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The effect of excess β-chain synthesis on cell-surface expression of allele-mismatched class II heterodimers *in vivo*

(transgenic mice/chain pairing)

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ABSTRACT **We have recently described 12 lines of H-$2^{s/s}$ mice carrying from 1 to 65 copies of an A^k_β transgene. The transgene was coexpressed with the endogenous allele, and A^k_β mRNA expression correlated well with transgene copy number. Overexpression of the transgene was associated with a variety of defects, including a significant reduction in I-A cell-surface expression. In this paper, we assess the effect of increased levels of A^k_β mRNA synthesis on I-A cell-surface expression in these mice. Crossing representatives from several lines of A^k_β mice to A^k_α transgenic mice demonstrated that the A^k_β mRNA was translated and expressed at high levels on the cell surface in association with A^k_α. In H-$2^{s/s}$ (A^s_α/A^s_β) mice carrying >10 copies of the A^k_β transgene, excess A^k_β mRNA and protein synthesis did drive cell-surface expression of the less favored A^s_α/A^k_β heterodimers. However, the highest levels of A^k_β detected on the cell surface were only 50–70% of those observed in [B10.A(4R) × nontransgenic]F_1 controls. Maximum levels of A^s_α/A^k_β cell-surface expression were accompanied by a significant reduction in A^s_α/A^s_β expression. Unpaired and improperly paired complexes were not detected intracellularly and appeared to be degraded quite rapidly. Thus, only a fraction of the chains competing for pairing reached the cell surface under conditions of asymmetric chain synthesis in these mice. This markedly reduced total Ia cell-surface levels in mice carrying >10 copies of the A^k_β transgene.**

The major histocompatibility complex class II (Ia) antigens are highly polymorphic cell-surface glycoproteins consisting of α and β chains noncovalently associated on the surface of specific cells in the immune system (1). Although a high degree of genetic diversity is maintained within a population, genetic diversity is limited within an individual, and the variety of class II heterodimers expressed on the cell surface is dependent on combinatorial association of the α and β gene products. Two class II isotypes are expressed in the mouse, I-A (Aα/Aβ) and I-E (Eα/Eβ). Theoretically, four distinct class II α/β heterodimers could be displayed on the cell surface in homozygous mice expressing all four gene products, but constraints on α/β chain pairing limit this possible heterogeneity.

In vitro studies have shown that polymorphic residues in the NH_2-terminal 50 amino acids of the α1 and β1 domains largely control both inter- and intraisotypic chain pairing (2–4). In these studies, at least one mixed heterodimer (A^b_α/A^k_β) was expressed on the cell surface at levels similar to those observed for haplotype-matched heterodimers, whereas other mixed heterodimers were expressed at low or barely detectable levels (A^k_α/A^b_β, A^k_α/A^d_β). In transfectants in which E_α, A^d_α, and A^d_β chains were allowed to compete for pairing, asymmetric chain synthesis was required for cell-surface expression of isotype-mixed E_α/A^d_β heterodimers (5).

In vivo, haplotype-matched α and β chains of the same isotype apparently pair more efficiently than do mixed chains. Preferential pairing of E^u_α with E^u_β results in a functionally significant decrease in E^u_α/E^k_β cell-surface expression in [B10.A(4R) × B10.PL]F_1 ($E^-_\alpha E^k_\beta/E^u_\alpha E^u_\beta$) mice (6). Mixed-isotype heterodimers have been observed on the cell surface only in mice in which excess E_α chain is synthesized. Low levels of isotype-mixed E^k_α/A^q_β and E^k_α/A^f_β heterodimers are expressed on the cell surface in mice with defective E_β chains (7). Similarly, E_α protein is expressed at low levels on the cell surface in mice carrying E^k_α or E^d_α transgenes (7, 8). Although the expression of haplotype-mixed heterodimers has been documented both functionally and biochemically in (H-$2^{k/k}$ × H-$2^{b/b}$)F_1 mice (9–11), more rigid pairing constraints have been observed in transgenic mice. A^k_α was not detected on the cell surface in association with A^b_β, A^s_β, or A^q_β in mice carrying A^k_α transgenes, and only low cell-surface levels of A^b_α/A^k_β and A^s_α/A^k_β have been observed in mice carrying A^k_β transgenes. However, high cell-surface levels of haplotype-matched A^k_α/A^k_β heterodimers have been observed in A^k_α/A^k_β double-transgenic mice (12–14).

