Brief Definitive Report

RESTRICTION MOLECULES INVOLVED IN THE INTERACTION OF T CELLS WITH ALLOGENEIC ANTIGEN-PRESENTING CELLS*

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T cells stimulated by a soluble antigen in vivo and restimulated in vitro respond to the stimulus by proliferation, provided that the stimulating and restimulating antigen-presenting cells (APC) share molecules controlled by the major histocompatibility complex (MHC) (1). The response of T cells to an antigen is thus restricted by the MHC molecules of the APC (in the mouse, the $A_\alpha A_\beta$ or A and $E_\alpha E_\beta$ or E molecules controlled by the H-2 complex) (2–5). The interaction of syngeneic T cells and APC is subject to two constraints. First, certain combinations of antigen and MHC molecules are not recognized by T cells, and this nonrecognition constitutes the basis for the identification of MHC-associated immune response (*Ir*) genes. Second, in strains carrying responder haplotypes (i.e., strains in which a given combination of MHC molecules and antigen is recognized by T cells), the MHC context of antigen recognition is remarkably constant. Thus, some antigens, such as poly($Glu^{40}Ala^{60}$) (GA) and lactate dehydrogenase B (LDH_B), are recognized by all responder strains in the context of the A molecule, whereas others, such as poly(Glu^{51}, Lys^{34}, Tyr^{15}) (GLT), are recognized (with the exception of two *H-2* haplotypes) in the context of the E molecule (5, 6). We have recently demonstrated that the first constraint does not operate when the T cells and the APC are allogeneic: not only are antigen-pulsed APC able to stimulate allogeneic T cells, but the stimulation occurs regardless of whether the T cell-APC combination is of the R-R, R-NR, NR-R, or NR-NR type (where R and NR are responder and nonresponder MHC haplotypes, respectively, of T cells and APC) (7–9). In this paper we provide evidence that the second constraint does not apply to allogeneic T cell-APC interactions, either; when the E molecule is expressed on the cell surface of the APC, T cells recognize the antigen in the context of both the A and E molecules.

Materials and Methods

Mice. All mice were obtained from our colony at the Max Planck Institute for Biology. 8–15-wk-old females and males were used.

Antigens. The random copolymers of amino acids GA (Miles-Yeda, Rehovoth, Israel) and GLT (a gift of Dr. P. H. Maurer, Thomas Jefferson University, Philadelphia, PA) were dissolved in distilled water (pH 8.1), aliquoted, and stored at −20°C. The ammonium sulfate

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precipitate of LDH_B (Boehringer, Mannheim, FRG) was dialyzed against culture medium, sterilized by γ-irradiation (3,000 rad) and stored at 4°C.

Monoclonal Antibodies. Ascites fluids were produced using the hybridomas 118-49R2 (anti-Ia.m1), B15-124R4 (anti-Ia.m2), B17-263 (anti-Ia.m3), B17-123R7 (anti-Ia.m4), 17/-27.R7 (anti-Ia.m5), 13/18 (anti-Ia.m7), B22–227.R19 (anti-Ia.m8) (10), P47-42 (anti-Ia.m9) (11), and 10-3.6.2 (anti-Ia.17) (12). Nonspecific inhibitory substances of low molecular weight occasionally present in ascites fuids were removed by ultrafiltration using Amicon XM-100A filters (Amicon Corp., Scientific Sys. Div., Lexington, MA) (13). The filtered antibodies were stored at −70°C.

Cell Cultures. The culture medium used was RPMI 1640 supplemented with 5% heat-inactivated horse serum (Gibco Europe Ltd., Paisley, United Kingdom), antibiotics, and 5×10^{-5} M 2-mercaptoethanol. Removal of alloreactivity, priming of T cells in vitro, and assaying of secondary T cell proliferation were performed as described previously (7). Briefly, splenic T cells from unprimed mice were co-cultured with allogeneic glass-adherent peritoneal cells for 3 d, and alloreactive T cells were removed by treatment with 5-bromo-2-deoxy-uridine (BUdR) and light (14). The surviving T cells were primed in bulk cultures with GA (40 μg/ml), GLT (40 μg/ml), or LDH_B (15 μg/ml) in the presence of fresh allogeneic APC for 7 d. Priming of T cells with antigen and syngeneic APC was done in the same way, including preculture with APC and BUdR plus light treatment. T cells were then distributed in flat-bottomed microculture plates at a density of 1×10^5 per well, together with 1×10^5 fresh APC, with or without antigen. Monoclonal antibodies at the appropriate dilutions were included in the same culture volume (0.2 ml). Proliferation was measured by incorporation of [^{3}H]thymidine on day 3 of culture. All determinations were done in triplicate, and the standard deviation did not exceed ±20% of the mean.

