Immunoglobulin (Ig) μ, κ Transgenic Mice Express Transgenic Idiotype on Endogenously Rearranged IgM and IgA Molecules by Secretion of Chimeric Molecules

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Summary

The sera of C57BL/6 mice transgenic for a μ_a allotype heavy (H) chain and κ light chain gene contained endogenous nontransgene immunoglobulin (IgM) (μ_b allotype) and IgA molecules which carried the idiotype expressed by the transgenically encoded IgM (μ_a) molecule. Serological analysis demonstrated that the presence of the transgenic idiotype on endogenous IgM and IgA was caused by the secretion of chimeric molecules that carried both chains encoded by the μ_a transgene and products of endogenously rearranged Ig μ_b or α genes. These and other results suggest that allelic exclusion of Ig gene rearrangement in μ, κ transgenic mice is not absolute, that B cells can secrete Igs composed of more than a single (H) chain type, and that endogenous isotype switching does not result in a complete silencing of transgene expression.

During ontogeny of B lymphocytes, somatic rearrangement of Ig genes occurs (1, 2). In this process, Ig H chain genes are formed by joining of a variable (V_H), a diversity (D_H) and a joining (J_H) gene segment, whereas Ig L chain genes are formed by bringing a V_L and a J_L gene segment in juxtaposition. Although two copies of each chromosome are present, B cells produce Ig molecules consisting of one species of L and of H chain only (3–5). Ontogenetic studies have demonstrated that no further rearrangements of the second allele occur after a functional reading unit is achieved on the first (6–8). Two mechanisms for this observed allelic exclusion have been proposed (9). One mechanism involves an active feedback from a functional Ig gene, or its product, which prevents further rearrangement of the other allele (6, 8, 10–12). The other mechanism is purely stochastic, proposing that the probability of a functional rearrangement is so low that the occurrence of two functional recombinations in one cell can be excluded statistically (13, 14).

To assess the mechanism responsible for allelic exclusion, a number of different rearranged Ig gene constructs were introduced into the germline of various mouse strains (for review see reference 15). Analysis of transgenic mice carrying rearranged κ L chain genes (16), rearranged μ H chain genes (17, 18), rearranged μ and κ genes (19–21) and rearranged δ H chain genes (22) has shown a varying, but never absolute inhibition of the rearrangement of endogenous Ig genes.

As a consequence of the incomplete allelic exclusion in Ig transgenic mice, flow cytometry analysis has shown the presence of cells carrying both transgene-encoded and endogenously rearranged Ig chains (21, 23, 24), and plasma cells producing both types of Ig chains have been demonstrated by immunohistology (21, 24). Moreover, analysis of hybridomas from Ig transgenic mice has indicated the presence of cells producing both transgene-encoded and endogenously rearranged μ and κ chains (18, 19, 22, 23), and serological analysis has suggested the presence of Ig molecules carrying both transgenic and endogenous μ allotype (18, 21, 23). Expression of transgenic idiotype on molecules encoded by rearranged endogenous Ig genes has been reported and ascribed to idiotypic networks (25) and interchromosomal isotype switching (26). Trans-splicing between transgene and endogenous sterile Ig gene transcripts (27) can also account for the expression of transgenic idiotype on endogenously rearranged Ig molecules.

In this study, we demonstrate the presence of transgene-encoded idiotype associated with endogenous rearranged IgM and IgA molecules in Sp6 μ, κ transgenic mice (19). Based on serological analysis of transgenic mice and of radiation bone marrow chimeras reconstituted with cells from transgenic and normal donors, we conclude that the observed idiotype expression can be ascribed to the presence of chimeric Ig molecules containing both transgene encoded μ chains and endogenously rearranged μ or α chains.

Materials and Methods

Reagents. The Sp603 antibody (28) and mAbs specific for murine IgM μ_a allotype, DS1, (29, 30), murine IgM μ_b allotype,

AF6.122, (31) and for Sp603 H chain idiotype, 20-5, (32) were purified from ascites or hybridoma culture supernatant on protein G-Sepharose (Pharmacia, Uppsala, Sweden). Biotinylation was performed using aminohexanoyl-biotin-*N*-hydroxysuccinimide ester (Zymed Laboratories, Inc., San Francisco, CA) as described (33). Purified DS1 was coupled to reacti-gel GF-2000 (Pierce Chemical Co., Rockford, IL) at a concentration of 1 mg protein per ml of gel. Purified Sp6 was used as an IgM μ_a standard, purified IgM-κ and purified IgA-κ (Pharmingen, San Diego, CA) as IgM μ_b and IgA standards, respectively. Unlabeled affinity-purified goat anti-mouse IgA and conjugates of this reagent and streptavidin with alkaline phosphatase and horseradish peroxidase (Southern Biotechnology Associates, Birmingham, AL), and alkaline phosphatase-conjugated monoclonal anti-mouse IgM (Zymed Laboratories, Inc.) were titrated for use in ELISA and employed at saturating dilutions. ABTS solution (Kierkegaard & Perry Laboratories, Inc., Gaithersburg, MD) was used as peroxidase substrate, whereas a 2 mg/ml solution of p-Nitrophenyl phosphate in AMP buffer (Sigma Chemical Co., St. Louis, MO) (34) was employed for alkaline phosphatase detection.

Mice. BALB/c and C57BL/6 Sp6 transgenic mice (19) were provided by M. C. Lamers (Max-Planck-Institut Fur Immunobiologie, Freiburg, Germany) and maintained at Bioqual (Rockville, MD). The Sp6 mice were generated by introduction of a construct containing rearranged μ and κ genes encoding an IgM molecule with antitrinitrophenyl specificity (Sp603) (28) and a bacterial gene for neomycin resistance under control of the SV40 promoter/enhancer in the germ line of Swiss albino mice. BALB/c and C57BL/6 Sp6 mice were obtained by extensive backcrossing of the Sp6 founder mice to the respective mouse strains.

Bone marrow chimeras were made by injecting irradiated (C57BL/6 × BALB/c)F_1 ($CB6F_1$) mice with 5×10^6 BALB/c Sp6 and 5×10^6 C57BL/6 T cell–depleted bone marrow cells as described (35).

