CLONAL CHARACTER OF F_1 HYBRID LYMPHOCYTE SUBSET RECOGNITION OF PARENTAL CELLS IN ONE-WAY MIXED LYMPHOCYTE CULTURES*, ‡

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F_1 hybrid lymphoid cells proliferate in vitro in one-way mixed lymphocyte cultures (MLC)[1] with nonproliferating parental target cells (1–3). This observation has been confirmed in some systems (3), but not all (4, 5). The effectiveness of this apparent cellular immunocompetence is limited, however. F_1 hybrid mice fail to reject grafts of parental tissue and do not react detectably to their parents in graft-vs.-host experiments. Transplanted parental tissue is not ignored in all situations. For example, tumors of parental origin grow more slowly in F_1 recipients than in the parental strain (6, 7). Irradiated F_1 mice resist parental bone marrow grafts (8, 9). F_1 hybrid rats injected with parental lymphoid cells produce serum antibodies specific for parental recognition structures (10). F_1 hybrid mice given repeated injections of parental cells are more resistant to induction to GVH with parental cells than uninjected animals (11). It seemed appropriate from these considerations to seek further evidence of specific recognition responses as the basis for the observed in vitro responses of F_1 toward parental cells.

The studies to be described define F_1 hybrid cell proliferation induced by parental cells in vitro in terms of proliferating clonal F_1 cell subsets which appear to respond independently to parental structures controlled by genes in or linked to the major histocompatibility locus (MHC) of the mouse.

Materials and Methods

Animals.—A/J, C57BL/6, CBA/J, Balb/c, DBAC/2, C57BL/10(B10), C57BL/10·BR (B10·BR), C57BL/10·D2(B10·2), C57BL/10·A(B10·A), and various F_1 hybrids derived from matings of these inbred strains were obtained either directly from Jackson Laborato-

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[1] *Abbreviations used in this paper:* BUdR, 5-bromodeoxyuridine; LD, lymphocyte defined; MCA, methylcholanthrene; MHC, major histocompatibility locus; MLC, mixed lymphocyte culture.

ries, Bar Harbor, Maine or derived from inbred lines maintained in this laboratory originating from Jackson stocks. In all experiments female F_1 cells were tested with female parental cells in MLC and all cells were taken from age-matched sources.

Induction and Maintenance of Tumors.—Fibrosarcomas were produced and maintained by techniques and procedures described in detail elsewhere (12, 13).

Preparation of Lymphoid Cell Suspension.—Thymus or spleen cells were taken after exsanguination via the abdominal aorta. As described previously (14), special care was taken to exclude lymph nodes adjacent to the thymus. Cell suspensions were prepared by gently pressing small tissue fragments suspended in RPMI-1640 culture medium (Grand Island Biological Co., Grand Island, N. Y.) through 60-mesh stainless steel screens and passing the resultant suspension through 23- and 27-gauge needles.

Cell Culture System.—A modification of methods previously reported (13–15) was employed in which mixtures of 1×10^6 responding cells and an equal number of target cells were cultured in 0.5-ml vol of medium (94% RPMI, 5% fresh human serum, and 1% penicillin-streptomycin) in 12 × 75-mm sterile plastic tubes (no. 2063, Falcon Plastics, Div. of Bio-Quest, Oxnard, Calif.). DNA synthesis in the target cells was blocked by incubating 15×10^6 cells with 0.1-ml mitomycin C (Nutritional Biochemicals Corporation, Cleveland, Ohio) at a concentration of 50 μg/ml. After 40 min of incubation at 37°C, the blocked cells were washed three times and recounted before addition to the culture tubes. All cultures were incubated for 96–120 h at 37°C in a humidified 5% CO_2-air environment. 1 day before the cultures were terminated, 0.5 μl of tritiated thymidine (sp act 1.9 Ci/mM, Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) was added to each culture. After the final 24 h of incubation, all cultures were washed once with 4-ml saline, 4-ml cold 5% TCA, and 4-ml cold absolute methanol. After the methanol wash, the tubes were inverted, allowed to dry thoroughly, and the TCA precipitates solubilized in 0.1 ml of NCS solubilizer (Amersham/Searle Corp., Chicago, Ill.). The solubilized material was transferred to scintillation vials by rinsing the culture tubes with two 2.5-ml rinses of scintillation fluid. All vials were counted in a Beckman LS-250 liquid scintillation counter (Beckman Instrumetnts, Inc., Fullerton, Calif.). Cultures were set up in quadruplicate, and the results are reported as the mean ± standard error for each group of four.