Collectively, these observations imply that *in vivo*, significant levels of mixed heterodimers are expressed on the cell surface only under conditions of asymmetric chain synthesis. We have addressed this question directly by analyzing 12 independent lines of H-$2^{s/s}$ mice carrying from 1 to 65 copies of an A^k_β transgene. Because mRNA expression correlated well with transgene copy number, we were able to assess the effects of increasing A^k_β mRNA synthesis on chain pairing and cell-surface expression in these mice. Cell-surface expression of mixed A^s_α/A^k_β heterodimers was driven by asymmetric chain synthesis. However, the process was inefficient, and only a fraction of the chains competing for pairing reached the cell surface. This markedly reduced total Ia cell-surface levels in mice carrying >10 copies of this transgene. High copy number (extreme overexpression) of the transgene was associated with additional defects that have been described in detail elsewhere (15).

MATERIALS AND METHODS

Mice. A^k_α transgenic mice (from the A^k_α-26 line; refs. 12 and 13) were provided by Diane Mathis and Christophe Benoist. Production of the A^k_β transgenic mice has been described (15). These mice carry 1–65 copies of an 11.6-kilobase (kb) fragment of the pI-A^k_β-1 genomic clone (16) containing 2–3 kb of 5′ sequence and 1–2 kb of 3′ sequence.

DNA Analysis. Mice carrying the transgene were identified by blotting (17) *Bgl* II-digested genomic DNA extracted from tail tissue onto Genatran (Plasco, Woburn, MA) filters. The filters were hybridized with an A^k_β cDNA clone (18) ^{32}P-labeled by random hexamer priming (19). Copy number was

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Abbreviations: NT, nontransgenic; 4R×NT, [B10.A(4R) × NT]F_1; NEPHGE, nonequilibrium pH-gradient electrophoresis; mAb, monoclonal antibody.

determined by comparing the intensity of the transgene band with that of the endogenous allele or to those of B10.A(4R) controls. DNA quantity was normalized using either the endogenous A^s_β bands or by reprobing with an A^k_α cDNA probe. Hybridization signal intensities were quantitated using an LKB Ultrascan XL laser densitometer.

RNA Extraction and Analysis. Tissues were homogenized in 5 M guanidine thiocyanate (Fluka), and RNA was centrifuged through a CsCl cushion following the method of Chirgwin *et al.* (20). RNA blot analysis was carried out following standard protocols (21).

For the RNase protection assays, an A^k_β-specific probe was constructed by cloning a 275-base pair (bp) *Eco*RI–*Taq* I fragment of an A^k_β cDNA clone (18), containing leader peptide and $\beta 1$ domain sequences, into the *Eco*RI and *Acc* I sites of the pGEM-1 vector. The plasmid was linearized with *Eco*RI, and A^k_β antisense probes were generated using SP6 polymerase purchased from Promega Biotec and [^{32}P]CTP (SP6 grade) from Amersham. The protection assays were done according to the method of Melton *et al.* (22).

Monoclonal Antibodies (mAbs). mAbs 10-3.6 (anti-$A_\beta{}^{s,k,f,r,u}$; ref. 23), 40.N (anti-$A_\beta{}^{k,f,r,u}$; ref. 24), MKS4 (anti-$A_\beta{}^{s,f,u}$; ref. 25), and 4D5 (anti-$A_\alpha{}^{s,b,f,p,q,r,u,v}$; ref. 26) were grown in ascites and purified by ammonium sulfate precipitation followed by protein A chromatography. Preparations were directly fluoresceinated or biotinylated (27). Conjugated RA3-6b2 (anti-B220; ref. 28) antibody was provided by Alan Stall and Nabuko Uchida (Stanford University).

Fluorescence-Activated Cell Sorter (FACS) Analysis. FACS analysis was done essentially as described by Hayakawa *et al.* (29). Cells were analyzed on a dual-laser FACS IV (Becton Dickinson Immunocytometry) equipped with a logarithmic amplifier. Dead cells were excluded by propidium iodide staining.

Protein Methods. Metabolic labeling of splenocytes was done essentially as described by Jones (30). For pulse-chase experiments, 7.5×10^7 spleen cells were incubated at 10^7 cells per ml in RPMI 1640 lacking methionine (supplemented with 5% dialyzed fetal calf serum) at 37°C in 5% CO_2. After 1 hr, cells were incubated for 10–30 min with 600–1000 μCi (1 Ci = 37 GBq) of [^{35}S]methionine (Amersham). Cells were washed in phosphate-buffered saline (PBS) containing 10 mM methionine and then incubated in complete medium containing 5-fold excess (10 mM) methionine, and samples were removed at the time points noted. Lysates were prepared in 0.5% Nonidet P-40 containing protease inhibitors and stored at −70°C. Immunoprecipitation was carried out using the mAbs 40.N, 10-3.6, and MKS4 adsorbed to fixed *Staphylococcus aureus*. Immunoprecipitates were analyzed under reducing conditions on $NaDodSO_4$/12.5% polyacrylamide gels.

