IMMUNE RESPONSES IN VITRO

VI. Genetic Control of the In Vivo-In Vitro Discrepancies in 19S Antibody Synthesis*

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Contact with an antigenic substance results in a complex sequence of cellular and molecular events, the consequence of which is the acquisition of specifically sensitized lymphoid cells. These sensitized cells can synthesize and secrete antibody or react with the antigen via cell-mediated-type reactions. Despite the complexity of immune-associated events, the orderly process and predictable nature of many biochemical parameters involved indicate that the response is efficiently regulated. Thus the controlling mechanisms most likely are also complex, and it is probable that the majority have yet to be described.

One experimental means of investigating biological control mechanisms is the use of genetically defined "mutant" animals. Such mutants possessing immunologically associated genetic differences have already played important roles in understanding the intricacies of the immune response. Guinea pigs which are either responders or nonresponders to synthetic antigens have facilitated the analysis of antigen recognition (1 and references therein). Rabbits have been instrumental in determining the cell source of genetic information for the protein chains of immunoglobulins via allotypic markers (2, 3). Different mouse strains have been used to study the genetic control of the magnitude of the antibody response (1 and references therein, 4–9), the genetic analysis of differential responses to determinants on the same immunogen (10–12), and the genetic mapping of immune response genes (1 and references therein). Even though the variety of antigens to which the response is genetically influenced in mice is rapidly being enlarged, the immunological cell type in which the genetic markers are expressed is just beginning to be clearly defined (12–15). Moreover, mutations have yet to be instrumental in defining cell functions or immunological control mechanisms.

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In a previous report (16), a culture system was described in which the in vitro responses of inbred strains of mice to sheep red blood cells (RBC)[1] did not always parallel their in vivo responses. Although only a limited number of strains was studied, the relationship of the in vivo-in vitro responses appeared to be strain specific. In the present report, the number of strains examined for this phenomenon has been extended. The results indicate that (*a*) the number of plaque-forming cells (PFC) expressed in certain strains after in vivo sensitization does not portray the potential number of PFC; (*b*) three types of strain-dependent in vivo-in vitro relationships exist; (*c*) a loss or impairment in vitro of some mechanism that regulates the extent of PFC production in vivo occurs; (*d*) this loss or impairment is under genetic control; and (*e*) the in vitro response is controlled by two unlinked autosomal genes. Of the possible explanations which may account for these findings, evidence is presented which suggests that the different in vitro responses are not a result of various culture artifacts.

Materials and Methods

Mice.—2–5-month old male inbred, F_1 hybrid, or backcross generation mice from our own colony were used for all experiments (female animals were also used in the backcross experiments). For all in vitro experiments, cells from the spleens of a minimum of three mice of the same age were pooled.

Culture Conditions.—The culture conditions have been described elsewhere (16). Briefly, the mice were killed by cervical dislocation and single cell suspensions were aseptically prepared from their spleens in cold phosphate-buffered saline (PBS). The cells were washed once, after which the mouse RBC were lysed by exposing the cells to distilled water for less than 5 sec. After this treatment, the cells were washed twice and suspended in 0.5 ml of PBS per spleen equivalent.

Unless indicated otherwise, triplicate cultures of $7.5–8.5 \times 10^6$ cells were incubated with 2×10^7 sheep RBC (SRBC) of either high (H)- or low (L)-stimulator type or 2×10^7 horse RBC (HRBC) as the antigen. Cells were cultured in 35-mm dishes containing 2 ml of a modified Eagle's-Hanks' medium supplemented to contain 5×10^{-5} M 2-mercaptoethanol (unless otherwise indicated) and 10% fetal calf serum. The cultures were incubated at 37°–38°C in a humidified atmosphere of 10% O_2–5% CO_2–85% N_2. The number of plaque-forming cells was assessed for each culture by a modified Jerne assay. Cell viability was determined on the day of assay by eosin exclusion. Data are expressed as PFC/10^6 viable cells. The standard error within triplicate cultures was in each case less than 15%.

RESULTS

Strain Dependency of PFC Responses.—Table I shows the in vitro and in vivo responses of 13 strains of mice to H-type SRBC. The in vivo responses of the strains fell into two classes: low (<500 PFC/10^6 cells) or high (>1500 PFC/10^6 cells). Low responders possessed the *b* and *ja* alleles at the major histocompatibility locus (*H-2*), whereas high responders possessed alleles *k*, *d*, *q*, and *bc*.

