

IN VIVO EXPRESSION AND FUNCTION OF HYBRID
Ia DIMERS ($E_{\alpha}A_{\beta}$) IN RECOMBINANT AND
TRANSGENIC MICE

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The MHC class II I-E molecules in mice consist of two polypeptide chains (α and β) expressed as a dimer on the surface of cells. Strains of H-2^b and H-2^s haplotypes do not express a surface I-E molecule because of a defect in the E_{α} gene (1, 2). The E_{β} chain, however, is synthesized and can be found in the cytoplasm. Additionally, H-2^f and H-2^g mice have been shown to lack I-E expression because of defective processing of both E_{α} and E_{β} RNA (2, 3). Only one recombinant, A.TFR5 ($I-A^f$, E_{α}^k), has been previously suggested to express a free E_{α} chain on the cell surface (4, 5).

The intact I-E molecule is known to participate in response to both foreign antigens and to self antigens. In the case of self antigens, the I-E-reactive V_{β} T cell subsets of $V_{\beta}11$ and $V_{\beta}17$ are known to be deleted in I-E⁺ mice (6-8). In addition, the I-E molecule plays a role in the deletion of T cell subsets ($V_{\beta}6$, $V_{\beta}8.1$, $V_{\beta}3$) reactive to the self antigen Mls-1^a and Mls-2^a (9-11).

Previous studies had not indicated occurrence of $E_{\alpha}A_{\beta}$ dimers in vivo (5). However, studies with transfected L cells have demonstrated the necessity of α/β dimers for class II molecule expression (12, 13). Further studies have also shown that a low frequency of $E_{\alpha}A_{\beta}$ dimers can be detected, especially in the absence of the E_{β} chains (14). In this report we examined recombinant mouse strains and transgenic mice with defective E_{β} genes, but with normal E_{α} molecules. By flow cytometry, we found low level expression in vivo of surface E_{α} molecule in B10.RFB2 (A_{β}^f , A_{α}^f , E_{β}^f , E_{α}^k) and B10.RQB3 (A_{β}^g , A_{α}^g , E_{β}^g , E_{α}^k). We also found E_{α} expression in H-2^g mice with the E_{α}^k transgene.

The E_{α} molecule in these strains can enhance clonal deletion of two V_{β} TCR-expressing T cell subsets. The $V_{\beta}11$ T cells were deleted in the E_{α} -expressing mice. $V_{\beta}6$ T cells, which are deleted in Mls-1^a mice and are also I-E regulated, were similarly shown to be fewer in E_{α} -expressing mice. In contrast, we found that only a very weak MLR could be generated between the B10.RQB2 (E_{α}^{-}) and B10.RQB3 (E_{α}^{+}) cells. This combination also showed no signs of skin graft rejection.

Materials and Methods

Mice. All mice were bred and maintained in our mouse colony and their H-2 genotypes are shown in Table I. The B6- E_{α}^k transgenic mice were the kind gift of Drs. C. Benoist and

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TABLE I
Intensity of Antibody Binding in B10 Recombinant and Congenic Strain

Strain	H-2 Molecules						Intensity of labeling*	
	K	A β	A α	E β	E α	D	14-4-4	17-3-3
B10.K	k	k	k	k	k	k	+	+
B10.M	f	f	f	f	f	f	-	-
B10.RFB2	f	f	f	f	k	b	+	-
B10.RFB3	f	f	f	f	p	b	+	-
B10.RQB2	q	q	q	q	q	b	-	-
B10.RQB3	q	q	q	q	k	b	+	-
B10.RFP	f	f	f	f	p	r	+	-

Origin of recombinants is detailed in reference 24.

* + +, Bright; + -, dull; - -, no staining.

D. Mathis (INSERM, CNRS, Strasbourg, France). The E α^k gene from this mouse was bred into the B10.Q (H-2 q) strain.

Flow Cytometry. The antibodies 14-4-4S and 17-3-3S (American Type Culture Collection, Rockville, MD) were purified by protein A from culture supernatant and biotinylated. PBL were isolated by Ficoll separation and stained with antibody at 4°C, followed by washing and labeling with streptoavidin-phycoerythrin at 4°C. A FACS IV (Becton Dickinson & Co., Mountain View, CA) was used to analyze the cells. Lymphocytes were gated on, using light scatter analysis, and resulting fluorescence is displayed on single fluorescence histograms.

The V β 11 antibody (RR3-15) and V β 6 antibody (44-22-1) were the kind gifts of Drs. Kanagawa (Lilly Research Laboratories, La Jolla, CA) and Hengartner (Institute of Pathology, University Hospital, Zurich, Switzerland). They were used as culture supernatants diluted 1:1 with buffer and incubated at 4°C with PBL. Cells were washed and stained with FITC-conjugated mouse anti-rat IgG (Accurate Chemical & Scientific Corp., Westbury, NY). The cells were then washed and labeled with biotinylated anti-Thy-1.2 (Becton Dickinson & Co.) followed by streptavidin-phycoerythrin (Tago Inc., Burlingame, CA). The FACS IV (Becton Dickinson & Co.) was again used and fluorescence intensities are displayed as dual-color counters. All dilutions and washes were done in PBS with 1% BSA (Sigma Chemical Co., St. Louis, MO) and 0.05% sodium azide.

