C57BL/6 hybrids. Inhibition (avidity) curves differed in the two strains and the F_1 hybrids tested. It is concluded that the number and avidity of TNP-RFC are under genetic control. Together with the postulated random (stochastic) expression of the V genes (genes of idiotypes?) in lymphocytes this suggests that the simplest mechanism of genetic control may be the ratio between the corresponding groups of V genes.

KEY WORDS: genetic control; avidity; Ig receptor; lymphocyte; antigen-binding cells.

New possibilities for the study of antibody diversity are provided by studies of the genetics of lymphocyte clones and clone formation in ontogeny [7-9, 13, 18, 19]. It has been shown that during embryonic development of mice (15th-19th days) antigen-binding lymphocytes of varied specificity appear almost simultaneously and that antigen-dependent positive selection of these lymphocytes is absent during prenatal and postnatal development. Definite quantitative proportions are maintained in inbred mice during development between antigen-binding cells of different specificity [7, 8, 19] and significant interlinear differences in the number of antigen-binding cells of a given specificity are found [7]. There is good reason to suppose [8, 9] that the appearance of different clonotypes [18] at different times of development depends not on the absence of Ig positive antigen-specific precursors of the clones of antibody-forming cells, but on other causes.

Differences in the number and avidity of antigen-binding B cells were studied in mice of different strains and their hybrids.

EXPERIMENTAL METHOD

Experiments were carried out on 2-month-old BALB/c, C57BL/6, (C57BL/6 × BALB/c)F_1, F_1 × BALB/c, and F_1 × C57BL/6 mice. Conjugates of trinitrophenyl (TNP) and dinitrophenyl (DNP) groups and also of sulfanylic acid (Sulf) with bovine serum albumin (BSA) – TNP_{24}-BSA and DNP_{23}-BSA – were used as inhibitors and for plotting avidity curves. Antibrain serum was obtained and tested by Golub's method [10] (cytotoxicity for thymocytes 96%, for spleen cells 30%). Anti-IgM and anti-IgG sera (Meloy) also were used. Donkey antirabbit anti-Ig serum (N. F. Gamaleya Institute of Epidemiology and Microbiology) was used in the indirect fluorescent antibodies method. TNP-conjugated sheep's erythrocytes (TNP-RBC), prepared by the method of Rittenberg et al. [16], were used for the rosette-formation test.

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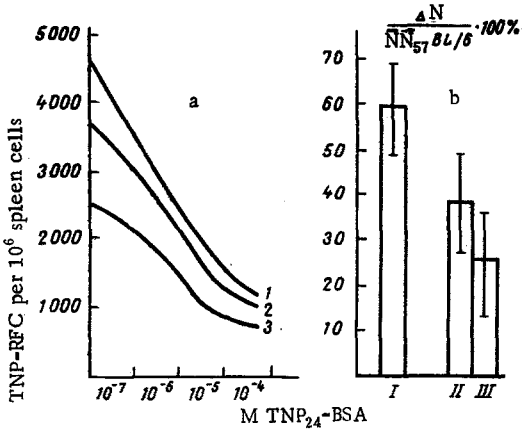

Fig. 1

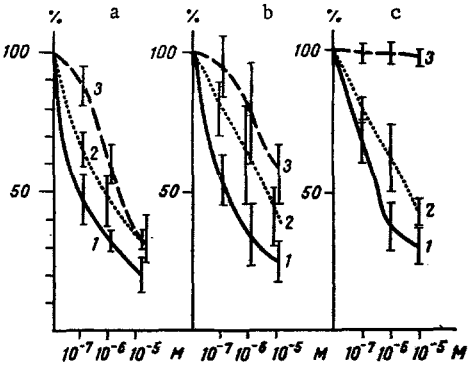

Fig. 2

Fig. 1. Comparison of distribution of TNP-RFC by avidity and by difference in number (N) of TNP-RFC in spleens of BALB/c, C57BL/6, and (BALB/c × C57BL/6)F_1 mice (in % of mean number of TNP-RFC in C57BL/6 mice). a) Avidity curves of TNP-RFC in typical experiment: 1) BALB/c, 2) (BALB/c × C57BL/6)F_1, 3) C57BL/6; b) difference in number of TNP-RFC in spleen between mice of two strains and F_1 hybrids in 10 experiments on 30 mice, analyzed by method of confidence intervals (with probability of 99%). I) Difference in number of TNP-RFC between BALB/c and C57BL/6; II) difference in number of TNP-RFC between BALB/c and F_1 hybrids; III) difference in number of TNP-RFC between F_1 hybrids and C57BL/6.

Fig. 2. Inhibition of TNP-RFC (in % of maximal number) by different concentrations of TNP_{24}-BSA (1), DNP_{23}-BSA (2), and $Sulf_{17}$-BSA (3): a) BALB/c; b) C57BL/6; c) (BALB/c × C57BL/6)F_1.