ELISA. 96-well plates (Titerek no. 77-127-05; Flow Laboratories, Zwanenburg, The Netherlands) were coated with 100 μl of a 5-μg/ml solution of specific capture antibody by incubating for 1 h at 37°C. Next, the plates were postcoated by adding 100 μl of PBS containing 1% BSA and 0.1% Tween 20 (PBT 1.0), and incubating for 30 min at 37°C. After postcoating, the plates were incubated with titrated concentrations of sera or standards diluted in PBT 1.0. After 1 h of incubation at 37°C and three washes with PBS containing 0.1% BSA and 0.1% Tween 20 (PBT 0.1), 100 μl of appropriately diluted biotinylated or enzyme-conjugated antiserum in PBT 1.0 were added to each well, after which the plates were incubated for 1 h at 37°C. Plates receiving biotinylated antibodies were washed three times with PBT 0.1 and received 100 μl of appropriately diluted enzyme-conjugated streptavidin and were incubated for 1 h at 37°C, whereas plates treated with enzyme-conjugated antisera were directly washed five times with PBT 0.1, after which 100 μl of substrate solution were added. After 30 min of incubation at 37°C, extinctions were determined spectrophotometrically (Titertek Multiskan; Flow Laboratories, McLean, VA).

Absorption Analysis. Fractions of 500 μl taken from 12-step twofold serial dilutions of individual sera in PBT 1.0 starting at a 1/200 initial dilution were incubated for 2.5 h at 4°C with 50 μl of a 1:1 suspension of anti-IgM μ_a gel in PBT 1.0, or with 37.5 μl of PBT 0.1 as a control. After incubation, the gel was spun down, and a variety of ELISAs were performed using 50 μl of the individual fractions. From the extinction values obtained by ELISA, the titers of the nonabsorbed control and the absorbed titration curves were calculated using logit transformation (36). From the titer of both curves the percentage of the titer remaining after absorption (percent remaining) was calculated according to: Percent remaining = $100\times$ ($\text{titer}_{\text{absorbed}}/\text{titer}_{\text{unabsorbed}}$).

Gelfiltration. Fractions of 200 μl of both pooled transgene positive and negative sera were spun down and separated by FPLC using a Superose 12 column (Pharmacia) in 200-μl fractions. The OD_{280} of the individual fractions was determined spectrophotometrically, after which they were tenfold diluted in PBT 1.0 and titrated out in various ELISAs.

Results

Serum IgM μ_a, μ_b and IgA Concentrations of C57BL/6 Sp6 Transgene-positive (tg^+)[1] and tg^- Mice. To investigate to what extent the introduction of a rearranged Ig construct encoding an IgM molecule of the μ_a allotype, characteristic of BALB/c mice, (19) influenced the production of endogenous IgM (carrying the μ_b allotype, characteristic of C57BL/6 mice) and IgA, we determined the concentration of these molecules in the sera of C57BL/6 Sp6 tg^+ and tg^- animals. As shown in Fig. 1, the ELISAs for each of these Igs allowed a specific determination. IgM μ_a was detected in the sera of BALB/c tg^+ and tg^- mice, (with endogenous μ chains expressing the μ_a but not the μ_b allotype) and in the serum of C57BL/6 tg^+ (capable of producing μ_a transgenic and endogenous μ_b chains), but not in the serum of C57BL/6 tg^- (endogenous μ_b only) (Fig. 1 *A*). IgM μ_b was detected in the sera of C57BL/6 tg^+ (μ_a and μ_b) and tg^- mice (endogenous μ_b only), but not in the sera of BALB/c tg^+ and tg^- (μ_a only) (Fig. 1 *B*). The assay for IgA detected purified IgA, but showed no reactivity with IgM μ_a or μ_b (Fig. 1 *C*). Using these assays the concentrations of IgM μ_a, μ_b, and IgA were determined in the sera of five individual C57BL/6 Sp6 tg^+, and tg^- (Table 1) mice. The data indicate that the presence of the transgene did not influence the amount of IgM μ_b detected, and that the serum concentrations of IgA were generally lower for tg^+ than for tg^- mice. These results support previous evidence for incomplete inhibition of rearrangement of endogenous Ig genes in Sp6 transgenic mice (19, 24, 32, 37, 38).

Expression of Transgenic Idiotype on Endogenously Rearranged IgM and IgA. To investigate the association of transgene-encoded idiotype with Ig molecules resulting from rearrangement of endogenous genes reported for other Ig transgenic mice (25, 26), the sera from the mice of Table 1 (C57BL/6 Sp6 tg^+/tg^-) were assayed for the association of transgenic idiotype with several serum Ig isotypes. The data for IgM μ_a, μ_b, and IgA are shown in Fig. 2. Using the 20-5 antibody, which recognizes an Ig H chain idiotype on the Sp603 antibody (32) from which the Sp6 transgenics were generated, transgenic idiotype could be detected in the sera of tg^+ mice in association with IgM μ_a, which is transgene encoded, (Fig. 2 *A*), but also with IgM μ_b (Fig. 2 *B*) and IgA (Fig. 2 *C*), which are endogenously encoded. Idiotype expression could not be detected in the sera of tg^- mice (Fig. 2, *D–F*). Moreover, the association of transgenic idiotype with

[1] *Abbreviations used in this paper:* bmc, bone marrow chimeras; PBT, PBS-Tween; tg, transgenic.

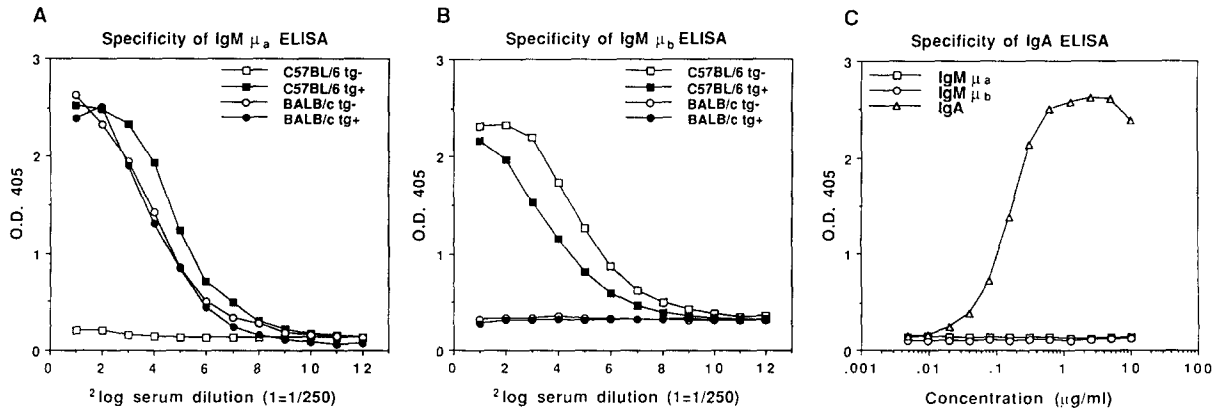

Figure 1. Test of the specificity of the reagents used for the detection of IgM μ_a, μ_b, and IgA. (*A* and *B*) Plates were coated with anti-IgM μ_a (*A*) or anti-IgM μ_b (*B*). Sera of tg^+ and tg^- C57BL/6 and BALB/c mice were added in titrated concentrations starting from a 1/250 initial dilution. IgM was detected using alkaline phosphatase-conjugated IgM. (*C*) Plates were coated with anti-IgA. Dilutions of purified IgM μ_a, μ_b, and IgA were added. IgA was detected using alkaline phosphatase-conjugated anti-IgA.

