Immunoglobulin (Ig) \( \mu, \kappa \) 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/\(z\), allotype heavy (H) chain and \( \kappa \) light chain gene contained endogenous nontransgene immunoglobulin (IgM) (\( \mu_\kappa \), allotype) and IgA molecules which carried the idiotype expressed by the transgenically encoded IgM (\( \mu_\kappa \)) 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 \( \mu_\kappa \) transgene and products of endogenously rearranged Ig \( \mu_\kappa \) or \( \alpha \) genes. These and other results suggest that allelic exclusion of Ig gene rearrangement in \( \mu, \kappa \) 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 (\( V_\alpha \)), a diversity (\( D_\alpha \)) and a joining (\( J_\alpha \)) gene segment, whereas Ig L chain genes are formed by bringing a \( V \), and a \( J \), 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 \( \kappa \) L chain genes (16), rearranged \( \kappa \) H chain genes (17, 18), rearranged \( \mu \) H chain genes (19–21) and rearranged \( \delta \) 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 \( \mu \) and \( \kappa \) chains (18, 19, 22, 23), and serological analysis has suggested the presence of Ig molecules carrying both transgenic and endogenous \( \mu \) 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 transgenic 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 endogenously rearranged IgM and IgA molecules in Sp6 \( \mu, \kappa \) 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 \( \mu \) chains and endogenously rearranged \( \mu \) or \( \alpha \) chains.

Materials and Methods

Reagents. The Sp603 antibody (28) and mAbs specific for murine IgM \( \mu_\kappa \), allotype, DS1, (29, 30), murine IgM \( \mu_\kappa \) allotype,
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 reac-ti-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 μκ standard, purified IgM-κ and purified IgA-κ (Phar-mingen, San Diego, CA) as IgM μκ 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 Bio-technology Associates, Birmingham, AL), and alkaline phosphatase-conjugated monoclonal anti-mouse IgM (Zymed Laboratories, Inc.) were titrated for use in ELISA and employed at saturating dilu-tions. 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 phos-phatase 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 injection of a construct containing rearranged μ and κ genes encoding an IgM molecule with antititinprophylol 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)F1 (C6F1) mice with 5 × 10⁶ BALB/c Sp6 and 5 × 10⁶ C57BL/6 T cell-depleted bone marrow cells as described (35).

ELISA. 96-well plates (Titertek 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 anti-serum 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 two-fold 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 μκ 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 × (titerabsorbed/titerunabsorbed).

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 OD₂₈₀ 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 μκ, μλ, and IgA Concentrations of C57BL/6 Sp6 Transgene-positive (tg +) and tg - Mice. To investigate to what extent the introduction of a rearranged Ig construct encoding an IgM molecule of the μκ allotype, characteristic of BALB/c mice, (19), influenced the production of endogenous IgM (carrying the μλ 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 μκ was detected in the sera of BALB/c tg + and tg - mice, (with endogenous μ chains expressing the μκ but not the μλ allotype) and in the serum of C57BL/6 tg + (capable of producing μκ transgenic and endog-enous μκ chains), but not in the serum of C57BL/6 tg - (endogenous μλ only) (Fig. 1 A). IgM μλ was detected in the sera of C57BL/6 tg + (μκ and μλ) and tg - mice (endog-enous μλ only), but not in the sera of BALB/c tg + and tg - (μκ and μλ) (Fig. 1 B). The assay for IgA detected purified IgA, but showed no reactivity with IgM μκ or μλ (Fig. 1 C). Using these assays the concentrations of IgM μκ, μλ, 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 μκ 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 μκ, μλ, 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 μκ, which is transgene encoded, (Fig. 2 A), but also with IgM μκ (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

Abbreviations used in this paper: bmc, bone marrow chimeras; PBT, PBS-Tween; tg, transgenic.
Specificity of IgM go ELISA

- C57BL/6 tg-
- C57BL/6 tg+
- BALB/c tg-
- BALB/c tg+

2 log serum dilution (1=1/250)

Specificity of IgM #b ELISA

- C57BL/6 tg-
- C57BL/6 tg+
- BALB/c tg-
- BALB/c tg+

2 log serum dilution (1=1/250)

