ANTIGEN-REACTIVE T CELL CLONES

I. Transcomplementing Hybrid I-A-Region Gene Products Function Effectively in Antigen Presentation

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One of the basic tenets of the clonal selection theory is that antigen-specific precursor cells bear recognition structures directed toward a single antigenic specificity. Although the concept of clonal selection has been demonstrated at the level of B cell responses, proof of clonally distributed antigen receptors on T cells is lacking. Recently, many laboratories have attempted to establish T cell clones with specific immunologic functions. The importance of immortalized T cell lines with such immunologic function in studying the T cell receptor repertoire, mechanisms of T cell activation, and cellular interactions between T cells and other immunocompetent cells is readily obvious. Initial attempts to derive immortalized T cell clones centered around the production of immunologically competent T cell hybridomas (1). There have been several reports of immunologically functional T cell hybridomas that mediate specific suppressor function (2–5). More recently, it has been possible to serially propagate cytotoxic T lymphocytes in culture with conditioned media obtained from supernates of lectin-stimulated lymphocytes (6). It has been possible to obtain clones of cytotoxic T cells (7–9) from these long-term cultured cytotoxic T lymphocytes. Another approach has been to develop alloreactive T cell lines that are propagated by repeated serial restimulation in vitro with alloantigen (10). This technique has allowed the generation of long-term cultures of alloreactive T cells from which alloreactive T cell clones have been isolated in both the murine (11–13) and human (14, 15) systems. Recently Schreier et al. (16) have demonstrated that it is possible to propagate antigen-specific murine T cells in vitro in the presence of antigen and major histocompatibility complex (MHC)[1]-compatible filler cells resulting in specific stepwise enrichment of antigen-induced proliferative cells. The data presented in this paper will outline the kinetics and specificity of such long-term antigen-reactive T cell lines and demonstrate our ability to isolate and characterize clones of antigen-reactive T cells that recognize antigen in specific association with selected sets of antigen-presenting cells. By studying clones of antigen-reactive T cells obtained from long-term-cultured immune lymph node cells from an F_1 mouse, it has been possible to isolate certain clones that recognize antigen only in association with antigen-

* Recipient of Research Career Development Award KO4 AI 00333 from the National Institutes of Health.

[1] *Abbreviations used in this paper:* B6, C57BL/6; $(B6A)F_1$; $(A/J \times B6)F_1$; BSA, bovine serum albumin; Con A, concanavalin A; FCS, fetal calf serum; FDA, fluorescein diacetate; GAT, synthetic random copolymer poly(L-Glu^{60},L-Ala^{30},L-Tyr^{10})$_n$; HOS, horse serum; [^{3}H]Tdr, [^{3}H]thymidine, KLH, keyhole limpet hemocyanin; LPS, lipolysaccharide; MHC, major histocompatibility complex: MLR, mixed lymphocyte reaction; PHA, phytohemagglutinin; PPD, purified protein derivative of *Bacillus tuberculosis.*

J. EXP. MED. © The Rockefeller University Press · 0022-1007/80/10/0759/12 $1.00 759
Volume 152 October 1980 759–770

presenting determinants of cells of the F_1 hybrid mouse, as well as clones that recognize antigen presented by cells of either of the parents and/or the F_1. Genetic mapping studies with recombinant congenic hybrid mice have suggested that the hybrid I-A-region-restricting element for T cell clones reactive to the synthetic random amino acid copolymer poly(L-Glu^{60},L-Ala^{30},L-Tyr^{10})$_n$ (GAT) is the product of transcomplementation between I-A^b and I-A^k regions.

Materials and Methods

Mice. A/J, C57BL/6 (B6), A/J × B6)F_1[(B6A)F_1], and BALB/c mice were purchased from The Jackson Laboratory, Bar Harbor, Maine or bred from the stock in our animal facilities, Mayo Clinic, Rochester, Minn. B10.A(4R), B10.A(5R), and A.TL mice were generously provided by Dr. Chella David, Mayo Medical School, Rochester, Minn. B10.MBR mice were generously provided by Dr. David Sachs, Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md. Hybrids between these recombinant congenic mice and The Jackson Laboratory stock listed above were bred in our animal breeding facilities at Mayo Clinic. Adult mice, aged 6–20 wk, were used in all experiments.