IgM μ_b (Fig. 2 *B*) and IgA (Fig. 2 *C*) could not be ascribed to crossreactivities between the ELISA reagents and the Sp603 antibody. Association of the transgenic idiotype with other Ig isotypes was not detected (data not shown).

Presence of Chimeric IgM and IgA Molecules in the Sera of C57BL/6 Sp6 tg^+ Mice. In previous reports the association of transgenic idiotype with endogenously rearranged Ig molecules was ascribed to idiotypic networks (25) and inter-

Table 1. *Ig Serum Concentrations*

Strain	Mouse	IgM μ_a	IgM μ_b	IgA
C57BL/6 Sp6 tg^+ mice	499	265*	2344	278
	2320	428*	1040	105
	2328	720*	564	50
	3320	983*	983	712
	3321	1419*	1030	5704
C57BL/6 Sp6 tg^- mice	497	0	2477	1604
	2322	0	1191	1191
	2329	0	935	935
	3324	0	729	729
	3325	0	642	642
(BALB/c Sp6 tg^+ + C57BL/6)→$CB6F_1$ mixed radiation bone marrow chimeras	464	28‡	3954	255
	465	26‡	5033	137
	467	8‡	861	230
	468	11‡	2686	87
	470	5‡	3455	70

Serum Ig concentrations were determined by ELISA comparing serum titration curves with titration curves of standards of known Ig concentrations. IgM μ_a was assayed using anti-IgM μ_a for coating and employing alkaline phosphatase-conjugated anti-IgM (a-IgM-AP) for detection. IgM μ_b was assayed using anti-IgM μ_b for coating and employing a-IgM-AP for detection. IgA was assayed using anti-IgA for coating and employing alkaline phosphatase conjugated anti-IgA for detection. (BALB/c Sp6 tg^+ + C57BL/6)→$CB6F_1$ radiation bone marrow chimeras were made as described in the experimental procedures.

* Serum concentration in μg/ml.

‡ Mice were bled 6 wk after bone marrow reconstitution.

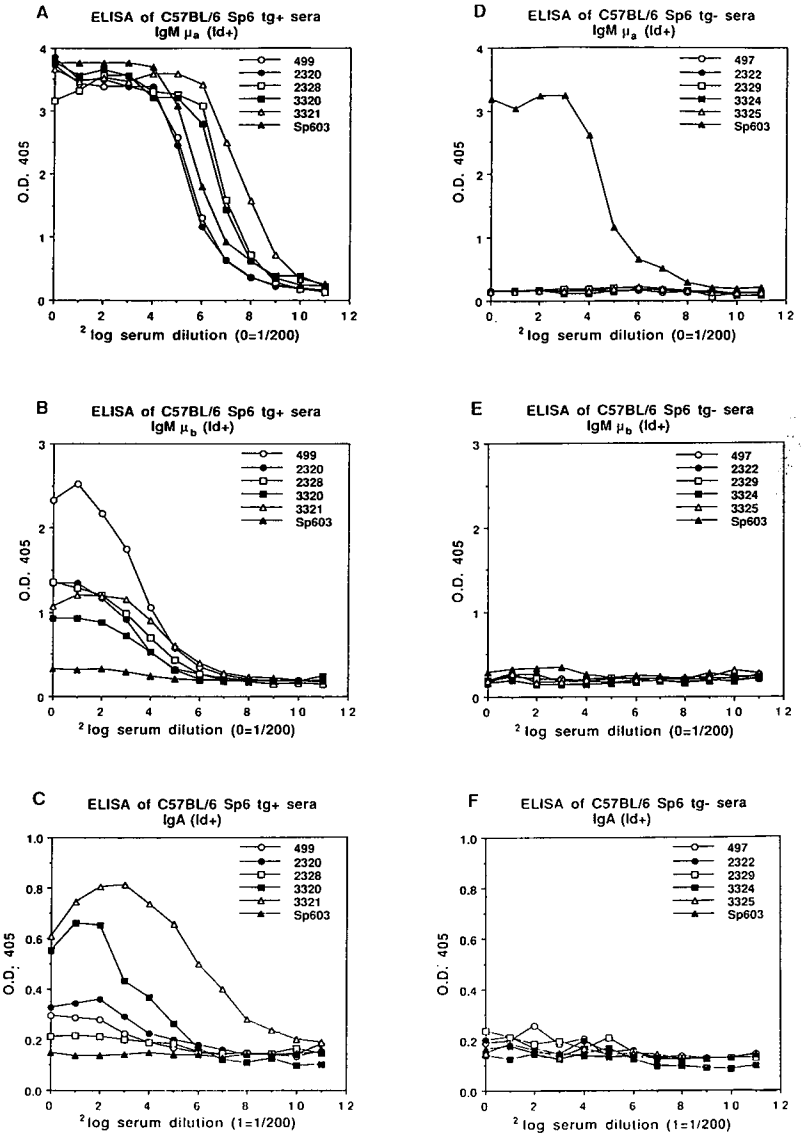

Figure 2. Demonstration of the association of transgenic idiotype with transgenic IgM μ_a and nontransgenic IgM μ_b and IgA in the sera of C57BL/6 Sp6 tg$^+$ mice. Plates were coated with the 20-5 mAb, which recognizes a H chain idiotype on the Sp603 IgM μ_a hybridoma from which the Sp6 transgenics were constructed. Sera of the five individual tg$^+$ (*A–C*) and tg$^-$ (*D–F*) C57BL/6 Sp6 mice assayed in Table 1 were added in titrated concentrations starting from an initial 1/200 dilution, as was a culture supernatant of the Sp603 hybridoma from an initial concentration of 14 μg/ml. (*A* and *D*) Transgenic idiotype positive (Id$^+$) IgM μ_a was detected using biotinylated anti-IgM μ_a, followed by alkaline phosphatase-conjugated streptavidin (sAV-AP). (*B* and *E*) Id$^+$ IgM μ_b was detected using biotinylated anti-IgM μ_b followed by sAV-AP. (*C* and *F*) Id$^+$ IgA was detected using alkaline phosphatase-conjugated anti-IgA.

