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Immunology

Antigen/major histocompatibility complex-specific activation of murine T cells transfected with functionally rearranged T-cell receptor genes

(gene transfer/antigen specificity/major histocompatibility complex restriction)

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ABSTRACT **The genes encoding the α and β chains of the T-cell antigen receptor isolated from a cytochrome *c*-specific, major histocompatibility complex (MHC)-restricted murine T-cell hybridoma were introduced into a mouse T-cell line of helper lineage by electroporation. In order to examine the contributions of those gene products to antigen and/or MHC specificity, the resultant transfectants were tested for functional antigen and/or MHC recognition. Only those transfectants that express both the introduced genes (α and β) contributed by the normal T cell can respond specifically to the appropriate antigen/MHC pair. None of the transfectants that express only one of the introduced genes (α or β) of the normal T cell, or paired hybrid genes (i.e., one gene from the normal T cell and the other from the fusion partner), can respond to the same combination of antigen and MHC product recognized by the donor T cell. However, one clone expressing the transfected genes that encode the α and β chains of the fusion partner shows reactivity to the antigen-presenting cells even in the absence of the antigens. These data suggest that the $\alpha\beta$ heterodimer of the T-cell receptor is required to define the fine specificity of a T cell.**

Thymus-derived (T)-lymphocyte recognition of products of the major histocompatibility complex (MHC) is a major factor controlling the vertebrate immune response. When responding to foreign antigens, T cells recognize the foreign molecules together with one of the self class I or class II MHC products expressed on the surface of the antigen-presenting cell (APC; for review, see ref. 1), a property termed MHC restriction. A relatively high proportion of T cells are capable of recognizing foreign- or allo-MHC products alone (2–5).

The T-cell antigen receptor responsible for MHC-restricted antigen recognition is a disulfide-linked heterodimer consisting of transmembrane α and β chains. Both α and β chains contain variable (V) and constant (C) regions homologous to immunoglobulin V and C regions (for review, see refs. 6–8). Recently, two sets of somatic cell genetic experiments indicated that a particular $\alpha\beta$ T-cell receptor heterodimer is essential for responses to a defined antigen plus MHC product (9) or MHC products alone (10). More direct evidence that the $\alpha\beta$ heterodimer alone may define the dual specificity (antigen plus MHC) of a given T-cell clone has also been reported (11, 12). Neither the mouse α chain nor the mouse β chain, alone or when expressed with a random human partner, mediates recognition of either specific antigen or MHC molecule (12).

The helper-T-cell response to cytochrome *c* in mice is a well-defined model for studying the T-cell response to restricted antigen and MHC determinants (for review, see ref. 1). Helper T (T_H) cells specific for cytochrome *c* or its carboxyl-terminal peptide analogues were raised in different MHC congenic strains of mice and several V_α and V_β genes were isolated and sequenced. It was shown that a single V_α gene segment ($V_\alpha 11.1$) is expressed in 14 of 19 murine T_H cells responding to cytochrome *c* (13–15). This observation, as well as others, has led to the proposal that the V_α gene segment plays a central role in controlling antigen specificity. We have examined the role the $V_\alpha 11.1$ gene from one cytochrome *c*-specific T_H hybridoma (V1.9.2) plays in generating cytochrome *c* specificity by transfecting it into a mouse cell line of the helper lineage singly and with homologous and heterologous β-chain genes. We show here that only transfectants expressing both the α and the β genes contributed by the V1.9.2 T_H cell reconstitute the antigen-specific, MHC-restricted fine specificity of the donor cells, emphasizing the importance of both α and β chains in antigen binding. One striking observation is that the expression of the α and β genes contributed by the fusion partner endows the recipient cell with the specificity to recognize particular APCs in the presence or absence of antigen, suggesting that these T-cell receptor molecules have allospecificity. These data provide direct evidence that the $\alpha\beta$ heterodimer of the T-cell receptor is required for the specific recognition of the antigen–MHC pair and strongly suggest that α and β chains are involved in the direct recognition of MHC molecules in the absence of antigen.

