ANTIGEN-SPECIFIC T-CELL FACTOR IN CELL COOPERATION

Mapping Within the *I* Region of the *H-2* Complex and Ability to Cooperate Across Allogeneic Barriers*

By M. J. TAUSSIG, A. J. MUNRO, ROSALIE CAMPBELL, C. S. DAVID AND N. A. STAINES

(From the Immunology Division, Department of Pathology, Cambridge, England, the Department of Human Genetics, University of Michigan, Ann Arbor, Michigan, and Searle Research Laboratories, High Wycombe, England)

Murine thymocytes, educated to various synthetic polypeptide antigens, e.g. poly(Tyr,Glu)-polyDLAla--polyLys [(T,G)-A--L],[1] poly(Phe,Glu)-polyDLAla--polyLys [(Phe,G)-A--L], produce in culture a corresponding antigen-specific "factor" capable of cooperating with bone marrow cells in vivo (1). This factor has been shown to be, at least in part, a product of the major histocompatibility (*H-2*) complex (2), in particular of the left-hand half of *H-2* which contains the *K* and *I* regions (3). We have now completed further mapping of the factor specific for (T,G)-A--L using region-specific anti-*H-2* sera, and have found that the gene(s) coding for the T-cell factor lies in the *I-A* subregion of *H-2*.

We have also studied in more detail the ability of the T-cell factor to cooperate with allogeneic bone marrow cells. Although previous experiments have shown that this is indeed possible (4, 5), other workers have found that "physiologic cooperation" between T cells and B cells requires that the interacting cells are identical for certain genes in the *I* region (6, 7). We now report systematic comparison of different combinations of B cells and T-cell factors [for (Phe,G)-A--L] from various strains, and have found no significant lessening of the response when factor and B cells are in allogeneic combination.

Materials and Methods

Preparation and Test of T-Cell Factor. The system used for the preparation of T-cell factor from "educated" T cells in vitro and its assay in combination with bone marrow cells in irradiated recipients was as previously described (1–5).

Antigens. The following multichain synthetic polypeptide were used as immunogens: (*a*) (T,G)-A--L, batch No. 1383, and (*b*) (Phe,G)-A--L, batch no. 223. For the determination of PFC to (T,G)-A---L (below) indicator red cells were coated with a related polypeptide, namely polyL(Tyr,Glu)-polyLPro--polyLLys, [(T,G)-Pro--L] batch no. 935.

All these materials were kindly supplied by Dr. Edna Mozes, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel. Their preparation and properties have been extensively described (8,9).

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[1] *Abbreviations used in this paper*: PFC, plaque-forming cells; (T,G)-A--L, poly(Tyr,Glu)-polyDLAla--polyLys; (Phe,G)-A--L, poly(Phe,Glu)-polyDLAla--polyLys; (T,G)-Pro--L, poly(Tyr,Glu)-polyPro--polyLys.

TABLE I
The Recombinant H-2 Haplotypes Used in These Experiments

Strain	Haplotype origin of region					
	K	*I-A*	*I-B*	*I-C*	*Ss*	*D*
B10.AQR	q	k	k	d	d	d
B10.A	k	k	k	d	d	d
A.TH	s	s	s	s	s	d
A.TL	s	k	k	k	k	d
B10.A (4R)	k	k	b	b	b	b

Hemolytic Plaque-Forming Cell Assay. Direct plaque-forming cells (PFC) to (T,G)-A--L were measured as previously described (3, 4) using as indicators sheep red blood cells (SRBC) coated with (T,G)-Pro--L. PFC to (Phe,G)-A--L were estimated with SRBC coated with (Phe,G)-A--L. Sensitization of SRBC was accomplished using chromium chloride, as described previously (3, 4).

Mice. Congenic-resistant strains on the C57BL/10ScSn (B10) background were used. B10, B10.D2, and B10.BR mice were obtained from the Laboratory Animal Centre, Carshalton, and bred at the Department of Pathology, Cambridge. B10.AQR and B10.A (4R) were the generous gift of Dr. H. Festenstein, London Hospital.

Strains A.TH, A.TL, and AQR used in the immunizations were bred at the Department of Human Genetics, University of Michigan, Ann Arbor, Mich. Table I shows the recombinant haplotypes of the strains used.

Sera. B10 anti-A/J and DBA/1 anti-B10.A sera were raised by repeated immunization with A/J or B10.A spleen cells over several weeks. These sera were used unabsorbed and after adsorption with B10.A (4R) or B10.AQR spleen cells respectively.

B10.D2 anti-B10.A serum was raised by first skin grafting on days 0 and 27, followed by inoculation of 1/4 spleen equivalent of B10.A cells i.p. on days 41, 48, 55, and 62. Mice were exsanguinated on day 69. Similarly, B10.A anti-B10.D2 serum was raised by repeated immunization with 1/4 spleen equivalent and 1/4 thymus equivalent of B10.D2 cells on days 0, 3, 7, 10, 14, 17, 21, and 24, after a single B10.D2 skin graft 5–11 wk previously. Mice were exsanguinated on day 34. These sera were used both unadsorbed and after adsorption with red cells to remove anti-*K* antibodies. The activity of these sera has been described in detail elsewhere.[2]

A.TH anti-A.TL and AQR anti-B10.A (10) were prepared by immunizations with splenic, thymic, and lymph node lymphocytes intraperitoneally for 4 wk, followed by monthly booster injections until a high cytotoxic titer was obtained.