chromosomal isotype switching (26). Other mechanisms that may explain the observed association include mRNA trans-splicing (27) and the formation of chimeric Ig molecules, which would be in concordance with the presence of IgM molecules of mixed allotype (18, 21, 23). To investigate the selective association of transgenic idiotype with nontransgene-encoded IgM μ_b and IgA, the sera analyzed in Fig. 2 were assayed for the presence of chimeric IgM molecules. Chimeric IgM μ_a/μ_b (Fig. 3 *A*) and IgA/μ_a (Fig. 3 *B*), but not IgA/μ_b (Fig. 3 *C*) were detected in the sera of tg$^+$ mice, and the observed results were not caused by crossreactivity of the ELISA reagents with the Sp603 antibody. No chimeric Ig molecules were detected in the sera of tg$^-$ mice (Fig. 3 *D–F*). A comparison of Figs. 2 *B* and 3 *A*, and of Figs. 2 *C* and 3 *B*, indicates a good correlation between the levels of idiotype expression and of chimeric Igs in the individual sera, with the exception of one serum (499) which displayed an unexplained deviation.

Idiotype Expression on Endogenous IgM and IgA by Chimeric Ig Molecules. To determine to what extent chimeric Ig molecules were responsible for the association of transgenic idiotype with endogenously rearranged Igs, the previously analyzed sera were absorbed with gel-coupled anti-IgM μ_a. To allow a quantitative comparison of a variety of Ig molecules, aliquots of individual dilutions of serum were incubated with the gel, after which both absorbed and unabsorbed sera were assayed by ELISA, and the percentage of the titer remaining after absorption (percent remaining) was calculated by division of the titer of the adsorbed serum by the titer of the corresponding unabsorbed serum. Next the mean value of the data of five individually tested sera was calculated. Fig. 4 demonstrates that the anti-μ_a gel absorbed 98% of IgM μ_a (Fig. 4 *A*), gave a minor absorption of IgM μ_b (Fig. 4 *B*), and did not bind IgA (Fig. 4 *C*). The absorption of the tg$^+$ sera resulted in an almost complete removal of total and transgenic idiotype positive (Id$^+$) IgM μ_a, chimeric IgM

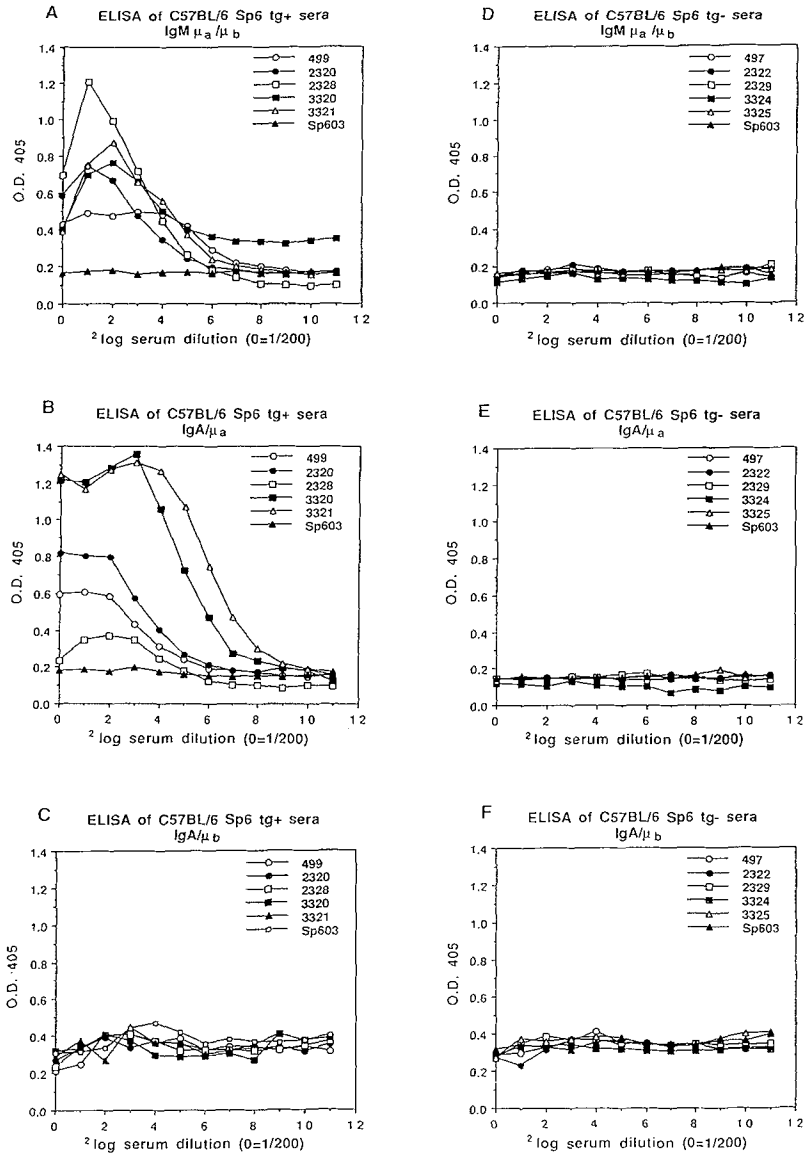

Figure 3. Demonstration of chimeric IgM μ_a/μ_b and IgA/μ_a molecules in the sera of C57BL/6 Sp6 tg^+ mice. Plates were coated with anti-IgM μ_b (*A*, *C*, *D*, and *F*), or anti-IgM μ_a (*B* and *E*). The sera of the five individual tg^+ (*A–C*) and tg^- C57BL/6 Sp6 mice assayed in Fig. 2 were added in titrated concentrations starting from an initial 1/200 dilution, as was a culture supernatant of the Sp603 hybridoma from an initial concentration of 14 μg/ml. (*A* and *D*) Chimeric IgM μ_a/μ_b molecules were detected using biotinylated anti-IgM μ_a, followed by alkaline phosphatase-conjugated streptavidin. (*B* and *E*) Chimeric IgA/μ_a molecules were detected using alkaline phosphatase-conjugated anti-IgA (a-IgA-AP). (*C* and *F*) Chimeric IgA/μ_b molecules were detected using a-IgA/AP.