[1] *Abbreviations used in this paper:* A cells, accessory cells; B cells, bone marrow-derived cells; CI, coupling index; HRBC, horse red blood cells; MET, mercaptoethanol; PFC, plaque-forming cells; PBS, phosphate-buffered saline; RBC, red blood cells; SRBC, sheep red blood cells; T cells, thymus-derived cells.

The magnitude of the responses of spleen cells from the same strains stimulated in vitro indicates that the strains were either high (>10,000), intermediate (4000–8000), or low responders (<3000). Comparison of the in vivo and in vitro responses of each strain permits a further classification. For convenience, this classification is referred to by a coupling index (CI), which is defined as the ratio of the response in vitro to that in vivo. A CI less than 1.5 was found for BALB/c, CBA, C3H, AKR, and 129, which indicates a tight in vitro-in vivo coupling. C57BL and WC strains exhibited the largest CI (>25) implying an uncoupled in vitro response. DBA/2 and DBA/1, while classified as high responders in vivo, exhibited intermediate responses in vitro; their CI of 3–5 indicates a partial in vivo-in vitro uncoupling.

TABLE I

Comparison of the Responses of Various Mouse Strains to H-Type SRBC

Mouse strain	H-2 allele	PFC/10^6 cells					Coupling index
		In vivo*	In vitro‡				
			Exp. 4-23	7-9	2-13	7-26	
WC (W/+)	*ja*	447 ± 113§	11,150 (48)‖	—	21,000 (47)	13,000	34
(+/+)	*ja*	437 ± 74		—	21,500 (45)		
C57BL/10Sn	*b*	450 ± 85	10,900 (42)	13,000	—	15,200	29
C57BL/6J	*b*	416 ± 70	10,300 (37)	—	12,900 (39)	16,000	31
C57BL/6J							
(Sl^d/+)	*b*	428 ± 180	14,000 (43)	—	11,000 (41)	15,500	32
(+/+)	*b*	430 ± 59		—	12,000 (44)		
C57BL/KA	*b*	291 ± 16	3,660 (56)	—	—	11,400	26
129/J	*bc*	2540 ± 435	1,370 (56)	1,750	—	1,870	0.7
DBA/2J	*d*	1575 ± 50	7,850 (35)	5,100	5,690 (45)	4,230	3.6
BALB/cHe	*d*	1820 ± 325	1,800 (30)	1,940	3,600 (38)	2,200	1.3
C3H/He	*k*	2100 ± 120	1,480 (38)	—	2,260 (32)	1,950	0.9
AKR/J	*k*	1970 ± 122	1,030 (30)	—	1,600 (31)	2,130	0.8
CBA/J	*k*	2455 ± 600	1,400 (30)	3,300	2,450 (46)	—	1.0
CBA_{t6t6}/J	*k*	3900 ± 100	—	—	—	3,090	0.8
DBA/1J	*q*	1770 ± 235	5,220 (28)	4,850	3,370 (40)	7,220	3.1

* Animals were injected (intraperitoneally) with 2.5×10^8 SRBC 4 days before assay.

‡ Determined after 4 days of culture.

§ Standard error.

‖ Numbers in parentheses represent per cent cell viability, i.e. (No. of viable cells recovered/No. of viable cells seeded) × 100.

The fluctuation in cell viability that appeared within experiments No. 4-23 and 2-13 was not strain associated; it was later found to be a result of the 20% error in the counting technique employed (17). In recent experiments (see Table IV) replicate counting of each sample has eliminated such fluctuations, thus resulting in comparable cell recovery for all strains.

Genetic Control.—Results presented in Table II indicate the type of genetic control involved in the in vivo and in vitro responses of three F_1 hybrids obtained by crossing a high in vitro responder (WC) to three low in vitro responders (AKR, C3H, and BALB/c). The in vitro as well as the in vivo responses of the three crosses were intermediate. The CI of each indicates that

TABLE II

Comparison of the Responses of Various F_1 Hybrids and Parental Strains to H-Type SRBC

Mouse strain	PFC/10^6 cells			Coupling index
	In vivo*	In vitro‡		
		Exp. 1-29	7-2	
WC	440	14,000	12,320	30
AKR	1970	400	—	<1
(WC × AKR)F_1	675	4,500	—	7
(AKR × WC)F_1	—	5,200	—	—
C3H	2100	—	690	<1
BALB/c	1820	—	1,720	<1
(WC × C3H)F_1	875	—	4,800	6
(WC × BALB/c)F_1	1280	—	6,000	5

* Animals were injected (intraperitoneally) with 2.5×10^8 SRBC 4 days before assay.