Antigens and Mitogens. GAT (lot 10) was purchased from Miles Laboratories, Inc., Miles Research Products, Elkhart, Ind. Bovine serum albumin (BSA), phytohemagglutinin (PHA), and concanavalin A (Con A) were obtained from Sigma Chemical Co., St. Louis, Mo. Lipopolysaccharide (LPS) was obtained from Difco Laboratories, Detroit, Mich. Purified protein derivative of *Bacillus tuberculosis* (PPD) was purchased from Connaught Laboratories, Toronto, Ontario, Canada, and keyhole limpet hemocyanin (KLH) was purchased from Schwartz/Mann Div., Becton Dickinson & Co., Orangeburg, N. Y.

Culture Medium. Complete culture media consisted of RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) that contained 10% fetal calf serum (FCS) (Microbiological Associates, Walkersville, Md.), 3×10^{-5} M 2-mercaptoethanol, 12 mM Hepes, 100 U/ml penicillin, and 100 μg/ml streptomycin. L-glutamine was added at a final concentration of 2×10^{-3} M before use. For secondary in vitro restimulation of immune lymph node cells, 10% horse serum (HOS) was used instead of FCS.

Immunization and Cell Cultures. Mice (four to five/group) were immunized with 100 μg GAT emulsified in complete Freund's adjuvant (Bacto-Adjuvant; Difco Laboratories) subcutaneously at the base of the tail (17). 7 d later, draining lymph nodes (inguinal and paraaortic) were removed, and single cell suspensions were made and cultured in Costar trays (3524, Costar, Data Packaging, Cambridge, Mass.) with 200 μg/ml GAT at a cell number of 6×10^6 cells/well in 1.5 ml RPMI-1640 HOS media. 4 d later, the cells were harvested, and blast cells were centrifuged on Ficoll-Paque at 2,000 rpm for 20 min. Cells from the interphase were collected, washed twice, and recultured with 5×10^6 irradiated (3,300 rad) syngeneic spleen cells (filler cells) at a concentration of 1×10^5 cells/ml in 2 ml RPMI-1640 FCS medium. After 14 d, the cells were harvested and viable recovered cells were counted using the vital stain flourescein diacetate (FDA) (18). Briefly, FDA was added to an aliquot of cells to give a final concentration of 5 μg/ml. After 3–5 min at room temperature, viable cells were brightly fluorescent when observed in a hemocytometer using a fluorescent microscope (Carl Zeiss, Inc., New York). 2×10^5 viable recovered cells were restimulated with 200 μg/ml GAT in the presence of 50×10^5 syngeneic irradiated filler cells in 2 ml RPMI-1640 FCS media. After 4 d, the cells were harvested, and blast cells were recultured with syngeneic irradiated filler cells in the absence of antigen at a concentration of 1×10^5 blast cells and 25×10^5 filler cells/ml in 2 ml RPMI-1640 FCS media for an additional 14 d. GAT-reactive T cells have been maintained by this 4-d antigen restimulation followed by 14-d resting culture and have been cultured over a long period of time (> 8 mo at the current time). In some experiments where large numbers of cells were required, cells were restimulated and recultured in an upright position in culture flasks (3024; Falcon Labware Div., Becton Dickinson & Co., Oxnard, Calif.) in a total vol of 15 ml at the same concentrations of GAT, GAT-reactive T cells, and filler cells.

Assay of Proliferative Response. 14 d after reculture, aliquots of viable recovered cells were assayed for their proliferative response. Variable numbers of GAT-reactive T cells were restimulated with GAT in the presence of filler cells in 0.2 ml RPMI-1640 FCS media/well in

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microtiter plates (3040; Falcon Labware Div., Becton Dickinson & Co.) for 48 or 72 h. 2 μCi of [^{3}H]thymidine ([^{3}H]TdR) (Research Products International Corp., Elk Grove Village, Ill.) were added 16 h before harvest. Cells were harvested on a filter paper, and radioactivity was counted using standard scintillation counting (19). Results were expressed as the mean of triplicate cultures. The standard deviation of each mean was within 10%.