Immunoadsorbents. These were prepared by coupling sera to CNBr-activated Sepharose as previously described (3).

Results

Mapping of Genes Coding for Factor. T-cell factor specific for (T,G)-A--L was passed through columns of region-specific anti-*H-2* sera immobilized on activated Sepharose, before transfer together with bone marrow cells and antigen into suitable irradiated recipients.

(*a*) Factor prepared in *H-2*^{k} (B10.Br) mice was passed through columns containing antisera specific for the *K* region of the *H-2*^{k} haplotype (anti-*K*^{k}; AQR anti-B10.A), or for the *I* (and *S*) regions of the same haplotye (anti-*I*^{k}; A-TH anti-A.TL). As shown in Table II, only the latter successfully removed the T-cell

[2] Staines, N. A., K. Guy, and D. A. L. Davies. 1975. The dominant role of Ia antibodies in the passive enhancement of *H-2* imcompatible skin grafts. Manuscript submitted for publication.

factor, indicating the factor contains a product of the *I* (or *S*) region. Further confirmation that the *K* region does not contribute to the factor was obtained by adsorption of an anti-*H-2*^{a} serum (DBA/ anti-B10.A) with B10.AQR spleen cells. After absorption this serum no longer removed the factor.

(*b*) Our previously published results (3) have shown the factor to be a product of the left-hand side of the recombinant *H-2*^{a} chromosome, i.e. of the *K, I-A or I-B* regions, and since the data above rule out the *K* region as coding for the factor, it remained to be determined which of the *I* subregions was responsible. This was accomplished by removing from an anti-*H-2*^{a} serum (B10 anti-A/J) the antibodies reacting with the *K* and *I-A* regions by absorption with B10.A (4R) spleen cells. Table III shows that after this absorption, the anti-*H-2*^{a} no longer removed the T-cell factor. These results are summarized in Fig. 1, from which it can be seen that the factor must be coded for, at least in part, by the *I-A* subregion.

(*c*) The following results confirmed that the factor is coded by the *I* region, and not by the *K* locus. Anti-*K,I* sera were rendered specific for the *I* region by

TABLE II

Removal of T-Cell Factor by Antisera Against the I Region of H-2

Factor*	Immunoadsorbent	Log_{10} mean PFC/spleen‡
—§	—	1.778
H-2^k (B10.BR)	—	4.436
	Anti-*K*^{k}‖	4,382
	Anti-*I*^{k}¶	2.225
Standard error		0.164

* Specific for (T,G)-A--L.
‡ Measured in B10 recipients.
§ Control—bone marrow cells transferred with antigen but without factor.
‖ AQR anti-B10.A.
¶ A.TH anti-A.TL.

TABLE III

Removal of T-Cell Factor by Antisera against the I Region of H-2

Factor*	Immunoadsorbent	Log_{10} mean PFC/spleen‡
—§	—	0.841
H-2^k (B10.BR)	—	3.643
	Anti-*K*^{k}, *I-A*^{k}, *I-B*^{k}‖	1.278
	Anti-*I-B*^{k}¶	3.662
Standard error		0.166

* Specific for (T,G)-A--L.
‡ Measured in B10 recipients.
§ Control—bone marrow cells transferred with antigen but without factor.
‖ B10 anti-A/J.
¶ B10 anti-A/J absorbed with B10.A (4R) spleen cells.

absorption with red cells. Mouse erythrocytes do not carry detectable Ia antigens (10), while they do carry the *K* and *D* molecules. Both antisera used were reactive only with the left-hand side of *H-2* (B10.A anti-B10.D2; B10.D2 anti-B10.A) and were absorbed with the appropriate red cells to remove antibodies reacting with the *K* gene product. Table IV shows that both antisera continued to remove the factor after absorption with red cells, indicating that the factor reacts with anti-*I* antibodies.

Ability of T-Cell Factor to Cooperate with Allogeneic Bone Marrow Cells. In order to determine whether there are any requirements for identity between the *H-2* types of the strain producing the factor and the strain donating the bone marrow cells, factor for (Phe,G)-A--L was produced in different strains [B10, B10.D2, B10.Br—all responders to (Phe,G)-A--L] and tested by transfer together with bone marrow cells obtained from each of the three strains. The results

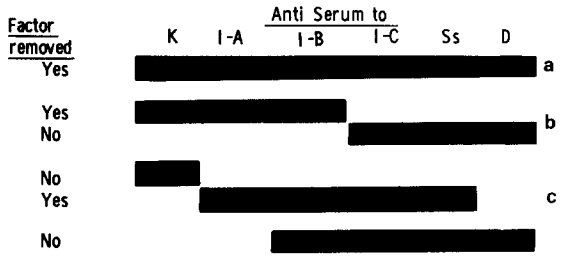

FIG. 1. Removal of T-cell factor with antisera against the *H-2* complex. The solid bars indicate the regions of the *H-2* complex against which the alloantisera were directed. Key: (*a*) ref. 2; (*b*) ref. 3; (*c*) this paper, Tables II and III.

