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

By Quirijn Vos and Richard J. Hodes

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) (\( \mu_\alpha \) 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 IgM and IgA genes. These and other results suggest that allelic exclusion of Ig gene rearrangement in /z, \( \kappa \) 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 (Vs) a diversity (Ds) and a joining (Js) 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 (17, 18), rearranged \( \mu \) and \( \kappa \) 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 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 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 \( \mu_\alpha \) 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-hydroxysuccinimid 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, standard, purified IgM χ, and purified IgA χ (Pharmingen, San Diego, CA) as IgM µ, and IgA standards, respectively. Unlabeled affinity-purified goat anti-mouse IgA and conjugates of this reagent and streptavidin 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-Institur 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 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).

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 + and tg -) were assayed for the association of transgenic idiotype with several serum Ig isotypes. The data for IgM, 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, 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 for other tg 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, µ, and IgA are shown in Fig. 2. Using the 20-5 antibody, which recognizes an Ig H chain idiotype on the Sp6 transgenics was generated, transgenic idiotype could be detected in the sera of tg + mice in association with IgM µ, which is transgene encoded, (Fig. 2 A), but also with IgM µ, (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 absorption (percent remaining) was calculated according to: Percent remaining = 100 x (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 µ, µ, 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 µ, allotype, characteristic of BALB/c mice, (19) influenced the production of endogenous IgM (carrying the µ, 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 Ig's allowed a specific determination. IgM µ, was detected in the sera of BALB/c tg + and tg - mice, (with endogenous µ chains expressing the µ, but not the µ, allotype) and in the serum of C57BL/6 tg + (capable of producing µ, transgenic and endogenous µ, chains), but not in the serum of C57BL/6 tg - (endogenous µ, only) (Fig. 1 A). IgM µ, was detected in the sera of C57BL/6 tg + (µ, and µ,) and tg - mice (endogenous µ, only), but not in the sera of BALB/c tg + and tg - (µ, only) (Fig. 1 B). The assay for IgA detected purified IgA, but showed no reactivity with IgM µ, or µ, (Fig. 1 C). Using these assays the concentrations of IgM µ, µ, 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, 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).

**Abbreviations used in this paper:** bmc, bone marrow chimeras; PBT, PBS-Tween; tg, transgenic.
IgM $\mu_\alpha$ (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 (23) and inter-

Table 1. Ig Serum Concentrations

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mouse</th>
<th>IgM $\mu_\alpha$</th>
<th>IgM $\mu_\beta$</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6 Sp6 $tg^+$ mice</td>
<td>499</td>
<td>265*</td>
<td>2344</td>
<td>278</td>
</tr>
<tr>
<td></td>
<td>2320</td>
<td>428*</td>
<td>1040</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>2328</td>
<td>720*</td>
<td>564</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>3320</td>
<td>983*</td>
<td>983</td>
<td>712</td>
</tr>
<tr>
<td></td>
<td>3321</td>
<td>1419*</td>
<td>1030</td>
<td>5704</td>
</tr>
<tr>
<td>C57BL/6 Sp6 $tg^-$ mice</td>
<td>497</td>
<td>0</td>
<td>2477</td>
<td>1604</td>
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<tr>
<td></td>
<td>2322</td>
<td>0</td>
<td>1191</td>
<td>1191</td>
</tr>
<tr>
<td></td>
<td>2329</td>
<td>0</td>
<td>935</td>
<td>935</td>
</tr>
<tr>
<td></td>
<td>3324</td>
<td>0</td>
<td>729</td>
<td>729</td>
</tr>
<tr>
<td></td>
<td>3325</td>
<td>0</td>
<td>642</td>
<td>642</td>
</tr>
<tr>
<td>(BALB/c Sp6 $tg^+$ + C57BL/6)→CB6F1 mixed radiation bone marrow chimeras</td>
<td>464</td>
<td>28‡</td>
<td>3954</td>
<td>255</td>
</tr>
<tr>
<td></td>
<td>465</td>
<td>26‡</td>
<td>5033</td>
<td>137</td>
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<td>467</td>
<td>8‡</td>
<td>861</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td>468</td>
<td>11‡</td>
<td>2686</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>470</td>
<td>5‡</td>
<td>3455</td>
<td>70</td>
</tr>
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</table>

