

ANALYSIS OF CROSS-REACTIVE ANTIGEN-SPECIFIC T CELL CLONES

Specific Recognition of Two Major Histocompatibility Complex (MHC) and Two Non-MHC Antigens by a Single Clone

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The recognition of an antigen by T lymphocytes is highly specific and has been shown to be associated with the recognition of determinants present on molecules encoded by genes in the major histocompatibility complex (MHC).¹ This concomitant recognition has been referred to as MHC restriction (1) and theories on the nature of the T cell receptor for antigen and immune response (Ir) gene control must take into account the phenomenon of MHC restriction (1, 2). Recently, studies at the clonal level have demonstrated that antigen-specific T cell clones are specific for antigen associated with self-MHC gene products (2). Several reports, however, have noted that T cells restricted to class I and class II antigens may also cross-react with other antigens, especially allogeneic MHC gene products (2-7). Data are presented here analyzing several T cell clones restricted to Class II antigens and recognizing either influenza viral antigens or myelin basic protein. Both of these clones are also stimulated by minor H antigens recognized in association with allo-MHC antigens. The specificity of the antigens stimulating these clones have been assessed by genetic and serological analysis.

Materials and Methods

T Cell Lines and Clones. The BRSR cell line consists of Ly1⁺2⁻ T cells specific for myelin basic protein (BP) of rat or mouse origin. This cell line was selected from (SJL/J × BALB/c)F₁ mice injected with BP in adjuvant (8). The cells were maintained in vitro for 9 months in propagation medium enriched with IL-2 (11) and then cloned using the limited dilution technique by seeding 0.25 cells per well (9). Nine clones were obtained out of 96 wells; all of them were responsive against BP and also against DBA/2 cells. BR-9 cloned cells, used in this study, as well as the BRSR cell line, were found to recognize BP in association with F₁ hybrid I-E molecules, subsequently referred to as I-E^{a/d} (manuscript in preparation).

The ABF cell line originated from spleen cells of B10.BR mice which were inoculated

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¹ *Abbreviations used in this paper:* BP, myelin basic protein; Con A, concanavalin A; IL-2, interleukin 2; mAb, monoclonal antibody; minor H, minor histocompatibility; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction.

intraperitoneally with 100 HA units of A/PR/8/34 type A influenza virus (PR8-virus). The spleen cells were selected in vitro for reactivity against PR8-virus by co-culturing 5×10^6 nucleated spleen cells from primed mice with 3×10^5 irradiated (1,500 rad) and virus-infected syngeneic stimulator cells in 2 ml of proliferation medium (11). After 5 d, cells were transferred into propagation medium (8), and after 3 mo in vitro, the ABF cell line was cloned as described above. 11 clones were obtained, all of which responded to PR8-infected syngeneic cells, and only 7 clones were found cross-reactive against DBA/2 cells. The AB-4 and AB-6 cloned cells used in this study and also the ABF cell line were found to recognize PR8-virus in association with I-A^k MHC antigens.

In Vitro Proliferative Response of T Cell Lines and Clones. Antigen-specific and mixed lymphocytes responses (MLR) were carried out in microculture wells, as previously described (8). Briefly, 2.5×10^4 responder cells were co-cultured with 5×10^5 irradiated syngeneic spleen cells in 0.2 ml of proliferation medium in the presence or absence of antigen (BP or PR-8 virus) for assessment of the proliferative response, or with 5×10^5 irradiated allogeneic spleen cells for the assessment of the MLR. Cultures were incubated for 64–68 h and for the last 18 h cultures were pulsed with 1 μ Ci of [³H]thymidine (specific activity 2.5 μ Ci/10 mM, Amersham, New England). Results were expressed as mean cpm of triplicate cultures. The standard deviation was between 5 and 15% of the mean cpm.