Cloning of GAT-reactive T Cells. (B6A)F_1 GAT-reactive T cells were induced as described above. After the fourth 2-wk interval of reculture, the cells were restimulated with 200 μg/ml GAT in the presence of syngeneic irradiated filler cells. 24 h later, the entire mixture of GAT-reactive T cells, filler cells, and GAT was mixed with 0.5% agar (1:2 vol/vol) and placed on a supporting layer of 0.5% agar as described previously (11). The concentration of cells in soft agar varied from 0.25 $\times$ 10^5 to 5 $\times$ 10^5 GAT-reactive T cells each in the presence of 50 $\times$ 10^6 filler cells. 5-7 d later, colonies were observed in the soft agar and these were picked and replated in individual microtiter wells with 1 $\times$ 10^6 irradiated synegeneic filler cells in the presence of 200 μg/ml of GAT. For subcloning procedures, the same techniques were utilized, but with the addition of 10% Con A-activated rat spleen cell supernate at each of these two initial steps (13). Approximately 7 d after this antigen restimulation, the cells were transferred to Costar wells with fresh irradiated filler cells (10 $\times$ 10^6/well) and fresh antigen, and subclones were removed from the influence of rat Con A supernate at this time. 7 d after this transfer, aliquots were removed from Costar wells and assayed as described above. Additionally, clones could be expanded in culture flasks as described above.

Results

Soluble-Antigen-Reactive T Cell Lines. B6 mice were immunized with the synthetic random amino acid copolymer (GAT). The immune lymph node cells were obtained and stimulated as described in Materials and Methods. 2 wk after the second in vitro restimulation, viable recovered cells were tested for their reactivity against a panel of selected antigens and mitogens in the presence of syngeneic irradiated spleen filler cells. As shown in Table I, these cells reacted specificially with GAT and did not recognize inappropriate antigens such as BSA or KLH. These cells did not react with PPD, despite the fact that regional draining lymph node cells obtained from the immune mouse at time 0 reacted strongly with PPD (data not shown). Although these cells did not respond to the T cell mitogen PHA nor to the B cell mitogen LPS, they demonstrated strong reactivity to the T cell mitogen Con A (Table I). Separate aliquots of these antigen-reactive cells were stained for cell surface markers by indirect

TABLE 1

Antigen Specificity and Mitogen Responsiveness

GAT-reactive T cells	Antigen or mitogen	μg/ml	^{3}H uptake
			cpm
B6	—	—	679
B6	GAT	200	22,271
B6	BSA	200	432
B6	KLH	100	717
B6	PPD	50	797
B6	PHA	2.5	1,175
B6	Con A	2	19,405
B6	LPS	50	2,270

B6 GAT-reactive cells were induced as described in Materials and Methods. After the third 2-wk interval of reculture, 1 $\times$ 10^4 viable recovered cells were assayed for reactivity on a selected panel of antigens or mitogens in the presence of 5 $\times$ 10^5 syngeneic irradiated filler cells. Proliferative response was assayed on day 2.

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immunofluorescence. 100% of these cells were Thy-1.2 positive and there were no demonstrable surface-immunoglobulin-positive cells (data not shown).

(B6A)F_1 GAT-reactive T cells were induced as described in Materials and Methods. Fig. 1 shows data obtained from an antigen dose-response study of such GAT-reactive T cells, demonstrating their broad range of antigen reactivity: the optimal concentration of GAT in the range studied was 100–200 μg/ml, although as little as 10 μg/ml of GAT would stimulate these cells quite well. Although GAT-reactive T cells showed vigorous proliferative responses in the presence of antigen and filler cells, they did not respond to GAT in the absence of filler cells (Table II). Furthermore, as reported by Schwartz et al. (20) and Schreier et al. (16), there existed a necessity of MHC-region identity between responding T cells and the irradiated spleen filler cells for such GAT-induced proliferative responses. The data presented in Table III in which similarly propagated strain A GAT-reactive T cells were restimulated in the presence

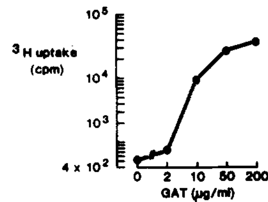

FIG. 1. Antigen dose-response of GAT-reactive T cells. 1 × 10^4 (B6A)F_1 GAT-reactive T cells were stimulated with various concentrations of GAT in the presence of 5 × 10^5 syngeneic filler cells. Proliferative response was assayed on day 3.