Clone Elimination Protocol.—Experiments involving attempts to eliminate reactive clones represent modification of the protocol described by Zoschke and Bach (16). 48 h after MLC were initiated, 5-bromodeoxyuridine (BUdR) at a final concentration of 5×10^{-5} M was added to each culture. The cultures were reincubated at 37°C for an additional 12 h, and exposed to light by positioning the culture tube rack over a fluorescent lamp such that the bottom of the tubes were 5 cm from the tube. After a 60-min exposure, the cells in each tube were washed once and resuspended in 0.5-ml fresh culture medium. This procedure was shown to eliminate proliferating cells in allogeneic systems, as previously described (16). At this time, a second mitomycin-blocked target cell population was added. Control cultures included re-addition of the same target cell population present during the first 36 h, cells from the other parent third-party (allogeneic) target cells, or no added target cells. All cultures were reincubated for an additional 72 h; [^{3}H]TdR was added during the final 24 h after which the cultures were terminated and prepared for scintillation counting as outlined above.

Chromosome Analysis of Proliferating Cells in MLC.—Spleen or lymph node cells taken from mice of the CBA/H-T6J and (CBA/CaJ × C57BL/6)F_1 strains were used in MLC. [^{3}H]TdR was added at the prescribed 72-h time period. 4 hours before the cultures were to be harvested for scintillation counting, 0.04 ml of colcemide was added to each. At the time of harvesting, 0.1-ml aliquots of each cell culture were collected and individually prepared for chromosome analysis according to the procedures of Moorehead et al. (17). The remaining 0.4 ml of cultured cells were prepared for scintillation counting. The microscopic slides of colcemide-treated cells were stained with Giemsas and systematically examined for metaphase plates. The number of chromosomes in each plate was counted and the presence or absence of

the T6 marker noted. None of over 6,000 metaphase plates examined from one-way MLC between (C57BL/6 × CBA/CaJ)F_1 and mitomycin-treated parent CBA/H-T6J contained the T6 marker.

RESULTS

F_1 Hybrid Stimulation by Parental Spleen Cells in One-Way MLC.—The original observation in which F_1 spleen cells were stimulated by parental cells (1) has been extended to F_1 hybrid combinations of available congenic lines on the C57BL/10 background. Representative data are given in Table I. Each combination shown is representative of data obtained from at least five identical experiments. These experiments extend the previously reported results (1) to multiple combinations; each F_1 shows significant DNA synthesis in response to parental cells. These observations may be interpreted to indicate that F_1 hybrid proliferation, in the presence of mitomycin-blocked parental cells, signifies stimulation by parental cell structures controlled by genes in or linked closely with the MHC chromosome region of the mouse.

Alternative interpretations of this basic observation have been explored extensively. It was first important to establish that proliferation in MLC was limited to F_1 cells. This was approached in two ways. First by including mitomycin-treated target cells alone as controls in each experiment and finding that these do not proliferate (see Table I). Secondly by examining MLC between F_1 hybrids resulting from matings between CBA/HeT6T6 with other strains and determining whether the F_1 or parental cell population proliferates. Table II illustrates the results of six experiments and confirms that proliferation is limited to the F_1 hybrid cells. Blocking efficacy has been further established by extensive dose-effectiveness studies of the mitomycin-blocking technique employed, as illustrated in Table III.

A second alternative interpretation is that the blocked parental cells are stimulated by allogeneic histocompatibility structures in the F_1 cell membrane and secrete a "blastogenic" factor which thereupon triggers F_1 proliferation. This mechanism would not depend upon DNA synthesis in the parental cell as a preamble to secretion, and the proliferation initiated would be nonspecific to the F_1 hybrid. Such factors have been reported in several in vitro systems (18–22). Target cells in MLC also release alloantigens into the supernatant fluid of MLC, but the power of such fluids to stimulate was specific to the alloantigen (23). This possible explanation was examined directly in experiments in which supernates from one-way F_1-parental interaction in MLC were added to syngeneic F_1 cells (Table IV) but without detectable mitogenic effect. Within the limitations of experimental design, such experiments, together with data to be reported below, appear to rule out a secreted nonspecific blastogenic factor but do not exclude the possibility of a close range cell-to-cell blastogenic effect.