EXPERIMENTAL RESULTS

The number (N) of cells forming rosettes with TNP-erythrocytes (TNP-RFC) inhibited by 10^{-4} M TNP_{24}-BSA (per 10^6 spleen cells) was compared in the spleens of 30 2-month-old BALB/c, C57BL/6, and (BALB/c × C57BL/6)F_1 mice. Ten experiments were carried out. Two mice of each strain and one F_1 hybrid mouse were used in each experiment. To exclude mistakes due to the fact that a fresh batch of TNP-RBC was used in almost every experiment (with the possible variation in concentration of TNP-groups of the erythrocyte surface) the difference in number (ΔN) of TNP-RFC within the same experiment was first found, and the results of all 10 experiments were then analyzed by the method of confidence intervals (with a probability of 99%).

In every case the mean value $\bar{N}$ of TNP-RFC (C57BL/6) was taken as 100%.

$$\frac{\Delta N}{\bar{N}_{C57BL/6}} \cdot 100 = \frac{N_{TNP\text{-}RFC(BALB/c)} - N_{TNP\text{-}RFC(C57BL/6)}}{\bar{N}_{TNP\text{-}RFC(C57BL/6)}} \cdot 100.$$

It will be clear from Fig. 1, which illustrates this relationship, that after summation of the results of 10 experiments (column I) the number of TNP-RFC in the spleen of the BALB/c mice was 60% greater than in the C57BL/6 mice. The difference in the number of TNP-RFC between the BALB/c and F_1 and F_1 and C57BL/6 mice was expressed in the same way in each experiment:

$$\frac{N_{TNP\text{-}RFC(BALB/c)} - N_{TNP\text{-}RFC(F_1)}}{\bar{N}_{TNP\text{-}RFC(C57BL/6)}} \cdot 100 \text{ [Fig. 1b, column II];}$$

$$\frac{N_{TNP\text{-}RFC(F_1)} - N_{TNP\text{-}RFC(C57BL/6)}}{\bar{N}_{TNP\text{-}RFC(C57BL/6)}} \cdot 100 \text{ [Fig. 1b, column III].}$$

Clearly differences between BALB/c and the F_1 hybrids (column II) and the difference between the F_1 hybrids and C57BL/6 mice (column III) were highly significant. As regards the TNP-RFC content in their spleen the F_1 hybrids thus occupied an intermediate position between the parental strains. The difference between columns II and III is not significant (Fig. 1), i.e., the possibility cannot be ruled out that the F_1 hybrids lay exactly midway between the parental strains for the number of TNP-RFC in their spleen.

The results of one experiment are shown in Fig. 1a as curves of inhibition of rosette formation by increasing concentrations of TNP_{24}-BSA. The specificity of inhibition of TNP-RFC is demonstrated by experiments with two closely similar haptens and one widely different hapten (Fig. 2). The curves of inhibition of TNP-RFC in the C57BL/6 mice (Fig. 2c) clearly differed significantly when TNP and DNP groups and also sulfanylic acid on the same carrier were used as inhibitors. This difference was much less marked in the BALB/c mice (Fig. 2a), Spleen cells of two strains of mice, binding TNP-RBC, thus differed not only in their number, but also in their avidity (during inhibition by $Sulf_{17}$-BSA).

In experiments on mixtures of spleen cells from 2-month-old mice of the two strains similar results were obtained (Fig. 3, I, II). In parallel tests mixtures of spleen cells from two types of hybrids were investigated: (F_1 ♂ × C57BL/6 ♀) – 10 mice and (F_1 ♂ × BALB/c ♀) – 10 mice. The difference in the number of TNP-RFC between them was 35% (Fig. 3, III-IV). Special experiments showed that anti-IgM serum inhibited TNP-RFC in a suspension of spleen cells of C57BL/6 mice by 88% and in a suspension from BALB/c mice by 84%, whereas the inhibition produced by anti-Ig serum was 91 and 89%, respectively. No significant difference in the number of Ig positive cells in the spleen of the C57BL/6 mice (32%) and BALB/c mice (31%) could be found by the luminescent antibodies method. In the cytotoxic test with antibrain serum 28% of the spleen cells died in the BALB/c mice and 26% in the C57BL/6 mice. After treatment with antibrain serum the number of TNP-RFC (per 10^6 spleen cells) increased by 26% (BALB/c) and by 24% (C57BL/6).