MATERIALS AND METHODS

Mice, Culture Medium, and Cell Lines. All mice were obtained from The Jackson Laboratory. Supplemented Dulbecco's modified Eagle's (DME) medium with the addition of antibiotic G418 (2 mg/ml) was used as the G418-containing medium. Hypoxanthine (15 μg/ml), aminopterin (0.2 μg/ml), and thymidine (8 μg/ml) were added to supplemented DME medium to make HAT-containing medium. The EL-4G-12 lymphoma cell line (16) and the L-cell transfectants (17) CA36.1.3 (expressing I-E^k molecules) and CA14.11.14 (expressing I-A^k molecules) were provided by R. T. Smith and B. Malissen, respectively. The V1.9.2 hybridoma was provided by D. Hansburg (Fox Chase Cancer Center, Philadelphia, PA). The interleukin 2 (IL-2)-dependent cell lines CTLL-2 and HT-2 (18) were provided by J. Kappler and P. Marrack.

Plasmid Construction. Two clones containing full-length cDNAs for V1.9.2 and BW5147 α chains, respectively, were isolated (15) from a cDNA library of the V1.9.2 T_H hybridoma. The inserts were separately subcloned into a pSV2neo-based vector to generate clones pV192αneo, pV192α(−)neo, and pBWαneo.

A genomic clone containing the rearranged V1.9.2 β-chain gene (provided by N. Lan) was isolated (15). A 7.7-kilobase (kb)

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Abbreviations: MHC, major histocompatibility complex; IL-2, interleukin 2; APC, antigen-presenting cell; T_H cell, helper T cell; HAT, hypoxanthine/aminopterin/thymidine; SV40, simian virus 40; PMA, phorbol 12-myristate 13-acetate; V, variable; C, constant; J, joining; D, diversity.

fragment containing the complete V1.9.2 β gene was then subcloned into a pSV2gpt-based vector, generating clone pV192βgpt. A cDNA clone containing a 1.2-kb fragment corresponding to a full-length BW5147 β gene (provided by R. Barth, California Institute of Technology, Pasadena, CA) was subcloned to generate clone pBWβgpt. Two fragments, one corresponding to the simian virus 40 (SV40) enhancer/promoter and the other containing the SV40 polyadenylylation sites, were introduced into pSV2neo (19) or pSV2gpt (20) to generate the pSV2neo- or pSV2gpt-based vectors.

Transfection. EL-4G-12 cells were transfected by electroporation as described by Potter *et al.* (21).

IL-2 Production. IL-2 production by T-cell transfectants was assayed as described by Shastri *et al.* (17).

RNA Preparation and Analysis. RNA was prepared from T cells according to Chirgwin *et al.* (22). Total RNA was electrophoresed in agarose/formaldehyde gels, blotted onto nitrocellulose filters, and hybridized in 50% formamide according to standard procedures (23). Posthybridization washes were in 2× SSC/0.1% $NaDodSO_4$ at room temperature, followed by 0.2× SSC/0.1% $NaDodSO_4$ at 50°C. (1× SSC is 0.15 M NaCl/15 mM sodium citrate, pH 7.)

DNA Probes. The V-specific fragment DNAs were isolated from the plasmids pUC-Vα1 (provided by J. Kobori, California Institute of Technology), pUC-V_β1 (provided by R. Barth), and pUC-V_α11 (provided by A. Winoto, Massachusetts Institute of Technology, Cambridge, MA), labeled with [γ-^{32}P]ATP by polynucleotide kinase, and purified by gel electrophoresis according to standard procedures (23). The probes, with a specific activity of 1–3 × 10^8 cpm/μg, were used for hybridization.

RESULTS

Construction of Recombinant DNAs Containing the T-Cell Receptor Genes and Gene-Transfer Experiments. The T-cell receptor genes used for gene-transfer experiments were isolated from the cloned murine T-cell hybridoma V1.9.2. This hybridoma was derived by the fusion of T-cell blasts, from a B10.A(5R) mouse immunized with synthetic peptides analogous to the carboxyl-terminal end of cytochrome *c* (DASp; see Fig. 2 for peptide structure), and the T-cell line BW5147. The V1.9.2 hybridoma responds strongly to an acetimidyl form of the DASp peptide (AmDASp), but not to the DASp peptide, when presented by B10.A(5R) spleen cells (expressing $E^k_\alpha E^b_\beta$ molecules) or B10.A spleen cells (expressing $E^k_\alpha E^k_\beta$ molecules) (24), thus exhibiting a heteroclitic response.