TABLE II

Requirements for Both Filler Cells and Antigen for T Cell Proliferation

GAT-reactive T cells	Filler cells	Antigen	^{3}H uptake
			cpm
(B6A)F_1	+	—	1,568
(B6A)F_1	+	GAT	53,199
(B6A)F_1	−	GAT	1,754

1 × 10^4 (B6A)F_1 GAT-reactive T cells were stimulated with 200 μg/ml of GAT in the presence or absence of 5 × 10^5 syngeneic filler cells. Proliferative response was measured on day 2.

TABLE III

MHC Requirements for Antigen Presentation

GAT-reactive T cells	Filler cells	MHC regions shared*	^{3}H uptake	
			Medium	GAT
			cpm	
A/J	A/J	All	727	13,363
A/J	B6	None	1,834	3,650
A/J	B10.A(4R)	KA	1,470	10,962
A/J	B10.A(5R)	JECSD	2,320	4,329
A/J	A.TL	ABJED	1,482	14,362

1 × 10^4 A/J GAT-reactive T cells were stimulated with 200 μg/ml GAT in the presence of irradiated filler cells from various strains of mice. The proliferative response was measured on day 2.

* The regions of the MHC that are shared between GAT-reactive T cells and filler cells.

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of a panel of antigen-presenting cells suggests that the ability of the antigen-presenting cells to effectively interact with the immune T cells resides in the I-A region of the MHC [i.e., the ability of B10.A(4R) and A.TL mice to present GAT effectively to the strain A GAT-reactive T cells]. Likewise, B6 GAT-reactive T cells could recognize antigen in association with B10.A(5R) antigen-presenting cells [but not B10.A(4R)], suggesting again the requirement for I-A- (or K) region sharing between the antigen-presenting cell and the T proliferative cell (data not shown).

Although such long-term-cultured GAT-reactive T cells showed antigen specificity on the limited panel studied, they still retained reactivity toward alloantigens as detected in mixed lymphocyte cultures. The data presented in Table III suggested that the long-term-cultured GAT-reactive T cells demonstrated proliferative responses against irradiated allogeneic spleen filler cells in the absence of antigen. We assayed their proliferative responses in the presence of irradiated spleen cells (in the absence of GAT) over 5 d of mixed lymphocyte culture to characterize the alloreactivity of such long-term GAT-reactive T cells. The kinetics of the proliferative response profile obtained from such GAT-reactive T cells was typical of primary mixed lymphocyte reaction (MLR) responsiveness: the peak response being on day 5 or later (data not presented).

Kinetics of Response of Soluble Antigen-Reactive T Cells. The data in Fig. 2 represent results obtained during an assay of several concentrations of GAT-reactive T cells in the presence of constant amounts of GAT and filler cells conducted on sequential days of culture. The magnitude of response is dependent upon the number of input antigen-primed cells; i.e., the actual counts per minute doubled as the number of input antigen-primed cells doubled. This suggests that it is possible to quantitate such GAT-reactive T cells. As can be seen from Fig. 2, the proliferative response peaked on day 4 with higher concentrations of GAT-reactive T cells. When lower numbers of input antigen-primed cells were used, the apparent logrithmic exponential growth pattern was maintained for longer periods of culture. This dose-response and kinetic pattern is very similar to that obtained from studying long-term-cultured alloreactive T cells (19).

Dose-Response of Filler Cells. To clarify the role of filler cells in the GAT induced proliferative responses, a titration experiment was performed in which the amounts of antigen and antigen-reactive T cells were held constant and the irradiated syngeneic spleen filler cells were titrated downward (Fig. 3). The optimal number of syngeneic filler cells was 1×10^6/well. However, as few as 1.5×10^4 filler cells/well provided significant antigen presentation for the GAT-reactive T cells in the presence of antigen.

