# GENETIC CONTROL OF THYMUS-DERIVED CELL FUNCTION

## I. In Vitro DNA Synthetic Response of Normal Mouse Spleen Cells Stimulated by the Mitogens Concanavalin A and Phytohemagglutinin*

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The influence of genetic factors could intervene at any level of the immune response from the recognition of antigen through the synthesis and secretion of specific immunoglobulins or the function of the effector cells of delayed hypersensitivity. Autosomal dominant immune response genes have been shown to exert quantitative and qualitative control over antibody production in mice and guinea pigs (1). Cell-mediated immunity is controlled in a qualitative, all-or-none fashion by these genes in guinea pigs (2). In both species, genetic mapping studies have shown a very close linkage between specific immune response genes and the major histocompatibility locus. Although the cell type(s) in which specific immune response genes function have not been determined directly, several observations in both mice and guinea pigs suggest that some of these genes are expressed in thymus-derived (T)[1] lymphocytes. (See reference 2 for a description of known histocompatibility-linked immune response genes and a review of the evidence that is consistent with their expression in thymus-derived cells.)

Lymphocyte transformation stimulated by the mitogens concanavalin A and phytohemagglutinin has been shown to be a property of thymus-derived lymphocytes that may be functionally related to the in vivo expression of delayed hypersensitivity by this cell type (3). Observations described here show that in several inbred mouse strains the mitogen-stimulated DNA synthetic responses of normal spleen cell populations differ. These responses are controlled by more than one autosomal dominant gene. No linkage of one of these genes to the *H-2* histocompatibility locus could be demonstrated.

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[1] *Abbreviations used in this paper*: Mouse strains: BALB/c = BALB/cJ, C3H = C3H/HeJ, AKR = AKR/J, C57BL/6 = C57BL/6J, $B6CF_1$ = (C57BL/6J × BALB/c)$F_1$ (female parent designated first), ($F_1$ × C57BL/6) backcross = offspring of a $B6CF_1$ female mated with a C57BL/6 male; Con A, concanavalin A; FCS, fetal calf serum; PHA, phytohemagglutinin; T cells, thymus-derived lymphocytes.

## *Materials and Methods*

*Mice.*—BALB/c, C3H/HeJ, AKR/J, and C57BL/6 mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. Adult mice of both sexes were maintained on standard laboratory chow and water *ad libitum* and were studied at 10–24 wk of age. (C57BL/6 × BALB/c)$F_1$ and ($F_1$ × C57BL/6) backcross animals were bred in our animal-care facility at Harvard Medical School. 7-12-wk-old B6C$F_1$ and backcross animals of both sexes were used.

*Media.*—RPMI-1640 supplemented with 100 units each of penicillin and streptomycin was used for all cultures. Media and separate antibiotic supplements (Microbiological Associates, Inc., Bethesda, Md.) mixed before use or RPMI-1640 with penicillin and streptomycin preadded (Associated Biomedic Systems, Inc., Buffalo, N. Y.) gave identical results, and both were used. Medium L-15 without glutamine (Microbiological Associates), supplemented with 10% fetal calf serum (FCS, various lots), was used as a diluent for cytotoxicity assays.

*Mitogens.*—Concanavalin A (Con A, lot No. 5594), obtained from ICN Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland, Ohio, was stored at 30 mg/ml in sterile saline at room temperature, since the mitogenic activity of Con A was found to decrease after a few days' storage in RPMI-1640 at 4°C. Such a preparation was used in the experiment described in Fig. 4. The shape of the Con A dose-response curve was nevertheless noted to change (shift to the left) after prolonged storage of the mitogen in saline at room temperature. The studies summarized in Fig. 5 were done using a 10-month-old preparation of Con A stored in saline, so the shape of the dose-response curve differed from the other experiments, which were carried out with the same Con A preparation at an earlier date.

Purified phytohemagglutinin (PHA) MR69 from Wellcome Research Laboratories, Beckenham, England (lot K1275, 13.2 mitogenic units and < 0.5 hemagglutinating units per milligram), was stored frozen in sterile saline at −20° or −70°C and was diluted in RPMI-1640 immediately before use. Its mitogenic properties did not appear to change with storage.

*DNA Synthetic Response.*—Mice were partially exanguinated from the orbital venous sinus and killed by cervical dislocation; and their spleens were removed under sterile conditions. Cell suspensions were prepared in RPMI-1640 plus antibiotics by pressing pieces of individual spleens through 250-gauge wire mesh (Industrial Wire Products Corp., Los Angeles, Calif.). Cells were washed once with medium, and tissue cultures were set up according to the method described by Stobo et al. (4). Cultures of 1.5 ml total volume in 12 × 75-mm plastic culture tubes (No. 2058, Falcon Plastics, Oxnard, Calif.) contained 1 million spleen cells in RPMI-1640 plus penicillin and streptomycin plus 5% FCS (lot No. 171170, Grand Island Biological Co. Grand Island, N. Y. or lot No. 378B, Colorado Serum Co., Denver, Colo.) and the appropriate concentration of mitogen. Triplicate cultures were prepared for each concentration of Con A or PHA. After 48-hr incubation at 37°C in an atmosphere of 95% air + 5% $CO_2$, 1 $\mu$Ci of thymidine-$^3$H (specific activity 1.9 Ci/mmole, Schwarz/Mann, Orangeburg, N. Y.) in 0.1 ml RPMI-1640 was added, and the cells were collected onto 0.45-$\mu$ Millipore (Millipore Corp., Bedford, Mass.) filters 18 or 24 hr later. After two washes with 1 mM unlabeled thymidine in phosphate-buffered saline, two 5-ml aliquots of 1 mM thymidine in ice-cold 5% trichloroacetic acid (TCA) were passed through each filter, followed by 5 ml of 95% ethanol. The precipitated material was solubilized in 5 ml of Aquasol (New England Nuclear, Boston, Mass.), and samples were counted on a Beckman model LS-250 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.) The DNA synthetic response stimulated by mitogens was defined as the increment in mean counts per minute of triplicate stimulated cultures compared with unstimulated cultures. The values reported under Results represent the means ± standard error for the DNA synthetic response determined for spleen cell populations from animals at each mitogen concentration.