RESULTS

mRNA Expression. From 14 founders, 12 independent lines carrying from 1 to 65 copies of the A^k_β transgene were established (Fig. 1). RNA blot analysis using A^k_β and A^k_α cDNA probes (which detect both transgenic and endogenous transcripts) indicated that expression of A_β mRNA was elevated in mice carrying >10 copies of the transgene (Fig. 2). The ratio of A_β to A_α mRNA progressively increased from 1 to >60 in mice carrying from 1 to 65 copies of the transgene (in comparison with nontransgenic controls). The relatively low levels of A_α message seen in mice carrying >50 copies of the transgene reflected the significantly reduced numbers of B cells in these animals (15).

RNase protection assays using a probe that distinguishes the endogenous A^s_β transcript from the A^k_β transcript demonstrated clearly that the excess A_β mRNA was a product of the transgene (Fig. 3). The ratio of A^k_β to A^s_β message was ≈1 in

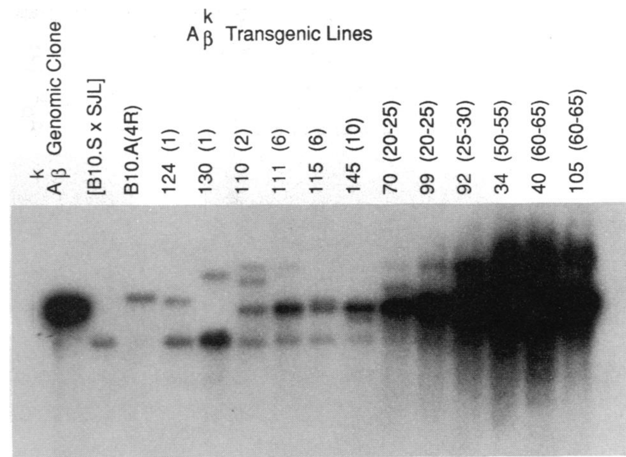


FIG. 1. Southern blot analysis of genomic DNA from a representative of each A^k_β transgenic line. DNA was digested with *Bgl* II, which cleaves the A^k_β transgene once, and filters were hybridized with a full-length A^k_β cDNA clone. A^k_β line numbers are directly above each lane; transgene copy numbers are in parentheses. Control lanes include B10.A(4R) (I-$A^{k/k}$) and (B10.S × SJL)F_1 (I-$A^{s/s}$) DNA, and the intact 11.6-kb A^k_β genomic clone.

[B10.A(4R) × nontransgenic (NT)]F_1 (4R×NT) control mice. In mice carrying one copy of the transgene, the ratio of A^k_β to A^s_β mRNA isolated from spleen tissue was 0.1 and progressively increased in mice carrying more copies of the transgene (Fig. 3*A*). Surveys of tissues from mice of several lines indicated that the transgene was coexpressed with the endogenous allele (Fig. 3*B*). No A^k_β message was seen in the absence of A^s_β message, and the ratio of A^k_β to A^s_β message was similar in all tissues examined within each mouse, including the thymus (data not shown).

Cell-Surface Expression. Cell-surface expression of the transgene and endogenous alleles was assessed by FACS analysis by using the mAbs MKS4, 40.N, and 10-3.6 (see *Materials and Methods*). mAb 40.N has been shown to recognize A^k_β in association with α chains other than A^k_α (31, 32), and mAb 10-3.6, which recognizes total Ia in these mice, can distinguish dissociated A_β chains (33). Total Ia levels were also confirmed with mAb 4D5 in several experiments (data not shown).

The A^k_β transgenic mice used for these experiments were on a B10.S × SJL (H-$2^{s/s}$) background. Control mice in each experiment included NT littermates (A^s_α/A^s_β), B10.A(4R) mice (A^k_α/A^k_β), and 4R×NT mice (A^k_α A^s_α/A^k_β A^s_β). The mean fluorescence values of Ia on mature B cells were obtained for each reagent and normalized to those seen in 4R×NT controls. Combined values obtained with mAbs MKS4 and 40.N coincided well with the value obtained for total Ia with mAb 10-3.6, and the mean fluorescence values obtained with mAb 10-3.6 on B10.A(4R), 4R×NT, and NT controls were quite similar (Fig. 4).