μ_a/μ_b and IgA/μ_a, and Id^+ IgA; a minor decrease of total (tot) IgA; and a major absorption of total IgM μ_b (Fig. 5 *A*). These data indicate that expression of transgenic idiotype on IgA is caused by chimeric IgA/μ_a molecules, since the essentially complete absorption of Id^+ IgA was similar to the absorption of IgA molecules coexpressing μ_a Ig chains, and only a very limited percentage of total IgA was absorbed out. The data on the expression of transgenic idiotype on IgM μ_b are less conclusive, since the absorption of both Id^+ and total IgM μ_b by anti-μ_a gel resembled that of all Ig molecules with μ_a chains. The specificity of this absorption was confirmed in a separate experiment that compared the effects of absorption with anti-μ_a gel to the absorption with a control (isotype matched) gel. Consistent with the results shown in Fig. 5 *A*, anti-μ_a gel absorbed significantly more total IgM μ_b from the tg^+ sera than did the control gel. In contrast, anti-μ_a gel had no greater effect than control gel on the titer of tg^- sera (data not shown). This suggests that the high absorption of total IgM μ_b from tg^+ sera is due to the fact that a significant proportion of these molecules contains μ_a Ig chains.

(BALB/c Sp6 tg^+ + C57BL/6)→$CB6F_1$ Radiation Bone Marrow Chimeras Express Transgenic Idiotype on IgA, but Not on IgM μ_b. To determine whether C57BL/6 Sp6 tg^+ mice express transgenic idiotype on IgM μ_b as a consequence of the production of chimeric IgM μ_a/μ_b molecules by individual B cells, radiation bone marrow chimeras (bmc) were generated by the cotransfer of BALB/c Sp6 tg^+ (μ_a) and C57BL/6 (μ_b) bone marrow cells into irradiated $CB6F_1$ recipient mice. In contrast to C57BL/6 Sp6 tg^+ mice, these chimeras have the genes encoding for IgM μ_a and μ_b in separate cells. Since the cells originating from the BALB/c Sp6 tg^+ mice not only possess the genes for transgenic and endogenous IgM μ_a, but also for the other Ig isotypes, it would be expected that the sera of the chimeras contain Id^+ IgA and chimeric μ_a/IgA, like the C57BL/6 Sp6 tg^+ mice.

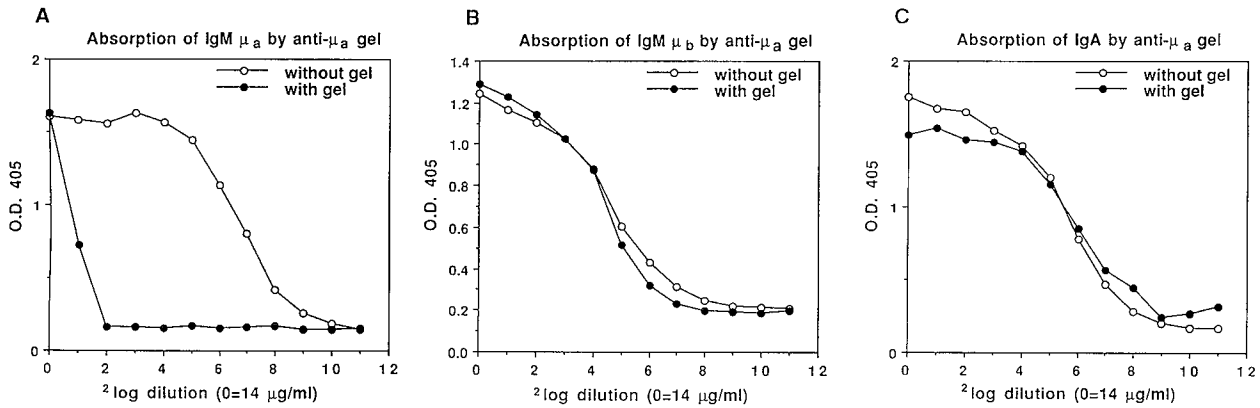

Figure 4. Test of the specificity of the absorption of gel-coupled anti-IgM μ_a. Aliquots taken from serial dilutions of IgM μ_a (*A*), IgM μ_b (*B*), and IgA (*C*) starting at an initial concentration of 14 μg/ml were incubated with gel-coupled anti-IgM μ_a. After incubation, the levels of the Igs from the incubated and unabsorbed control dilutions were determined by ELISA. (*A*) IgM μ_a was assayed by an ELISA using anti-IgM μ_a for coating and employing alkaline phosphatase-conjugated anti-IgM for detection. (*B*) IgM μ_b was assayed by an ELISA using anti-IgM μ_b for coating and employing alkaline phosphatase-conjugated anti-IgM for detection. (*C*) IgA was assayed by an ELISA using anti-IgA for coating and employing alkaline phosphatase-conjugated anti-IgA for detection.

However, if Id$^+$ IgM μ_b, or chimeric IgM μ_a/μ_b molecules in serum reflect the production of chimeric molecules by individual B cells, these molecules should not be present in the mixed bone marrow reconstituted mice. Before assaying the presence of these Ig molecules, the serum concentrations of IgM μ_a, μ_b, and IgA of five individual radiation bone marrow chimeras were determined (Table 1). A comparison of these levels with those of the C57BL/6 Sp6 tg$^+$ mice (Table 1) indicated that the chimeras had comparable levels of serum IgA, decreased levels of IgM μ_a, and comparable levels of IgM μ_b.