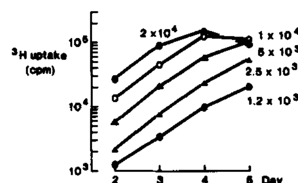

FIG. 2. Kinetic response of GAT-reactive T cells. Various numbers of $(B6A)F_1$ GAT-reactive T cells were restimulated with 200 μg/ml GAT in the presence of 5×10^5 syngeneic filler cells. The proliferative response was assessed serially for 5 d by [^{3}H]TdR uptake after an overnight pulse with 2 μCi of [^{3}H]TdR.

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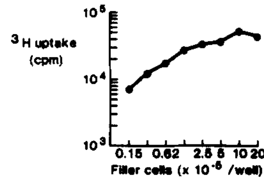

FIG. 3. Filler cell dose-response. 1×10^4 (B6A)F_1 GAT-reactive T cells were stimulated with 200 μg/ml of GAT in the presence of various numbers of syngeneic filler cells. The proliferative response was assessed on day 3 by [^{3}H]TdR uptake.

Recognition of a Unique F_1 Antigen-presenting Determinant. Previous studies of in vitro secondary MLR have suggested that there exists a unique hybrid MLR-stimulating determinant(s) on (B6A)F_1 mice (21, 22). Additionally, studies of the recognition of *Listeria monocytogenes*-immune T cells in a murine system suggested the possibility that there were subpopulations of T cells which uniquely saw *L. monocytogenes* as presented in association with F_1 cells (23). To look for such a unique F_1 antigen-presenting determinant, we induced (B6A)F_1 GAT-immune T cells and tested their response against antigen presented by either parental A/J or parental B6, a mixture of A/J and B6 cells, or the (B6A)F_1 splenic filler cells. The proliferative response of such (B6A)F_1-immune GAT-reactive T cells to antigen in association with syngeneic (B6A)F_1 filler cells was higher in three separate experiments than that observed by antigen presentation either of parent A/J or B6, or an equal mixture of both (data not shown). These data suggest that there exist clones in the (B6A)F_1 immune cells that proliferate in response to antigen associated with a unique F_1 determinant(s). To prove this hypothesis, we serially restimulated these (B6A)F_1 GAT-reactive T cells and cloned them in soft agar.

GAT-reactive T Cell Clones. (B6A)F_1 immune GAT-reactive T cells were cloned in soft agar as described in Materials and Methods. The initial results of this cloning experiment are presented in Table IV. The serially restimulated (B6A)F_1 GAT-reactive T cells responded to antigen in association with parental A/J, B6, and (B6A)F_1 filler cells. They also retained alloreactivity as evidenced by their response to allogeneic BALB/c filler cells. The colonies obtained from soft-agar cloning of this parental population can be classified into several types according to their reactivity patterns. The first type would be represented by colonies that recognized GAT presented on either or both parental filler cells A/J and B6, but not as well as they responded to GAT presented on syngeneic (B6A)F_1 filler cells (data not shown). The most likely interpretation of the reactivities of colonies of this type was that they were not truly cloned and that they contained contaminant cells that were in the area surrounding the colony we were attempting to isolate from soft agar. Subcloning experiments on these contaminated colonies are reported below and support this interpretation. The second type would be represented by colonies 6-35 in Table IV that reacted to antigen equally well in the presence of either B6 or (B6A)F_1 filler cells. The third type would be exemplified by colonies 3-43 that reacted to antigen only in the presence of (B6A)F_1 filler cells. These colonies did not respond to antigen in the presence of either parental filler cell or an equal mixture of cells from parent A/J and B6 (data not shown), suggesting that our hypothesis is correct: that there exist unique antigen-presenting structures on F_1 cells that allow selected clones of F_1-immune GAT-reactive T cells to respond to antigen. None of these colonies retained alloreac-

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TABLE IV
Colonies of GAT-reactive T Cells