*Antisera.*—Anti-$\theta$C3H was prepared by immunizing AKR/J mice with C3H/HeJ or C3HeB/FeJ thymus cells. Batch I was prepared by immunizing AKR/J mice with 20 million C3H/HeJ thymus cells intraperitoneally. These animals received five weekly booster doses of

10 million cells and a single additional injection 10 wk after the fifth boost. Batch II was prepared by immunizing AKR/J mice with 15 million C3HeB/FeJ thymus cells and 0.2 NIH antigenic units of killed *Bordetella pertussis* vaccine (V-1035, Control 5CN98A, Eli Lilly & Co., Indianapolis, Ind.), followed by five weekly booster doses of 10 million cells and, after 1 month, by two weekly booster doses of 10 million C3H/HeJ thymus cells. All animals were bled 1 wk after the last injection.

Monospecific anti-$H\text{-}2^d$ [anti-31, prepared by immunizing (C57BL/6 × A)$F_1$ against B10.D2] ascitic fluid was the generous gift of Dr. Henry Winn. Multispecific anti-$H\text{-}2^d$ was prepared in collaboration with Dr. Eric Martz, by immunizing C57BL/6 mice with P815 mastocytoma (DBA/2 origin) cells. Both anti-$H\text{-}2^d$ preparations were specifically cytotoxic for BALB/c and B6C$F_1$ lymph node cells, but not for C57BL/6 lymph node cells. ($F_1$ × C57BL/6) backcross animals were designated $H\text{-}2^{bd}$ if both anti-$H\text{-}2^d$ sera were specifically cytotoxic to their lymph node cells. In all animals tested, the monspecific and multispecific anti-$H\text{-}2^d$ sera gave identical typing results.

*Cytotoxicity Tests.*—Equal volumes of cell suspension, 5 million cells/ml in L-15 + 10% FCS, and diluted antiserum were mixed and incubated for 15 min at 37°C. The cells were washed once in L-15 + FCS, resuspended in 1:32 normal guinea pig serum (lot No. 1051401, BBL, BioQuest Div., Becton, Dickinson & Co., Cockeysville, Md.), and incubated at 37°C for 30 min. After another wash in L-15 + FCS, the cells were resuspended in 0.02% trypan blue in L-15 without FCS and added immediately to a hemocytometer chamber. The percentage of dead (blue) cells was determined by counting 100 or more lymphoid cells under high-power magnification. Antiserum dilutions maximally lytic for spleen cells were chosen. A modified cytotoxicity test in which the washing steps were omitted and the incubation temperatuse was 25–27°C was used with batch I anti-$\theta$C3H. Normal mouse serum was obtained from (AKR/J × C3H/HeJ)$F_2$ mice.

## RESULTS

*Mitogen-Stimulated DNA Synthetic Responses of Spleen Cells from Various Inbred Mouse Strains.* –Preliminary studies of the DNA synthetic response of BALB/c spleen cells stimulated by Con A in a culture system containing 5% FCS from a single lot (No. 171170) indicated that peak responsiveness occurred at 1 $\mu$g/ml Con A. Different lots of fetal calf serum resulted in different mitogen-stimulated responses for the same spleen cell population. Therefore, comparisons can be made only among responses obtained with the same lot of FCS. Spleen cells from several individual animals of various strains were cultured under these conditions (Table I). Cells from BALB/c mice ($H\text{-}2^d$) responded nearly three times as well as C57BL/6 ($H\text{-}2^b$) cells, as illustrated by the difference in thymidine-$^3$H incorporation between Con A–stimulated and unstimulated cultures. AKR and C3H (both $H\text{-}2^k$) cells showed an intermediate response that was significantly different from those of both BALB/c and C57BL/6. It should be noted that the responses of AKR and C3H cells were not significantly different even though thymidine-$^3$H incorporation by unstimulated AKR spleen cells was nearly four times greater than by C3H cells (Table I).

*Mitogen-Stimulated Responses of BALB/c, C57BL/6, and B6C$F_1$ Cells.*—Data presented in Fig. 1 show the dose-response curves of the Con A–stimulated DNA synthetic response of spleen cells from BALB/c, B57BL/6, and

$B6CF_1$ mice. C57BL/6 spleen cells responded least well at all doses of Con A, and no significant differences were detected between BALB/c and $B6CF_1$ cells at any Con A dose. In these and subsequently presented studies, FCS lot No. 378B was used.