Serum Ig concentrations were determined by ELISA comparing serum titration curves with titration curves of standards of known Ig concentrations. IgM $\mu_\alpha$ was assayed using anti-IgM $\mu_\alpha$ for coating and employing alkaline phosphatase-conjugated anti-IgM (α-IgM-AP) for detection. IgM $\mu_\beta$ was assayed using anti-IgM $\mu_\beta$ for coating and employing α-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)→CB6F1 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.
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 \(\mu_\alpha\) and IgA, the sera analyzed in Fig. 2 were assayed for the presence of chimeric IgM molecules. Chimeric IgM \(\mu_\alpha/\mu_\beta\) (Fig. 3 A) and IgA/\(\mu_\alpha\) (Fig. 3 C), but not IgA/\(\mu_\beta\) (Fig. 3 B), 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 \(\mu_\alpha\). 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-\(\mu_\alpha\) gel absorbed 98% of IgM \(\mu_\alpha\) (Fig. 4 A), gave a minor absorption of IgM \(\mu_\beta\) (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 \(\mu_\alpha\), chimeric IgM

![Figure 2](image-url)

**Figure 2.** Demonstration of the association of transgenic idiotype with transgenic IgM\(\mu_\alpha\) and nontransgenic IgM\(\mu_\beta\) 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 \(\mu_\alpha\) 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 \(\mu\)g/ml. (.4 and D) Transgenic idiotype positive (Id\(^+\)) IgM \(\mu_\alpha\) was detected using biotinylated anti-IgM \(\mu_\alpha\), followed by alkaline phosphatase-conjugated streptavidin (sAV-AP). (B and E) Id\(^+\) IgM \(\mu_\beta\) was detected using biotinylated anti-IgM \(\mu_\beta\) followed by sAV-AP. (C and F) Id\(^+\) IgA was detected using alkaline phosphatase-conjugated anti-IgA.
μ0/μe and IgA/μe, and Id+ IgA; a minor decrease of total (tot) IgA; and a major absorption of total IgM μb (Fig. 5 A). These data indicate that expression of transgenic idio-
type on IgA is caused by chimeric IgA/μe molecules, since the essentially complete absorption of Id+ IgA was similar to
the absorption of IgA molecules coexpressing μe Ig
chains, and only a very limited percentage of total IgA was absorbed out. The data on the expression of transgenic idio-
type on IgM μb are less conclusive, since the absorption of both Id+ and total IgM μb by anti-μe gel resembled that of
all Ig molecules with μe chains. The specificity of this ab-
sorption was confirmed in a separate experiment that com-
pared the effects of absorption with anti-μe gel to the ab-
sorption with a control (isotype matched) gel. Consistent
with the results shown in Fig. 5 A, anti-μe gel absorbed
significantly more total IgM μb from the tg+ sera than did
the control gel. In contrast, anti-μe 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 μb from
tg+ sera is due to the fact that a significant proportion of
these molecules contains μe Ig chains.

To determine whether C57BL/6 Sp6 tg+ mice express transgenic idio-
type on IgM μb as a consequence of
the production of chimeric IgM μb/μb molecules by indi-
vidual B cells, radiation bone marrow chimeras (bmc) were
generated by the cotransfer of BALB/c Sp6 tg+ (μb) and
C57BL/6 (μb) bone marrow cells into irradiated CB6F1 re-
cipient mice. In contrast to C57BL/6 Sp6 tg+ mice, these
chimeras have the genes encoding for IgM μb/μb in sep-
arate cells. Since the cells originating from the BALB/c Sp6
tg+ mice not only possess the genes for transgenic and en-
dogenous IgM μb, but also for the other Ig isotypes, it
would be expected that the sera of the chimeras contain Id+
IgA and chimeric IgM μb/μb, like the C57BL/6 Sp6 tg+ mice.
However, if Iγ Mμβ, or chimeric Iγ Mμα/μβ 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 Iγ Mμα, Iγ Mμβ, and Iγ A 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 Iγ A, decreased levels of Iγ Mμα, and comparable levels of Iγ Mμβ.

