ANTIGEN-REACTIVE T CELL CLONES

II. Unique Homozygous and (High Responder × Low Responder)F_1 Hybrid Antigen-presenting Determinants Detected Using poly(Tyr, Glu)-poly D, L-Ala--poly Lys-reactive T Cell Clones*

By MASAO KIMOTO AND C. GARRISON FATHMAN‡

From the Department of Immunology, Mayo Clinic, Rochester, Minnesota 55901

The recognition by T cells of soluble antigen is one of the important steps in the initiation of immune response. Antigen recognition is a complex event and requires, in addition to immunocompetent T cells and antigen, a second cell type that can present antigen to the T cell in order for the T cell to be stimulated (1, 2). Such antigen-presenting cells must share selected regions of the major histocompatibility complex (MHC)[1] with the responding T cells in order to efficiently present antigen (3, 4). Thus, immune T cells are restricted by MHC determinants expressed on antigen-presenting cells as well as by antigen specificity. To study immunocompetent cellular interactions in depth, precise characterization of such restricting determinants is essential.

Clones of antigen-reactive T cells derived from murine systems have provided powerful tools for analyzing the nature of restricting determinants expressed on antigen-presenting cells. These clones of antigen-reactive cells have allowed us to examine the restricting determinants in the absence of effects of contaminant cells that might exert inhibitory or nonspecific allogeneic effects. In a previous paper, we showed that the poly(L-Glu^{60}, L-Ala^{30}, L-Tyr^{10}) (GAT)-reactive T cell clones derived from (C57Bl/6 × A/J)F_1 [(B6A)F_1] mice could be divided into at least three groups in terms of the restricting determinants that allowed antigen recognition (2, 3). The first group recognized antigen expressed on parent A/J (A) cells and (B6A)F_1 cells; the second group recognized antigen presented on C57Bl/6(B6) and (B6A)F_1 antigen-presenting cells, whereas the third group's recognition of antigen was restricted by determinants expressed only on the hybrid (B6A)F_1 cells which were not expressed on cells of either parent A or B6. Genetic mapping studies allowed us to show that the restricting determinants uniquely expressed on such F_1 cells were the products of transcomplementation within the I-A subregion of the mouse MHC (3). Data presented in this paper will suggest that there exist poly(Tyr, Glu)-poly D, L-Ala--poly Lys [(T,G)-A--L]-reactive T cell clones derived from (high responder × low responder)F_1 [(B6A)F_1] mice that can use transcomplementing hybrid I-A region products as antigen-presenting determinants. Thus neither I-A product independently

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; Con A, concanavalin A; FCS, fetal calf serum; GAT, poly(L-Glu^{60},-L-Ala^{30}, L-Tyr^{10}); HOS, horse serum; [^{3}H]Tdr, [^{3}H]thymidine; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; OVA, ovalbumin.

confers nonresponsiveness (i.e., neither the alpha nor beta chain of H-2^k confers low responsiveness). Additionally, evidence presented in this paper will show that the recognition of (T,G)-A--L by certain T cells clones derived from B6 mice is restricted by determinants uniquely expressed on homozygous B6 cells that are not expressed on semisyngeneic (B6A)F_1 cells. These determinants are very similar to "homozygous" alloantigenic determinants, recognized by murine alloreactive T cell clones, which are present on cells from strain B6, but not on semisyngeneic (B6A)F_1 stimulator cells (5).

Materials and Methods

Mice. A/J (A), C57Bl/6(B6), and (B6 × A)F_1 [(B6A)F_1] mice were purchased from The Jackson Laboratory, Bar Harbor, Maine, or bred from the stock in our animal facilities. B10.A(4R) and B10.A(5R) mice were generously provided by Dr. Chella David, Mayo Medical School. B10.MBR breeding stock mice were generously provided by Dr. David Sachs, Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md. The breeding stock of B6.C-H-2^{bm12} (bm12) was derived from the colony of Dr. Roger Melvold, Northwestern Medical School, Chicago, Ill. Hybrids between these recombinant congenic mice and the Jackson stock listed above were bred in our animal breeding facilities at The Mayo Clinic. Adult mice aged 6–20 wk were used in all experiments.