TABLE I

*Concanavalin A–Stimulated DNA Synthesis by Spleen Cells From BALB/c, C3H/HeJ, AKR/J, and C57BL/6 Mice*

| | Mouse strain | | | |
|---|---|---|---|---|
| | BALB/c | C3H/HeJ | AKR/J | C57BL/6 |
| *H-2* specificity | $H\text{-}2^d$ | $H\text{-}2^k$ | $H\text{-}2^k$ | $H\text{-}2^b$ |
| Number tested | 24 | 10 | 9 | 14 |
| Thymidine-$^3$H incorporation by unstimulated spleen cells* | 2122 ± 404 | 1559 ± 282 | 6033 ± 2089 | 1154 ± 258 |
| Viability of unstimulated cultures after 72 hr‡ | 79.5% ± 0.9 | 77.7% ± 3.5 | 78.0% ± 4.5 | 79.8% ± 3.9 |
| Concanavalin A–stimulated thymidine-$^3$H incorporation§ | 69,600 ± 2811 | 42,082 ± 7803 | 44,935 ± 5858 | 25,597 ± 2717 |
| *P* values of Con A–stimulated responses‖ | | | | |
| cf. BALB/c | — | <0.001 | <0.001 | <0.001 |
| cf. C57BL/6 | <0.001 | <0.05 | <0.005 | — |

* Thymidine-$^3$H incorporation data are expressed as mean ± standard error of TCA-precipitable cpm for triplicate cultures of 1 million spleen cells. Cultures of 1.5 ml containing 5% fetal calf serum (lot No. 171170) in RPMI-1640 were incubated at 37°C in 95% air + 5% $CO_2$ for 72 hr with 1 μCi of thymidine-$^3$H (specific activity 1.9 Ci/mmole) added for the final 24 hr. Adult mice of both sexes were used.

‡ Viability was determined by trypan blue exclusion on cultures from three or four separate spleens.

§ Data are expressed as mean ± standard error of the increment of thymidine-$^3$H incorporation in cpm of cultures stimulated by 1.0 μg/ml Con A compared with incorporation of unstimulated cells from the same spleen.

‖ Student's two-tailed *t* test.

Similar differences between BALB/c and C57BL/6 cells were observed when the PHA-stimulated DNA synthetic response for these spleen cells was studied (Fig. 2). BALB/c cells again responded significantly better than C57BL/6 cells at PHA concentrations of 0.5 μg/ml or greater. As with Con A, the response of $B6CF_1$ cells was not significantly different from that of BALB/c cells, but it was significantly different ($P < 0.025$) from that of C57BL/6 at PHA concentrations of 1 μg/ml or higher.

(Table II contains the *P* values for a two-tailed comparison by Student's *t*

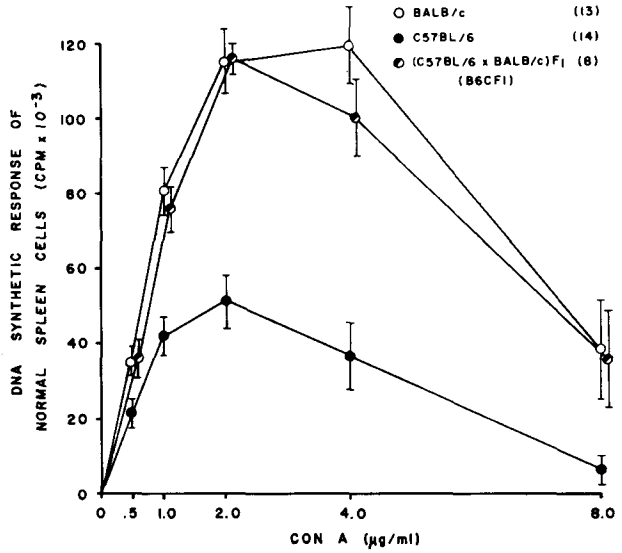


FIG. 1. Concanavalin A-stimulated DNA synthetic responses of normal spleen cells from BALB/c, C57BL/6, and B6CF$_1$ adult mice of both sexes. Numbers in parentheses represent the number of spleens tested. Data, mean ± standard error, are expressed as the difference in TCA-precipitable cpm between Con A–stimulated and unstimulated cultures. 1 million spleen cells were incubated at 37°C in 5% FCS (lot No. 378B) in RPMI-1640 under 95% air + 5% $CO_2$ for 72 hr total with 1 µCi of thymidine-$^3$H (specific activity 1.9 Ci/mmole) added for the final 24 hr. Cultures of cells from separate spleens were done in triplicate at each Con A concentration.