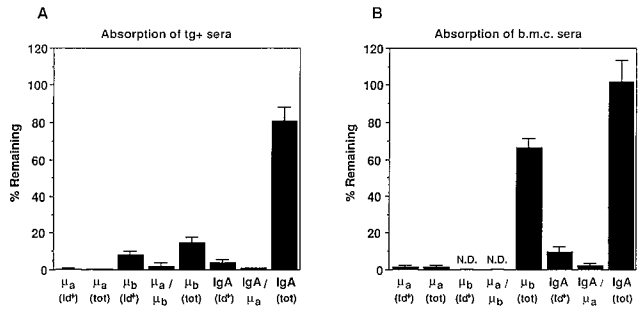

Figure 5. Absorption of sera from C57BL/6 Sp6 transgene positive (tg$^+$) and radiation-mixed bone marrow chimeras of BALB/c Sp6 tg$^+$ and C57BL/6 donors (b.m.c.) using gel-coupled anti-IgM μ_a. Aliquots taken from serial dilutions of five individual tg$^+$ and b.m.c. mice were incubated with gel-coupled anti-μ_a. After absorption, the incubated and unabsorbed control fractions were analyzed by a number of different ELISAs. Transgenic idiotype positive (Id$^+$) IgM μ_a was assayed using the antiidiotypic antibody 20-5 for coating and employing biotinylated anti-IgM μ_a (a-μ_a-bio) followed by alkaline phosphatase-conjugated streptavidin (sAV-AP) for detection. Total (tot) IgM μ_a was assayed using a-μ_a for coating and employing alkaline phosphatase-conjugated anti-IgM (a-IgM-AP) for detection. Id$^+$ IgM μ_b was detected using 20-5 for coating and employing biotinylated anti-IgM μ_b (a-μ_b-bio) followed by sAV-AP for detection. Chimeric IgM μ_a/μ_b was assayed using a-μ_b for coating and employing a-μ_a-bio followed by sAV-AP for detection. IgM μ_b (tot) was assayed using a-μ_b for coating and employing a-IgM-AP for detection. Id$^+$ IgA was assayed using 20-5 for coating and employing alkaline phosphatase-conjugated anti-IgA (a-IgA-AP) for detection. Chimeric IgA/μ_a was assayed using a-μ_a for coating and a-IgA-AP for detection. IgA (tot) was assayed using a-IgA for coating and a-IgA-AP for detection. Titers of absorbed and unabsorbed sera were calculated using log-logit transformation of the ELISA data. The percentage of the titer remaining after absorption (*% remaining*) was calculated by dividing the titer after absorption by the titer of the unabsorbed serum. Data represent the mean ± SEM of sera from five individually tested animals. (*A*) Absorption of sera from tg$^+$ mice. (*B*) Absorption of sera from b.m.c. mice. B.m.c. were made as described in Materials and Methods.

To determine whether chimeric IgA/μ_a molecules were the cause of Id$^+$ IgA, the chimeric sera were absorbed with gel coupled anti-μ_a. The results of the absorption (Fig. 5 *B*) show that the gel removed total and Id$^+$ IgM μ_a, Id$^+$ IgA, and chimeric IgA/μ_a; bound a part of the total IgM μ_b; and absorbed little total IgA. Taken together, the data on the bone marrow chimeras extend the evidence that chimeric Ig molecules produced by individual cells are the cause of transgenic idiotype expression on endogenously rearranged Igs. Moreover, the limited extent of IgM μ_b absorption from the sera of the chimeras further supports the notion that a significant part of all IgM μ_b molecules in the sera of C57BL/6 Sp6 tg$^+$ mice is present as IgM μ_a/μ_b chimeric molecules.

Chimeric IgA/μ_a Molecules Coelute with IgM in Gelfiltration. To biochemically characterize chimeric IgA/μ_a molecules, sera of Sp6 tg$^+$ mice were analyzed by electrophoresis and gel filtration. The electrophoresis experiments were unsuccessful, because of a failure to achieve adequate separation of IgA and IgM under nondenaturing conditions. In the gel filtration experiment, pooled sera from Sp6 tg$^+$ and tg$^-$ mice were separated and the OD_{280} of the individual fractions was determined (Fig. 7, *A* and *E*). Using ELISA, the