A third possible alternative is that F_1 hybrid response is a result of expression of recessive genes governing recognition. The F_1 parental combinations within congenic lines shown in Table I indicate that each combination was stimulatory.

TABLE I

*Proliferation of F_1 Hybrid Spleen Cells in One-Way MLC with Target Cells of Parental Origin**

F_1 hybrid	Mitomycin-blocked target cell	[^{3}H]Thymidine incorporation
		mean cpm ± SE
(A/J × C57BL/6J)	—	1,354 ± 326
(A/J × C57BL/6J)	(A/J × C57BL/6J)	1,733 ± 288
(A/J × C57BL/6J)	A/J	7,263 ± 142
(A/J × C57BL/6J)	C57BL/6J	9,077 ± 204
—	A/J	38 ± 9
—	C57BL/6J	51 ± 14
(BALB/cJ × A/J)	—	846 ± 51
(BALB/cJ × A/J)	(BALB/cJ × A/J)	1,686 ± 133
(BALB/cJ × A/J)	BALB/cJ	3,927 ± 262
(BALB/cJ × A/J)	A/J	2,589 ± 101
—	BALB/cJ	89 ± 12
—	A/J	47 ± 4
(C3H/HeJ × DBA/2J)	—	502 ± 157
(C3H/HeJ × DBA/2J)	(C3H/HeJ × DBA/2J)	926 ± 75
(C3H/HeJ × DBA/2J)	C3H/HeJ	3,725 ± 299
(C3H/HeJ × DBA/2J)	DBA/2J	4,137 ± 654
—	C3H/HeJ	24 ± 6
—	DBA/2J	75 ± 23
(C57BL/6J × CBA/J)	—	217 ± 44
(C57BL/6J × CBA/J)	(C57BL/6J × CBA/J)	890 ± 72
(C57BL/6J × CBA/J)	C57BL/6J	5,422 ± 319
(C57BL/6J × CBA/J)	CBA/J	4,203 ± 512
—	C57BL/6J	97 ± 4
—	CBA/J	83 ± 12
(C57B10 × C57B10·A)	—	1,206 ± 427
(C57B10 × C57B10·A)	(C57B10 × C57B10·A)	1,640 ± 145
(C57B10 × C57B10·A)	C57B10	4,930 ± 650
(C57B10 × C57B10·A)	C77B10·A	3,554 ± 210
—	C57B10	49 ± 10
—	C57B10·A	93 ± 7
(C57B10·BR × C57B10·A)	—	2,338 ± 94
(C57B10·BR × C57B10·A)	(C57B10·BR × C57B10·A)	3,183 ± 138
(C57B10·BR × C57B10·A)	C57B10·BR	3,274 ± 275
(C57B10·BR × C57B10·A)	C57B10·A	3,576 ± 353
—	C57B10·BR	91 ± 9
—	C57B10·A	75 ± 2
(C57B10·BR × C57B10)	—	2,610 ± 853
(C57B10·BR × C57B10)	(C57B10·BR × C57B10)	5,640 ± 1,580
(C57B10·BR × C57B10)	C57B10·BR	4,280 ± 637
(C57B10·BR × C57B10)	C57B10	7,401 ± 719
—	C57B10·BR	124 ± 24
—	C57B10	65 ± 5

* 1×10^6 F_1 spleen cells were cocultured with an equal number of mitomycin-blocked target cells in 0.5 ml of culture medium. [^{3}H]thymidine was added at 72 h and the cultures were terminated at 96 h. The data are presented as mean values ± SE for four replicate tubes.