Colony	Antigen	—	GAT	GAT	GAT	—
	Filler cells	$(B6A)F_1$	A/J	B6	$(B6A)F_1$	BALB/c
				cpm		
Parental uncloned population		704	1,835	7,111	16,282	4,107
6		941	719	8,498	11,727	253
18		618	1,065	18,981	21,091	380
28		356	737	15,583	12,049	523
35		887	729	22,598	23,915	630
3		661	510	520	3,398	169
17		1,245	1,945	2,039	17,708	1,160
36		554	500	655	5,079	308
43		261	270	824	24,927	473

$(B6A)F_1$ GAT-reactive T cells were cloned in soft agar as described in Materials and Methods. 0.5×10^4 to 2×10^4 GAT-reactive T cell clones were stimulated with 200 μg/ml GAT in the presence of A/J, B6, or $(B6A)F_1$ filler cells. Clones were also assayed for their reactivity toward allogeneic BALB/c irradiated spleen cells. The proliferative response was assayed on day 3 by $[^3H]$TdR uptake. The proliferative responses of the parental population from which these colonies were derived is included as control.

tivity when tested on BALB/c irradiated spleen cells. Selected colonies were serially propagated by restimulation with antigen and syngeneic filler cells. These colonies were assayed 4 or 6 wk later, and retained their specificity with time (data not shown).

Subcloning with GAT-reactive T Cell Colonies. From our original soft agar cloning of F_1-immune GAT-reactive T cells, we obtained many colonies that exhibited reactivities not readily identifiable as the response of single clones. These colonies had reactivity for antigen presented by either (or both) parental filler cells but much better reactivity for antigen presented by the syngeneic $(B6A)F_1$ filler cells. The most likely interpretation of the reactivity of colonies of this type is that they were not truly cloned. In an attempt to prove this hypothesis, we subcloned one of these apparently contaminated colonies in soft agar utilizing procedures outlined in Materials and Methods. The colony selected for subcloning, colony 5, exhibited predominant reactivity for GAT presented in association with $(B6A)F_1$ cells, although it had moderate reactivity for GAT in association with parental A/J antigen-presenting cells (Table V). Subclones were obtained as outlined in Materials and Methods. The reactivity profile of several of these subclones obtained from soft agar is presented in Table V. These data support our hypothesis that there exist unique antigen-presenting determinants on F_1 cells recognized by selected clones of F_1-immune GAT-reactive T cells. Additionally, the reactivity of subclone 12-5-a-24 suggests that our interpretation of contaminant clones that give rise to the apparently mixed reactivity patterns of certain primary colonies is correct.

Genetic Mapping of F_1-specific Antigen-presenting Determinants. The data presented above have suggested that certain T cell clones derived from F_1-immune mice recognize antigen only in association with antigen-presenting determinants that are unique to the F_1 cells. To genetically map the genes controlling the expression of such antigen-presenting determinants expressed uniquely on $(B6A)F_1$ antigen-presenting cells, we assayed the response of one of the GAT-reactive subclones to GAT in the

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TABLE V

Subclones of GAT-reactive Colony 12-5

Subclone number	A/J		B6		A/J + B6		(B6A)F1	
	Medium	GAT	Medium	GAT	Medium	GAT	Medium	GAT
					cpm			
12-5*	238	2,080	144	350	570	2,722	253	22,521
12-5-a-2	251	281	281	349	481	355	303	10,897
12-5-a-27	235	248	261	316	756	574	435	15,971
12-5-a-31	426	348	351	338	755	1,207	519	15,654
12-5-a-34	307	283	99	194	201	374	155	17,012
12-5-a-24	396	16,221	270	1,174	959	17,747	162	10,590

* Parental colony.

One of the contaminant colonies obtained in the soft-agar cloning procedure described in Table IV was subcloned in soft agar in the presence of 10% rat Con A supernate as described in Materials and Methods. The reactivity of the parental colony 12-5 is included for comparison. 1×10^4 cells were restimulated with 200 μg/ml GAT in the presence of A/J, B6, equal mixtures of A/J + B6, and (B6A)F_1 filler cells. The total number of filler cells/well was 1×10^6. The proliferative response was assayed on day 2 by [^{3}H]TdR uptake.