TABLE IV

Removal of T-Cell Factor by Antisera against the I Region of H-2

Factor*	Immunoadsorbent	Log_{10} mean PFC/spleen
—‡	—	1.104
$H\text{-}2^d$ (BALB/c)	—	4.690
	Anti-K^d, I^d §	2.146
	Anti-I^d ‖	1.518
Standard error		0.200
—‡	—	1.122
$H\text{-}2^k$ (B10.BR)	—	4.863
	Anti-K^k, I^k **	2.079
	Anti-I^k ‡‡	2.785
Standard error		0.184

* Specific for (T,G)-A--L.

‡ Control—bone marrow cells transferred with antigen but without factor.

§ B10.A anti-B10.D2.

‖ B10.A anti-B10.D2 absorbed with B10.D2 RBC.

¶ Measured in B10 recipients.

** B10.D2 anti-B10.A.

‡‡ B10.D2 anti-B10.A absorbed with B10.A RBC.

(Table V) clearly show that the factor from each of these strains functioned equally well in combination with allogeneic or syngeneic bone marrow. We were unable to find any evidence of the type of restrictions reported for T cell-B cell interaction (6, 7).

Discussion

The results described here further define the genes coding for a T-cell product specific for (T,G)-A--L, and show that they lie in the *I-A* subregion of the *H-2* complex (Fig. 1). This mapping has been accomplished using antisera raised specifically against *I*-region differences between otherwise congenic strains, or rendered specific for different regions of *H-2* by absorption. This takes the mapping of the (T,G)-A--L specific factor as far as is possible with the recombinants presently available.

It is, of course, also important that the *I-A* subregion controls the level of immune response to the synthetic polypeptides built on A--L, of which (T,G)-A--L is one (11). Although the genes for the (T,G)-A--L specific molecule map in this region, it does not automatically follow that responsiveness is always associated with a lack of this product. For example, low responders to (T,G)-A--L of $H\text{-}2^k$ haplotype produce the specific T-cell factor, but their bone marrow cells do not respond to active factor and antigen (4). On the other hand, some low responder strains fail to make factor, e.g. SJL (12, 13) and B10.M (unpublished observations), although bone marrow cells of B10.M are responsive. This would suggest that at least two genes in the *I-A* region must be involved in genetic control of

TABLE V
Ability of T-Cell Factor to Cooperate with Allogeneic Bone Marrow Cells

Factor*	Bone marrow cells	Log_{10} mean PFC/spleen‡
—§	B10	1.380
	B10.Br	1.204
	B10.D2	1.079
B10	B10	4.792
	B10.BR	4.929
	B10.D2	4.880
B10.BR	B10	4.322
	B10.BR	4.146
	B10.D2	4.176
B10.D2	B10	3.903
	B10.Br	4.491
	B10.D2	4.255
Standard error		0.342

* Specific for (Phe,G)-A--L.

‡ Measured in recipients syngeneic for bone marrow cells.

§ Controls—bone marrow cells transferred with antigen but without factor.

response to (T,G)-A--L, one coding for the T-cell factor (T-cell receptor?) while the other codes for the ability of B cells to respond to the factor/antigen signal. We are currently pursuing experiments designed to test this two-gene hypothesis.

We have also found that the T-cell factor is able to cooperate with allogeneic bone marrow cells as effectively as with syngeneic cells. Experiments testing cooperation between T cells and B cells—as opposed to T-cell factor and bone marrow cells—have indicated that the interacting cells must share certain *H-2* specificities, notably those of the *I*-region (6, 7). Our results show that triggering of bone marrow cells by the T-cell factor is not limited in this way, and thus infer that the histocompatibility restrictions on cooperation between cells may not be related to the triggering event itself. For example, cell approximation may require certain basic similarities in the surfaces of the cells involved; alternatively it might be necessary to avoid the recognition of B-cell *I* antigens by cooperating T cells. The process of B-cell triggering, which we are able to study in isolation using the soluble T-cell factor instead of T cells, seems not to require *I*-region identity, but is brought about by interactions between molecular structures which are common to all responder strains. These interacting structures are probably located in the T-cell receptor/factor molecule and on a site on B cells (or macrophages) where the T-cell signal is accepted.

Summary

Further mapping of the mouse T-cell factor specific for poly(Try,Glu)-polyDLAla--polyLys is reported. It is shown to be a product of the *I-A* subregion of the *H-2*complex by the use of antisera either raised specifically against or made specific, by absorption, for different regions of the *H-2* complex. The factor cooperates across allogeneic barriers, e.g., when factor produced by one strain is combined with bone marrow cells of other *H-2* incompatible strains.

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