Antigens. (T,G)-A--L (lot MC9) was purchased from Miles Laboratories, Elkhard, Ind. Bovine serum albumin (BSA) and ovalbumin (OVA) were obtained from Sigma Chemical Co., St. Louis, Mo.

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

Immunization and Cell Culture. Cell cultures were done (with slight modification) as described previously (3). Briefly, mice were immunized with 100 μg (T,G)-A--L in complete Freund's Adjuvant (CFA; Difco Laboratories, Detroit, Mich.) subcutaneously at the base of the tail. 7 d later, cells from draining lymph nodes were cultured with 200 μg/ml (T,G)-A--L at a cell number of 6×10^6 cells/well in 1.5 ml RPMI-1640 HOS media. 4 d later, 2×10^5 blast cells were centrifuged on Ficoll-Paque (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) and cells from the interphase were recultured with 10×10^6 syngeneic irradiated (3,300 rad) filler cells in 2 ml complete media in Costar wells (Costar, Data Packaging, Cambridge, Mass.). 14 d later, 2×10^5 viable recovered cells were stimulated with 200 μg/ml (T,G)-A--L in the presence of 10×10^6 syngeneic filler cells in 2 ml complete medium. (T,G)-A--L reactive T cells were maintained by this 4-d antigen restimulation, followed by 14 d resting culture. In some experiments where large numbers of cells were needed, (T,G)-A--L-reactive T cells were restimulated and recultured in an upright position in culture flasks (Falcon 3013 or 3024, Falcon Labware, Div. of Becton, Dickinson and Co., Oxnard, Calif.) in a total volume of 6 or 16 ml at the same concentration of (T,G)-A--L, (T,G)-A--L-reactive T cells, and filler cells.

Cloning of (T,G)-A--L-reactive T Cell Clones. Long-term cultured (T,G)-A--L-reactive T cells were stimulated with 200 μg/ml (T,G)-A--L in the presence of 80×10^6 syngeneic filler cells in 6 ml complete culture media in an upright positioned culture flask (Falcon 3013). The concentration of T cells stimulated was varied from 0.3×10^5 to 1×10^5 cells in a flask. After 36 h incubation, cells in the flask were harvested, spun down, and all but 1 ml of supernate was removed by suction. The 1-ml cell suspension was mixed with 2 ml of 0.5% agar media and placed on a supporting layer of 0.5% agar as described previously (5). Colonies observed after 5–7 d were picked and expanded as previously described (3). For subcloning procedures, concanavalin A (Con A)-activated rat spleen cell supernate was used as described previously (3, 5). Clones could be expanded by the same procedure as that for long-term cultured cell

lines. However, we found that the removal of antigen followed by resting culture was not absolutely necessary and that clones could be restimulated every 2 wk without removing antigen.

Subcloning by Limiting Dilution. 1×10^5 cells from (T,G)-A--L-reactive colonies were stimulated with 200 μg/ml (T,G)-A--L with 80×10^6 syngeneic filler cells. After 36 h incubation, cells were harvested, counted, and plated at two different concentrations in microtiter plates (Falcon 3040) to give either 0.3 or 1 viable cell/well with 1×10^6/well fresh syngeneic filler cells in a total volume of 0.2 ml in complete media with 200 μg/ml of (T,G)-A--L and 25% Con A supernate. 10–14 d later, wells showing positive growth were transferred to Costar wells and expanded in a similar manner as described for soft agar subcloning. Cloning efficiency was ~40%, indicating that each subclone was derived from a single cell.

Assay of Proliferative Response. After 10–14 d of culture, aliquots of viable recovered cells were assayed for their proliferative responses. 1×10^4 (T,G)-A--L-reactive T cells were restimulated with 200 μg/ml (T,G)-A--L in the presence of filler cells in 0.2 ml complete media/well in microtiter plates (Falcon 2040) for 48 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 filter paper and radioactivity was counted using standard scintillation counting (6). Results were expressed as the mean of triplicate culture. The standard deviation of each mean was within 10%.