Although T-cell receptor molecules have not been detected on the surface of the fusion partner BW5147, the α- and β-chain loci of this tumor cell line are rearranged and transcribed. Upon fusion with normal T cells, the BW5147-derived receptor molecules can be expressed on the hybridoma surface (9, 10). Therefore, T-cell hybridomas can express as many as four different receptors due to combinatorial association between α and β chains contributed by the normal T cell and the fusion partner. The antigen fine specificity of the V1.9.2 hybridoma (i.e., the heteroclitic reactivity toward AmDASp instead of DASp) could be explained, for example, by the combination of the V1.9.2 α and BW5147 β chains. To test this, both the V1.9.2 T-cell receptor genes and the BW5147 T-cell receptor genes were subcloned and used in the transfection experiments.

To be able to examine the contribution of the individual α and β chains to the observed antigen and MHC specificity of the V1.9.2 hybridoma, we decided to subclone the two pairs of genes into different selectable expression vectors—i.e., pSV2neo- and pSV2gpt-based vectors, respectively. The pSV2neo and pSV2gpt vectors were modified by inserting two DNA segments, one containing SV40 enhancer and promoter and the other containing SV40 polyadenylylation sites, to ensure the expression of the introduced α or β genes. A genomic fragment that contains the complete rearranged V1.9.2 β gene and a full-length BW5147 β cDNA clone were used in this study (Fig. 1 *b* and *e*). Because of the length of the introns between the C_α gene segment and rearranged $V_\alpha J_\alpha$ segments (25–27), full-length V1.9.2 and BW5147 α cDNA clones were used rather than genomic DNA fragments (Fig. 1 *a* and *d*). One construct in which the V1.9.2 α gene is opposite to the SV40-driven transcriptional orientation was also isolated and served as a control in the transfection experiments (Fig. 1*c*).

The plasmid constructs were individually transfected or cotransfected in $\alpha\beta$ combinations into the EL-4G-12 lymphoma cells by electroporation (21). The HAT-sensitive EL-4G-12 cell line was chosen as the recipient because of its ability to secrete IL-2 upon stimulation with T-cell mitogens (16). From several independent electroporation experiments, drug-resistant clones were isolated by appropriate selection. The resultant transfectants obtained from these gene-transfer experiments are summarized in Table 1 and described below in detail.

Function of Transfected Genes. To determine which combinations of the introduced T-cell receptor genes were expressed and responsive to specific antigen/MHC, all the G418- and HAT-resistant clones (double transfectants) and some of the G418- or HAT-resistant clones (single transfectants) were screened for responses to the DASp or AmDASp antigens together with appropriate APCs [spleen cells from B10.A(5R) mice]. The mitogen PMA was used in the screening experiments as a nonspecific stimulator to ensure that the transfectants retained the ability to secrete IL-2. As shown in