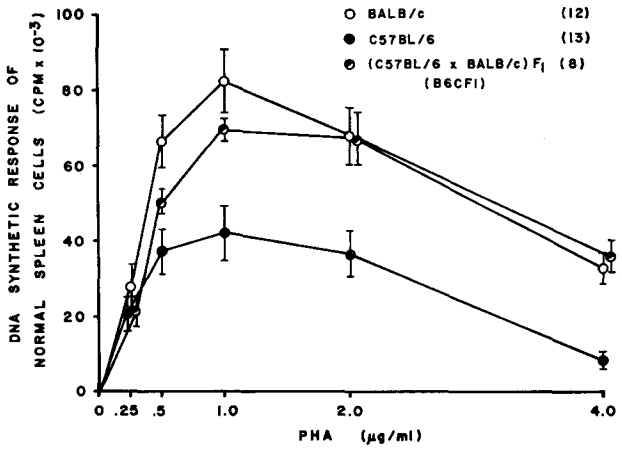


FIG. 2. Phytohemagglutinin-stimulated DNA synthetic responses of normal spleen cells from BALB/c, C57BL/6, and B6CF$_1$ adult mice of both sexes. Experimental details were identical to those described under Fig. 1, with PHA used in place of Con A.

test of the arithmetic mean DNA synthetic responses and unstimulated thymidine-$^3$H incorporation for the data plotted in Figs. 1 and 2.)

*Effects of Total Culture Time and Cell Number.* –The differences between BALB/c and C57BL/6 spleen cell DNA synthetic responses stimulated by Con A or PHA were shown not to be due to differences in optimal culture times for the two cell types. Cultures were incubated for 48, 72, 96, and 120 hr with 1.33 μg/ml Con A or 0.33 μg/ml PHA, and 1 μCi of thymidine-$^3$H was added for the final 18 hr. In each case, BALB/c spleen cells incorporated a significantly higher number of cpm compared with C57BL/6 cells.

TABLE II

*Statistical Analysis of the Differences in the In Vitro DNA Synthetic Responses among BALB/c, C57BL/6, and B6CF$_1$ Spleen Cells Stimulated by Various Concentrations of Con A or PHA*

| | *P* values of Con A–stimulated responses | | | | | |
|---|---|---|---|---|---|---|
| | | Con A dose (μg/ml) | | | | |
| | Unstimulated | 0.50 | 1.0 | 2.0 | 4.0 | 8.0 |
| BALB/c cf. C57BL/6 | >0.20 | <0.05 | <0.001 | <0.001 | <0.001 | <0.025 |
| BALB/c cf. B6CF$_1$ | >0.40 | >0.90 | >0.60 | >0.80 | >0.20 | >0.90 |
| B6CF$_1$ cf. C57BL/6 | >0.50 | <0.10 | <0.001 | <0.001 | <0.001 | <0.02 |
| | *P* values* of PHA–stimulated responses | | | | | |
| | | PHA dose (μg/ml) | | | | |
| | Unstimulated | 0.25 | 0.5 | 1.0 | 2.0 | 4.0 |
| BALB/c cf. C57BL/6 | >0.10 | >0.40 | <0.01 | <0.005 | <0.01 | <0.001 |
| BALB/c cf. B6CF$_1$ | >0.30 | >0.50 | >0.10 | >0.20 | >0.95 | >0.60 |
| B6CF$_1$ cf. C57BL/6 | >0.60 | >0.90 | >0.10 | <0.025 | <0.02 | <0.001 |

* Comparison by Student's *t* test of mean responses presented in Figs. 1 and 2.

When increasing numbers of C57BL/6 cells were cultured under identical conditions, 4 million C57BL/6 spleen cells showed a Con A–stimulated DNA synthetic response similar to that of 1 million BALB/c cells (Fig. 3). Similarly, 0.5 million BALB/c cells responded to Con A better than 1 million C57BL/6 spleen cells (Fig. 4), but the response was not directly proportional to cell number. Similar cell dose phenomena were observed in the case of PHA-stimulated responses.

*Theta-Positive Cells in BALB/c, C57BL/6, and B6CF$_1$ Spleens.*—Since Con A and PHA stimulation of DNA synthesis is predominantly a property of thymus-derived cells, the proportions of θ-positive cells in BALB/c, C57BL/6, and B6CF$_1$ spleens were determined, using two batches of anti-θ serum and two separate cytotoxicity testing techniques (Table III). Regardless of the method used for calculating the proportion of θ-positive cells, BALB/c spleens had the highest value. However, the only significant difference at the 0.01 level was

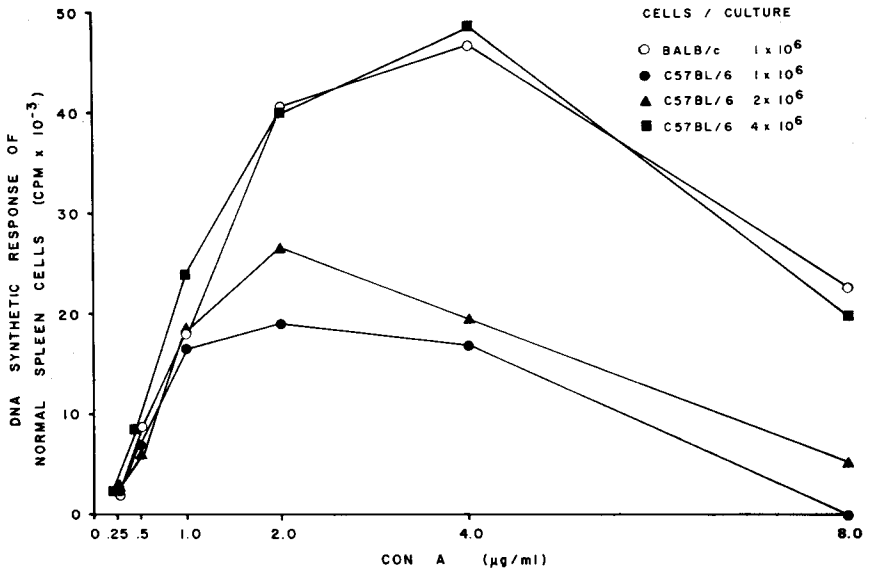


FIG. 3. Concanavalin A–stimulated DNA synthetic responses of 1 million BALB/c spleen cells and varying numbers of C57BL/6 spleen cells.Culture conditions were as described under Fig. 1 except that thymidine-$^3$H was added for the final 18 hr.