TABLE VI

Transcomplementation in I-A of Hybrid Antigen-presentating Determinants

Experiment	Filler cells	MHC region K	A	B	J	E	C	S	D	Medium	GAT
1	A/J	k	k	k	k	k	d	d	d	178	340
	B6	b	b	b	b	b	b	b	b	309	767
	A/J + B6	k/b	k/b	k/b	k/b	k/b	d/b	d/b	d/b	377	896
	(B6A)F_1	k/b	k/b	k/b	k/b	k/b	d/b	d/b	d/b	361	16,878
	B10.A(4R)	k	k	b	b	b	b	b	b	436	955
	[B10.A(4R) × B6]F_1	k/b	k/b	b/b	b/b	b/b	b/b	b/b	b/b	370	13,146
2	(B6A)F_1	k/b	k/b	k/b	k/b	k/b	d/b	d/b	d/b	1,547	35,350
	[B10.MBR × A.AL]F_1	b/k	k/k	k/k	k/k	k/k	k/k	k/k	q/d	788	1,055

Subclone 12-5-a-2 (Table V) was assayed on filler cells from various strains and hybrid mice in a manner similar to that described for Table III. The MHC region of these mice have been included for clarity of presentation. Data are presented as the proliferative response assayed on day 2 by [^{3}H]TdR uptake.

presence of filler cells derived from several congenic and recombinant F_1 mice as listed in Table VI. As can be seen from these data, this subclone proliferated in response to GAT presented not only by syngeneic (B6A)F_1 filler cells but also in response to GAT presented by [B10.A(4R) × B6]F_1 antigen-presenting cells. A/J, B6, B10.A(4R), and (B10.MBR × A.AL)F_1 spleen cells could not effectively present antigen to this subclone. These data suggest that the expression of the unique hybrid antigen-presenting determinant present on (B6A)F_1 spleen cells is controlled by genes mapping within the I-A subregions of the MHC. Furthermore, the inability of A/J, B6, B10.A(4R), and (B10.MBR × A.AL)F_1 spleen cells to present antigen suggests that

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the determinant utilized for antigen presentation to this particular clone of GAT immune T cells is the product of transcomplementation between the I-A loci of strain A/J and strain B6.

Discussion

Despite many reports that have demonstrated the feasibility of maintaining long-term-cultured alloreactive T cells either by repeated stimulation with alloantigen (10) or by supplementation with exogenous growth factor (6), it has until recently been difficult to culture conventional soluble antigen-specific T cells for long periods of time. Ben-Sasson et al. (24) succeeded in maintaining antigen-specific guinea pig T lymphocytes in culture by repeated restimulation with antigen-pulsed peritoneal macrophages for up to 5 wk. Recently, reports by Schreier et al. (16) and Watson (25) have demonstrated that it is possible to culture murine antigen-specific T cells in the presence of syngeneic irradiated spleen filler cells and conditioned media obtained from lectin-stimulated lymphocytes. Studies by Schreier et al. (M. Schreier. Personal communication.) have suggested that it is possible to isolate clones of murine T cells reactive with determinants present on sheep erythrocytes. Studies by Sredni et al. (26) have suggested that it is possible to isolate antigen-specific T cell clones directly from immune mice and propagate them in vitro by utilizing specific antigen, filler cells, and supernates from lectin-stimulated lymphocytes. The results described in this report clearly demonstrate that it is possible to culture soluble antigen-reactive T lymphocytes for long periods of time and to derive clones of antigen-reactive T cells in the presence of antigen and irradiated syngeneic spleen filler cells without using any additional growth supporting factors such as culture supernates of lectin-stimulated lymphocytes.