Results

The response of in vivo primed T cells to GA and LDH_B is restricted by the A molecule and that of to GLT by the E molecule (6). As shown in Table I, this selective restriction applies to the proliferative response of T cells primed in vitro as well. Thus the responses to GA and LDH_B are only inhibited with A-specific antibodies, whereas the anti-GLT response is selectively inhibited with the E-specific Ia.m7 antibody. In the allogeneic T cell-APC interactions, this constraint on the MHC context of recognition no longer holds (Table II, Fig. 1). Of the eight combinations tested for the anti-GA response, the APC did not express cell-surface E molecules in three, and in

TABLE I

Restriction Molecules in Syngeneic T Cell-APC Interactions

T cell and APC		Antigen	Response*		Percent inhibition of response by‡		Restriction molecule
Strain	H-2		Δ cpm	(S.I.)	Anti-A	Anti-E§	
B10.D2	*d*	GA	3,572	(13.3)	75 (Ia.m5)§	6	A
CBA	*k*	GA	4,754	(14.4)	51 (Ia.m5)	0	A
BALB/c	*d*	LDH_B	3,069	(4.9)	83 (Ia.m4)	5	A
B10.RIII	*r*	LDH_B	10,604	(4.1)	64 (Ia.m4)	7	A
B10.D2	*d*	GLT	6,082	(13.5)	5 (Ia.m4)	84	E

* Δ cpm represents cpm in cultures with antigen and APC minus cpm in cultures with APC and without antigen; stimulation index (S.I.) indicates cpm in cultures with antigen and APC divided by cpm in cultures with APC but without antigen. The background cpm in cultures without antigen were in the range 290–3435.

‡ Percent reduction of cpm in the presence of antibodies (final dilution 1:600).

§ The E molecule was tested using Ia.m7-specific antibody. The specificity recognized by each A-specific antibody is given in parentheses.

TABLE II

Restriction Molecules in Allogeneic T Cell-APC Interactions

T cell		APC		Antigen	Response*	Percent inhibition of response by‡		Restriction molecule
Strain	*H-2*	Strain	*H-2*			Anti-A	Anti-E§	
					Δ *cpm* (*S.I.*)			
C57BL/10	*b*	B10.Q	*q*	GA	11,338 (8.4)	76(Ia.m9)§	—‖	A
BALB/c	*d*	B10.BR	*k*	GA	14,829 (13.3)	98(Ia.m2)	NT¶	A (E not tested)
A.CA	*f*	B10.Q	*q*	GA	12,332 (9.9)	76 (Ia.m9)	—	A
B10.BR	*k*	B10.D2	*d*	GA	2,970 (4.7)	38 (Ia.m5)	73	A + E
CBA	*k*	B10.Q	*q*	GA	7,487 (3.6)	71 (Ia.m9)	—	A
A.SW	*s*	BALB/c	*d*	GA	11,121 (29.2)	87 (Ia.m8)	NT	A (E not tested)
A.SW	*s*	BALB/c	*d*	GA	6,010 (7.5)	91(Ia.m5)	43	A + E
A.SW	*s*	C3H.NB	*p*	GA	14,151 (4.4)	80(Ia.m3)	81	A + E
B10.A	*a*	BALB/c	*d*	LDH_B	14,022 (8.6)	87(Ia.m8)	85	A + E
B10.M	*f*	B10.WB	*j*	LDH_B	2,383 (3.7)	68(Ia.m3)	56	A + E
B10.M	*f*	B10.BR	*k*	LDH_B	3,812 (4.2)	59(Ia.m1)	67	A + E
DBA/1	*q*	C57BL/10	*b*	LDH_B	8,829 (5.1)	80(Ia.m5)	—	A
DBA/1	*q*	BALB/c	*d*	LDH_B	5,326 (7.4)	51(Ia.m5)	50	A + E
A.SW	*s*	B10.D2	*d*	LDH_B	5,074 (12.6)	81(Ia.m3)	59	A + E
A.SW	*s*	B10.Q	*q*	LDH_B	18,759 (8.2)	79(Ia.m3)	0**	A
BALB/c	*d*	A.CA	*f*	GLT	7,767 (5.7)	81(Ia.17)	6**	A
B10.D2	*d*	B10.M	*f*	GLT	15,513 (6.2)	NT	8**	A
B10.D2	*d*	B10.BR	*k*	GLT	9,061 (2.6)	85(Ia.m1)	0	A
BALB/c	*d*	B10.Q	*q*	GLT	11,024 (11.3)	90(Ia.m9)	18**	A
B10.BR	*k*	B10.D2	*d*	GLT	6,683 (14.6)	63(Ia.m5)	91	A + E
A.SW	*s*	BALB/c	*d*	GLT	18,867 (5.2)	86(Ia.m3)	94	A + E

* See footnote to Table I. The background cpm in cultures without antigen were in the range 325–4213.
‡ Percent reduction of cpm in the presence of antibodies (final dilution 1:600 or 1:640).
§ See footnote to Table I.
‖ Molecule is not expressed on the cell surface; antibody inhibition was not done.
¶ Not tested.
** Molecule is not expressed on the cell surface; antibody inhibition was done.