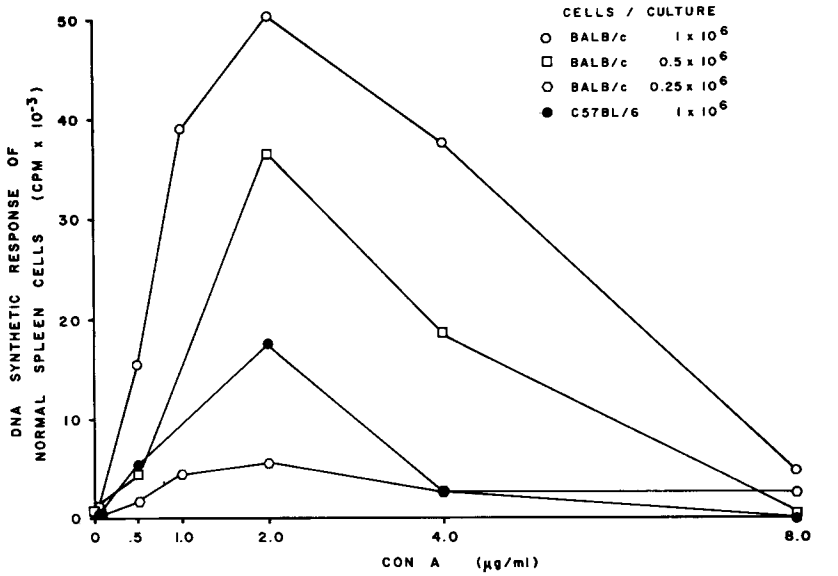


FIG. 4. Concanavalin A–stimulated DNA synthetic responses of 1 million C57BL/6 spleen cells compared with varying numbers of BALB/c spleen cells. Culture conditions were as described under Fig. 1 except that the Con A preparation had been stored in RPMI-1640 at 4°C for 5 days.

between the cytotoxic index of BALB/c compared with $B6CF_1$ spleen cells. This difference depended on the fact that more $B6CF_1$ than BALB/c cells were dead in control tubes with normal mouse serum. We were unable to demonstrate a statistically significant correlation between the proportion of $\theta$-positive cells and the peak Con A– or PHA-stimulated DNA synthetic response of spleen cells from individual BALB/c, C57BL/6, or $B6CF_1$ mice.

TABLE III

*Θ-Positive Cells in Spleens from BALB/c, C57BL/6, and $B6CF_1$ Mice*

| | Mouse strain | | | | | |
|---|---|---|---|---|---|---|
| | BALB/c | | C57BL/6 | | $B6CF_1$ | |
| | anti-$\theta$* | CI‡ | anti-$\theta$ | CI | anti-$\theta$ | CI |
| Anti-$\theta$C3H serum§ | | | | | | |
| Batch I (5 spleens) | 35.6‖ ± 4.4 | 30.4 ± 4.3 | 28.6 ± 3.7 | 18.4 ± 1.8 | 27.8 ± 2.5 | 19.5 ± 2.1 |
| Batch II (5 spleens) | 39.8 ± 2.2 | 29.7 ± 3.4 | 34.8 ± 5.4 | 24.0 ± 6.4 | 36.0 ± 1.6 | 20.3 ± 2.6 |
| Combined values (10 spleens) | 37.7 ± 2.4 | 30.1 ± 2.6 | 31.7 ± 3.2 | 21.2 ± 3.3 | 31.9 ± 2.0 | 19.9 ± 1.6 |

* Per cent dead cells with anti-$\theta$C3H serum prepared by immunizing AKR/J mice with C3H/HeJ (batch I) or C3HeB/FeJ (batch II) thymus cells.

‡ CI = cytotoxic index =

$$100 \times \frac{\% \text{ dead cells with anti-}\theta \text{ serum} - \% \text{ dead cells with NMS.}}{100 - \% \text{ dead cells with normal mouse serum (NMS)}}$$

§ Separate cytotoxicity techniques were used on different spleen cell populations. For batch I, cells (0.3 million/ml) plus antiserum or NMS (1:60) plus guinea pig complement (1:24) were incubated at 25–27°C for 45 min. For batch II, cells (2.5 million/ml) plus antiserum (1:40) were incubated at 37°C for 15 min, washed with medium L-15 + 10% fetal calf serum, resuspended in 1:32 guinea pig complement (absorbed with agarose and/or shown to be noncytotoxic at this dilution), incubated at 37°C for 30 min, washed as before, and resuspended in freshly prepared 0.02% trypan blue in medium L-15. Percentage of dead (blue) cells was determined immediately in a hemocytometer chamber under high-power magnification.