TABLE II

*T6 Chromosomes in Cells Comprising F_1 Hybrid Parent MLC's**

F_1 hybrid	Target cell‡	Number of metaphases without T6 chromosomes	Number of metaphases with T6 chromosomes	[^{3}H]Thymidine incorporation
				mean cpm ± SE
(CBA/CaJ × C57BL/6)F_1	—	0	0	305 ± 54
(CBA/CaJ × C57BL/6)F_1	(CBA/CaJ × C57BL/6)F_1[M]	0	0	295 ± 62
(CBA/CaJ × C57BL/6)F_1	CBA/H-T6J[M]	140	0	2,416 ± 341
—	CBA/H-T6J[M]	0	0	93 ± 8
(CBA/CaJ × C57BL/6)F_1	CBA/H-T6J	102	129	75,511 ± 3,059

* 1×10^6 F_1 spleen cells were cocultured with an equal number of mitomycin-blocked target cells in 0.5 ml of culture medium. [^{3}H]thymidine was added at 72 h, colcemide at 92 h, and the cultures were terminated at 96 h. The data are presented as mean values ± SE for four replicate tubes; the chromosomal data represent examination of over 1,000 morphologically intact metaphase plates.

‡ Target cells with the suffix [M] were mitomycin blocked. Mitomycin-blocked cells alone had [^{3}H]TdR incorporation values of less than 100 cpm.

TABLE III

*Effectiveness of Blocking of DNA Synthesis in Spleen Cells Treated with Varying Amounts of Mitomycin C**

Cell combination tested in MLC	Concentration of mitomycin (μg) used to treat C57BL/6J target spleen cells	[^{3}H]Thymidine incorporation
		mean cpm ± SE
(C57BL/6J × CBA/J) + C57BL/6J	0	59,424 ± 2,019
(C57BL/6J × CBA/J) + C57BL/6J[M]	10	14,511 ± 832
(C57BL/6J × CBA/J) + C57BL/6J[M]	25	2,340 ± 212
(C57BL/6J × CBA/J) + C57BL/6J[M]	50	3,172 ± 198
(C57BL/6J × CBA/J) + C57BL/6J[M]	100	2,754 ± 72
(C57BL/6J × CBA/J) + C57BL/6J]M]	200	2,097 ± 215
C57BL/6J	0	539 ± 22
C57BL/6J[M]	10	290 ± 74
C57BL/6J[M]	25	117 ± 18
C57BL/6J[M]	50	33 ± 4
C57BL/6J[M]	100	56 ± 7
C57BL/6J[M]	200	47 ± 9
C57BL/6J + PHA(0, 25 μl)	0	90,644 ± 8,743
C57BL/6J[M] + PHA(0, 25 μl)	10	7,140 ± 1,229
C57BL/6J[M] + PHA(0, 25 μl)	25	867 ± 256
C57BL/6J[M] + PHA(0, 25 μl)	50	153 ± 35
C57BL/6J[M] + PHA(0, 25 μl)	100	78 ± 11
C57BL/6x[M] + PHA(0, 25 μl)	200	116 ± 29

* Cultures containing 1×10^6 spleen cells were taken from a pool of 30×10^6 cells which had been incubated for 30 min at 37°C with the indicated amount of mitomycin C in 0.5 ml of culture medium and washed three times. These were incubated as target cells with 1×10^6 F_1 hybrid cells, alone, or with PHA (0, 25 μl) as indicated for 72 h. [^{3}H]TdR was added for the final 24 h. The data are mean values ± SE of four replicate tubes.

TABLE IV

*Effect of F_1 Hybrid-Parental Culture Supernatant Fluids upon Syngeneic F_1 Hybrid Spleen Cells**

Source of culture supernate	Volume supernate tested	F_1 hybrid test cell	[^{3}H]Thymidine incorporation
	μl		*mean cpm ± SE*
(A/J × C57BL/6J) + A/Jm	—	(A/J × C57BL/J)	371 ± 78
(A/J × C57BL/6J) + A/Jm	10	(A/J × C57BL/J)	239 ± 44
(A/J × C57BL/6J) + A/Jm	50	(A/J × C57BL/J)	416 ± 70
(A/J × C57BL/6J) + A/Jm	100	(A/J × C57BL/J)	390 ± 87
(A/J × C57BL/6J) + A/Jm	250	(A/J × C57BL/J)	113 ± 17
(A/J × C57BL/6J) + A/Jm	500	(A/J × C57BL/J)	201 ± 49
(C57BL/6 × DBA/2J) + DBAm	—	(C57BL/6J × DBA/2J)	535 ± 93
(C57BL/6 × DBA/2J) + DBAm	10	(C57BL/6J × DBA/2J)	416 ± 35
(C57BL/6 × DBA/2J) + DBAm	50	(C57BL/6J × DBA/2J)	690 ± 83
(C57BL/6 × DBA/2J) + DBAm	100	(C57BL/6J × DBA/2J)	612 ± 21
(C57BL/6 × DBA/2J) + DBAm	250	(C57BL/6J × DBA/2J)	552 ± 66
(C57BL/6 × DBA/2J) + DBAm	500	(C57BL/6J × DBA/2J)	627 ± 60

* In these experiments the culture supernates from various F_1 parent one-way MLC were added to cultures of syngeneic F_1 hybrid cells in the volumes indicated. The indicator cultures were then incubated 72 h; the final 24 h in the presence of [^{3}H]TdR.