Blocking with Monoclonal Anti-Ia Antibodies. Monoclonal antibody (mAb) 10.2.16 (Salk Cell Distribution Center, La Jolla, CA) is an anti-Ia-17 murine antibody with specificity for the I-A^{k,s,f,r,u} subregion gene product (10). M5/114.2 is a rat mAb with specificity for I-A^d, I-E^d, I-E^k, and I-A^b molecules (11). Supernatants from in vitro passaged cells were used at a final concentration of 10–15%.

Results

Antigen-specific T Cell Lines Cross-reactive with Minor Histocompatibility (H) Alloantigens. Two T cell lines of different mouse strain origin and with different antigenic specificity, BRSR and ABF, were tested for alloreactivity against a panel of cells with varying H-2 haplotypes. Both cell lines responded specifically to the immunizing antigen, and both lines were depleted of alloreactivity against the majority of the H-2 haplotypes tested (Table I). Some of the 25 mouse strains tested are listed in Table I. The BRSR cell line was depleted of alloreactivity against B10.BR, B10.D2, C57BL/6, A.TH, and B10.S irradiated spleen cells. The ABF cell line also did not react against irradiated spleen cells from these strains. Both cell lines showed consistent reactivity against some allogeneic cells; however, each line also displayed a unique alloreactivity pattern (Table I). The BRSR cell line showed alloreactivity against NZB, DBA/2, and to a lesser extent, to B6D2F₁ and DBA/1. The ABF cell line showed consistent reactivity against DBA/2 and (SJL/J \times BALB/c)F₁ and to a lesser extent also against B6D2F₁ and BALB/c. The alloreactivity of both cell lines appeared to be directed at least in part to minor histocompatibility (H) antigens since cells from B10.D2 mice (H-2^d) failed to stimulate, while cells from other strains that share the H-2^d haplotype (DBA/2, NZB, and B6D2F₁ mice) showed consistent stimulation. Thus, BRSR and ABF cell lines that are, respectively, specific for BP in association with an I-E^{s/d} hybrid gene product (unpublished data), and for the influenza virus, PR-8, in association with I-A^k gene products, appeared to cross-react with minor alloantigens of DBA/2, B6D2F₁, and to a lesser extent with antigens on the cells of other strains of mice.

Clonal Analysis of the Cross-reactivity with the DBA/2 Minor H Alloantigen. The reactivity of both cell lines to the priming antigens and to various alloantigens required us to clone these cell lines. We have cloned them using the limiting dilution technique and have focused on their cross-reactivity with DBA/2, which was observed for both

TABLE I
Cross-reactivity of Antigen-specific Long-term T Cell Lines

Irradiated [§] spleen cells	Proliferative responses of T cell lines (mean cpm × 10 ⁻³)*							
	BRSR (anti-BP) [‡]				ABF (anti-PR8-Virus) [‡]			
	Response to antigen:			Mixed lymphocyte response	Response to antigen:			Mixed lymphocyte response
None	BP [¶]	Con A	None		PR8-virus**	Con A		
(SJL/J × BALB/c)F ₁	0.4	34.2	42.1 ^{††}	—	—	—	—	26.4
B10.BR				1.1	0.3	38.3	41.2	—
BALB/c				0.5				4.4
B10.D2				0.7				1.9
C57BL/6J				0.8				1.3
NZB				19.5				ND
DBA/2				27.6				39.5
DBA/1				4.2				1.4
(C57BL × DBA/2)F ₁				8.6				11.7
A.TH				0.3				0.5
B10.S				0.4				1.2

* Mean cpm of triplicate cultures, standard duration was usually 5-15% of mean cpm.

[†] 2.5 × 10⁴ responders per culture.

[§] 5 × 10⁵ irradiated spleen cells per culture.

[¶] BP = 125 µg/ml, concanavalin A (Con A) = 2.5 µg/ml.

** 5 HA units.

^{††} Highly significant responses were underlined.