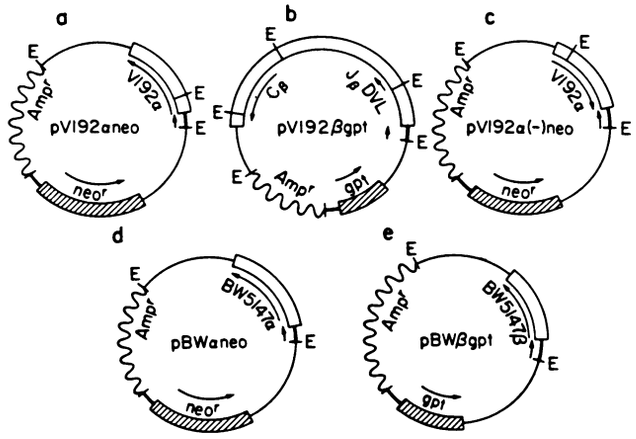

FIG. 1. Recombinant DNA constructs. (*a*) A full-length V1.9.2 α-chain cDNA clone was removed from a λgt10 clone by partial digestion with *Eco*RI and subcloned into a pSV2neo-based vector. (*b*) A *Sal* I–*Hin*dIII genomic DNA fragment containing the complete rearranged V1.9.2 β-chain gene [including constant (C), joining (J), diversity (D), variable (V), and leader (L) segments] was subcloned into a pSV2gpt-based vector. (*c*) The same V1.9.2 α cDNA clone used in *a* was subcloned into a pSV2neo-based vector in the opposite orientation with respect to the SV40 enhancer/promoter. (*d* and *e*) Two cDNA clones containing full-length BW5147 α and β genes, respectively, were subcloned into pSV2neo-based (*d*) and pSV2gpt-based (*e*) vectors separately. The *Pvu* II–*Hin*dIII fragments, corresponding to the SV40 enhancer/promoter, and the *Rsa* I–*Eco*RI fragment, containing the SV40 polyadenylylation sites, were introduced into pSV2neo or pSV2gpt at the *Eco*RI site to generate the pSV2neo- or pSV2gpt-based vector. The α or β gene was then subcloned into the modified vector at the junction of the two inserted segments. Open boxes indicate the inserts containing the T-cell receptor genes. The pBR322 DNA is represented by a wavy line. SV40 sequences are represented by solid lines, and the SV40 enhancer/promoter region is represented by a heavier solid line. Abbreviations: E, *Eco*RI site; Amp^r, ampicillin-resistance gene; neo^r, the aminoglycoside 3′-phosphotransferase gene; gpt, *E. coli* xanthine (guanine) phosphoribosyltransferase gene. Arrows indicate direction of transcription.

Table 1. Isolated T-cell transfectants

Plasmid DNA	No. of drug-resistant clones	No. of analyzed/ responsive clones	Designation of analyzed clones
pV192αneo (A)	>100	6/0	T-V192α-1 to -6
pV192βgpt (B)	>100	6/0	T-V192β-1 to -6
pBWαneo (C)	>100	NT	None
pBWβgpt (D)	>100	NT	None
A + B	17	17/7	T-V192αβ-1 to -17*
A + D	10	10/0	T-V192αBWβ-1 to -10
C + B	15	15/0	T-BWαV192β-1 to -15
C + D	4	4/1	T-BWαβ-1 to -4†
pV192α(−)neo + B	4	4/0	T-V192α(−)β-1 to -4
pV192α(−)neo + D	5	5/0	T-V192α(−)BWβ-1 to -5

Plasmid DNAs were introduced into EL-4G-12, a HAT-sensitive lymphoma cell line, by electroporation. After 48 hr, drug-resistant clones were selected in the presence of G418 for pV192αneo or pBWαneo transfections, HAT for pV192βgpt or pBWβgpt transfections, and G418 plus HAT for cotransfection. Drug-resistant clones were screened for specific IL-2-production response induced by antigen (AmDASp or DASp peptide) plus APCs [spleen cells from B10.A(5R) mouse] or APCs alone. IL-2 production by T-cell transfectants were assayed as described in the legend to Fig. 2. All the analyzed clones have retained the ability to produce IL-2 upon nonspecific stimulation with the mitogen phorbol 12-myristate 13-acetate (PMA) a property of the untransfected EL-4G-12 cells. NT, not tested.

*Transfectants T-V192αβ-1 to -7 are the seven clones that respond to AmDASp peptide in the context of B10.A(5R) APCs. They do not respond to DASp peptide in association with the appropriate APCs or to APCs alone (see Fig. 2).

†T-BWαβ-1 is the clone that responds to B10.A(5R) APCs in the presence or absence of either of the two antigens (see Fig. 2).

Table 1 and Fig. 2, of the 17 double transfectants that received α and β genes of V1.9.2 cell, 7 exhibited the same response phenotype as the donor V1.9.2 hybridoma. Reproducibly they responded to the AmDASp peptide, but not to the DASp peptide, in association with APCs. They did not respond to either of the two antigens alone or to APCs alone. Neither the tested single transfectants nor the double transfectants that received the hybrid αβ paired genes (i.e., one of the two genes derived from the V1.9.2 cell and the other from the fusion partner BW5147 cell) responded to either of the two antigens presented by B10.A(5R) APCs. Interestingly, one of the clones transfected with BW5147 α and β genes (T-BWαβ-1) showed reactivity with the B10.A(5R) APCs alone. The presence of either of the two antigens did not inhibit or enhance this reactivity (Fig. 2). This transfectant also exhibited a strong response to spleen cells from B10.A mice but not from B6 mice (data not shown), consistent with the proposition these genes encode an alloresponse against MHC molecules, presumably I-E^k. We provisionally defined this transfectant as an alloreactive clone.