These data, together with those to be described, render it quite unlikely that recessive gene expression is the explanation of the observed phenomena.

Augmentation of F_1-Parental MLC by Tumor-Bearing Mice.—Inbred and congenic mice bearing methylcholanthrene (MCA)-induced fibrosarcomas (12, 13) or after treatment with BCG (24) have expanded T- and B-lymphocyte subpopulations and a greatly augmented capacity to express primary alloantigen recognition in MLC. It was of interest, therefore, to examine tumor-bearing F_1 congenic mice for analogous augmentation of alloantigen recognition. (B10·BR × B10)F_1 mice bearing MCA tumors developed in that combination were assessed for their responsiveness to parental spleen cells. The resultant data are illustrated by the experiment shown in Fig. 1. The dose-response relationship between responding F_1 and target-parental cells indicated significantly elevated levels of responsiveness to parent in the tumor-bearing animals. These data provide further evidence that F_1 recognition responses toward parental antigens are similar to those between allogeneic cells in the same system.

Evidence for Separate Parental Recognition Subsets in F_1 Hybrids.—Several types of evidence indicate that proliferative responses to alloantigens in MLC are clonal with respect to subsets of responding cells (25–28). Two experimental approaches were made to detect separate subsets of recognition cells in the F_1 hybrid responsive for each set of parental alloantigens. The first depended upon the prediction that such subsets should respond independently and therefore additively in one-way MLC. This appears to be the case, as illustrated in Table V. Responses to parental cells at peak target to responding cell ratios

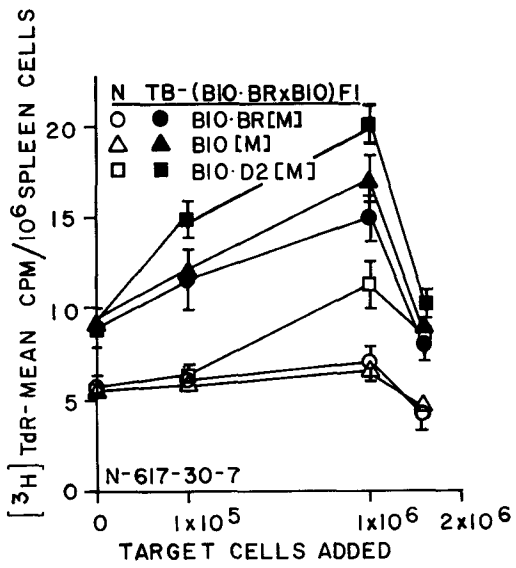

FIG. 1. Dose-response relationship of normal and tumor-bearing F_1 hybrid recognition of parent in one-way mixed culture. Spleen cells from normal (N) and tumor-bearing (TB) (B10·BR × B10)F_1 mice were tested for reactivity to several concentrations of blocked parental or alloantigenic cells (B10·D2). The values given represent means and standard errors of four replicate cultures.

TABLE V

*Effect of Mixtures of Both Parental Cells as Target Cells on Proliferation in MLC**

F_1 hybrid	Target cell‡	Ratio of reacting cells to target cells	[^{3}H]Thymidine incorporation
			mean cpm ± SE
(C57BL/6J × CBA/J)	—	—	210 ± 8
(C57BL/6J × CBA/J)	(C57BL/6J × CBA/J)[M]	1:0.5	297 ± 62
(C57BL/6J × CBA/J)	(C57BL/6J × CBA/J)[M]	1:1	771 ± 38
(C57BL/6J × CBA/J)	CBA/J[M]	1:0.5	2,300 ± 124
(C57BL/6J × CBA/J)	CBA/J[M]	1:1	2,577 ± 322
(C57BL/6J × CBA/J)	C57BL/6J[M]	1:0.5	2,100 ± 208
(C57BL/6J × CBA/J)	C57BL/6J[M]	1:1	1,853 ± 431
(C57BL/6J × CBA/J)	C57BL/6J[M] + CBA/J[M]	1:0.5	2,407 ± 194
(C57BL/6J × CBA/J)	C57BL/6J[M] + CBA/J[M]	1:1	5,516 ± 411