Results

Long-term Cultured (T,G)-A--L-reactive T Cells. Following serial restimulation of long-term cultured (T,G)-A--L-reactive cells derived from B6 mice, antigen specificity of these lines was tested using a limited panel of native as well as synthetic antigens. The data presented in Table I show the reactivity of 1×10^4 (T,G)-A--L-reactive T cells stimulated with antigen in the presence of 1×10^6 irradiated syngeneic B6 filler cells. These data suggest that such cells respond to (T,G)-A--L, but do not respond to inappropriate antigens such as BSA, keyhole limpet hemocyanin (KLH), or OVA.

Several other characteristics of long-term cultured (T,G)-A--L-reactive T cell lines, including surface phenotype, antigen dose-response curves, the requirement of antigen and filler cells for proliferation, mitogen responsiveness, alloreactivity, and kinetic responses, are similar to those previously published for long-term cultured GAT-reactive T cells (3).

Homozygous Antigen-presenting Determinants on B6 Cells. Long-term culture lines of (T,G)-A--L-reactive T cells derived from B6 mice were cloned in soft agar and the reactivities (antigen recognition on a panel of filler cells) of the soft agar colonies

TABLE I

Antigen Specificity of (T,G)-A--L-reactive T Cells

Antigen	^{3}H uptake
μg/ml	*cpm*
—	1,690
(T,G)-A--L, 200	23,513
BSA, 200	2,066
KLH, 200	858
OVA, 200	1,619

1×10^4 long-term cultured (T,G)-A--L-reactive T cells derived from B6 mice were stimulated with antigen in the presence of 1×10^6 irradiated B6 filler cells. Proliferative responses were assayed on day 2 by [^{3}H]TdR uptake.

obtained are listed in Table II. Colonies 1.g to 1.p in Table II exhibited equal response to (T,G)-A--L in the presence of either B6 or (B6A)F_1 antigen-presenting filler cells. These colonies did not respond to (T,G)-A--L presented by strain A filler cells, showing that syngeneic or semisyngeneic antigen-presenting cells are necessary for antigen-induced T cell proliferation. Colonies 1.c and 1.d in Table II showed totally unexpected restriction specificities. They responded to (T,G)-A--L in the presence of B6 filler cells, but showed very weak (1.d) or no (1.c) response to (T,G)-A--L in the presence of semisyngeneic (B6A)F_1 antigen-presenting cells. Therefore, colony 1.d was subcloned in soft agar in an attempt to isolate T cell clones which recognized antigen presented by unique homozygous antigen-presenting determinants present on parental cells not expressed on semisyngeneic F_1 cells. The data presented in Table III shows the reactivities of subclones obtained from colony 1.d. All of the subclones listed in Table III recognized (T,G)-A--L in association with B6 antigen-presenting cells yet did not respond to (T,G)-A--L in the presence of (B6A)F_1 antigen-presenting cells. Table IV presents mixing experiments that prove that the failure of responsiveness of these clones to (T,G)-A--L in association with (B6A)F_1 filler cells was not due to suppressor effects by determinants present on semisyngeneic (B6A)F_1 filler cells. In these experiments, 1:1 mixtures of A and B6 cells, or (B6A)F_1 and B6 filler cells, showed antigen-presenting ability equal to that of B6 filler cells alone. Furthermore,

TABLE II

Colonies of (T,G)-A--L-reactive T Cells from B6 Mice

Colony	MED*	(T,G)-A--L	(T,G)-A--L	(T,G)-A--L
	(B6A)F_1	A	B6	(B6A)F_1
			cpm	
1.g	199	168	6,987	6,607
1.n	223	554	5,178	5,312
1.p	377	800	11,594	13,890
1.c	191	131	13,066	355
1.d	363	683	19,960	4,514

B6 (T,G)-A--L-reactive T cells were cloned in soft agar as described in Materials and Methods. 1×10^4 (T,G)-A--L-reactive cloned cells were stimulated with 200 μg/ml (T,G)-A--L in the presence of 1×10^6 A, B6, or (B6A)F_1 filler cells. Proliferative responses were assayed on day 2 by [^{3}H]TdR uptake.

* MED, medium.