The strict requirement for MHC identity between antigen-reactive T cells and antigen-presenting cells has been well documented in antigen-driven T cell proliferative assays (27) and immune T cell macrophage-binding studies (23). More detailed mapping in antigen-driven murine T cell proliferative responses has shown that identity is required only at the I-A subregion of the MHC between filler cells and antigen-reactive T cells for proliferation in the presence of certain antigens (20). As seen in Table III, strain A/J GAT-reactive T cells respond to antigen in association with A/J B10.A(4R), or A.TL filler cells, thus demonstrating the requirement for the I-A-subregion identity between T cells and antigen-presenting cells in this system. These data support the concept that T cells do not recognize foreign antigen per se, but must recognize this antigen in association with cell surface Ia antigens (whether as altered self or dual recognition) to be activated.

Dual clonality of F_1 immune T cells has been reported in antigen-induced T cell proliferative responses (16, 28), helper T cell assay (29) and immune T cell macrophage binding assay (23). In addition to T cell subpopulations in such F_1 immune populations that recognize antigen in association with cells of one or the other of the parental types, the possibility has been suggested that there exists a third group of T cells that recognize antigen in association with an F_1-specific determinant (16, 23). The existence of an F_1 hybrid determinant has been demonstrated in the study of alloreactive T cells (21, 22), and their clones (11-13). Biochemical analysis of hybrid determinants (30), as well as serological identification of such hybrid determinants has been demonstrated (31). The data presented in this report clearly demonstrate that there

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exist antigen-presenting determinants expressed only on (B6A)F_1 cells that do not exist on either parental cell. Furthermore, these data support the existence T cell clones in F_1-immune mice that recognize antigen in association with such F_1-specific antigen-presenting determinants. The expression of the F_1 antigen-presenting determinant for (B6A)F_1 anti-GAT responses was shown to be controlled by the I-A region of the murine MHC by analyzing the effectiveness of antigen presentation by cells derived from congenic recombinant hybrid mice (Tables III and VI). We suggest that the most likely possibility is that the (B6A)F_1 antigen-presenting determinant recognized by the T cell clones described in this report is the hybrid I-A molecule consisting of either $A_\alpha^k A_\beta^b$ or $A_\alpha^b A_\beta^k$ molecules formed by transcomplementation between I-A loci in the F_1. These data support the concept that I-A α- and β-chains can freely associate in such F_1 cells to create hybrid products that are expressed on the surface as Ia antigens that function in antigen presentation. Such free combinatorial association of α- and β-chains could result in expansion of the immunological capacity of F_1 animals if each Ia antigen created by such association functions as an antigen-presenting determinant and, as suggested by Benacerraf (32), exhibits antigen-recognition specificity unique to that molecular association.

Summary

Studies in our laboratory and elsewhere have shown that it is possible to propagate antigen-specific murine T cells in vitro with resultant specific stepwise enrichment of antigen-induced proliferative cells. The proliferative responses of these T cells are antigen specific and dependent upon the presence of antigen-presenting cells (spleen cells) that share the I-A subregion with the proliferating T cell. Using techniques of soft-agar cloning, it has been further possible to isolate clones of antigen-reactive T lymphocytes from such long-term cultures. Data suggesting that these were clones of antigen-reactive T cells were obtained by studying the recognition of antigen in association with antigen-presenting cells with a panel of such clones of antigen-reactive T cells. Proof of clonality was obtained by subcloning.

Clones derived from F_1-immune mice can be divided into three separate categories: one clone recognizes antigen in association with antigen-presenting determinants of parent A and the F_1; the second type recognizes antigen in association with antigen-presenting determinants of parent B and the F_1; and the third type recognizes antigen only in association with antigen-presenting determinants of the F_1 mouse. Genetic studies on the major histocompatibility complex requirements for antigen presentation to such F_1-reactive T cell clones suggests that the hybrid antigen-presenting determinant in this system results from transcomplementation of products of the I-A region of haplotypes a and b.

These studies support the concept developed in our laboratory that there exist unique F_1 hybrid determinants on (A/J × C57BL/6)F_1 cells and suggest that these determinants can be utilized physiologically by hybrid mice in immunocompetent cellular interactions.

We wish to thank Ms. Katherine Goad for her excellent technical assistance in this project and Mrs. Shirley Behnken for secretarial assistance in the preparation of the manuscript.

Received for publication 30 April 1980 and in revised form 2 June 1980.

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