* 1×10^6 F_1 spleen cells were cultured with 0.5×10^6 or 1×10^6 mitomycin-blocked cells from one of the parents or with equivalent aliquots from both parents. The final culture vol was 0.5 ml in all instances. [^{3}H]TdR was added at 72 h and the cultures were terminated at 96 h. The data are given for four replicate tubes.

‡ Mitomycin-blocked cells alone always gave [^{3}H]TdR incorporation values of less than 100 cpm.

were indeed additive when both were added to F_1 responding cells in five such experiments involving three-strain combinations.

The second approach was to eliminate proliferating F_1 cells in MLC containing one parent through treatment with BUdR and light as described by Zoschke and Bach (16) and then test for residual responsiveness to the other parent. Table VI gives the results of representative experiments of this type involving different strain combinations. Elimination of the proliferating population stimulated by one parent failed reciprocally to affect responsiveness to the other, while readdition of the same parental cells resulted in no F_1 proliferation. The demonstrated adequacy of the mitomycin block of parental cells precludes allogeneic interactions between these cells. Dose-response titrations were included to eliminate the possibility of inhibitory target-responding cell ratios. These data may be interpreted to substantiate the hypothesis that F_1 proliferation in the presence of each parent is clonal; that is, generated in individual subsets of lymphoid cells. Moreover the data effectively rule out several of the alternatives considered. For example, the effects of nonspecific blastogenic factors are excluded since such factors should trigger responses in both responding subsets rather than individually specific ones in sequence. Recessive gene expression also seems unlikely to be expressed in individual cell subsets.

DISCUSSION

Experiments reported here confirm and extend the basic observation that F_1 hybrid mouse lymphoid cells proliferate in MLC with blocked parental cells. Several alternative explanations of the experimental observations were explored, including inadequacy of mitomycin C block of target cells, the possibility that nonspecific blastogenic factors produced by the blocked parent induce proliferation in the F_1, and that recessive gene expression might be involved. No direct experimental support was found for any of these alternative interpretations. The data do not rigorously exclude that blocked parental cells might initiate F_1 proliferation as a result of recognition responses on their own part. If true, this recognition response must occur in cells incapable of DNA synthesis and somehow be communicated directly and specifically as a proliferation inducing stimulus to an unblocked nonrecognizing F_1 cell. In view of the apparently clonal nature of the F_1 response it would additionally require that the antigenic sites of the recognized structure on the F_1 hybrid cell be clonally represented as well. While conceivable, this possibility has no experimental support at present. On the other hand, direct evidence is presented indicating that F_1 is in recognition of parent, is clonal, and involves separate subsets of responding cells having specificity directed toward each parent. Moreover the F_1-parent MLC appears in every way tested to be analogous to MLC reactions between allogeneic cells.

Interpreted in this way, consideration must be given (*a*) to the characteristics of and control over the recognition process and the responding cell subsets and

TABLE VI

*Clonal Character of F_1 Hybrid Subsets Stimulated by Parental Cells in One-Way MLC**