‖ Mean ± standard error.

*Mitogen-Stimulated Responses of ($F_1$ × C57BL/6) Backcross Animals and Relation to H-2 Specificity.*—Spleen cells from 11 10-wk-old ($F_1$ × C57BL/6) backcross littermates were tested for their in vitro DNA synthetic responses to Con A and PHA. Lymph node cells from each animal were tested for the presence of the $H\text{-}2^d$ allele with both monospecific anti-31 and multispecific anti-$H\text{-}2^d$ antisera. Data presented in Fig. 5 show that the Con A–stimulated DNA synthetic responses of these littermates varied over more than a sixfold range. This variability may be compared with the responses of spleen cells from

BALB/c or C57BL/6 littermates cultured on the same day, which normally varied by less than 10% at each mitogen dose. Individual responses were not correlated with sex or with the presence of the *H-2*$^d$ specificity characteristic of BALB/c and B6CF$_1$ cells. Because a different Con A preparation was used,

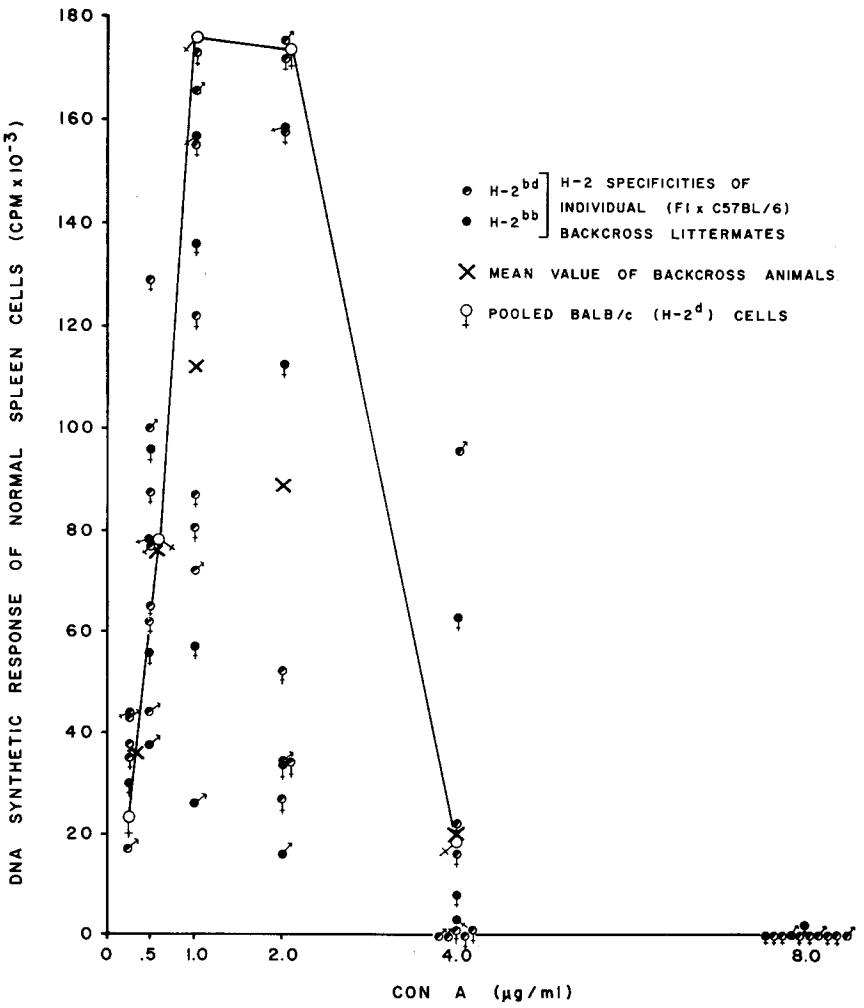


FIG. 5. Concanavalin A–stimulated DNA synthetic responses of spleen cells from individual (B6CF$_1$ × C57BL/6) backcross littermates. *H-2* specificities were determined on lymph node cells of each mouse by cytotoxicity tests with monospecific anti-31 antibody (anti-*H-2*$^d$) plus rabbit complement and with multispecific anti-*H-2*$^d$ antiserum (C57BL/6 anti-DBA/2) plus guinea pig complement. The sex of individual backcross animals is indicated by the appropriate symbol. Culture conditions were as described under Fig. 1 except that the Con A preparation used here had been stored in saline solution at room temperature for approximately 10 months.

the curves and points in Fig. 5 cannot be directly compared with those in Fig. 1; but it appears that the range of responses for backcross animals was equal to, or greater than, the range of responses of BALB/c and C57BL/6 cells.