TABLE II
Cross-reactive T Cell Clones Recognize the Priming Antigen in Association With Self-Ia and DBA/2 Minor H Antigens in Association With Allo Ia Antigen

Irradiated spleen cells	Blocking mAb	Proliferative response of Cloned T cell line (Mean cpm × 10 ⁻³)*		
		BR-9	AB-4	AB-6
DBA/2	None	28.4	17.4	0.5
	10.2.16	26.3	16.5	0.5
	M5/114.2	3.8	0.4	0.4
DBA/1	None	0.2	0.3	0.3
	D2.GD	12.7	18.0	2.2
B10.GD	None	14.3	16.9	0.2
	10.2.16	2.2	0.2	0.3
	M5/114.2	0.3	0.2	0.4
B10.D2	None	0.2	0.3	0.2
B10.BR	None	ND [‡]	0.3	0.4
B10.BR-PR8 [§]	None	ND	23.1	30.1
	10.2.16		0.8	1.6
	M5/114.2		29.6	32.9
(SJL/J × BALB/c)F ₁	None	0.3	ND [‡]	ND
(SJL/J × BALB/c)F ₁ -BP [¶]	None	38.7	ND	ND
	10.2.16	41.3		
	M5/114.2	42.7		

* See legend of Table I.

[‡] ND, not done.

[§] B10.BR irradiated spleen cells were infected with PR8 virus.

[¶] (SJL/J × BALB/c)F₁ irradiated spleen cells were conjugated with BP.

lines. All of the clones that had been obtained from the BRSR line responded to DBA/2 cells, as well as to the priming BP antigen. From the ABF cell line, all the clones obtained responded to PR8-virus, but only 7 clones (out of 11) were also reactive against DBA/2. No clones were obtained from either line that reacted only with DBA/2. Table II shows the alloreactivity of three representative clones. BR-9

cloned cells derived from the BRSR line responded strongly against BP in the presence of syngeneic accessory cells, and also cross-reacted with DBA/2 antigens, on DBA/2 and D2.GD stimulator cells. AB-4 and AB-6 cloned cell lines both reacted against the specific antigen, influenza virus, but only the AB-4 clone cross-reacted with DBA/2 and D2.GD. The cross-reactive responses of BR-9 and AB-6 were roughly comparable to their response to the specific antigen. The lack of cross-reactivity of the virus-specific AB-6 cloned cells suggests that the stimulation by DBA/2 cells is due to specific antigen recognition rather than to nonspecific stimulation. The lack of clones from either line that were reactive against DBA/2 H antigens only, demonstrates that the cross-reactivity of ABF or BRSR lines could not be due to contaminating alloreactive cells. Furthermore, cloning of the ABF cell line in the presence of irradiated DBA/2 cells and interleukin 2 (IL-2) resulted in five clones that were reactive against DBA/2 H antigens as well as against PR8-virus antigens (data not shown).

The data presented in Table II also confirms that the alloreactivity of BP or virus-specific T cells against DBA/2 and D2.GD must involve minor H antigens of DBA/2, and are not directed only to the MHC antigens, since H-2 identical B10.D2 and B10.GD cells failed to stimulate proliferative responses (Table II).

Dual Specificity of T Cell Clones: Recognition of Conventional Antigen Associated with Self-Ia Antigen and Minor Alloantigen Associated with Allo I-A^d Antigen. It has been previously demonstrated (8) that minor H alloantigens are recognized by cloned T cells in association with the MHC gene products syngeneic to the responder T cells. Genetic analysis and antibody-blocking experiments were therefore performed to determine whether MHC antigens were involved in the response to DBA/2. As indicated above, BR-9 and AB-4 cloned cells, specific for BP and PR8 virus, respectively, cross-react with DBA/2 and D2.GD but not with DBA/1, B10.GD, or with B10.D2 cells (Table II), suggesting that BR-9 and AB-4 might require both minor H antigens and the I-A^d MHC antigens present on DBA/2 and D2.GD cells. The D2.GD strain that shares only the K^d and I-A^d MHC antigens with DBA/2 and also expresses DBA/2 background antigens, stimulated the BR-9 and AB-4 clones (Table II). Furthermore, the M5/114.2 mAb with specificity for I-A^d, but not the 10.2.16 mAb with specificity for I-A^k, could block the in vitro stimulation of BR-9 and AB-4 cloned T cells by DBA/2 and D2.GD irradiated cells. On the other hand, M5/114.2 mAb could not block the stimulation of BR-9 cloned cells by BP plus (SJL/J × BALB/c)F₁ accessory cells nor the stimulation of AB-4 cloned cells by PR8 virus plus B10.BR syngeneic accessory cells. 10.2.16, however, does block effectively the response of AB-4 or AB-6 to PR8 virus (Table II). Thus, those T cell clones that are specific for BP associated with I-E^{s/d} and PR8-virus associated with I-A^k (Table II) appear to also recognize DBA/2 minor H antigens in association with allo I-A^d. Furthermore, the serological analysis demonstrated that the allo I-A^d MHC epitopes recognized by these clones are not shared with self-I-A epitopes (11, 12).