‡ Determined after 4 days of culture.

the in vitro responses were all partially uncoupled from their respective in vivo responses.

The number of genes involved in the control of the in vitro responses was determined with a high and low responder (WC and AKR). The distributive profile is shown in Fig. 1 for the in vitro responses of the two parental strains, F_1 hybrids, and backcross animals. The minimum difference between the responses of the two parental strains was fivefold. The responses of F_1 hybrids were intermediate to those of the parental strains and did not overlap the range of either parental response. Although the lack of overlap is not readily apparent for the responses of WC and F_1 animals, this is a result of combining the data from three independent experiments, in which the responses of parent, F_1 hybrid, and backcross animals were lower in one experiment than in the other two. The responses of all backcross animals in which AKR was the parent fell within either the F_1-response range or the AKR-response range. Although not clearly shown (again because three independent experiments were com-

bined), within an experiment the responses of the other backcross animals fell within the range encompassed by either the F_1 or the WC responses.

Table III summarizes the results of six experiments in which the segregation of the in vitro responses of backcross animals is correlated to the sex and coat color of the animals. The responses of both backcross generations were found to

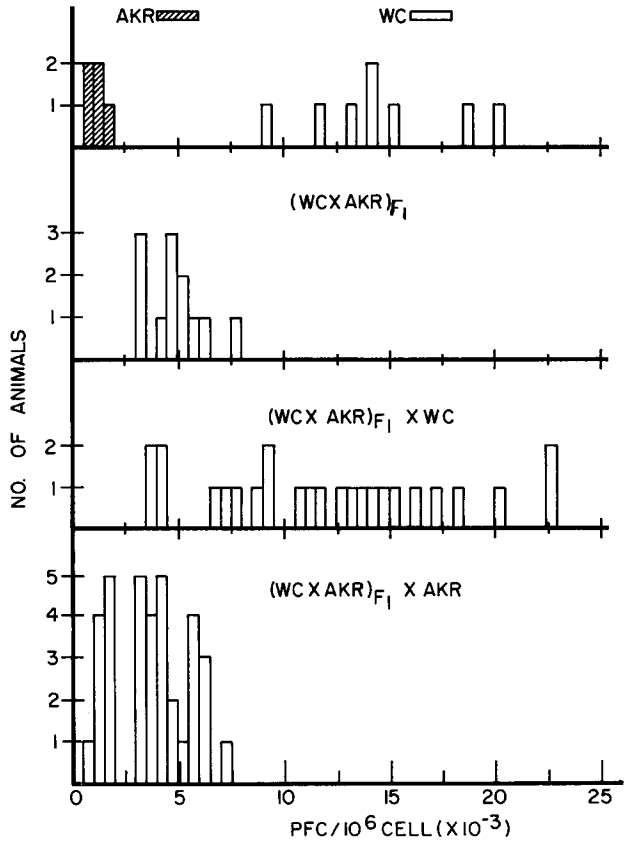

FIG. 1. The immune responses of animals falling within intervals of 500 PFC/10^6 cells.

segregate as expected for two unlinked genes.[2] The responses of the backcross animals were associated with neither sex nor coat color.

Hypothesis for In Vivo-In Vitro Discrepancies.—Various hypotheses can be advanced to explain the differences in the relationships of the in vivo and

[2] The segregation data for the backcross descendants of WC were independent of the stimulator-type of SRBC (H or L). Preliminary experiments (footnote 3) indicate that the in vitro responses of the other backcross descendants may segregate differentially against the two types of SRBC.

in vitro PFC responses. The following experiments will focus on one possible explanation: the phenomenon is an artifact of the culturing technique and therefore has no biological significance. Various parameters tested that could differentially affect the responses of high, intermediate, and low responders were the kinetics of the response, the dose-response relationships, and the requirement for certain culture components.

Kinetics of the Responses.—To ascertain whether the culture medium altered the response kinetics of different types of responders, the number of PFC was determined at different time intervals for a high, intermediate, and two low

TABLE III

Correlation of Sex and Coat Color to the In Vitro Responses Against SRBC in Backcross Descendants of WC and AKR

Backcross and sex	Coat color	Number of backcross animals responding like		
		WC	(WC × AKR)F_1	AKR
F_1 × WC*				
Female	Black	7	3	0
Male	"	19	4	0
Not determined‡	"	10	5	0
Total		36	12	0
F_1 × AKR§				
Female	Black	0	2	1
	White	0	0	0
Male	Black	0	12	4
	White	0	9	5
Total		0	23	10

‡ Both males and females were used; the sex was not recorded.