MLC and Skin Grafts. 3 × 10⁵ lymph node cells (LNC) were cultured with irradiated stimulator spleen cells as described earlier (15). Means and SEM were calculated from triplicate cultures.

Skin grafts were performed as described earlier (16). Briefly, tail skin from B10.RQB3 was grafted on the back of B10.RQB2 mice, and observed daily for signs of rejection.

Results

E α Expression. The E α -specific mAb 14-4-4S (17) stained B10.K PBL brightly. B10.M showed no labeling, while the recombinant strain B10.RFB2 with a nonexpressed E β^f and a functional E α^k showed low level E α expression (Fig. 1 A). Strain B10.RFP with a functional E β^k also had similar levels of E α expression. Strain B10.RQB2 (E α^-) showed no labeling, while B10.RQB3 (E α^k) gave low level of E α expression (Fig. 1 B). Both of these strains have nonfunctional E β^q chains, and differ only at the E α gene. The B10.Q-E α^k transgenic mice confirmed these results. Fig. 1 C shows low level expression in the B10.Q-E α^k transgenic mice as compared with B10.K and a B10.Q-E α^- sibling.

E β Expression. Because the recombination in these mice lies within the E β gene (18), we checked for the possibility of low levels of E β being expressed with the E α

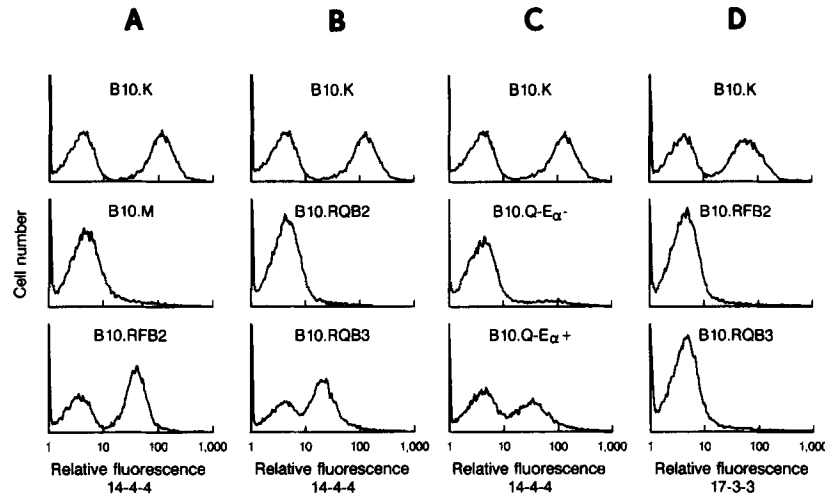


FIGURE 1. Expression of E_{α} and E_{β} molecules labeled with 14-4-4 (E_{α}) and 17-3-3 (E_{β}). (A) $H-2^f$ recombinants; (B) $H-2^g$ recombinants; (C) E_{α}^k transgenics; (D) E_{β} expression in recombinants.

molecule. PBLs were labeled with the E_{β} -specific antibody 17-3-3S (17). Fig. 1 D shows 17-3-3 staining of B10.K, but no detectable expression of E_{β} was seen in B10.RFB2 or B10.RQB3. Table I summarizes the E_{α} and E_{β} screening results.

Clonal Deletion of TCR $V_{\beta}11^+$ T Cells. The percent of TCR $V_{\beta}11^+$ T cells in the blood is shown in Table II. Bill et al. (6) have shown that the I-E molecule is necessary in the deletion of $V_{\beta}11^+$ T cells. In B10.RFB2 the low level of E_{α} expression decreases the percentage of $V_{\beta}11^+$ T cells to $\sim 25\%$ of that found in B10.M. Similarly, E_{α}^+ B10.RQB3 shows approximately half the level of $V_{\beta}11^+$ T cells compared with E_{α}^- B10.RQB2. B10.Q- E_{α}^+ transgenic mice also had consistently lower levels of $V_{\beta}11$ cells while the E_{α}^- full siblings were similar to the B10.Q parent.

Clonal Deletion of TCR $V_{\beta}6^+$ T Cells. TCR $V_{\beta}6^+$ cells are clonally deleted in the context of Mls-1^a and I-A and/or I-E molecules. F₁ crosses were made between DBA/1 (Mls-1^a, I-E⁻) and strains of mice listed in Table II. DBA/1 ($H-2^g$) was chosen because

TABLE II
Level of V_{β} T Cell Expression

Strain	E_{α} Expression*	Percent $V_{\beta}11^{\dagger}$	Percent $V_{\beta}6$ in DBA/1 F ₁ cross [†]
B10.K	++	0.5 ± 0	0.3 ± 0.1
B10.M	--	4.0 ± 0.1	2.4 ± 0.1
B10.RFB2	+-	0.9 ± 0.1	0.6 ± 0
B10.RQB2	--	6.5 ± 0.3	4.9 ± 0.1
B10.RQB3	+-	2.9 ± 0.2	1.7 ± 0.1
B10.Q- E_{α}^+	+-	4.4 ± 0.1	2.3 ± 0.2
B10.Q- E_{α}^-	--	6.8 ± 0.1	4.7 ± 0.3

* ++, Bright; +-, dull; --, no staining.