these the GA was recognized in the context of A molecules; in the remaining combinations the E molecule was expressed by the APC, and in the three cases tested, the GA was recognized in the context of both A and E molecules. Of the seven allogeneic T cell-APC combinations tested for the anti-LDH_B response, the APC expressed both A and E molecules in five, and in all five combinations both molecules provided the context for LDH_B recognition. Finally, in all of the six combinations tested for the anti-GLT response, the A molecule provided the context for GLT recognition (in three of the six combinations, the E molecule was expressed on the APC, and in two of these three combinations it served as restriction element for GLT). In summary, in 10 out of 11 combinations, where both the A and the E molecules were expressed on the surface of the APC, both were used as restriction elements, regardless of whether in syngeneic T cell-APC combinations only the A or the E molecules were used for the context of recognition. Thus, the constraints on the context of recognition observed in syngeneic T cell-APC interactions do not apply when the T cells and the APC are allogeneic.

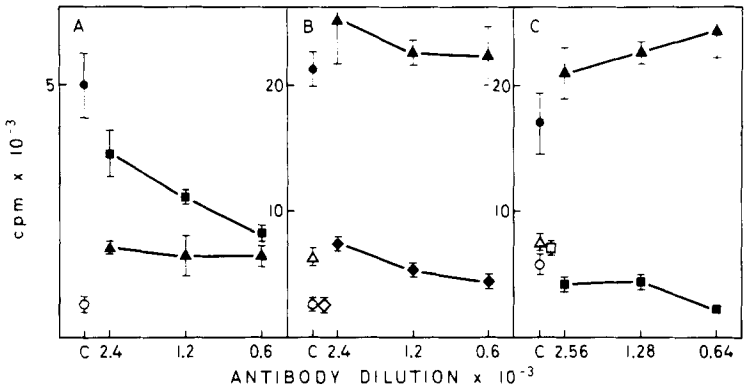

FIG. 1. Inhibition with monoclonal Ia-specific antibodies of T cell proliferation to antigens presented by allogeneic APC. (A) Response of B10.M T cells to LDH_B on B10.BR APC (A + E restricted). (B) Response of A.SW T cells to LDH_B on B10.Q APC (A restricted, APC do not express E molecules). (C) Response of B10.D2 T cells to GLT on B10.BR APC (A restricted, APC express E molecules). The antibodies used were anti-Ia.m1 (■), anti-Ia.m3 (◆), and anti-Ia.m7 (▲). The controls (C) include antigen + APC (●), medium + APC (○), and medium + antibodies (other open symbols). Vertical bars represent ± SD.

Discussion

The remarkable constancy observed in syngeneic T cell-APC interaction with respect to channelling of a response via either A or E molecules can theoretically be explained in one of three ways. First, the germ line repertoire of T cell receptors contains only one type of T cells, either anti-A + X or anti-E + X (where X represents the foreign antigen). Second, the germ line repertoire contains both anti-A + X and anti-E + X T cells, but either the anti-A + X or the anti-E + X cells are eliminated (or not expanded) during T cell ontogeny. Third, the antigens fail to form an immunogeneic complex with one of the two restriction molecules on the APC. The data presented in this communication help to choose among these three possibilities. It is clear from these and previous experiments (7), and from the recent work of Clark and Shevach (15) that selective restriction cannot be a failure of either the A or the E molecule to form immunogeneic complexes with certain antigens. Furthermore, selective restriction cannot result from the lack of anti-A + X or anti-E + X clones from the germ line repertoire, because both kinds of clone are found in T cell responses restricted by allogeneic MHC molecules (Fig. 1, Table II). In fact, the allogeneic MHC-restricted responses appear to reveal a germ line-type, unselected T cell repertoire. Thus, by exclusion we suggest that both nonresponsiveness and selective restriction are probably the result of elimination (or tolerance) of self-reactive clones from the T cell repertoire (16). The data do not explain, however, why the response to a given antigen uses invariably the same restriction molecule in all responder haplotypes (5, 6). Identification of the self antigens that cause "holes" in the T cell repertoire would probably help to resolve this problem.

Summary

The proliferative responses of T cells, depleted of alloreactive cells, were tested upon stimulation by antigens presented on allogeneic antigen-presenting cells (APC). Restriction molecules involved in these responses were identified by inhibition of T

cell proliferation with monoclonal antibodies against $A(A_\alpha A_\beta)$ and $E(E_\alpha E_\beta)$ molecules of the APC. The responses to all three antigens tested [Poly($Glu^{40}Ala^{60}$) (GA), lactate dehydrogenase B (LDH_B), and poly(Glu^{51}, Lys^{34}, Tyr^{15}) (GLT)] were A plus E restricted when the allogeneic APC expressed both molecules, and only A restricted when the APC did not express cell surface E molecules. In contrast, when T cells and APC are syngeneic, the same antigens are recognized only in the context of either A molecules (GA and LDH_B) or E molecules (GLT). The data indicate that the immune response gene control of these responses is not associated with either a failure of antigen presentation, or the lack of certain T cell specificities from the germ line repertoire, but probably with selective somatic elimination (tolerance) of certain clones from the T cell repertoire.

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