* The segregation data for this backcross are a composite of responses to both H- and L-type SRBC.

§ The segregation data for this backcross are for responses to H-type SRBC only.

responders. As shown in Fig. 2, the shapes of the curves are similar for the four strains. During the first 24 hr the number of PFC increased three- to fivefold. The maximum rate of increase occurred between days 1 and 3 and thereafter declined. The maximum response occurred between days 4 and 5. In addition to the differences in magnitude and rate, the number of background PFC/10^6 spleen cells in unimmunized animals was strain associated; 0.2–0.4 PFC/10^6 cells were found in WC and DBA/2 compared to 2–5 PFC/10^6 cells present in BALB/c and CBA.

Number of Cells Cultured.—In vitro antibody synthesis against RBC requires three cell types: thymus-derived (T), bone marrow-derived (B), and accessory (A) cells (18–20). The possibility that the high, intermediate, and low in vitro responses of various strains are a consequence of differential survival in culture

of these cell types was examined by determining the dose-response dependency for different types of responders. As shown in Table IV, as the number of cells cultured was increased from 2.8–2.9 $\times$ 10^6 to approximately 7 $\times$ 10^6, the number of PFC increased similarly for all strains irrespective of the antigen. It is clear that the number of cells required for a maximal response to occur was

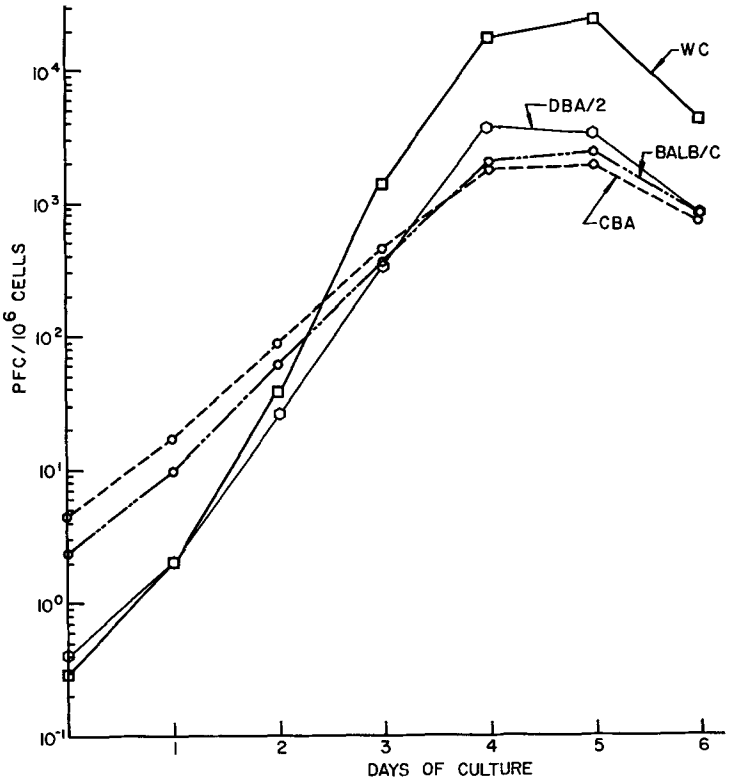

FIG. 2. Kinetics of PFC production in vitro. WC, DBA/2, BALB/c, and CBA spleen cells were cultured with H-type SRBC for indicated times.

similar for different types of responders and similar for the three antigens tested.

Fig. 3 presents a log-log plot of selected data from Table IV. The slopes of lines in such a plot have been used to estimate the number of cell types interacting in a response (20). Transitions in the slopes of such curves have been interpreted to mean that an upper limit to the degree of interaction between cells has been reached (21). It would be expected, therefore, that major differences in the distributions of T, B, and A cells in the various responders would be indicated by different slopes and by different cell inputs at which transitions

occur. As shown, the slopes and points of transitions were similar for the responses of WC, DBA/2, and CBA against either H- or L-type SRBC. Likewise, the slopes and points of transition were similar for all responses against HRBC. The slopes of the responses against HRBC were greater than those found for SRBC at cell input levels less than 5×10^6 but were similar at higher cell inputs. Variations in the responses at cell-input levels greater than 8.5×10^6