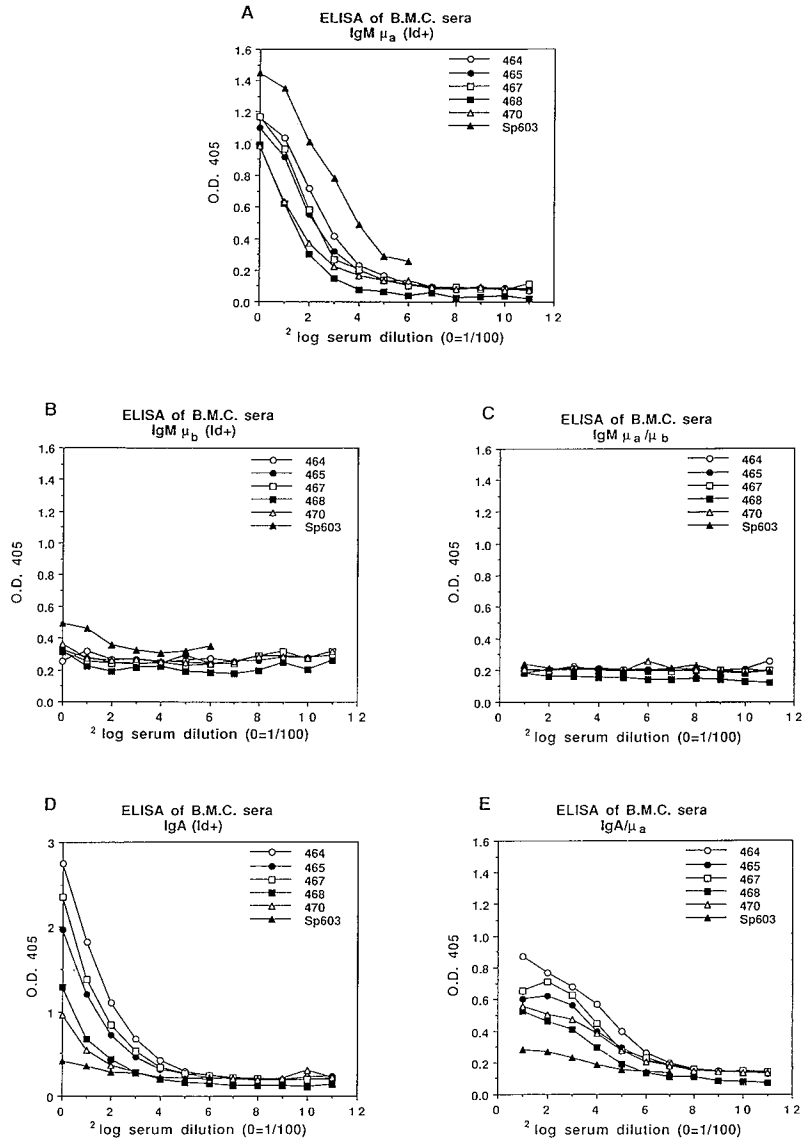

Figure 6. The sera of (BALB/c Sp6 tg^+ + C57BL/6)→ $CB6F_1$ mixed radiation b.m.c. contain chimeric IgA/μ_a molecules and show the association of transgenic idiotype with IgA, but have no chimeric IgM μ_a/μ_b molecules or IgM μ_b molecules carrying the transgene-encoded idiotype. B.m.c. mice were made by injecting irradiated $CB6F_1$ mice with 5×10^6 BALB/c Sp6 tg^+ and 5×10^6 C57BL/6 T cell–depleted bone marrow cells and were bled 6 wk after bone marrow reconstitution. The levels of various Ig molecules were determined by ELISA. Plates were coated with antiidiotypic antibody 20-5 (*A*, *B*, and *D*), anti-IgM μ_b (*C*), or anti-IgM μ_a (*E*). Sera of five individual b.m.c. mice were added at titrated concentrations starting from an initial 1/100 dilution, as was a culture supernatant of the Sp603 starting from an initial concentration of 400 ng/ml. (*A*) Transgenic idiotype positive) (Id^+) IgM μ_a was detected using biotinylated anti-IgM μ_a (a-μ_a-bio), followed by horseradish peroxidase-conjugated streptavidin (sAV-PO). (*B*) Id^+ IgM μ_b was detected using biotinylated anti-IgM μ_b followed by sAV-PO. (*C*) Chimeric IgM μ_a/μ_b was detected using a-μ_a-bio followed by sAV-PO. Id^+ IgA (*D*) and chimeric IgA/μ_a (*E*) were detected using horseradish peroxidase-conjugated anti-IgA.

concentrations of total IgM and IgA (Fig. 7, *B* and *F*), the titers of Id^+ IgM and IgA (Fig. 7, *C* and *G*), and of chimeric IgA/μ_a (Fig. 7, *D* and *H*) in each of the fractions were determined. The data show that the gel filtration separated IgM and the various molecular weight forms of IgA (39) (Fig. 7, *B* and *F*). The analysis of the expression of transgenic idiotype on IgM and IgA (Fig. 7, *C* and *G*) indicated that Id^+ IgA coeluted with IgM in contrast to total IgA. The Id^+ IgA eluted in the same fractions as the IgA/μ_a molecules. These findings indicate that Id^+ IgA molecules result from the incorporation of endogenous α chains in transgenic IgM μ_a molecules.

Discussion

The allelic exclusion of endogenous Ig gene rearrangements in transgenic mice with a variety of Ig gene constructs is never absolute (15). As a consequence of this incomplete feedback inhibition Sp6 μ, κ transgenic mice rearrange endogenous Ig genes and synthesize their products (19, 32). In the present study, we demonstrated the selective association of a transgene-encoded Ig H chain idiotype with endogenous IgM and IgA molecules in the serum of Sp6 mice, and sought to identify the mechanism responsible for this expression.

Two mechanisms for the expression of transgene-encoded idiotype on endogenously rearranged Ig molecules have been reported. First, it has been suggested that idiotypic networks could induce the expression of transgenic idiotype on endogenously rearranged Ig molecules (25, 26). Although the precise details of this mechanism remained unaddressed, it was hypothesized that antiidiotypic regulatory cells would play a crucial part in the recruitment of Id^+ cells from the population of B cells carrying the products of endogenously rearranged genes. It is unclear, however, why in a population of B lymphocytes expressing transgenically encoded idiotype, a small number of cells carrying this idiotype as a result of