[†] Mean percent ± SEM of peripheral blood T cell labeled with V_{β} antibody. $n \geq 3$ from individual mice.

it is I-E⁻ and previously shown to have little influence on the clonal deletion of T cells reactive to Mls-1^a (19). (DBA/1 × B10.RFB2)F₁ expresses ~25% of the level of V β 6⁺ T cells compared with (DBA/1 × B10.M)F₁ (Table II). (DBA/1 × B10.RQB3)F₁ showed <50% of the V β 6⁺ cells found in (DBA/1 × B10.RQB2)F₁ mice. (B10.Q-E α ⁺ × DBA/1)F₁ mice also deleted 50% of V β 6⁺ T cells compared with (B10.Q-E α ⁻ × DBA/1)F₁ mice.

E α Molecule Is a Weak Alloantigen. B10.RQB2 (E α ⁻) LNC responded well in an MLR to MHC differences (B10.K) but no significant response was seen against B10.RQB3 (E α ⁺) from the grafted or ungrafted mice ($p > 0.01$, data not shown). B10.RQB3 tail skin, although rejected in 10–14 d when grafted onto B10.K, was not rejected when grafted onto three B10.RQB2 mice after 50 d.

Discussion

FACS analysis has shown in vivo expression of the E α molecule on the cell surface in I-A^g and I-A^f recombinant mouse strains that do not express E β . The possibility of low levels of transcribed E β carrying E α to the cell surface was discounted by demonstrating the lack of binding of an E β -specific antibody (17-3-3) in these strains. The observation that E α is expressed in transgenic mice also confirms that E β is not required for the E α ^k surface expression. Since E α ^k chain cannot be expressed on the membrane by itself, it must be pairing with the A β ^k and A β ^j chains, resulting in low level surface expression. The existence of an E α + A β hybrid molecule has recently been shown in a BALB/c lymphoma (20). We were not able to pick up this hybrid molecule by immunoprecipitation. The low level of expression is presumably due to the very low affinity binding of E α and A β as compared with A α binding to A β . Previous studies showing E α chain expression have also acknowledged the possibility of low affinity binding, which prevents precipitation of the A β chain with E α in vivo (5, 15). This hybrid molecule has not so far been detected in vivo in mice expressing E β . The excess cytoplasmic E α chain in strains lacking E β may increase the association of E α with A β . Our results confirm in vivo previous studies done in vitro with transfected L cells and in B cell lymphomas.

The E α + A β hybrid molecule does function in vivo in the thymus by deleting T cells expressing V β 6 and V β 11 TCRs. Both of these TCRs have been shown to require the involvement of the I-E molecule, in presentation of self antigens or in recognition of the I-E molecule. We have shown the E α chain is significant in these processes.

The expression of E α , however, does not lead to strong allorecognition in stimulating an MLR or in graft rejection. Previous studies have shown differences at the intact I-E molecule to be weakly antigenic in allograft rejection (21, 22) and to stimulate a moderate MLR (15). The lower E α expression in these recombinant mice compared with other I-E⁺ strains might lead to the limited response. However, this seems unlikely since LPS-stimulated spleen cells with increased E α expression are still not effective in stimulating a strong primary MLR (data not shown). The allorecognition against an intact I-E molecule may be predominantly directed against the polymorphic E β molecule while the E α molecule shows a great deal of homology between various haplotypes (23). It is possible that the responder mice we used are tolerant to the E α molecule or the T cells with highly I-E-reactive TCRs are missing, such as V β 17 (7). It will be interesting to see whether we can generate E α -specific antibodies in this strain combination.

In conclusion, we have shown that the E_{α} molecules can be expressed in vivo as a hybrid $E_{\alpha}A_{\beta}$ dimer and function in the thymus by clonally deleting T cells but cannot be recognized as an alloantigen in graft rejection. Studies are currently underway to see whether this hybrid molecule plays a role in immune tolerance and evasion of tumor destruction.

Summary

We have found cell surface expression of an E_{α} molecule in recombinant and transgenic mouse strains lacking an E_{β} molecule. Flow cytometry has shown low level expression of E_{α} in B10.RQB3 (I-A^qE_α^k) and B10.RFB2 (I-A^fE_α^k) mice. We have also found that B10.Q (H-2^q) mice can express the E_{α} ^k transgene. Since these strains do not have functional E_{β} chains, we propose that the $E_{\alpha}A_{\beta}$ hybrid dimers are formed in low numbers and can be picked up by FACS analysis. So far we have not been able to identify these hybrid molecules by cytotoxicity or immunoprecipitation. The E_{α}/A_{β} molecule can function in vivo during thymic selection in the clonal deletion of two V_{β} TCR subsets, $V_{\beta}11$ and $V_{\beta}6$, which have been shown to interact with the intact I-E molecule.

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