As shown in Fig. 4, low levels of A^k_β were detected on mature B cells from mice carrying from 1 to 6 copies of the transgene. The highest cell-surface levels of A^k_β (50–70% of 4R×NT values) were seen in mice carrying from 20 to 30 copies of A^k_β. However, no individual animal expressed A^k_β on the cell surface at levels equal to those observed in 4R×NT control mice. In addition, cell-surface expression of the endogenous A^s_β chain was reduced significantly more than expected (to ≤15% of nontransgenic, I-$A^{s/s}$, values) in mice carrying >20 copies of the transgene. Mean fluorescence values obtained with mAb 10-3.6 confirmed that cell-surface expression of total Ia was markedly reduced in these mice. A^k_β cell-surface expression varied from 10% to 40% of 4R×NT levels, A^s_β was reduced to 15–30% of 4R×NT levels (7–15%

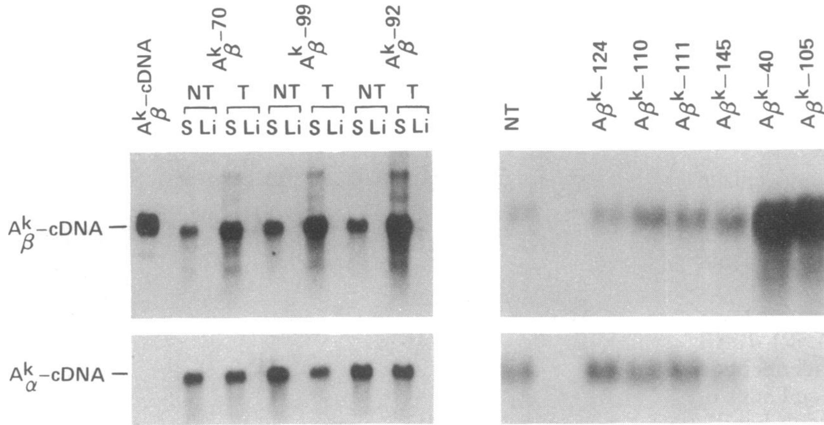
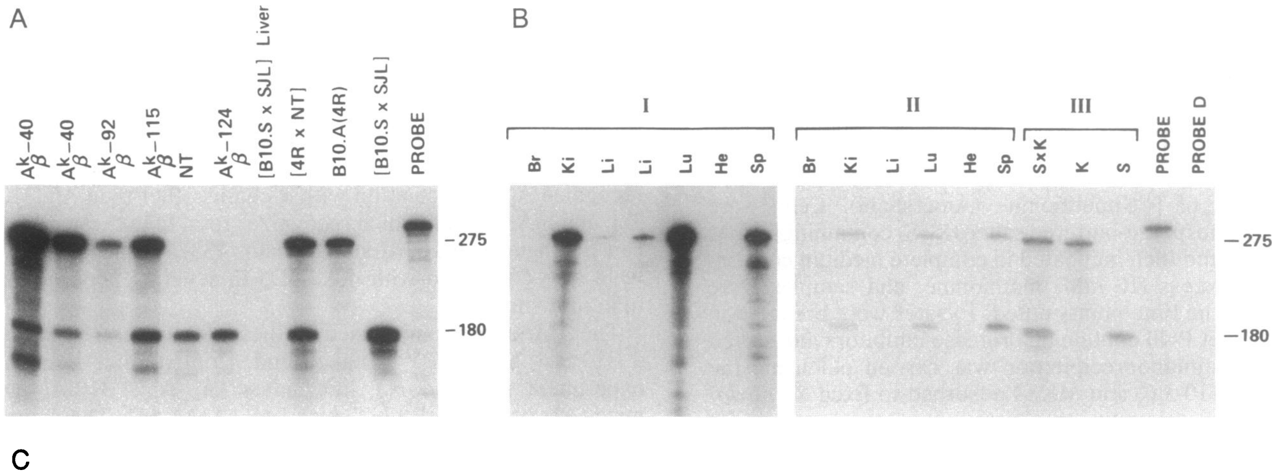


FIG. 2. Northern (RNA) blot analysis of spleen (S) RNA from A^k_β transgenic mice and controls. Fifteen micrograms of total RNA was run in each lane; liver (Li) RNA was run as a control. Filters hybridized with an A^k_β cDNA probe were stripped and then hybridized with A^k_α cDNA probe as noted. Copy numbers of the A^k_β mice are as follows: A^k_β-70, 20–25; A^k_β-99, 20–25; A^k_β-92, 25–30; A^k_β-124, 1; A^k_β-110, 2; A^k_β-111, 6; A^k_β-145, 10; A^k_β-40, 60–65; and A^k_β-105, 60–65.

of nontransgenic levels), and total Ia cell-surface expression ranged from 15% to 40% of control levels in mice carrying from 50 to 65 copies of the transgene. The reduction in cell-surface Ia did not affect cell-surface expression of other proteins. Cell-surface levels of B220, IgM, IgD, and class I on Ia^+ B cells were similar to those of controls in all transgenic lines.

The above data indicated that pairing of A^k_β with A^s_α was inefficient but could be driven by excess A^k_β mRNA and protein synthesis. Breeding A^k_β mice to A^k_α transgenic mice demonstrated that the transgene was expressed more efficiently on the cell surface when the haplotype-matched A^k_α pairing partner was present. The A^k_α mice used (from the A^k_α-26 line) carry 20 copies of A^k_α and express excess A_α mRNA. No A^k_α protein is expressed on the cell surface in association with either A^s_β or A^b_β, and cell-surface expression of the endogenous alleles is not affected by the excess of A^k_α mRNA expression (refs. 12 and 13; data not shown).