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

To determine whether chimeric Iγ A/μα molecules were the cause of Iγ γ Mμα, Iγ Mμβ, and Iγ A 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 Iγ A, decreased levels of Iγ Mμα, and comparable levels of Iγ Mμβ.

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

To determine whether chimeric Iγ A/μα molecules were the cause of Iγ γ Mμα, the chimeric sera were absorbed with gel coupled anti-μα. The results of the absorption (Fig. 5 B) show that the gel removed total and Iγ γ Mμα, Iγ γ A, and chimeric Iγ A/μα; bound a part of the total Iγ Mμα; and absorbed little total Iγ A. 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 Iggs. Moreover, the limited extent of Iγ Mμα absorption from the sera of the chimeras further supports the notion that a significant part of all Iγ Mμα molecules in the sera of C57BL/6 Sp6 tg+ mice is present as Iγ Mμα/μα chimeric molecules.

Chimeric IgA/μα Molecules Coelute with Iγ M in Gelfiltration. To biochemically characterize chimeric Iγ A/μα 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 Iγ A and Iγ M under nondenaturing conditions. In the gel filtration experiment, pooled sera from Sp6 tg+ and tg- mice were separated and the OD280 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)→ CB6F1 mixed radiation b.m.c. contain chimeric IgA/μκ molecules and show the association of transgenic idiotype with IgA, but have no chimeric IgM/μκ molecules or IgM/μκ 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 μκ (C), or anti-IgM μκ (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 μκ was detected using biotinylated anti-IgM μκ (a-μκ-bio), followed by horseradish peroxidase-conjugated streptavidin (sAV-PO). (B) Id+ IgM μκ was detected using biotinylated anti-IgM μκ followed by sAV-PO. (C) Chimeric IgM μκ/μκ was detected using a-μκ-bio followed by sAV-PO. Id+ IgA (D) and chimeric IgA/μκ (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/μκ (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/μκ molecules. These findings indicate that Id+ IgA molecules result from the incorporation of endogenous α chains in transgenic IgM μκ 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 feed-back inhibition Sp6 μκ 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 "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 μκ 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 $\mu$/$\mu$ and IgA/$\mu$ chimeric molecules in the sera of $\text{tg}^+$ 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/$\mu$ molecules were responsible for the expression of transgenic idiotype on IgA. The same absorption experiment suggested that IgM $\mu$/$\mu$ chimeric molecules were responsible for the expression of transgenic idiotype on IgM $\mu$.

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 $\text{tg}^+$ bone marrow and normal C57BL/6 bone marrow. Analysis of the sera of these chimeras demonstrated the presence of Id+ IgA and chimeric $\mu$/$\mu$ molecules, but the absence of both Id+ IgM $\mu$ and chimeric IgM $\mu$/$\mu$. These findings indicate that the association of transgenic idiotype with IgM $\mu$ depends on the expression of transgenic $\mu$, and endogenous $\mu$, genes in the same cells. Moreover, absorption from $\text{tg}^+$, $\text{tg}^-$, and radiation bone marrow chimeras sera indicated that a considerable part of the IgM $\mu$ of C57BL/6 Sp6 $\text{tg}^+$ mice was present as IgM $\mu$/$\mu$ chimeric molecules. Finally, gel filtration demonstrated that Id+ IgA and IgA/$\mu$ 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 $\mu$, 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.

We wish to thank Dr. Rinus Lamers for his help in obtaining the Sp6 mice and the Sp603 and 20-5 hybridomas; Dr. James Kenny for reagents and helpful suggestions; Dr. David Segal for performing gel filtration; and Drs. Jay Berzofsky and Ron Germain for critically reading the manuscript.

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Received for publication 11 May 1992.

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