F_1 hydrid	First target cells added	Second target cells added	[^{3}H]Thymidine incorporation
			mean cpm ± SE
(C57BL/6J × CBA/J)	—	—	376 ± 42
(C57BL/6J × CBA/J)	(C57BL/6J × CBA/J)	—	118 ± 11
(C57BL/6J × CBA/J)	—	(C57BL/6J × CBA/J)	338 ± 71
(C57BL/6J × CBA/J)	(C57BL/6J × CBA/J)	(C57BL/6J × CBA/J)	210 ± 18
—	(C57BL/6J × CBA/J)	—	31 ± 4
—	—	(C57BL/6J × CBA/J)	55 ± 12
—	(C57BL/6J × CBA/J)	(C57BL/6J × CBA/J)	72 ± 16
(C57BL/6J × CBA/J)	CBA/J	—	263 ± 20
(C57BL/6J × CBA/J)	—	CBA/J	1,094 ± 135
(C57BL/6J × CBA/J)	CBA/J	CBA/J	408 ± 74
—	CBA/J	—	81 ± 14
—	—	CBA/J	27 ± 5
—	CBA/J	CBA/J	68 ± 17
(C57BL/6J × CBA/J)	C57BL/6J	—	435 ± 119
(C57BL/6J × CBA/J)	—	C57BL/6J	855 ± 76
(C57BL/6J × CBA/J)	C57BL/6J	C57BL/6J	215 ± 44
—	C57BL/6J	—	97 ± 32
—	—	C57BL/6J	45 ± 18
—	C57BL/6J	C57BL/6J	87 ± 17
(C57BL/6J × CBA/J)	CBA/J	C57BL/6J	3,025 ± 218
(C57BL/6J × CBA/J)	C57BL/6J	CBA/J	2,097 ± 109
—	CBA/J	C57BL/6J	108 ± 9
(A/J × C57BL/6J)	—	—	791 ± 55
(A/J × C57BL/6J)	(A/J × C57BL/6J)	—	524 ± 91
(A/J × C57BL/6J)	—	(A/J × C57BL/6J)	454 ± 65
(A/J × C57BL/6J)	(A/J × C57BL/6J)	(A/J × C57BL/6J)	431 ± 63
—	(A/J × C57BL/6J)	—	77 ± 22
—	—	(A/J × C57BL/6J)	34 ± 5
—	(A/J × C57BL/6J)	(A/J × C57BL/6J)	80 ± 16
(A/J × C57BL/6J)	A/J	—	325 ± 87
(A/J × C57BL/6J)	—	A/J	976 ± 101
(A/J × C57BL/6J)	A/J	A/J	612 ± 56
—	A/J	—	75 ± 7
—	—	A/J	—
—	A/J	A/J	48 ± 11
(A/J × C57BL/6J)	C57BL/6J	—	232 ± 87
(A/J × C57BL/6J)	—	C57BL/6J	704 ± 66
(A/J × C57BL/6J)	C57BL/6J	C57BL/6J	316 ± 72
—	C57BL/6J	—	95 ± 16
—	—	C57BL/6J	89 ± 12
—	C57BL/6J	C57BL/6J	61 ± 18
(A/J × C57BL/6J)	A/J	C57BL/6J	2,421 ± 203
(A/J × C57BL/6J)	C57BL/6J	A/J	2,970 ± 468
—	A/J	C57BL/6J	84 ± 27
(BALB/cJ × A/J)	—	—	1,249 ± 114
(BALB/cJ × A/J)	(BALB/cJ × A/J)	—	868 ± 35
(BALB/cJ × A/J)	—	(BALB/cJ × A/J)	1,077 ± 151

* 1×10^6 F_1 spleen cells were cultured with equal numbers of mitomycin-blocked target cells from one parent in 0.5-ml medium. The cultures were first incubated 48 h, then 5×10^{-5} M BUdR was added for 12 h followed by exposure to light for one h and subsequently removal of the BUdR. 1.0×10^6 blocked cells from the second parent or the first parent again were added to these cultures and they were reincubated for an additional 48 h. [^{3}H]-thymidine was added at 96 h and the cultures were terminated at 120 h. The data given are for four replicate tubes.

TABLE VI—*Concluded*

F_1 hybrid	First target cells added	Second target cells added	[^{3}H]Thymidine incorporation
			mean cpm ± SE
(BALB/cJ × A/J)	(BALB/cJ × A/J)	(BALB/cJ × A/J)	650 ± 113
—	(BALB/cJ × A/J)	—	26 ± 3
—	—	(BALB/cJ × A/J)	72 ± 16
—	(BALB/cJ × A/J)	(BALB/cJ × A/J)	99 ± 14
(BALB/cJ × A/J)	BALB/cJ	—	702 ± 125
(BALB/cJ × A/J)	—	BALB/cJ	1,633 ± 240
(BALB/cJ × A/J)	BALB/cJ	BALB/cJ	857 ± 119
—	BALB/cJ	—	63 ± 10
—	—	BALB/cJ	44 ± 20
—	BALB/cJ	BALB/cJ	91 ± 13
(BALB/cJ × A/J)	A/J	—	453 ± 124
(BALB/cJ × A/J)	—	A/J	929 ± 57
(BALB/cJ × A/J)	A/J	A/J	846 ± 112
—	A/J	—	57 ± 18
—	—	A/J	93 ± 9
—	A/J	A/J	71 ± 12
(BALB/cJ × A/J)	BALB/cJ	A/J	2,394 ± 308
(BALB/cJ × A/J)	A/J	BALB/cJ	2,841 ± 414
—	BALB/cJ	A/J	117 ± 32

(*b*) to the nature of the membrane structures to which the specificity of these subsets is directed. This interpretation implies further that the proliferating clones represent self-recognition cell subsets.