We analyzed offspring from three $A^k_\alpha \times A^k_\beta$ pairs carrying 1, 10, and 65 copies of A^k_β and 20 copies of A^k_α (Fig. 4). Mice carrying 1 copy of A^k_β expressed 6% 4R×NT levels of A^k_β in association with A^s_α, whereas double-transgenic mice expressed 29% 4R×NT levels of A^k_β. Similarly, mice carrying 10 copies of A^k_β expressed 50% 4R×NT levels of A^k_β in association with A^s_α but 250% 4R×NT levels when excess A^k_α was present. Finally, mice carrying 65 copies of A^k_β and 20 copies of A^k_α (in which the α chain was probably limiting) expressed 10-fold (1000%) more A^k_β on the cell surface than did 4R×NT controls. These observations suggested that A^k_α/A^k_β chain pairing was four to five times more efficient than was pairing



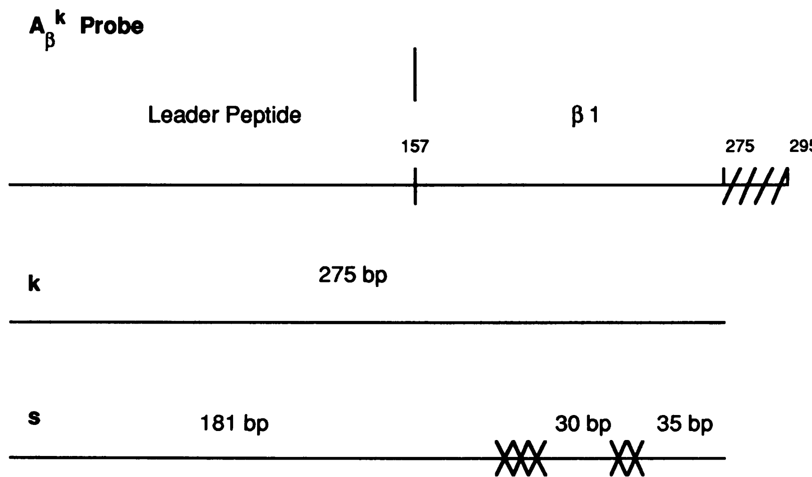


FIG. 3. Comparison of A^k_β and A^s_β message levels using RNase protection. RNase protection assays were carried out using a ^{32}P-labeled antisense probe containing 275 bp of A^k_β sequence and 20 bp of polylinker. Five to 15 μg of total RNA was used in each lane. (*A*) Protection of A^k_β and A^s_β message by using spleen RNA isolated from B10.S × SJL (I-$A^{s/s}$), B10.A(4R) (I-$A^{k/k}$), and 4R×NT (I-$A^{s/k}$) control mice and A^k_β transgenic mice carrying the following copy numbers of A^k_β: A^k_β-124, 1; A^k_β-115, 5; A^k_β-92, 25–30; and A^k_β-40, 60–65. (*B*) Protection of RNA from tissues from an A^k_β-40 mouse carrying 60–65 copies of A^k_β (I), an A^k_β-111 mouse carrying 6 copies of A^k_β (II), and spleen RNA from control mice (III). Br, brain; Ki, kidney; Li, liver; Lu, lung; He, heart; and Sp, spleen. Probe D, unprotected probe digested with RNase. (*C*) Expected protection of the A^k_β and A^s_β messages. /, Polylinker; X, expected cleavage sites.

Mean Fluorescence

Mice		40.N ■	MKS4 □	10-3.6 ◆
4R x NT		[1]	[1]	[2]
	4R	1.63 ± .20	0.03 ± .02	2.10 ± .44
	NT	0.02 ± .01	2.34 ± .40	2.24 ± .40
α	β			
0	1-2	0.06 ± .03	2.14 ± .55	1.96 ± .76
0	6	0.14 ± .07	1.83 ± .67	1.60 ± .55
0	10	0.51 ± .12	0.94 ± .22	1.30 ± .22
0	20-30	0.53 ± .13	0.32 ± .07	0.88 ± .16
0	50-65	0.28 ± .10	0.22 ± .07	0.52 ± .16
20	1	0.29 ± .08	1.56 ± .28	1.9 ± .29
20	10	2.62	1.23	4.23
20	65	11.7 ± 2.13	0.36 ± .10	11.0 ± 1.65