Thus, by transfecting cells with V1.9.2 α and β genes, we are able to isolate clones that have the same reactivity as the V1.9.2 parent line. After transfection with BW5147 α and β genes, we isolated one putative alloreactive clone. No clone that can specifically react to DASp in association with B10.A(5R) APCs was identified with this antigen-response screening assay.

Transcription of Transfected α and β Genes. To determine which transfectants expressed α and/or β genes, we analyzed their RNA transcripts by blot hybridization analyses using probes specific for the V_α and V_β genes of the V1.9.2 and BW5147 cell lines. Total cytoplasmic RNAs were analyzed using a $V_\alpha 11.1$-specific probe from V1.9.2 hybridoma cells (Fig. 3*a*). The untransfected EL-4G-12 cells (lane 1) and cells that received the V1.9.2 α and β genes but fail to respond to AmDASp/APC (lane 2) did not show hybridization. All AmDASp/APC-reactive clones and some of the transfectants

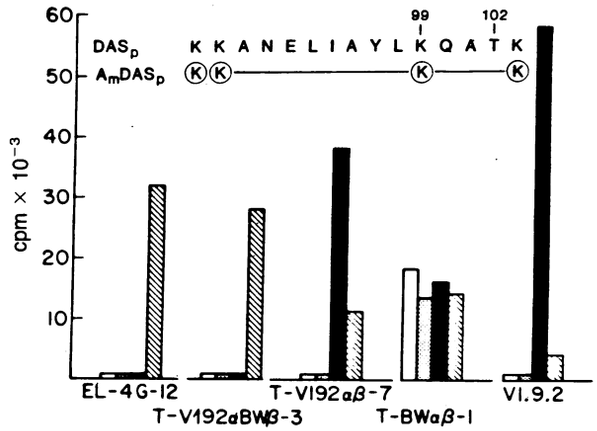

FIG. 2. IL-2 production of T-cell transfectants in response to antigen and/or MHC. The following stimulation protocols were employed: APCs [B10.A(5R) spleen cells] alone (open bars); 20 μM DASp peptide plus APCs (stippled bars); 20 μM AmDASp peptide plus APCs (solid bars); or PMA (20 ng/ml) (hatched bars). DASp and AmDASp antigens (shown at top in standard one-letter amino acid symbols) are synthetic derivatives of the moth cytochrome *c* carboxyl-terminal peptide. A circled K denotes an acetimidylated lysine and a dash denotes identity. About 10^5 T cells were cultured in 0.2 ml of medium with 5×10^5 mitomycin C-treated spleen cells (APCs) in the presence or absence of antigen. After 24 hr, 50 μl of the culture supernatant was added to 5×10^3 cells of the IL-2-dependent cell line CTLL-2 or HT-2, to a final volume of 100 μl. Results are expressed as the amount of [^{3}H]thymidine incorporated into the IL-2-dependent cells during the last 4 hr of a 24-hr culture. None of the tested T cells produced any detectable IL-2 when stimulated by either of the two antigens alone. Similar results were obtained by using 10 μM antigen (data not shown). Each bar represents the mean of [^{3}H]thymidine incorporation of IL-2-dependent CTLL-2 cells from triplicate cultures. The same pattern of response was obtained when the IL-2 dependent cell line HT-2 was used (data not shown). The results obtained from all the nonresponsive clones listed in Table 1 are similar to that obtained from clone T-V192αBWβ-3. The patterns of IL-2-production responses among each individual transfectant T-V192αβ-1 to -7 are the same, and the results for T-V192αβ-7 are shown as an example for the seven responsive clones.

belonging to the T-V192αBWβ category, as well as some of the single transfectants that received only the V1.9.2 α gene, showed one distinct transcript of 2.8 kb, which is larger than the authentic V1.9.2 α transcript of 1.8 kb (lane 3). One example of each case is shown in Fig. 3*a* (lanes 4–6). Presumably, this