TABLE III

Subclones of Colony 1.d from B6 Mice

Subclone	MED (T,G)-A--L		MED (T,G)-A--L		MED (T,G)-A--L	
	A		B6		(B6A)F_1	
			cpm			
1.d.2	66	289	491	3,166	135	183
1.d.13	54	140	87	3,487	82	198
1.d.14	289	121	553	5,953	101	801
1.d.15	357	470	579	8,593	160	991

Subclones from colony 1.d in Table II were obtained in soft agar as described in Materials and Methods. 1×10^4 cells of selected subclones were assayed as described in the legend in Table II.

TABLE IV

A or (B6A)F_1 Filler Cells Are Not Suppressive to Antigen Presentation by Unique Homozygous Antigen-presenting Determinants on B6 Cells

Filler cells	1.d.15	
	MED	(T,G)-A--L
A	199	277
B6	226	10,384
(B6A)F_1	130	638
A + B6	NT*	10,651
(B6A)F_1 + B6	NT	10,908

1×10^4 cells from B6-derived (T,G)-A--L-reactive subclone 1.d.15 were stimulated with 200 μg/ml (T,G)-A--L in the presence of A, B6, (B6A)F_1, 1:1 mixture of A + B6 and (B6A)F_1 + B6 filler cells. Total number of filler cells was 1×10^6/well. Proliferative responses were measured on day 2.

* NT, not tested.

TABLE V

Homozygous Antigen-presenting Determinants Expressed on B6 Cells Controlled by I-A

Filler cells	MHC region*								1.d.15		1.d.17	
	K	A	B	J	E	C	S	D	Med	(T,G)-A--L	Med	(T,G)-A--L
Experiment 1												
A	k	k	k	k	k	d	d	d	159	182	160	592
B6	b	b	b	b	b	b	b	b	244	14,460	366	11,953
(B6A)F_1	k/b	k/b	k/b	k/b	k/b	d/b	d/b	d/b	111	288	458	703
B10.A(4R)	k	k	b	b	b	b	b	b	393	1,179	838	1,338
B10.MBR	b	k	k	k	k	k	k	q	296	900	363	842
B10.A(4R) × B6	k/b	k/b	b/b	b/b	b/b	b/b	b/b	b/b	171	1,120	194	1,366
Experiment 2												
B6	b	b	b	b	b	b	b	b	818	15,482	NT‡	NT
(B6A)F_1	b/k	b/k	b/k	b/k	b/k	b/k	b/k	b/k	511	1,944	NT	NT
B10.A(5R)	b	b	b	k	k	d	d	d	325	12,675	NT	NT
[B10.A(5R) × B6]F_1	b/b	b/b	b/b	k/b	k/b	d/b	d/b	d/b	395	10,956	NT	NT

1×10^4 cells from B6-derived (T,G)-A--L-reactive clones (1.d.15 and 1.d.17) were stimulated with 200 μg/ml (T,G)-A--L in the presence of 1×10^6 filler cells from various strains of mice and their F_1s as listed. Proliferative responses were assayed on day 2.

* MHC regions of filler cells are listed for convenience of interpretation.

‡ NT, not tested.

titration experiments of semisyngeneic (B6A)F_1 antigen-presenting cells showed that the lack of response of clone 1.d.15 to (T,G)-A--L associated with (B6A)F_1 antigen-presenting cells was not due to an inappropriate number of filler cells (i.e., any number of filler cells, from 1×10^6 to 1.5×10^4 (B6A)F_1 cells/well, could not present (T,G)-A--L to this clone) (data not presented).

To study whether the expression of such a homozygous antigen-presenting determinant was controlled by the H-2 region of the mouse MHC, filler cells from various congenic strains of mice and F_1 mice derived from these lines were used as antigen-presenting cells; these data are presented in Table V. The ability of B10.A(5R) and [B10.A(5R) × B6]F_1 cells to present antigen and the inability of antigen presentation by (B6A)F_1, B10.A(4R), B10.MBR, or [B10.A(4R) × B6]F_1 filler cells suggested that the homozygous antigen-presenting determinants expressed on B6 antigen-presenting cells were controlled by the I-A subregion of the MHC. These results clearly indicated that there existed homozygous antigen-presenting determinants on B6 cells controlled by the I-A subregion of the MHC that were not present on semisyngeneic cells.