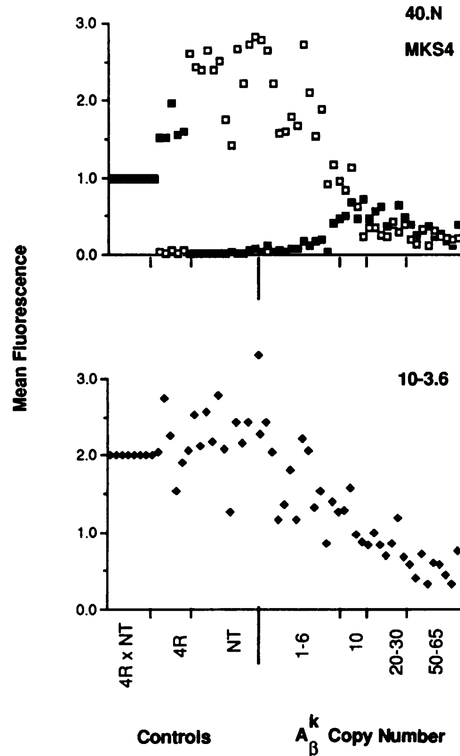


FIG. 4. Relative Ia cell-surface levels on mature B cells from A^k_β transgenic mice. Mice were stained for Ia with mAb 40.N (A^k_β), mAb MKS4 (A^s_β), and mAb 10-3.6 (A^k_β and A^s_β; total Ia). Mean fluorescence values obtained with each reagent were normalized to those observed on 4R×NT B220$^+$ cells in the same experiment. mAb 40.N and mAb MKS4 mean fluorescence intensities were assigned a value of 1.0, and mAb 10-3.6 was assigned a value of 2.0 in 4R×NT controls. Numbers in the table are means of all values obtained for A^k_β and A^k_β/A^k_α double-transgenic mice; when only two mice were analyzed no SEM is given. Copy numbers of the A^k_β and A^k_α transgenes are indicated at left.

of A^k_β with A^s_α. Expression of the transgene in thymic tissue and on macrophages paralleled that observed on B cells (ref. 15, data not shown).

Protein Analysis. Clearly, excess A^k_β mRNA that was translated and expressed on the cell surface in double-transgenic mice did not reach the cell surface in single transgenic animals (Fig. 4). To determine whether excess A^k_β protein was being sequestered intracellularly in the A^k_β mice, spleen cells from representatives of each copy-number range were metabolically labeled with [^{35}S]methionine, and Ia complexes were immunoprecipitated with mAbs 40.N, MKS4, and 10-3.6. Quantitatively, the results agreed with the FACS data and appeared to reflect both decreased cell-surface expression of Ia and a reduction in the number of B cells in mice carrying >20 copies of the A^k_β transgene. No excess A^k_β protein was immunoprecipitated from the A^k_β high-copy-number lines, nor was excess A^k_β protein (or any additional protein) observed in total cell lysates analyzed by nonequilibrium pH-gradient electrophoresis (NEPHGE) (data not shown).

These observations implied that excess A^k_β protein and much of the endogenous A^s_α/A^s_β protein was being degraded intracellularly in the high-copy A^k_β mice. Pulse-chase experiments suggested that degradation occurred rapidly, before formation of epitopes recognized by the antibodies used. When spleen cells from A^k_β-92 mice (which carry 25–30 copies of A^k_β) were incubated with [^{35}S]methionine for 10 min and samples were taken at 0, 10, and 20 min, no excess protein was observed at any early time point (Fig. 5*A*). The total amount of Ia immunoprecipitated (with a mixture of mAbs 10-3.6, 40.N, and MKS4) from the A^k_β-92 cells was consistent with the 50% reduction in cell-surface Ia and 10% reduction in B-cell number observed in these mice. This experiment was repeated several times and was confirmed in the A^k_β-145 line (which has an ≈40% reduction in cell-surface Ia and control numbers of B cells). Thus, excess A^k_β protein appeared to be degraded before the formation of epitopes recognized by these antibodies or was folded aberrantly so that these epitopes were destroyed. mAb 10-3.6 has been shown to immunoprecipitate dissociated A^k_β chains (33) and should have immunoprecipitated all properly folded A^k_β chains present.

No significant differences in Ia turnover were observed in several experiments using several lines of mice and up to 6 hr of chase time (Fig. 5*B*). In addition, no gross structural abnormalities in A^k_β protein structure were observed when immunoprecipitates from several lines of mice were analyzed by NEPHGE (data not shown).

DISCUSSION

Because A^k_β mRNA expression correlated with transgene copy number, we were able to assess competition of A^k_β with A^s_β for pairing with A^s_α *in vivo*. Excess mRNA and protein synthesis were accompanied by a significant increase in A^s_α/A^k_β cell-surface expression in mice carrying >10 copies of the transgene. Our results augment those from transfected cells in which various combinations of A^d_β, E^d_β, A^d_α, and E_α chains were allowed to compete for pairing and cell-surface expression (5). In this experiment, asymmetric chain synthesis drove cell-surface expression of the less favored I-A/I-E interisotypic heterodimers, although such heterodimers were not expressed under balanced (physiologic) conditions of chain synthesis.