In experiments on C57BL/6 and BALB/c mice and F_1 hybrids and in back-cross experiments the number of B lymphocytes binding TNP-RBC (TNP-RFC) in the spleen was thus found to be under genetic control. Comparison of the distribution of TNP-RFC by avidity in the two strains of mice and the F_1 hybrids (using TNP_{24}-BSA as inhibitor) and comparison of the TNP-RFC of the two strains by avidity in parallel tests with three inhibitors suggest that TNP-positive splenic lymphocytes in the mice of the strains studied are not identical in avidity. The results of experiments in which a suspension of spleen cells was treated with antibrain serum and complement and by inhibition of TNP-RFC by anti-IgM serum indicate that, under the experimental conditions used, T lymphocytes are virtually absent among the rosette-forming cells in mice of both strains. No significant difference could be found between the number of Ig-positive cells in the spleen of C57BL/6 and BALB/c mice. This factor, consequently, could not influence the interlinear differences in the number of splenic TNP-RFC. The population of TNP-RFC inhibited by 10^{-4} M TNP_{24}-BSA was taken to consist of TNP-specific cells. TNP-RFC and RBC-RFC of low avidity were thus excluded. However, their contribution was small (10% of TNP-RFC for BALB/c and 15% for C57BL/6). Inhibition of rosette formation by anti-IgM serum showed that not less than 83-84% of TNP-RFC was due to the presence of IgM-receptors on the surface of the TNP-specific B lymphocytes.

It follows from these results that TNP-RFC, inhibited by 10^{-4} TNP-BSA, consists mainly of a population of IgM-positive B cells of varied avidity. The avidity of each of them is evidently a function of two variables: the affinity of the Ig-receptors and their number. There is evidence that a definite contribution to the avidity of a lymphocyte is made by the affinity of its receptors and not by their number [1, 3, 4, 14, 15, 17]. An important argument is the parallel increase in the number of highly avid antigen-binding lymphocytes [1, 14, 15], cells synthesizing high-affinity antibodies [3], and the concentration of high-affinity antibodies in the blood during the immune response. Another important fact is that the same cells [8] may have low avidity for one and high avidity for another hapten. Analysis of interlinear differences in the number and avidity of TNP-RFC suggests that they are under genetic control. It is evidently a question of two (nonidentical for the affinity of their Ig-receptors) sets of clones of B lymphocytes. Hence it follows that qualitative differences exist between the set of V_{TNP} genes, expressed as Ig receptors, in BALB/c and C57BL/6 mice. When discussing the regular quantitative relationships between antigen-binding cells, interlinear differences, and the results of unpublished back-cross experiments [7], the authors concerned consider it unlikely that a small number of structural genes can control both the whole set of antigen-binding cells, with their differing specificity, and also the sequence of expression of this set in the course of development.

The results now obtained can be explained as follows. The writers postulated previously [2] that during random (stochastic)* antigen-independent expression of the V genes (idiotype genes?) in the composition of the Ig receptors the relative number of different groups of clones in the B-lymphocyte population will be proportional to the relative number of the corresponding V genes in the same population. Genetic control of the number of antigen-binding B lymphocytes of a given specificity and, evidently, of the distribution of lymphocytes of that specificity by the affinity of the receptors, in conjunction with the postulated random expression of V genes,

*The random mechanism of expression of the V genes has been postulated on several occasions previously [12].

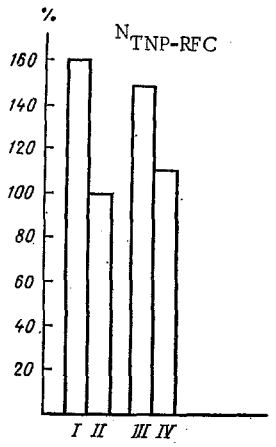

FIG. 3. Comparison of mixtures of spleen cells from two strains of mice and their hybrids in same experiment. I) BALB/c (5 mice); II) C57BL/6 (5 mice); III) (F_1 × C57BL/6) hybrids (10 mice); IV) (F_1 × BALB/c) hybrids (10 mice). Number of TNP-RFC per 10^6 spleen cells in C57BL/6 mice taken as 100%.

suggests that the genes controlling the number of groups of clones of B lymphocytes are structural genes of the V segments of immunoglobulins. In other words, if different V genes are expressed almost simultaneously in ontogeny and if there is no mechanism of selective expression of particular genes, the simplest mechanism of genetic control of the ratio between the groups of B lymphocytes of different specificity may be a definite quantitative ratio between the corresponding groups of V genes. In the present case the high relative number of TNP-RFC in the population of B lymphocytes probably reflects a high relative number of V_{TNP} genes. Interlinear differences in the avidity of TNP-positive lymphocytes and in the presence of crossing-over with "near" (DNP) and "remote" (sulfanylic acid [8]) haptens suggest that these are populations of different clones. Possibly the high relative number of TNP-positive lymphocytes is due to the presence of a large number of antigens, important in evolution, cross-reacting with TNP and DNP groups.

The point of view under discussion is in good agreement with the latest observations on genetic control over idiotypes and corresponds more closely to certain variants of the germ line theory [5, 6] than the mutation theory [11] of antibody diversity. However, its essential link, namely the hypothesis of genetic control of avidity of the B cells, requires more sophisticated experimental techniques and, in particular, the use of anti-idiotypic antisera and comparison of the B cells from different strains of mice at the height of the immune response with respect to affinity of the antibodies formed by them. Investigations along these lines are in progress by the writers at present.

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