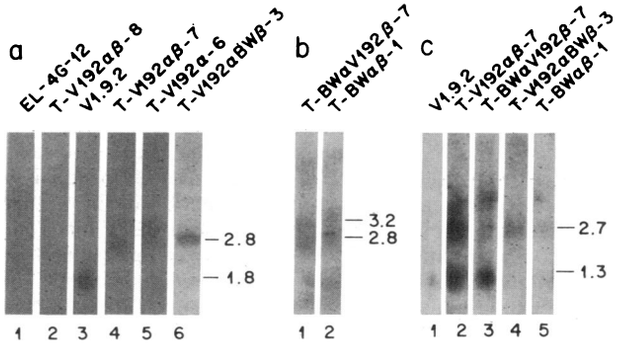

FIG. 3. RNA blot analysis of transfectants for expression of the V1.9.2 α gene (*a*), the BW5147 α gene (*b*), and the V1.9.2 or BW5147 β genes (*c*). Total cytoplasmic RNA (20 μg) was electrophoresed in a 1% agarose gel containing 2.2 M formaldehyde, transferred to nitrocellulose filters, and hybridized with probes specific for $V_\alpha 11.1$ (*a*), $V_\alpha 1$ (*b*), or $V_\beta 1$ (*c*), respectively. The relative sizes (in kb) of the hybridizing RNAs are indicated to the right of each autoradiogram.

2.8-kb RNA is the result of new RNA initiation and/or termination sites. An increase in the transcript size was also observed in previous studies (12, 28) of transfectants expressing T-cell receptor cDNAs cloned into similar vectors.

A probe specific for the V_α gene of BW5147 ($V_\alpha 1$) (29) demonstrated that no BW5147 α RNA was expressed either in the untransfected EL-4G-12 cells or in some of the T-BWαV192β and T-BW$\alpha\beta$ transfectants (data not shown). At least two transfectants (T-BWαV192β-3 and -7) and the alloreactive clone, T-BW$\alpha\beta$-1, did show hybridization. Again, the two larger transcripts (2.8 kb and 3.2 kb, Fig. 3*b*) that hybridized to the probe probably resulted from a combination of different RNA initiation and/or termination sites of the introduced DNA.

Since both the V1.9.2 and BW5147 cells use the same $V_\beta 1$ gene segment (30), most of the RNAs of the transfectants analyzed above were examined using a $V_\beta 1$-specific probe (Fig. 3*c*). No $V_\beta 1$ RNA was detected in EL-4G-12 cells, in cells transfected with only the V1.9.2 α gene, or in the nonresponder clone T-V192$\alpha\beta$-10 (data not shown). All the other tested transfectants had the $V_\beta 1$ transcripts. Cells transfected with the β gene of V1.9.2, derived from a genomic DNA fragment, gave a band at 1.3 kb, which is the normal size for a complete *VDJC* transcript (lane 1) and an additional transcript of 2.7 kb (lanes 2 and 3). Transfectants generated with the cDNA of BW5147 β gene gave a larger $V_\beta 1$ transcript (2.7 kb, lanes 4 and 5). Again, the larger transcripts were probably the result of new RNA initiation and/or termination sites.

The results of antigen-stimulation assays and RNA blot analysis suggest that only transfectants receiving and expressing both α and β genes of V1.9.2 T-cell receptor can recognize the AmDASp peptide associated with APCs, the functional property of the V1.9.2 hybridoma. None of the transfectants that express the V1.9.2 α or β gene alone (paired with the other gene contributed by either the recipient cell or BW5147 introduced by gene transfer) can respond to AmDASp and/or MHC.

Functional Recognition by Transfectants T-V192$\alpha\beta$-1 to -7 Is Antigen-Specific and MHC-Restricted. To further extend the results that demonstrate the AmDASp-reactive transfectants do respond to antigen/MHC in the same manner as V1.9.2 hybridoma, we tested all seven transfectants for recognition specificity in detail.