Consistent with other studies (12, 13), pairing of the haplotype-matched A^k_β and A^k_α chains was at least 4–5 times more efficient than was pairing of A^k_β with A^s_α in our transgenic mice. Although asymmetric synthesis of A^k_β clearly resulted in increased cell-surface expression of the less favored α/β heterodimer, maximum levels of A^s_α/A^k_β were accompanied by a significant decrease in A^s_α/A^s_β expression. At best, 50–70% of 4R×NT levels of A^k_β were observed on the cell surface in any line of mice. Extremely high levels of A^k_β mRNA and protein synthesis did not lead to a further increase in A^s_α/A^k_β cell-surface expression but rather interfered with expression of the endogenous A^s_α/A^s_β complex. These observations suggest that only relatively low levels of mixed heterodimers can be driven by asymmetric chain synthesis

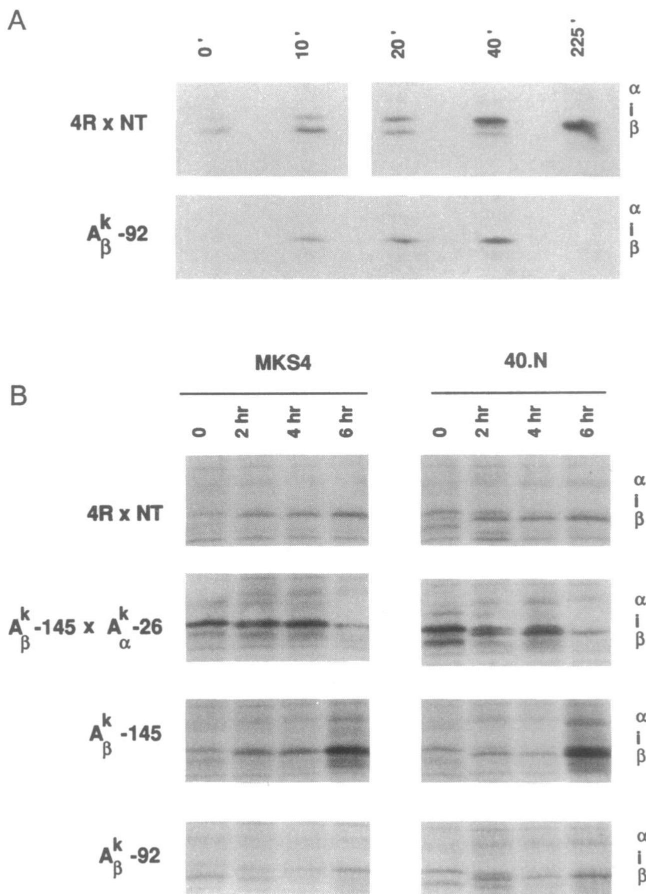


FIG. 5. Immunoprecipitation of Ia from control and transgenic lines of mice. (*A*) Spleen cells (7.5×10^7) from 4R×NT and A^k_β-92 mice (25–30 copies of A^k_β) were pulsed for 10 min with [^{35}S]methionine, and samples were removed at the indicated chase times. Total Ia from each sample was immunoprecipitated with a mixture of mAbs 40.N, MKS4, and 10-3.6. (*B*) Spleen cells (7.5×10^7) from 4R×NT, A^k_β-145 × A^k_α-26 (10 copies A^k_β, 20 copies A^k_α), A^k_β-145 (10 copies A^k_β) and A^k_β-92 (25–30 copies A^k_β) mice were pulsed for 30 min with [^{35}S]methionine, and samples were removed at the indicated chase times. Each sample was divided, and A^s_α/A^s_β and A^s_α/A^k_β complexes were immunoprecipitated with mAbs MKS4 and 40.N. All immunoprecipitates were analyzed on 12.5% acrylamide gels, and the positions of the α, β, and invariant (i) chains are indicated at right.

without a significant loss of those chains competing for pairing.

Improperly paired and unpaired proteins were not detected intracellularly, and pulse-chase experiments suggested that they were degraded quite rapidly. These data are not consistent with the idea that α and β chains have relatively long half-lives and accumulate in the endoplasmic reticulum before pairing and transport; nor do they support a model in which a rate-limiting transport or processing event occurs after dimer formation (5). It was the formation of α/β heterodimers that appeared to be limiting in the A^k_β mice, and this process was very inefficient under conditions of extreme overexpression and asymmetric chain synthesis. The amount of Ia expressed on the cell surface appeared to be limited primarily by the number of α and β chains that paired correctly. A 5- to 6-fold increase in total Ia was accommodated on the cell surface in A^k_α/A^k_β double transgenic mice.