Analysis of the Cross-reactive DBA/2 Minor H Antigens. The availability of two independent antigen-specific T cell clones that are both cross-reactive with DBA/2 antigens allowed us to determine (a) whether AB-4 and BR-9 clones recognize the same DBA/2 antigens, (b) whether DBA/2 antigens are cross-reactive with BP or PR8 virus antigens, and (c) whether there is a requirement for a specific association between the MHC antigens and the non-MHC antigens. Table III shows that the BR-9 clone that is cross-reactive with DBA/2 antigens could not be stimulated with

TABLE III
Stimulation of Cross-reactive T Cell Clones Requires the Association of a Specific Non-MHC Antigen With a Specific MHC Antigen

Irradiated spleen cells	Proliferative Response of Responder T Cells (cpm $\times 10^{-3}$)				
	Antigen	BRSR Line	BR-9 Clone	ABF Line	AB-4 Clone
(SJL/J \times BALB/c)F ₁	None	0.3	0.4	14.4	8.9
	BP	24.6	22.4	14.6	8.6
	PR8-virus	0.4	0.3	14.9	9.1
B10.BR	None	0.2	ND	0.1	0.2
	PR8-virus	0.3		15.8	16.5
	BP	0.2		0.2	0.2
DBA/2	None	19.5	12.7	21.8	26.4
B10.D2	None	ND [†]	0.4	ND	0.4
	BP		0.3		0.5
	PR8-virus		0.5		0.5

* See legend for Table I.

[†] ND, not done.

syngeneic (SJL/J \times BALB/c)F₁ or with allogeneic B10.D2 or B10.BR cells infected with PR8 virus. Similarly, the AB-4 clone that is also cross-reactive with DBA/2 antigens could not be stimulated in vitro by BP in association with syngeneic B10.BR cells, B10.D2, or (SJL/J \times BALB/c)F₁ cells. The failure of (SJL/J \times BALB/c)F₁ cells infected with PR8 virus to stimulate the syngeneic BR-9 cells, and the failure of BP associated with B10.BR cells to stimulate AB-4 cells demonstrate that BP and PR8 virus are not cross-reactive. Similarly, the failure of both BP and PR8 virus associated with I-A^d (B10.D2 cells) to stimulate BR-9 or AB-4 cells, respectively, suggests (a) that the virus or BP do not cross-react with DBA/2 minor H antigens, (b) that these clones recognize different determinants on the DBA/2 minor H antigens, and (c) that there is a requirement for a specific association between the different MHC antigens and the non-MHC antigens to stimulate the cells.