Hybrid Antigen-presenting Determinants Detected by (T,G)-A--L-reactive T Cell Clones

Derived from (B6A)F_1 Mice. In a previous paper, we demonstrated that there existed unique hybrid antigen-presenting determinants present on (B6A)F_1 cells which were not expressed on either of the parental cells, which functioned to restrict recognition of GAT by GAT-primed T cell clones (3). The immune response to (T,G)-A--L has been shown to be controlled by immune response genes that map to the I-A subregion of the MHC (7, 8). Certain mouse strains have been classified as low responders and others as high responders. Mice with haplotype H-2^a are low responder mice, whereas mice with haplotype H-2^b and F_1 derived between these two [(B6A)F_1] are high responders to (T,G)-A--L (9). We therefore decided to study whether there existed clones of antigen-reactive T cells in F_1 mice derived between high responder by low responder [(H-2^a × H-2^b)F_1] mice which could respond to antigen in association with unique F_1-specific hybrid antigen-presenting determinants consisting of hybrid products derived from low responder and high responder parents. (T,G)-A--L-reactive T cell colonies from (B6A)F_1 mice were derived from soft agar cloning experiments as previously described and their reactivities are shown in Table VI. Colonies 2.a to 2.k in Table VI showed equal proliferative responses to (T,G)-A--L in association with B6 and (B6A)F_1 antigen-presenting cells. Colonies 2.d and 2.e reacted to (T,G)-A--L much better in association with (B6A)F_1 antigen-presenting cells than they did to antigen associated with or presented by B6 antigen-presenting cells, suggesting that there existed T cell clones in colonies 2.d and 2.e which responded to (T,G)-A--L in association with F_1 specific antigen-presenting determinants. To prove this, colony 2.e was recloned not only in soft agar but by limiting dilution (see Materials and Methods). The reactivities of subclones obtained from colony 2.e are shown in Table VII. Most of the subclones derived from colony 2.e showed reactivity to (T,G)-A--L only in association with (B6A)F_1 antigen-presenting cells and not in association with either A or B6 antigen-presenting cells as exemplified by colonies 2e.LD.2, 2e.SA.12, and 2e.SA.13 (and many others not included). Several colonies, as exemplified by colony 2e.SA.2 (and others not included), reacted equally well to (T,G)-A--L presented both by B6 and (B6A)F_1 antigen-presenting cells. These results suggested that clones of T cells existed that reacted to (T,G)-A--L in (high responder × low responder)F_1 mice in association with unique F_1-specific antigen-presenting determinants composed of products of both high responder and low responder haplotypes. Using filler cells from various congenic recombinant strains of mice and F_1s derived from these strains,

TABLE VI

Colonies of (T,G)-A--L-reactive T Cells from (B6A)F_1 Mice

Colony	MED	(T,G)-A--L	(T,G)-A--L	(T,G)-A--L
	(B6A)F_1	A	B6	(B6A)F_1
2.a	350	124	7,010	7,490
2.b	569	514	15,109	16,957
2.c	796	489	6,104	7,718
2.k	239	237	7,944	5,829
2.d	198	209	4,130	7,930
2.e	413	329	2,899	7,150

Long-term cultured (T,G)-A--L-reactive T cells derived from (B6A)F_1 mice were cloned in soft agar as described in Materials and Methods. 1×10^4 cells from (T,G)-A--L-reactive clones were stimulated with 200 μg/ml (T,G)-A--L in the presence of 1×10^6 A, B6, (B6A)F_1 filler cells. Proliferative responses were assayed on day 2.

TABLE VII

Subclones of Colony 2e

Subclone	MED (T,G)-A--L		MED (T,G)-A--L		MED (T,G)-A--L		MED (T,G)-A--L	
	A		B6		A + B6		$(B6A)F_1$	
2e.LD 2	149	171	248	484	342	792	367	12,453
2e.SA 12	124	165	109	366	134	191	88	12,159
2e.SA 13	89	76	62	100	54	155	25	11,004
2e.SA 2	496	768	738	4,035	773	3,823	248	3,137

Subclones from colony 2e in Table VI were obtained in soft agar (SA) as well as by limiting dilution (LD) as described in Materials and Methods. 1×10^4 cells from (T,G)-A--L-reactive T cell clones were stimulated with (T,G)-A--L in the presence of 1×10^6 A, B6, 1:1 mixture of A + B6 and $(B6A)F_1$ filler cells. Proliferative responses were measured on day 2.