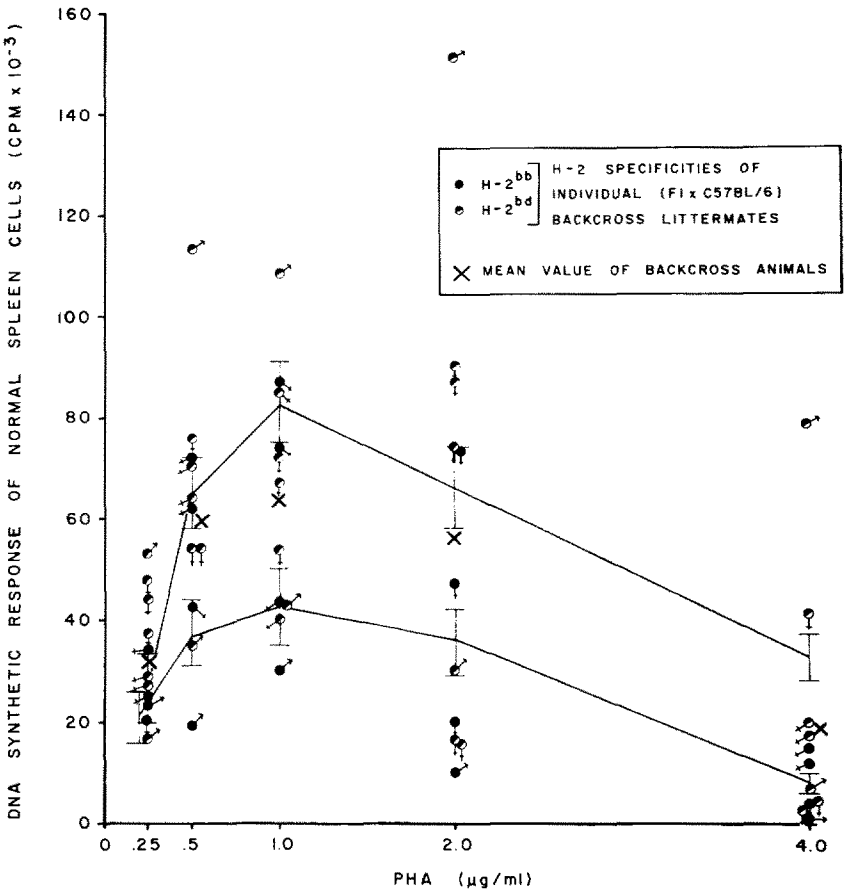


FIG. 6. PHA-stimulated DNA synthetic responses of spleen cells from individual ($B6CF_1$ × C57BL/6) backcross littermates. Culture conditions were identical to those described under Fig. 1, with PHA used in place of Con A. These spleen cells are from the same animals described in Fig. 5. The mean responses of BALB/c and C57BL/6 spleen cells from Fig. 2 are repeated for comparison.

PHA-stimulated DNA synthetic responses of spleen cells from the same backcross animals are shown in Fig. 6. In this case the average response of backcross animals showed the same relation to PHA dose as that of BALB/c and C57BL/6 cells. Like the Con A–stimulated responses, the individual ($F_1$ × C57BL/6) backcross spleen cell responses were unrelated to the sex or *H-2*

specificity of the backcross animal cells. Individual backcross animals' responses spanned a range greater than that of BALB/c and C57BL/6 cells, while the average response for all backcross animals fell in between the C57BL/6 and BALB/c levels at PHA concentrations of 0.5 $\mu$g/ml or greater.

The mean DNA synthetic response of ($F_1 \times$ C57BL/6) backcross littermate spleen cells with *H-2*$^{bd}$ specificity compared with those with *H-2*$^{bb}$ specificity were not significantly different ($P > 0.10$) at any dose of Con A or PHA.

When individual backcross littermates were ranked according to the thymidine-$^3$H incorporation of unstimulated spleen cells compared with the peak Con A– or PHA-stimulated DNA synthetic response, a significant ($P < 0.001$)

TABLE IV

*Rank Correlation of Peak Con A– and PHA-Stimulated DNA Synthetic Responses with Thymidine-$^3$H Incorporation by Unstimulated Spleen Cells from ($F_1 \times$ C57BL/6) Backcross Littermates**

| | Correlation with thymidine-$^3$H incorporation by unstimulated cells | | |
|---|---|---|---|
| | $r$‡ | $t$§ | $P$ value |
| Unstimulated | 1.000 | — | — |
| Con A–stimulated‖ | 0.900 | 6.882 | <0.001 |
| PHA-stimulated | 0.873 | 6.145 | <0.001 |

* 11 backcross animals described in Figs. 5 and 6.

‡ Spearman's rank correlation coefficient, $r = 1 - \frac{6 \Sigma d^2}{n^3 - n}$ where d = rank difference for each of n = 11 backcross littermate spleens.

§ Student's $t = r \sqrt{\frac{n - 2}{1 - r^2}}$ with n – 2 degrees of freedom ($n \geq 10$).

‖ Rank correlation coefficient for peak Con A–stimulated and peak PHA-stimulated DNA synthetic response was 0.827 ($P < 0.005$).

correlation was observed in both cases. The correlation between the peak Con A– and the peak PHA-stimulated DNA synthetic response was slightly less significant ($P < 0.005$) (Table IV).

## DISCUSSION

The observations reported here show that a constant number of spleen cells from different inbred mice display statistically significant differences in the degree of mitogen-stimulated DNA synthesis in vitro. Since the in vitro DNA synthetic response to Con A and PHA can be partially or completely blocked by pretreatment of the cells with anti-$\theta$ serum and complement (4), some of the genes that control this phenomenon must be expressed in, or regulated by, thymus-derived cells.