Specificity of IgA ELISA

- C57BL/6 tg-
- C57BL/6 tg+
- BALB/c tg-
- BALB/c tg+

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-
chromosomal isotype switching (26). Other mechanisms that may explain the observed association include mRNA transsplicing (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 \(\mu\) and IgA, the sera analyzed in Fig. 2 were assayed for the presence of chimeric IgM molecules. Chimeric IgM \(\mu\_\mu\) (Fig. 3 A) and IgA/\(\mu\) (Fig. 3 B), but not IgA/\(\mu\) (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 \(\mu\). 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-\(\mu\) gel absorbed 98% of IgM \(\mu\) (Fig. 4 A), gave a minor absorption of IgM \(\mu\) (Fig. 4 B), and did not bind IgA (Fig. 4 C). The absorption of the \(\text{tg}^+\) sera resulted in an almost complete removal of total and transgenic idiotype positive (Id+) IgM \(\mu\), chimeric IgM
μ/μ and IgA/μ, and Id* IgA; a minor decrease of total (tot) IgA; and a major absorption of total IgM μ, (Fig. 5 A). These data indicate that expression of transgenic idiotype on IgA is caused by chimeric IgA/μ, molecules, since the essentially complete absorption of Id* IgA was similar to the absorption of IgA molecules coexpressing μ, 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, are less conclusive, since the absorption of both Id* and total IgM μ, by anti-μ, gel resembled that of all Ig molecules with μ, chains. The specificity of this absorption was confirmed in a separate experiment that compared the effects of absorption with anti-μ, gel to the absorption with a control (isotype matched) gel. Consistent with the results shown in Fig. 5 A, anti-μ, gel absorbed significantly more total IgM μ, from the tg+ sera than did the control gel. In contrast, anti-μ, 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, from tg+ sera is due to the fact that a significant proportion of these molecules contains μ, Ig chains.

\[ \text{(BALB/c Sp6 tg+ + C57BL/6)} \rightarrow \text{CB6F1 Radiation Bone Marrow Chimeras Express Transgenic Idiotype on IgA, but Not on IgM μ,)} \]

To determine whether C57BL/6 Sp6 tg+ mice express transgenic idiotype on IgM μ, as a consequence of the production of chimeric IgM μ/μ, molecules by individual B cells, radiation bone marrow chimeras (bmc) were generated by the cotransfer of BALB/c Sp6 tg+ (μ, and C57BL/6 (μ, bone marrow cells into irradiated CB6F1 recipient mice. In contrast to C57BL/6 Sp6 tg+ mice, these chimeras have the genes encoding for IgM μ, and μ, 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, but also for the other Ig isotypes, it would be expected that the sera of the chimeras contain Id* IgA and chimeric μ/μ, like the C57BL/6 Sp6 tg+ mice.
Figure 4. Test of the specificity of the absorption of gel-coupled anti-IgM μa. Aliquots taken from serial dilutions of IgM μa (A), IgM μb (B), and IgA (C) starting at an initial concentration of 14 μg/ml were incubated with gel-coupled anti-IgM μa. After incubation, the levels of the IgGs from the incubated and unabsorbed control dilutions were determined by ELISA. (A) IgM μa was assayed by an ELISA using anti-IgM μa for coating and employing alkaline phosphatase-conjugated anti-IgM for detection. (B) IgM μa was assayed by an ELISA using anti-IgM μa 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 μa, or chimeric IgM μa/μb 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 μa, μb, 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 μa, and comparable levels of IgM μb.

The sera of the chimeras were then assayed for the expression of transgenic idiotype on transgenic IgM μa and on endogenous Ig isotypes, and for the presence of chimeric Ig molecules. The data demonstrate transgenic idiotype on IgM μa (Fig. 6 A) and on IgA (Fig. 6 D), and the presence of chimeric IgA/μa molecules (Fig. 6 E). In contrast, the sera did not contain Id⁺ IgM μa (Fig. 6 B) or chimeric IgM μa/μb (Fig. 6 C).