TABLE VIIIA

Hybrid Antigen-presenting Determinants of (High Responder × Low Responder)F_1 Controlled by I-A

Filler cells	MHC region*								2e.LD.2		2e.SA.12		2e.SA.13	
	K	A	B	J	E	C	S	D	MED	(T,G)-A--L	MED	(T,G)-A--L	MED	(T,G)-A--L
A/J	k	k	k	k	k	d	d	d	90	127	94	115	132	174
B6	b	b	b	b	b	b	b	b	228	545	243	833	237	696
A + B6‡	k/b	k/b	k/b	k/b	k/b	d/b	d/b	d/b	215	678	224	973	183	582
$(B6A)F_1$	k/b	k/b	k/b	k/b	k/b	d/b	d/b	d/b	115	14,889	109	11,442	257	22,959
B10.A(4R)	k	k	b	b	b	b	b	b	692	733	334	578	300	350
$[10.A(4R) \times B6]F_1$	k/b	k/b	b/b	b/b	b/b	b/b	b/b	b/b	1,455	16,654	421	13,385	665	22,958
$(B10.MBR \times A.AL)F_1$	b/k	k/k	k/k	k/k	k/k	k/k	k/k	q/d	439	287	74	214	130	277
$(bm12 \times B10.A)F_1$	b/k	b/k	b/k	b/k	b/k	b/d	b/d	b/d	692	1,585	118	578	368	16,609

1×10^4 cells from (T,G)-A--L-reactive T cell clones (2e.LD.2, 2e.SA.12, and 2e.SA.13) were stimulated with 200 μg/ml (T,G)-A--L in the presence of 1×10^6 filler cells from various strains of mice and their F_1s. Proliferative responses were assayed on day 2.

* MHC regions of filler cells are listed for convenience of interpretation.

‡ 1:1 mixture of A and B6 filler cells.

it was possible to show that (high responder × low responder)F_1-specific antigen-presenting determinants were controlled by the I-A subregion of the mouse MHC (Table VIIIA). Also included in the data presented in Table VIIIA is a study using $[B6.C\text{-}H\text{-}2^{bm12}$ (bm12) × B10.A]F_1 cells as antigen-presenting cells. The mutant mouse bm12 has been shown to have a mutation which results in greatly reduced or absent expression of $I\text{-}A^b$ region gene products expressed on the surface of Ia-bearing cells (10). Studies from our laboratory have suggested mutation in bm12 results in the failure of expression of the normal $I\text{-}A^b_\beta$-chain expressed on the cell surface (11). Transcomplementation allows expression of hybrid I-A molecules of phenotype $A^b_\alpha A^k_\beta$ (11). Using $(bm12 \times B10.A)F_1$ cells as antigen-presenting cells to F_1-specific (T,G)-A-L-reactive T cell clones restricted by the hybrid I-A antigen-presenting determinants, it was possible to identify at least two types of F_1-specific (T,G)-A--L-reactive T cell clones. Data presented in Table VIIIA show that clone 2e.SA.13, but not clones 2e.LD.2 and 2e.SA.12, react to (T,G)-A--L in association with $(bm12 \times B10.A)F_1$ cells despite the fact that all three of these clones use $(B6A)F_1$ hybrid antigen-

presenting determinants. The most likely explanation of these results is that clone 2e.SA.13 uses the $A^b_\alpha A^k_\beta$ product, whereas clones 2e.LD.2 and 2e.SA.12 use $A^k_\alpha A^b_\beta$ products as restricting determinants. These data suggest that both possible combinations of transcomplementing I-A products can be used as restricting determinants for antigen recognition of T cell clones. Data presented in Table VIIIB showing the inability of F_1 cells from this mutant [(bm12 × B10.A)]F_1 to present (T,G)-A--L to clones of (T,G)-A--L-reactive T cells whose recognition is restricted by I-A products on B6 and (B6A)F_1 cells suggests additionally that the normal I-A^b product ($A^b_\alpha A^b_\beta$) is not reconstituted by this transcomplementation. These data support our earlier findings with alloreactive T cell clones which suggested that the $A^b_\alpha A^b_\beta$ MLR-stimulating determinant was not present on cells of (bm12 × B10.A)F_1 mice (11).