TABLE IV

Cell Dose Dependency of Responses to RBC

Stain	Number of cells cultured ($\times 10^{-6}$)	Cell recovery %	PFC/10^6 cells cultured with*		
			H-type SRBC	L-type SRBC	HRBC
WC	2.85	39	2120	440	350
	4.75	40	8200	1510	2520
	7.60	57	12100	1630	3010
	8.55	54	11400	1810	3500
	10.4	48	11200	1360	4200
CBA	2.84	32	325	—	8
	4.72	40	845	—	160
	7.55	48	1300	—	295
	8.55	55	1510	—	144
	10.4	54	1560	—	340
C3H/DiSn	2.86	35	—	74	30
	5.10	37	—	111	126
	6.90	43	—	200	226
	8.55	44	—	345	270
	9.70	45	—	216	318
DBA/2	2.94	37	—	630	137
	4.90	40	—	1155	1800
	6.85	43	—	1700	2350
	7.90	57	—	1750	1950
	9.80	56	—	695	630

* Determined against indicated RBC after 4 days of culture.

cells are most likely a result of differences in the lots of culture medium since the responses of WC and CBA against HRBC and H-type SRBC were determined in an independent experiment.

Culture Components.—The culture systems generally used by other investigators to study "primary" antibody synthesis (22, 23) have failed to show the strain-dependent in vivo-in vitro response dichotomy observed under the present culture conditions. Even though many of the ingredients of this culture medium have been shown to be important for maximum antibody synthesis (16), the distinguishing component is the reducing agent, mercaptoethanol (MET). Fig. 4 shows the effect of various concentrations of MET in the medium

on the number of PFC produced by a low and a high responder strain against H-type SRBC and HRBC. The optimal concentration of MET was 2.5–5.0 × 10^{-5} M for the response of both strains to both antigens. Although a slight difference in optimal concentration is evident for the two antigens, repeated experiments would be necessary to establish this conclusively.

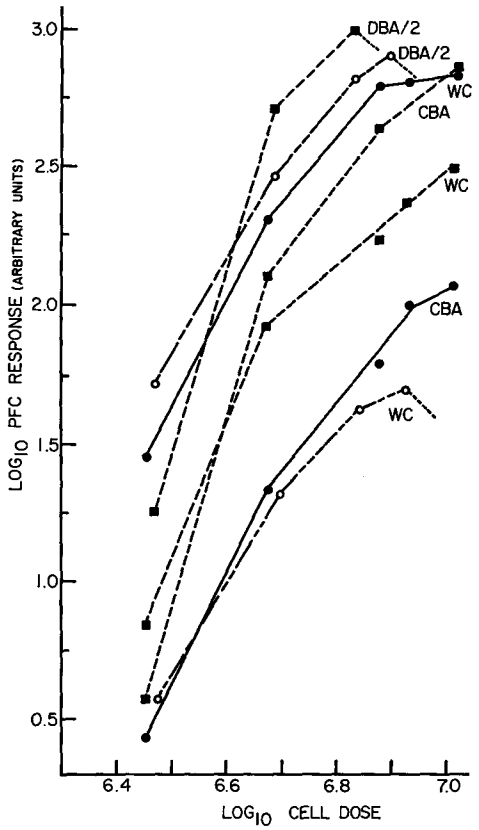

FIG. 3. Dose-response curves for WC, DBA/2, and CBA against HRBC (■----■), H-type SRBC (●——●), and L-type SRBC (○----○).

Other components of the medium (nonessential amino acids, glutamine, nucleic acid precursors) which are necessary to maximize the response (16), similarly affected the responses of various strains to SRBC.[3]

DISCUSSION

Genetic Control.—The 13 strains tested against H-type SRBC can be classified as high, intermediate, or low responders in vitro. These responses contrast with

[3] Click, R. E., and L. Benck. Unpublished results.

those found in vivo; thus an additional classification can be made based on the relationship of the in vivo and the in vitro responses. The in vitro responses of BALB/c, C3H, CBA, CBA_{t6t6}, AKR, and 129 were low and were tightly coupled to their respective in vivo responses (CI < 1.5). The responses of DBA/2 and DBA/1 were intermediate in vitro and were partially coupled to their high in vivo responses (CI = 3–6). All C57 and WC strains were low in vivo responders and high in vitro responders; thus these strains had the highest