Discussion

Two T cell proliferative clones generated to different antigens have been shown to also recognize DBA/2 minor H alloantigens in association with allo I-A^d antigens. Furthermore, experiments using mAb to I-A antigens (Table II) not only confirmed that these clones recognize DBA/2 minor H antigens in association with I-A^d, but also make it unlikely that the I-A^d epitope recognized by the AB-4 clone is shared by the I-A^k antigen (11). The data also suggest the BR-9 and AB-4 clones recognize different determinants on DBA/2 cells (Table III). This is based on the assumption that if the BR-9 and AB-4 cells recognize the same DBA/2 minor H antigen associated with I-A^d MHC antigen, it would be expected that the association of DBA/2 minor H antigen with I-A^d generates a recognition unit that would mimic both the BP + I-E^{s/d} and PR8 virus + I-A^k recognition units. If this were the case then both the BP + I-E^{s/d} and the PR8-virus + I-A^k recognition units should stimulate the BR-9 and AB-4 clones reciprocally. Since the AB-4 clone could not be stimulated in vitro by BP associated with (SJL/J \times BALB/c)F₁ cells, and the BR-9 clone could not be stimulated by PR8 virus-infected B10.BR cells, it is most likely that AB-4 and BR-9 clones recognize different DBA/2 minor H alloantigens.

The data directly demonstrated that a T cell clone may recognize two pairs of determinants, each pair consisting of an MHC and a non-MHC determinant. The

data in Table III, however, showing that the AB-4 T cell clone is cross-reactive with (BALB/c \times SJL/J) F_1 antigens in addition to DBA/2 antigens indicate that AB-4 cloned cells may recognize a third I-A antigen (since this cross-reactivity could not be blocked by mAb anti-I-A^k or -I-A^d; data not shown), and possibly also another minor H alloantigen.

What are the implications of these cross-reactivities for the nature of the T cell receptor? Of major interest are the findings demonstrating a requirement for a specific association between MHC antigens and the non-MHC antigens to stimulate a T clone. For example, the AB-4 cloned cell line recognizes two different MHC antigens, I-A^k and I-A^d, each in association with a distinct unrelated non-MHC antigen, i.e., PR8 virus and DBA/2 minor H antigen, respectively. I-A^d (B10.D2 cells) associated with PR8 virus or with BP could not stimulate AB-4 cells or BR-9 cells (Table III). A cross-reactivity that is similar in nature to that presented here has been obtained with a cytotoxic T cell clone derived from a chimera (6). The authors of that study have interpreted the cross-reactivity as supporting the "altered self" hypothesis, to explain the associative recognition of antigen with MHC gene products by T cells. Our data, demonstrating the recognition of four antigens, using more defined antigens and two clones that were derived from mice primed in vivo to very different antigens, support their interpretation. The specific associative recognition of non-MHC antigen with the MHC antigens by the AB-4 or BR-9 cloned cells strongly supports the "altered self" hypothesis, i.e., a single T cell antigen receptor. It appears rather difficult to explain these cross-reactivities by the dual recognition model, since this model would predict either cross-reactivity for both receptors or that more than two receptors are expressed by a single clone cell population. The data (Table III) argue against the former possibility since the two MHC and two non-MHC antigens are not independently recognized by these T cell clones.

The fact that the BRSR and BR-9 cells which are specific for self-antigens and induce experimental autoimmune encephalomyelitis (8) can also be stimulated with DBA/2 minor H alloantigen, warns of the possibility of the inverse reaction. Thus, following allotransplantations, it may also be possible to activate autoimmune clones, although clinical manifestations may be masked by the effects of immunosuppressive therapy.

Summary

Two T cell clones, one specific for I-E^{s/d} plus myelin basic protein (BP) and another specific for I-A^k plus influenza virus have been demonstrated to cross-react with DBA/2 cells. Genetic and serological analyses have shown that each clone recognizes its respective priming antigen in association with self-major histocompatibility complex (MHC) determinants and each recognizes DBA/2 minor H antigens in association with allo I-A^d MHC antigens. Further analysis of these clones suggests (*a*) that the allo I-A^d MHC epitopes recognized by these clones are not shared with self-I-A epitopes, (*b*) that the virus or BP antigens do not cross-react with DBA/2 minor H antigens, (*c*) that these clones recognize different determinants on the DBA/2 minor H antigens, and (*d*) that there is a requirement for a specific association between the different MHC antigens and the non-MHC antigens to stimulate these clones. This

specific associative recognition argues strongly for the "altered self" hypothesis.

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