To determine whether chimeric IgA/μa molecules were the cause of Id⁺ IgA, the chimeric sera were absorbed with gel coupled anti-μa. The results of the absorption (Fig. 5 B) show that the gel removed total and Id⁺ IgM μa, Id⁺ IgA, and chimeric IgA/μa, bound a part of the total IgM μa, 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 lgs. Moreover, the limited extent of IgM μa absorption from the sera of the chimeras further supports the notion that a significant part of all IgM μa molecules in the sera of C57BL/6 Sp6 tg⁺ mice is present as IgM μa/μb chimeric molecules.

Chimeric IgA/μa Molecules Coelute with IgM in Gelfiltration. To biochemically characterize chimeric IgA/μa 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 OD₂₅₀ of the individual fractions was determined (Fig. 7, A and E). Using ELISA, the
ELISA of B.M.C. sera

| IgM μα (Id+) |
|------------|
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |

ELISA of B.M.C. sera

| IgM μβ |
|------------|
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |

ELISA of B.M.C. sera

| IgA μα |
|------------|
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |

ELISA of B.M.C. sera

| IgA μβ |
|------------|
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |
| 0.00 | 0.20 | 0.40 | 0.60 | 0.80 | 1.00 | 1.20 | 1.40 | 1.60 |

Figure 6. The sera of (BALB/c Sp6 tg + + C57BL/6) → CB6F1 mixed radiation b.m.c. contain chimeric IgA/μα molecules and show the association of transgenic idiotype with IgA, but have no chimeric IgM/μα, μβ molecules or IgM μβ molecules carrying the transgene-encoded idiotype. B.m.c. mice were made by injecting irradiated CB6F1 mice with 5 × 10⁶ BALB/c Sp6 tg + and 5 × 10⁶ 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 μβ (C), or anti-IgM μα (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 μα was detected using biotinylated anti-IgM μα (a-μα-bio), followed by horseradish peroxidase-conjugated streptavidin (sAV-PO). (B) Id + IgM μβ was detected using biotinylated anti-IgM μβ followed by sAV-PO. (C) Chimeric IgM μα/μβ was detected using a-μβ-bio followed by sAV-PO. Id+ IgA (D) and chimeric IgA/μα (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/μα, μβ (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/μα molecules. These findings indicate that Id+ IgA molecules resulted from the incorporation of endogenous α chains in transgenic IgM μα 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 μα, k 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...
the rearrangement of endogenous Ig genes would be selectively expanded. Indeed, on the basis of the "idiotype 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 μκ and IgA molecules detected in Sp6 transgenic sera.

Figure 7. Gelfiltration shows the coelution of transgenic idiotype-associated (Id⁺) IgA and chimeric IgA/μκ 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 OD280 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-μκ 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 anti-idiotypic antibody 20-5 for coating and employing a-IgM-AP and a-IgA-AP for detection, respectively. (D and H) Chimeric IgA/μκ was assayed using anti-IgM μκ for coating and a-IgA-AP for detection.
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 $\mu/$ $\mu_b$ and IgA/$\mu_b$ chimeric molecules in the sera of Sp6 $tg^+$ 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/ $\mu_b$ molecules were responsible for the expression of transgenic idiotype on IgA. The same absorption experiment suggested that IgM $\mu/\mu_b$ chimeric molecules were responsible for the expression of transgenic idiotype on IgM $\mu_b$.

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 chimera 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 IgM $\mu_b/\mu_b$ and chimeric IgM $\mu_b/\mu_b$. These findings indicate that the association of transgenic idiotype with IgM $\mu_b$ depends on the expression of transgenic $\mu_b$ and endogenous $\mu_b$ genes in the same cells. Moreover, absorption from $tg^+$, $tg^-$, and radiation bone marrow chimera sera indicated that a considerable part of the IgM $\mu_b$ of C57BL/6 Sp6 $tg^+$ mice was present as IgM $\mu/\mu_b$ chimeric molecules. Finally, gel filtration demonstrated that Id + IgA and IgA/$\mu_b$ had the same molecular weight as IgM, which suggests that the expression of transgenic idiotype on IgA depends on the integration of $\alpha$-H chains in transgene-encoded IgM $\mu_b$ 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 $\mu, \kappa$ 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 $\alpha$-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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