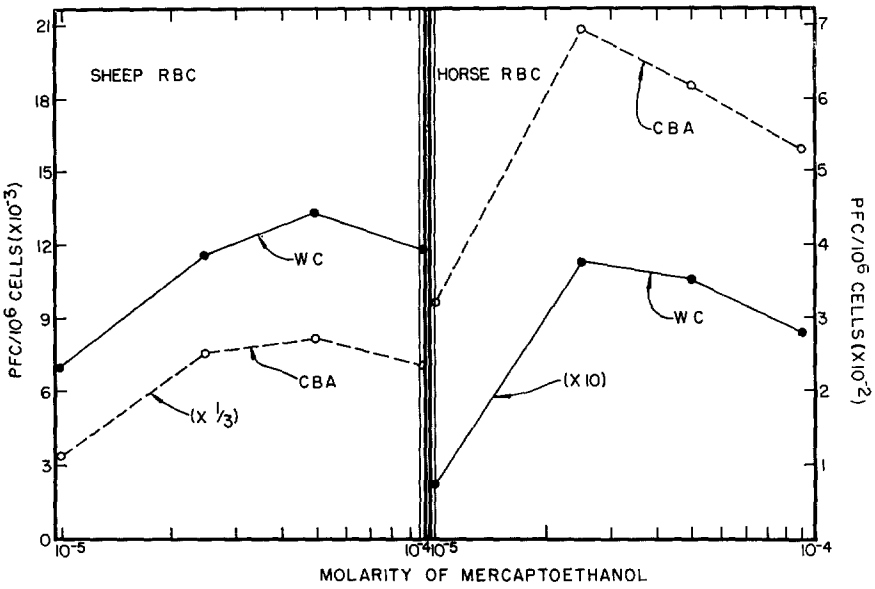

FIG. 4. PFC production at various concentrations of mercaptoethanol. WC and CBA spleen cells were cultured 4 days in the presence of either H-type SRBC or HRBC. It should be noted that the PFC response of CBA to SRBC is one-third that of the ordinate scale and the PFC response of WC to HRBC is 10 times that of the ordinate scale.

coupling indices (CI > 25) (all these responses to SRBC as well as those to HRBC have previously been demonstrated to be antigen specific [16]).

The type of genetic control of the in vivo and in vitro responses to SRBC appears to depend on gene dosage as suggested by the intermediate responses of (WC × AKR)F_1, (WC × C3H)F_1, and (WC × BALB/c)F_1 hybrids. Results obtained with other F_1 hybrids[3] suggest that other types of control also exist in vivo; e.g., (WC × 129)F_1 hybrids do not respond intermediate to that of the two parental strains but, rather, respond as high as the 129 parent. The different controlling element operative in this F_1 hybrid compared to the other three F_1 hybrids indicates that there are at least two kinds of high in vivo responders.

The genetic control of the in vitro response to SRBC was further analyzed

with backcross animals. The segregation of the in vitro responses of ($F_1 \times$ WC) animals was that expected for two unlinked genes; this finding is consistent with the report that three genes control the in vivo response to SRBC in BALB/c and NZB mice (24). The 2.3:1 segregation for the in vitro response of ($F_1 \times$ AKR) animals indicates that the alleles of each locus show dominance in their control of the response. The results also indicate that the products of the two genes interact in some manner since (*a*) a single dominant allele at either locus resulted in a response similar to that in which both loci possessed a dominant allele, and (*b*) the presence of two dominant alleles at either locus resulted in a maximum response independent of the allele distribution at the other locus. This conclusion must be somewhat tentative, since an additive effect on the response by the dominant alleles of the two genes most likely would not have been detectable because of the difficulty in differentiating a response intermediate to that of the F_1 hybrids and parents. The findings do, however, rule out control by a single gene or by two genes, one of which is expressed recessively (see appendix in reference 25 for analysis of genetic data).

The independent segregation of the sex, coat color, and in vitro responses of the backcross animals indicate that the genes controlling these phenotypes were not linked. The in vivo and in vitro responses were also not influenced by heterozygosity at the *W* locus or the *Steel-Dickie* locus.

Mechanisms Underlying the In Vivo-In Vitro Response Discrepancies.— Various hypotheses can be advanced to explain the in vivo-in vitro dichotomy and the controlling mechanisms of the unlinked genes. Selective cell growth or function does not adequately explain the strain differences. Pertinent findings leading to this conclusion are the following. (*a*) Except for the rate and magnitude of PFC production, the kinetics of the in vitro responses of various strains were indistinguishable from one another as well as from their respective in vivo responses.[4] (*b*) The number of cells required to optimize the in vitro responses was similar for all classes of responders against H- and L-type SRBC and HRBC. (*c*) The relative distribution of T, B, or A cells in the spleens of various responders was similar as shown by the slopes and transition points of the log dose-log response curves. (*d*) The cell viability after 4 days of culture was relatively constant within an experiment for different strains (variations observed were not strain associated). (*e*) The dependency of the response on MET was similar for different responders and independent of the antigen.