The panel of transfectants was tested for IL-2 production in the presence of various concentrations of antigen and/or APCs bearing different MHC molecules. The untransfected EL-4G-12 cells did not respond to any of the tested APCs in the presence or absence of either of the two antigens (AmDASp and DASp peptides), even at antigen concentrations up to 200 μM (data not shown). The donor V1.9.2 hybridoma responded strongly to the AmDASp peptide, but not to the DASp peptide, in the context of spleen cells from B10.A(5R) mice or B10.A mice (Fig. 4*a*), similar to the results previously obtained (24). In addition, the V1.9.2 hybridoma showed a good response to the AmDASp peptide, but not to the DASp peptide, when presented by L-cell transfectants CA36.1.3 (expressing I-E^k molecules). It failed to respond to either of the two antigens presented by spleen cells from B6 mice (expressing I-A^b molecules) or BALB/c mice (expressing both the I-A^d and I-E^d molecules) (data not shown) or by L-cell transfectants CA14.11.14 (expressing I-A^k molecules) (Fig. 4*a*). The seven reactive transfectants showed the same antigen and MHC fine specificity as the V1.9.2 hybridoma described above, although the levels of IL-2 production were slightly different among these positive clones. Therefore, the transfectants expressing complete V1.9.2 α and β genes respond to the combination of AmDASp peptides and I-E^k molecules, as does the donor V1.9.2 hybridoma. One example of the IL-2-production responses of the seven positive transfectants is shown in Fig. 4*b*.

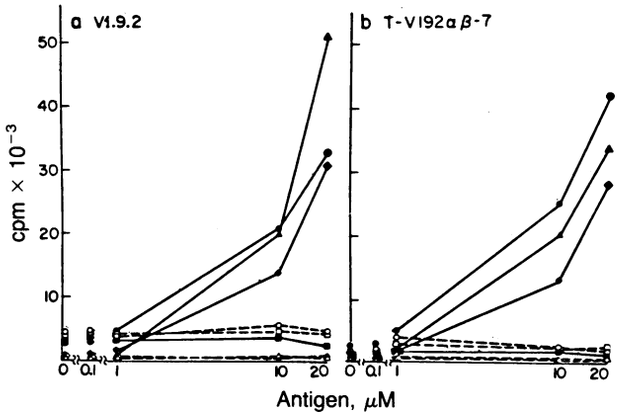

FIG. 4. Antigen specificity and MHC restriction of the V1.9.2 and T-V192$\alpha\beta$-7 cells. The V1.9.2 hybridoma (*a*) or T-V192$\alpha\beta$-7 (*b*) cells (10^5) were cultured with the indicated concentration of AmDASp peptide (▲, ◆, ●, and ■; solid lines) or DASp peptide (△, ◇, ○, and □; broken lines) presented by spleen cells from B10.A(5R) mouse (▲, △) or B10.A mouse (◆, ◇) or by L-cell transfectant CA36.1.3 (●, ○) or CA14.11.14 (■, □). IL-2 production by T cells was assayed as described in the legend to Fig. 2. Each point represents the mean of triplicate cultures.

Further evidence for the direct involvement of the I-E^k molecules in the specific functional antigen/MHC corecognition was obtained from antibody-blocking studies. The IL-2 production of the V1.9.2 hybridoma and the transfectants induced by AmDASp peptide in the presence of the I-E^k-expressing L-cell transfectants was inhibited by the monoclonal anti-I-E^k antibody 10B, but not by the monoclonal anti-H-2K^k antibody 84.17.2 (17) (Table 2). This demonstrates that it is the same MHC molecules that restrict antigen recognition by both the V1.9.2 hybridoma and the positive transfectants.

DISCUSSION

Our data confirm the recent observation that the T-cell receptor $\alpha\beta$ heterodimer can transfer specific antigen/MHC recognition from one class II-restricted T_H cell to another (12). Transfection of the EL-4G-12 cells with the α- and/or β-chain genes of V1.9.2 and BW5147 cells generated cells that expressed the introduced gene messages. Only transfectants in which both α and β genes of the V1.9.2 T-cell receptor are transcribed can respond specifically to the AmDASp peptide presented by appropriate MHC molecules, as the V1.9.2 hybridoma. Transfectants expressing only V1.9.2 α or β gene message show no detectable

Table 2. Effect of anti-MHC antibodies on the IL-2-production response of the T-V192$\alpha\beta$-7 transfectant