Discussion

The results reported here showed the following: (*a*) there exist homozygous antigen-presenting determinants which are uniquely expressed only on parental B6 cells and not on semisyngeneic (B6A)F_1 cells; (*b*) there exist T cell clones from (high responder × low responder)F_1 mice which react to antigen in association with hybrid antigen-presenting determinants derived from products of both high responder and low responder parents; and (*c*) the expression of these determinants is controlled by the I-A subregion of the mouse MHC.

The existence of unique homozygous antigen-presenting determinants present only on B6 parental cells [not present on semisyngeneic (B6A)F_1 cells] cannot be explained by our present knowledge of murine MHC genetics. Previously it has been shown that products encoded by the H-2 region of the mouse are codominantly expressed on the surface of F_1 cells (12). The possibility that suppression by certain products expressed on either A or (B6A)F_1 cells interfered with antigen-presenting ability of the semisyngeneic (B6A)F_1 cells, compared with B6 antigen-presenting cells, to such B6 (T,G)-A--L-reactive clones, was ruled out by cell-mixing experiments (Table IV). The existence of such homozygous antigen-presenting determinants might be an argument against the hypothesis that Ia antigens and Ir genes (as defined by the ability of T cells to recognize antigen under I region restriction) are identical (13). However, the existence of such unique homozygous determinants has already been reported by Hengartner and Fathman (5) in their study of alloreactive T cell clones in which several alloreactive clones derived from strain A lymph node cells repeatedly

TABLE VIIIB

(B6A)F_1-derived (T,G)-A--L-reactive T Cell Clones Restricted by $A^b_\alpha A^b_\beta$

Clone	MED (T,G)-A--L		MED (T,G)-A--L		MED (T,G)-A--L		MED (T,G)-A--L	
	A		B6		(B6A)F_1		(bm12 × B10.A)F_1	
2a.2	320	736	1,027	14,827	197	13,521	201	392
2a.5	184	1,941	4,562	24,890	166	19,181	637	499
2a.8	398	810	684	5,658	508	5,923	210	382
2a.10	109	322	254	5,538	400	5,561	236	326

Colony 2a inTable VI was subcloned in soft agar. 1×10^4 cells from selected subclones were stimulated with 200 μg/ml (T,G)-A--L in the presence of 1×10^6 A, B6, (B6A)F_1, and (bm12 × B10.A)F_1 filler cells. Proliferative responses were assayed on day 2.

stimulated by B6 spleen cells could be restimulated only by B6 cells and not by semisyngeneic (B6A)F_1 stimulator cells. Additionally, Ishikawa and Dutton (14) have reported that determinants controlled by the K region of haplotype H-2^k mice detected by F_1 anti-parent cytotoxic T cells are expressed only on homozygous cells and not on heterozygous cells. The phenomenon of hybrid resistance demonstrated in experiments by Cudkowicz and Bennet (15) has demonstrated a similar paradox in which cells from F_1 mice recognize determinants on parental cells. These results suggest that Ia and H-2 determinants which might function as antigen-presenting determinants as well as MLR-stimulating determinants and target determinants for cytotoxic T cells are controlled in a more complex manner than had been initially recognized. It is possible that T cells recognize determinants and/or restricting elements for antigen recognition or targets of CML which are not detected by antibody. To date, there have been no reports of serologically demonstrable Ia or H-2 determinants present only on homozygous cells and not on semiallogenic cells.