The most conclusive argument against the possibility that culture artifacts underlie the in vivo-in vitro discrepancies comes from studies now in progress[3] in which the type of in vitro responses to SRBC and to HRBC segregate independently in backcross animals. In these experiments all culture parameters, as well as parameters related to cell-type distributions for each backcross animal, are constant.

[4] Lovchik, J. C., and R. E. Click. Manuscript in preparation.

If the genetically controlled discrepancy of in vivo and in vitro responses cannot be accounted for by culture artifacts, it must be associated in some manner with the regulation of the in vivo responses. A similar conclusion has been suggested to explain the parallel segregation of the ability to respond to many unrelated antigens in Swiss mice selectively bred for their responses to sheep and pigeon RBC (26). Even though it is not known whether these two genetically controlled systems are related, two possible explanations for the findings are (*a*) strains differ in their physiological milieu in which lymphoid cells function; or (*b*) strain variations are a result of differential sensitivity of the lymphoid cells to a common suppressive factor. Irrespective of whether either alternative is correct, the results indicate a loss or impairment in vitro of some mechanism that regulates the extent of antibody synthesis in vivo. Since this mechanism is strain dependent and genetically controlled, it differs from other types of control mechanisms postulated for 19S antibody synthesis, e.g., 7S antibody feedback (27, 28) or immunosuppression by the factors elaborated as a consequence of antigen competition (29). Thus, at least two in vivo control mechanisms must be considered operative in controlling the 19S response to an antigen (at least SRBC). One, which all strains possess, controls the kinetics of 19S synthesis (or the time at which 19S antibody synthesis is "shut off"), and the other, which is strain dependent, regulates the magnitude of the response.

Relationship of the Loss of Control In Vitro to Other Genetic Markers.—It is not the purpose of this report to define the mechanism by which the in vivo and in vitro responses to SRBC are genetically controlled, yet further understanding of the phenomenon could be accelerated if systems analogously controlled were known. Observations that must be considered in an attempt to correlate the present classifications to other parameters are the following. (*a*) All high in vivo responder strains showed either tightly coupled or only partially coupled in vitro responses and possessed the *H-2* alleles *k*, *d*, *q* and *bc*. (*b*) All low in vivo responders possessed the *H-2* alleles *b* and *ja* and their in vitro responses were uncoupled. (*c*) The in vitro responses and the CI were not always associated with the *H-2* alleles, as demonstrated by the responses of BALB/c and DBA/2, both of which possessed the *d* allele. (*d*) The strain classification based on the CI correlated positively with the *Ig-1* alleles (30) controlling the allotypes of the 7S γ_{2A}-globulins, that is, all strains possessing the *a* and *d* alleles had a CI of <1.5, those having the *c* allele had a CI of 3–6, and those having the *b* allele had CI > 25. It should be pointed out that the accuracy of these correlations must await further documentation in other strains and backcrosses in which the segregation of the responses, the *H-2* alleles, and the *Ig-1* alleles can be determined independently.

Most other immune responses that are genetically controlled are linked to various *H-2* alleles (1 and references therein, 4–12), and not to the allotype of the gamma-globulins (5, 31). Of these, only those responses linked to the *H-2*

alleles *b* and *k*, such as those against (T, G)-A--L, (H, G)-A--L (4) and the mouse male transplantation antigen (32) correlate with the present system. However, this correlation with the response to the synthetic polypeptides is most likely fortuitous since the amount of 7S (rather than 19S) antibody synthesized was used to classify the strains against the polypeptides, whereas the present system is based on 19S antibody synthesis.