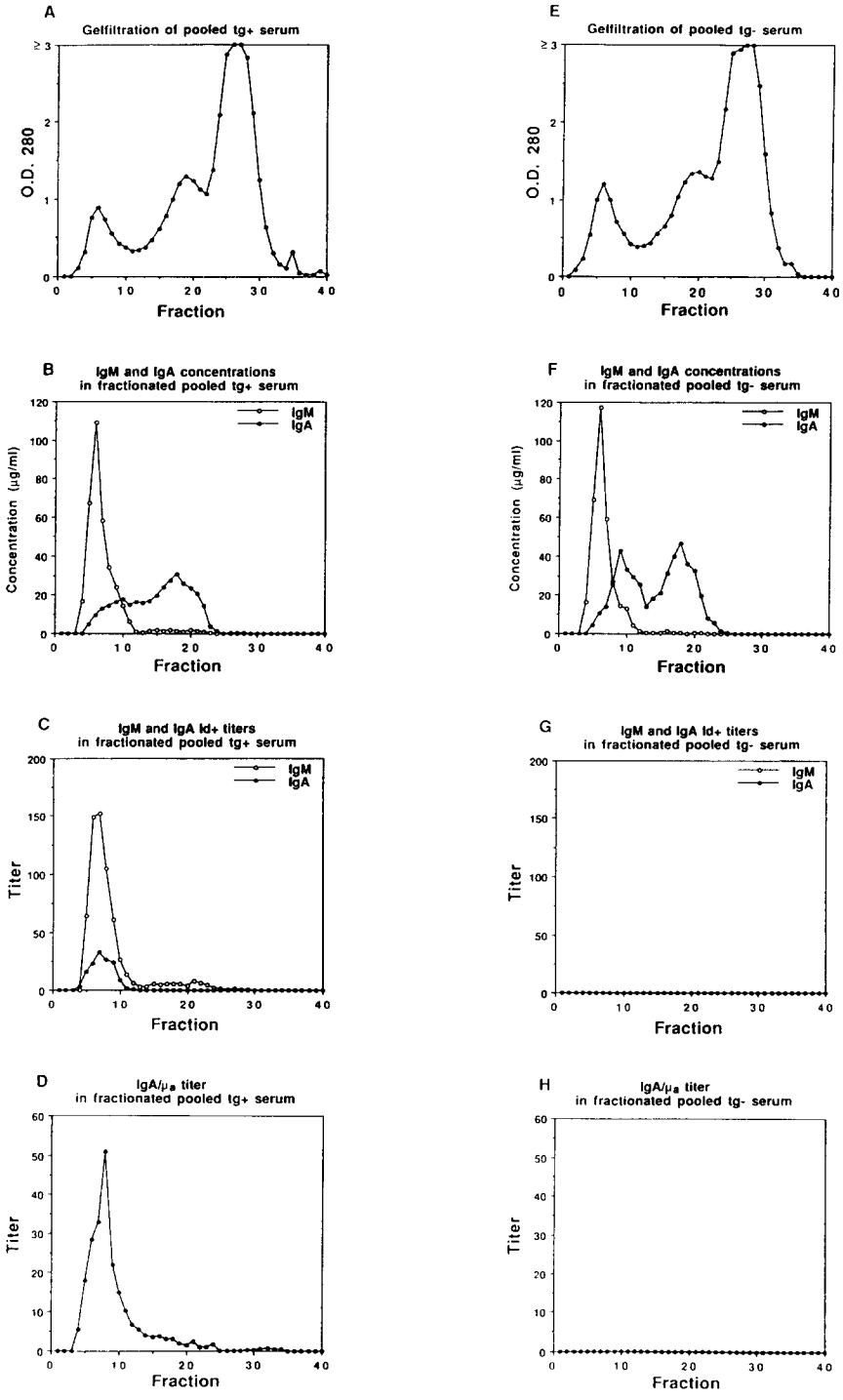

Figure 7. Gelfiltration shows the coelution of transgenic idiotype-associated (Id^+) IgA and chimeric IgA/μ_a with IgM. 200 μl of pooled Sp6 transgene positive (tg^+) (*A–D*) and transgene negative (tg^-) sera (*E–H*) were separated by FPLC using a Superose 12 column into 200-μl fractions. The OD_{280} of the individual fractions was determined spectrophotometrically (*A* and *E*) and the levels of various Igs were determined by ELISA. (*B* and *F*) IgM was determined using anti-μ_a for coating and employing alkaline phosphatase-conjugated anti-IgM (a-IgM-AP) for detection. IgA was assayed using anti-IgA for coating and employing alkaline phosphatase-conjugated anti-IgA (a-IgA-AP) for detection. (*C* and *G*) Id^+ IgM and IgA were assayed using antiidiotypic antibody 20-5 for coating and employing a-IgM-AP and a-IgA-AP for detection, respectively. (*D* and *H*) Chimeric IgA/μ_a was assayed using anti-IgM μ_a for coating and a-IgA-AP for detection.

the rearrangement of endogenous Ig genes would be selectively expanded. Indeed, on the basis of the "idiotypic network theory" (40) it would be speculated that the immune system of Ig transgenic mice would actively suppress the overexpression of the transgene-encoded idiotype.

Interchromosomal isotype switching (26) is the second mechanism previously reported to cause expression of transgenic idiotype on endogenously rearranged Ig molecules. In that study, the immunization of μ tg^+ mice with specific antigen resulted in the presence of Id^+ IgG molecules. Immunization of the Sp6 μ, κ transgenic mice with TNP, the hapten recognized by the Sp603 antibody, did not result in an increased formation of specific antibodies or in detectable amounts of Ig class switching (32). This finding suggested that interchromosomal isotype switching may not occur in all Ig transgenic mice, and raised doubt whether this mechanism can account for the Id^+ IgM μ_b and IgA molecules detected in Sp6 transgenic sera.

To find the mechanism responsible for the expression of transgenic idiotype on endogenous nontransgene Ig molecules, further serum analysis was performed. This analysis showed the presence of IgM μ_a/μ_b and IgA/μ_a chimeric molecules in the sera of tg^+ mice at titers that showed good correlation with the titers of Id^+ IgM and Id^+ IgA, respectively. Next, employing immunoabsorption we investigated the extent to which the chimeric Ig molecules were responsible for the expression of transgenic idiotype on nontransgene Ig molecules. This experiment demonstrated that IgA/μ_a molecules were responsible for the expression of transgenic idiotype on IgA. The same absorption experiment suggested that IgM μ_a/μ_b chimeric molecules were responsible for the expression of transgenic idiotype on IgM μ_b. To determine whether these chimeric Ig molecules were the synthesized products of individual cells with more than one functionally rearranged Ig H chain gene, we generated radiation bone marrow chimeras which were reconstituted with a mixture of equal amounts of BALB/c SP6 tg^+ bone marrow and normal C57BL/6 bone marrow. Analysis of the sera of these chimeras demonstrated the presence of Id^+ IgA and chimeric μ_a/IgA molecules, but the absence of both Id^+ IgM μ_b and chimeric IgM μ_a/μ_b. These findings indicate that the association of transgenic idiotype with IgM μ_b depends on the expression of transgenic μ_a and endogenous μ_b genes in the same cells. Moreover, absorption from tg^+, tg^-, and radiation bone marrow chimera sera indicated that a considerable part of the IgM μ_b of C57BL/6 Sp6 tg^+ mice was present as IgM μ_a/μ_b chimeric molecules. Finally, gel filtration demonstrated that Id^+ IgA and IgA/μ_a had the same molecular weight as IgM, which suggests that the expression of transgenic idiotype on IgA depends on the integration of α H chains in transgene-encoded IgM μ_a molecules.

These data demonstrate that the formation of chimeric molecules is another mechanism by which Ig transgenic mice can express transgenic idiotype on nontransgene Igs. Since incomplete allelic exclusion has been observed in a variety of Ig transgenic mice, it is possible that the expression of a transgenic H chain idiotype on endogenous nontransgene molecules is a phenomenon that is not restricted to the Sp6 mouse. Based on the absence of preferential Ig chain association detected in hybrid hybridomas, quadromas, of matched isotype (41) it may be concluded that cells producing more than one species of H and L chain can associate these chains randomly in a complete Ig molecule. Indeed, the presence of IgM molecules of mixed allotype in the sera of Ig transgenic mice other than the Sp6 has been suggested (18, 21, 23).

The selective expression of transgenic idiotype on endogenous IgM and IgA molecules indicates that only those H chains that possess the 18 extra amino acids at their carboxyl end, that allow them to bind to J chain (42), can be integrated in chimeric Ig molecules.

Our data support previous experimental evidence for the incomplete allelic exclusion observed in Sp6 μ, κ transgenic mice (19, 24, 32). Moreover, based on the previous detection of endogenous IgM on a small percentage of all B cells, they support evidence for the disproportional secretion of endogenous molecules, which has been explained by preferential selection (37, 38) and by silencing of transgene expression in the final stage of B cell differentiation (21). Immunoabsorption data suggested a difference in the extent of Id^+ Igs in the population of endogenous IgM (85%) and IgA (19%) molecules. This may be caused by a silencing of the transgene after isotype switching, or a restriction to the incorporation of α chains in pentameric Ig molecules.

Combined with earlier reports on Sp6 transgenic mice (19, 24, 32), the identification of a T cell clone expressing two distinct TCRs (43), and the report of double transgenic mice with T cells that express two functional receptors (44), the data presented here demonstrate that individual lymphocytes expressing more than one type of antigen receptor can exist within the immune system.

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