The hybrid antigen-presenting determinants on (B6A)F_1 cells used by (T,G)-A--L-reactive T cell clones for antigen restriction described in this paper are similar to those detected in systems using GAT-reactive T cell clones (3). Genetic mapping studies have suggested that these determinants are composed of transcomplementing products within the I-A subregion. The existence of T cell clones in (high responder × low responder)F_1 mice that can respond to antigen presented on the hybrid determinants composed of products of both high and low responder parents suggests that phenotypic high responsiveness of (high responder × low responder)F_1 mice comes not only from products of the high responder parental genome but also is allowed by the ability of F_1 mice to create new antigen-presenting determinants which function effectively for such F_1 mice. The F_1 specific hybrid determinants which present antigen to (T,G)-A--L-reactive T cell clones are composed of the following configurations: $A^b_\alpha A^k_\beta$ and $A^k_\alpha A^b_\beta$. The experiments using the F_1 derived between the mutant bm12 and B10.A as antigen-presenting cells suggested that both of these two types of hybrid molecules are used as antigen-presenting determinants for (T,G)-A--L-reactive clones derived from (B6A)F_1 mice. Thus, one F_1 specific (T,G)-A--L-reactive clone (2e.SA.13) can be stimulated by (T,G)-A--L in association with (B6A)F_1 and (bm12 × B10.A)F_1 cells equally well, whereas two other F_1 specific (T,G)-A--L-reactive clones (2e.LD.2 and 2e.SA.12) can be stimulated with (T,G)-A--L only in association with (B6A)F_1 cells, not in association with (bm12 × B10.A)F_1 cells (Table VIIIA). These data show that at least two new determinants are created in F_1 mice which function as antigen-presentation (restricting) determinants.

The existence of T cell clones that recognize antigen in association with F_1 specific antigen-presenting determinants could be taken as support of the hypothesis of maturation of T cell restriction proposed by Zinkernagel (16). According to this hypothesis, T cells learn specificity for self during maturation in the thymus, or lymphoreticular system, since neither parent should be able to provide genomic material which would allow recognition of unique hybrid F_1 determinants. Although unlikely, an alternative possibility, that each chain of I-A can be recognized individually by T cell clones must be considered. Such combinatorial association of "recognition units" on the F_1 T cell might allow genomic recognition of "hybrid I-A molecules." T cells that recognize F_1 specific determinants as self must have the ability of acquiring such recognition, and it is conceivable that this would be accomplished

by maturation in the environment of the thymus or lymphoreticular system. Longo and Schwartz (17) have recently shown that B10.A + B10.A(18R) [B10.A × B10.A(18R)]F_1 chimeric spleen cells can respond to GLϕ when transferred to acutely irradiated [B10.A × B10.A(18R)]F_1 mice along with T cell-depleted bone marrow cells as antigen-presenting cells from F_1 mice, suggesting that the nonresponder phenotype can be converted to responder phenotype during maturation in the responder environment. These results, along with thymus grafting experiments of Zinkernagel et al. (18, 19) and the data presented in this paper, allow us to suggest that antigen-induced proliferating T cells, as well as cytotoxic effector T cells, are given specificity for self in association with radioresistant portions of the thymus epithelium and lymphoreticular system. It is reasonable to assume that F_1 T cells which recognize F_1 specific antigen-restricting determinants learn specificity for F_1 determinants during maturation in the thymus or lymphoreticular system, providing a natural counterpart for these chimeric experiments.

Summary

Using murine (T,G)-A--L-reactive T cell clones, we have demonstrated the existence of unique homozygous antigen-presenting determinants expressed on C57Bl/6 mice, controlled by the I-A subregion of the murine major histocompatibility complex (MHC), which are not expressed on semisyngeneic (C57Bl/6 × A/J)F_1 [(B6A)F_1] cells. Additionally, we were able to demonstrate that there exist (T,G)-A--L-reactive clones in F_1 mice derived between low responder and high responder parents [(B6A)F_1] that recognize antigen in association with transcomplementing hybrid I-A subregion determinants expressed uniquely on (B6A)F_1 cells not expressed on cells of either of the parental strains. These data suggest that phenotypic high responsiveness exhibited by (higher responder × low responder)F_1 mice was not simply controlled by the high responder parental genome, but was controlled at the phenotypic level of expression of antigen-presenting determinants. Such antigen-presenting determinants can be created by complementation using products of the low responder as well as high responder genome. The significance of the existence of such F_1 specific hybrid antigen-presenting determinants for T cell specificity and recognition of self was discussed.

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