Spleen cells from mice of *H-2* specificities *H-2*$^d$ (BALB/c), *H-2*$^b$ (C57BL/6),

and *H-2*$^k$ (AKR and C3H) differed significantly in their responses to an optimum level of Con A. AKR and C3H spleen cells responded similarly when the measure of response was the increment in thymidine-$^3$H incorporation of Con A–stimulated compared with unstimulated cultures, although the unstimulated incorporation by AKR cells was much greater than that of C3H cells. Thus, any relationship between *H-2* specificity and mitogen–stimulated response might best be demonstrated by measuring the increment in DNA synthesis of stimulated compared with unstimulated cultures.

The response of B6CF$_1$ spleen cells stimulated by Con A and PHA was not significantly different from that of cells from the high responder BALB/c parent. This observation plus the similarity of responses between cells from male and female B6CF$_1$ animals, suggests that autosomal dominant genes play a major role in regulating this response. The dose-response curve for B6CF$_1$ cells stimulated by Con A is nearly identical to that of BALB/c cells. Thus, no gene dose effect has been observed for Con A stimulation.

The response curves of B6CF$_1$ and BALB/c cells to PHA were not the same, but the differences were not significant. However, the differences between B6CF$_1$ and C57BL/6 cell responses were statistically significant ($P < 0.025$) for doses of 1 $\mu$g/ml or higher.

Spleen cells from littermates of a backcross of the B6CF$_1$ animals to the low-responder C57BL/6 strain varied widely in their response to both Con A and PHA. Therefore, neither response is controlled by a single dominant gene. There was also no obvious correlation of mitogen-stimulated response with sex or *H-2* specificity of the backcross animals. These observations rule out the possibility that the Con A– or PHA–stimulated DNA synthetic responses are controlled by single dominant genes linked to the *H-2* locus. The data are consistent with polygenic control, and the possibility of linkage between some pertinent genes and histocompatibility loci remains open.

Genetic control of mitogen-stimulated DNA synthesis in vitro could operate at many levels. Strain differences that affect the ability of the cells to function optimally under the culture conditions employed may be irrelevant to the mitogenic response as a measure of thymus-derived cell function. Although no strain differences in viability of unstimulated cultures were observed, the requirements of maximally stimulated cultures may differ among strains. A genetically controlled difference in the cell cycle length, as has been noted in other genetically controlled responses of mouse cells,[2] could also account for the differences observed.

The relationship between cell number and mitogenic response for both the low-responder C57BL/6 and the high-responder BALB/c strains suggests the possibility of genetic differences in the proportion of an essential cell type in the spleen; but although high-responder BALB/c spleens also contained the highest

[2] Biozzi, G. Personal communication.

proportion of $\theta$-positive cells, no simple relationship between the proportion of $\theta$ positive cells and the mitogenic response was observed.

The mitogen-stimulated DNA synthetic responses of individual backcross animals were positively correlated with the level of thymidine-$^3$H incorporation by unstimulated spleen cells. A positive correlation between PHA- and Con A–stimulated responses was thus an expected consequence. Since the binding of Con A and PHA on the lymphocyte cell surface is inhibited by different sugars, which suggests different receptors (5–7), one might conclude that the genetic differences observed here reflect genetic control of DNA synthesis by, or its stimulation in, thymus-derived cells at a level other than antigen or mitogen recognition. These genetically controlled differences in the response of T cells to mitogens may therefore be the counterpart of the selection obtained by Biozzi et al. (8) at the B cell level in their high and low antibody-producing mouse lines. These investigators have demonstrated that these two lines of mice differ primarily in the rate of division of the precursors of antibody-producing cells.[2] If this interpretation is correct, the genetic differences observed in this study could be used to select high- and low-responder lines for functions of thymus-derived cells.

SUMMARY

Concanavalin A– or phytohemagglutinin-stimulated DNA synthetic responses of 1 million normal mouse spleen cells in vitro were significantly different among various inbred strains. BALB/cJ (*H-2*$^d$) responded better than C57BL/6J (*H-2*$^b$) spleen cells, and the responses of C3H/HeJ or AKR/J (both *H-2*$^k$) cells were intermediate. These responses, measured as the increment in thymidine-$^3$H incorporation of mitogen-stimulated compared with unstimulated cultures, varied according to the number of cells cultured or the mitogen concentration. BALB/c spleens had the highest proportion of $\theta$-positive cells, but no direct relationship between the proportion of $\theta$-positive cells and the DNA synthetic response was observed. (BALB/cJ × C57BL/6)$F_1$ spleen cells responsed as well as BALB/c cells. Responses of spleen cells from ($F_1$ × C57BL/6) backcross littermates varied over a range equal to, or greater than, that of BALB/c and C57BL/6 cells. There was no correlation between *H-2* specificity (*H-2*$^{bd}$ or *H-2*$^{bb}$) or sex and the mitogen-stimulated DNA synthetic response of backcross animals. Con A– and PHA-stimulated responses of individual backcross animals were positively correlated with the level of thymidine-$^3$H incorporation by unstimulated spleen cells. These results are consistent with autosomal dominant, non– *H-2*–linked, polygenic control of the mitogen-stimulated in vitro DNA synthetic response of mouse spleen cells.

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