The F_1 recognition subsets for parental cells have been characterized as widely represented in lymphoid tissues, including the immunologically competent subpopulation of the thymus. These characteristics, taken with direct evidence that they carry the Thy-1 (θ) membrane antigen, indicated that the responding cells belong to a subclass of T cells not different from those which respond in allogeneic MLC.

The existence of specific subsets of responding T cells for each set of parental cell structures indicates that the recognition structures are not necessarily codominantly expressed in the F_1 hybrid, as is the case of membrane alloantigens. This provides a strong argument against the hypothesis that the phenomenon is explicable solely in terms of allogeneic membrane nonconformity, the allogeneic stimulation hypothesis of Lafferty et al. (29).

MLC reactions appear to involve at least two sequential elements. Initiation or permission is controlled by one set of gene loci termed lymphocyte defined (LD) in the mouse (30) or MLC in man (31). These loci are within or linked to the MHC, particularly in the *Ir-1A* gene region. It is not known whether the LD locus is polymorphic or whether its phenotype is expressed upon the recognition or the target cell, or both, or at what level specificity is expressed. The second step, cytotoxic cell generation, is thought to depend upon serologically defined structures on target cells determined by genes at the K or D or 4 and LA regions of the MHC (30). While it is conceivable that both steps

are involved in F_1-parental MLC, the proliferation step is the only effect thus far demonstrated. Efforts are being made to test for cytotoxic cell generation.

The structures on parental cells which appear to stimulate proliferation of the responsive T-cell subsets are not defined by these experiments except to limit them as determined by or linked to genes in the MHC locus. This provides a range of possibilities inherent in the expression of an estimated 1,000–2,000 loci gene including the histocompatibility antigens determined serologically, LD locus products, idiotypic and other antigenic structures determined by the *Ir* loci, virus associated structures such as X-1 (Sato et al.) or Friend-Moloney-Rauscher viruses, (32, 33) or even possibly expressions of S-tropic C-particle viruses incorporated into the genome in the IX linkage group (34). The data do not permit any precise conclusion as to which of these many structures might be involved.

The experiments reported here and correlated with various types of evidence of in vivo responsiveness (8, 11) permit speculation that F_1 hybrids and perhaps all heterozygotes carry clones of lymphoid cells capable of self-recognition and the immunologic consequences thereof. Although such a self-responding system is probably controlled or moderated in vivo through some mechanism of self-tolerance, it may have a major role in repair and disposal of normal cells in so-called autoimmune disorders and in the process of oncogenesis.

SUMMARY

Proliferation of F_1 hybrid lymphocytes in mixed lymphocyte cultures is stimulated by mitomycin-blocked parental cells. The demonstration of this phenomenon using F_1 hybrids derived from congenic lines of mice establishes that the stimulation is controlled by genes in or closely linked to the major histocompatibility locus chromosome region. In agreement with the finding that tumor-bearing mice have an increased capacity for primary alloantigen recognition, it was observed that the F_1 hybrid response to parent was also augmented by tumor bearing.

Chromosomal analysis of dividing cells in one-way mixed cultures confirms that F_1 cells, and not the blocked parental cells, enter mitosis. Stimulation of F_1 cells by a soluble mediator liberated by the parental cells was not observed and mitomycin blocking of parental cells seems to be a completely effective blocking agent ensuring that parental cells can not enter DNA synthesis.

The specificity and clonal nature of F_1 recognition of parent was demonstrated using a 5-bromodeoxyuridine-suicide procedure. Distinct clones of lymphocytes in F_1 spleen cell populations seem to recognize one or the other parent, but not both, in such experiments. These observations and others in tumor systems suggest that most or all heterozygous organisms may possess potentially self-reactive clones of lymphocytes.

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