		[^{3}H]Thymidine incorporation, cpm × 10^{-3}		
T cells	Stimulus	No antibody	10B	84.17.2
EL-4G-12	AmDASp + APCs	0.6	0.5	0.6
	APCs	0.5	0.4	0.4
T-V192$\alpha\beta$-7	AmDASp + APCs	22.5	6.7	18.8
	APCs	1.6	1.4	1.5
V1.9.2	AmDASp + APCs	17.1	4.1	14.1
	APCs	2.5	2.2	2.2

T cells were stimulated to produce IL-2 in the presence of AmDASp peptide (10 μM) plus APCs (CA36.1.3) or of APCs alone. Cultures received either no antibody, antibody 10B (anti-I-E^k, 4 μg/ml), or antibody 84.17.2 (anti-H-2K^k, 4 μg/ml). The monoclonal antibodies were kindly provided by M. Pierres. IL-2 production was assayed as described in the legend to Fig. 2. Values for thymidine incorporation are means of triplicate cultures. Cells were able to produce IL-2 when stimulated by PMA in the presence of either of the two antibodies (data not shown).

response to specific antigen/MHC. Therefore, these data strongly suggest that both the α and β chains of a T-cell receptor are required for defining the antigen fine specificity and MHC restriction of a T cell.

Analysis of other transfectants that transcribed the introduced single V1.9.2 α or β gene, or the paired hybrid genes (i.e., one gene from the V1.9.2 cell and the other from the BW5147 cell) allowed us to determine whether the combinatorial association between α and β chains plays a role in defining the heteroclitic reactivity of the V1.9.2 hybridoma. These studies also allowed us to examine the ability of individual chains of the T-cell receptor from the V1.9.2 cell to mediate functional antigen or MHC recognition independently. All the analyzed transfectants that expressed only one of the V1.9.2 α and β gene messages or messages of the paired hybrid genes failed to respond to antigen (AmDASp or DASp peptides) alone, to MHC molecules of B10.A(5R) APCs alone, or to both antigen and MHC molecules together. Thus, the following observations can be made. First, the heteroclitic specificity to AmDASp/MHC of the V1.9.2 hybridoma is not due to combinatorial association between α and β chains contributed by the normal T cell (V1.9.2) and the fusion partner (BW5147). Second, V1.9.2 and BW5147 cells use the same $V_\beta 1$ segment but a different *D–J* region in β chains (30), yet transfectants that transcribe the V1.9.2 α gene and the BW5147 β gene do not respond to the antigen/MHC combination that stimulates the T-V192$\alpha\beta$ transfectants and the V1.9.2 hybridoma. This is direct evidence that the junctional region (*V–D–J*) in β chain can be important for the specificity in antigen/MHC recognition. This observation correlates with results obtained from DNA sequence analysis of genes isolated from T cells with different specificity (14, 31) and suggests that *V–D–J* joining could be one of the important mechanisms for generating the diversity of the T-cell repertoire. Third, the hybrid T-cell receptors containing one V1.9.2 chain cannot mediate detectable recognition either of cytochrome *c*-related antigen or of MHC (I-E^k) molecules, although the V_α segment of V1.9.2 ($V_\alpha 11.1$) was shown to be associated with a large fraction of the T-cell responses of mice to cytochrome *c* (14, 15). We cannot, however, rule out the possibility that (*i*) the level of expression of the introduced T-cell receptor gene products on the surface of the recipient cells might be too low to permit effective responses or (*ii*) the structure of the EL-4G-12 or BW5147 partner chain might sterically hinder the V1.9.2 T-cell receptor chain from binding to the antigen or MHC molecules alone.

The expression of messages of both the α and the β genes of BW5147 has endowed the recipient cell (T-BW$\alpha\beta$-1) with the ability to recognize MHC molecules. Whether this reactivity is due to the expression of a receptor made up of α and β chains from BW5147, or a combination of receptor chains of BW5147 and EL-4G-12, is not known. Study of the functional properties of the single transfectants generated in this study would answer this question. Regardless, the generation of an alloreactive clone by *in vitro* gene transfer demonstrates that the transfection experiment has provided a means to directly examine the role of T-cell receptor molecules in alloreactive T cells.

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