The one immune response that may be genetically related to the immunoglobulin allotype is that in which selective breeding for differential responsiveness to RBC resulted in the segregation of genes concerned with the general regulation of immunoglobulin synthesis irrespective of the antigen (26). Three different allotypic phenotypes were detected in mice of the high responder lines. One resembled the BALB/c prototype, the second that of C57BL, and the third was comparable to that of a (BALB/c $\times$ C57BL)F_1 cross in which both types of allotypic specificities were present. Of these three, the C57BL prototype was not one of those present in the high in vivo responder strains described in the present studies. In contrast to the high responder lines, all mice of the low responder lines exhibited an identical immunoglobulin phenotype which was different from those found in mice of the high responder lines. This allotype was probably a result of a crossover between the heavy-chain linkage groups of DBA/2 and NH prototypes. Likewise, all low responder strains in the present studies possessed only one allotypic allele, *b*. However, this allele (C57BL prototype) was found in the high responder selectively bred lines. Many interpretations can be advanced to explain these differences; e.g., the two studies were measurements of different genetic mechanisms of control (hemolysin was used in the present classification, whereas hemagglutinin titers against both SRBC and pigeon RBC were used to select lines in the breeding studies); alternatively, the apparent correlation of the allotype markers and the control of antibody synthesis may be fortuitous.

One genetically controlled phenomenon that does not appear to be related to the present in vivo-in vitro dichotomy is hybrid resistance to hematopoeitic tissue grafts (33). This conclusion is based on the facts that strains 129, C57BL, and WC which have different CI's have all been assigned the same hybrid resistance allele (*Hh-1*^{a}). This interpretation may, however, be incorrect due to some pecularities of 129^3 and WB (a strain closely related to WC) (34) and will be dealt with more extensively in a later publication.

A different type of hybrid resistance recently described (35) correlates better with the present findings. In this type of resistance, which is controlled by two unlinked genes, strains of mice possessing the same or slight modifications of the alleles at the *H-2* locus, e.g., 129 and C57BL, differ in their resistance to a third strain which possesses an unrelated *H-2* allele. As demonstrated by the present results these two strains also differ in their CI's.

Another genetically controlled system to which the in vivo-in vitro phenomenon may have relevance is the susceptibility to disease, specifically to

neoplasia. An example showing a close association with the present controlling system is the differential susceptibility of strains to Gross and Friend virus-induced leukemia, which is controlled by two unlinked genes for each virus (36–38). C57BL, which is refractory to the action of most strains of these two viruses, is the lowest responder in vivo, yet the highest in vitro, whereas most strains possessing CI's less than 6 (129 being an exception) are generally susceptible to leukemia induction by one of the two viruses.

Significance of the Present Mechanism of Control for Other Immune Systems.— The results of the present report indicate that (*a*) in certain strains the magnitude of the PFC response arising in vivo does not portray the potential number of PFC (a similar conclusion has been reached from results obtained from cell transfer experiments) (39); and (*b*) the in vitro loss or impairment of some mechanism that regulates the extent of antibody synthesis in vivo is under genetic control. These two findings limit the conclusions derived from classification of strains based on their in vivo responses. For example, if the observed similarity of 19S antibody synthesis by low and high responder mice to (T,G)-A--L is not an accurate portrayal of the response potential of these strains, a reconsideration of the hypothesis that the gene defect in nonresponders may lie in the marrow-derived precursors of antibody-forming cells instead of the favored hypothesis that the *Ir-1* gene is exerted through thymus-derived cells must be entertained. Further, until the importance of the present findings is extended to the control of 7S synthesis, other classifications based on 7S antibody synthesis may also be misleading.

The validity of the limiting dilution technique in enumerating immunocompetent precursors cell frequency can be questioned in view of the control mechanisms described in the present report. If it is assumed that the suppressive environment of recipient mice is unaffected by X-irradiation and, further, that the suppressive effect is similar irrespective of the number of cells injected, then the frequency of cells participating in the events of immune responses would be underestimated; e.g., an underestimation up to 30-fold could occur in some strains in which SRBC were used as the antigen (40). If, on the other hand, it is assumed that the suppressive mechanism affects T, B, or A cells differentially or that the degree of suppression is dependent on the number of cells inoculated, then the dependency of the response frequencies on the number of cells transplanted may not be described by the Poisson model. In fact, such findings have been described for transplanted marrow cells in (C57BL × WB)F_1 and (C3H × C57BL)F_1 hybrids (40).

SUMMARY

The finding that the relationship of the in vitro and in vivo responses of different strains of mice is under genetic control indicates that at least two mechanisms must operate under in vivo conditions to control 19S antibody synthesis. One is involved in the termination of 19S antibody synthesis; the

other has a regulatory role on the magnitude of the response. In light of these findings, various concepts based on other genetically controlled immune responses and on the limiting dilution technique should be reassessed. Furthermore, the suppressive in vivo mechanism may be an important type of control in the resistance or susceptibility to the establishment or maintainance of neoplasms.

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