Collectively, these mice have provided the opportunity to extend *in vitro* transfection studies of α/β chain pairing to a well-defined *in vivo* system. By crossing various copy numbers of the A^k_β transgene onto other haplotypes, pairing, expression, and both qualitative and quantitative effects of various A_α/A^k_β heterodimers on T-cell selection and the response to specific antigens can also be assessed.

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1. Travers, P. & McDevitt, H. O. (1987) in *The Antigens*, ed. Sela, M. (Academic, New York), Vol. 7, pp. 147–211.
2. Braunstein, N. S. & Germain, R. H. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2921–2925.
3. Sant, A. J., Braunstein, N. S. & Germain, R. N. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8065–8069.
4. Germain, R. N., Bentley, D. M. & Quill, H. (1985) *Cell* **43,** 233–242.
5. Sant, A. J. & Germain, R. N. (1989) *Cell* **57,** 797–805.
6. McNicholas, J. M., Murphy, D. B., Matis, L. A., Schwartz, R. H., Lerner, E. A., Janeway, C. A., Jr., & Jones, P. P. (1982) *J. Exp. Med.* **155,** 490–507.
7. Anderson, G. D. & David, C. S. (1989) *J. Exp. Med.* **170,** 1003–1008.
8. Mineta, T., Seki, K., Matsunaga, M. & Kimoto, M. (1990) *Immunology* **69,** 385–390.
9. Kimoto, M. & Fathman, C. G. (1980) *J. Exp. Med.* **152,** 759–770.
10. Sredni, B., Matis, L. A., Lerner, E. A., Paul, W. E. & Schwartz, R. H. (1981) *J. Exp. Med.* **153,** 677–693.
11. Beck, B. N., Frelinger, J. G., Shigeta, M., Infante, A. J., Cummings, D., Hammerling, G. & Fathman, C. G. (1982) *J. Exp. Med.* **156,** 1186–1194.
12. Le Meur, M., Waltzinger, C., Gerlinger, P., Benoist, C. & Mathis, D. (1989) *J. Immunol.* **142,** 323–327.
13. Fehling, H. J., van Ewijk, W., Pasquali, J. L., Waltzinger, C., Le Meur, M., Gerlinger, P., Benoist, C. & Mathis, D. (1990) *J. Immunol.* **144,** 2865–2874.
14. Anderson, G. D., Banerjee, S. & David, C. (1989) *J. Immunol.* **143,** 3757–3761.
15. Gilfillan, S., Aiso, S., Michie, S. A. & McDevitt, H. O. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7319–7323.
16. Germain, R. N., Norcross, M. A. & Margulies, D. H. (1983) *Nature (London)* **306,** 190–194.
17. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–513.
18. Estess, P., Begovich, A. B., Koo, M., Jones, P. P. & McDevitt, H. O. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3594–3598.
19. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
20. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
22. Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. & Green, M. R. (1984) *Nucleic Acids Res.* **12,** 7035–7056.
23. Oi, V. T., Jones, P. P., Goding, J. W., Herzenberg, L. A. & Herzenberg, L. A. (1978) *Curr. Top. Microbiol. Immunol.* **81,** 115–129.
24. Pierres, M., Devaux, C., Dosseto, M. & Marchetto, S. (1981) *Immunogenetics* **14,** 481–495.
25. Kappler, J. W., Skidmore, B., White, J. & Marrack, P. (1981) *J. Exp. Med.* **153,** 1198–1214.
26. Beck, B. N., Buerstedde, J.-M., Krco, C. J., Nilson, A. E., Chase, C. G. & McKean, D. J. (1986) *J. Immunol.* **136,** 2953–2961.
27. Goding, J. W. (1976) *J. Immunol. Methods* **13,** 215–226.
28. Coffman, R. L. & Weissman, I. L. (1981) *Nature (London)* **289,** 681–683.
29. Hayakawa, K., Hardy, R. R., Parks, D. R. & Herzenberg, L. A. (1983) *J. Exp. Med.* **157,** 202–218.
30. Jones, P. P. (1980) in *Selected Methods of Cellular Immunology*, eds. Mishell, B. & Shiigi, S. (Freeman, San Francisco), pp. 398–470.
31. Frelinger, J. G., Shigela, M., Infante, A. J., Nelson, P. A., Pierres, M. & Fathman, C. G. (1984) *J. Exp. Med.* **159,** 704–715.
32. Landais, D., Beck, B. N., Buerstedde, J.-M., Degraw, S., Klein, D., Koch, N., Murphy, D., Pierres, M., Tada, T., Yamamoto, K., Benoist, C. & Mathis, D. (1986) *J. Immunol.* **137,** 3002–3005.
33. Kupinski, J. M., Plunkett, M. L. & Freed, J. H. (1983) *J. Immunol.* **130,** 2277–2281.