

ALLELIC EXCLUSION IN TRANSGENIC MICE CARRYING
MUTANT HUMAN IgM GENES

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Allelic exclusion is a mechanism that permits expression of only one of the two sets of parental Ig alleles (1-5). How this occurs is not known, but experiments in transgenic mice suggest that it is mediated by the membrane-bound form of the μ heavy chain (6). It is likely that exclusion operates by inhibiting V/DJ or D/J recombination of the nonexpressed allele (4, 5). Nevertheless, most mature B cell lines and many spleen B cells carry two completely rearranged heavy chain genes (5). These rearranged genes might be accounted for if only one were capable of producing a functional heavy chain, the other having rearranged aberrantly (7). Alternatively, it is possible that the functional Ig product of one allele suppresses a postrearrangement step in the expression of the second. To test the latter hypothesis, we set out to determine whether two functionally intact heavy chain genes could be expressed simultaneously in primary B lymphocytes in vivo. For this purpose we created strains of transgenic mice that carry either of two forms of a human μ gene. One strain carries a gene encoding only membrane-bound human μ heavy chains (6). Another, similar to that produced by Storb et al. (8), carries a human μ gene that can only direct the synthesis of secreted IgM heavy chains. We have examined the expression of transgenic and endogenous heavy chains in both types of mice and in their hybrid progeny.

Materials and Methods

Transgenic Mice. DNA used for pronuclear injection was from a human μ gene whose membrane exons were deleted by Sal I digestion (9). Sal I was also used to linearize the insert and eliminate vector sequences (Fig. 1 A). Pronuclei of fertilized one-cell mouse eggs derived from FVB/N mice were injected and transferred to pseudopregnant females as previously described (10). Two lines of transgenic mice carrying the secreted gene were produced (TG.ST and TG.SU).

DNA and RNA Isolation. DNA from mouse tails and RNA from tissues were prepared as previously described (10). Poly(A)⁺ RNA was selected by oligo(dT) chromatography (11).

DNA and RNA Analysis. DNA was denatured in 0.3 M NaOH, spotted onto nylon filters (New England Nuclear, Boston, MA), and a Pvu II human μ fourth constant region (μ CH4) probe (see Fig. 1) was used to identify transgenic mice. To distinguish between the μ transgenes, DNA was digested with Pst I. RNA samples were electrophoresed in 1% agarose and 0.6% formaldehyde gels and transferred to nitrocellulose for hybridization assays. RNase protection assays were performed as described by Melton et al. (12). Probes illustrated in

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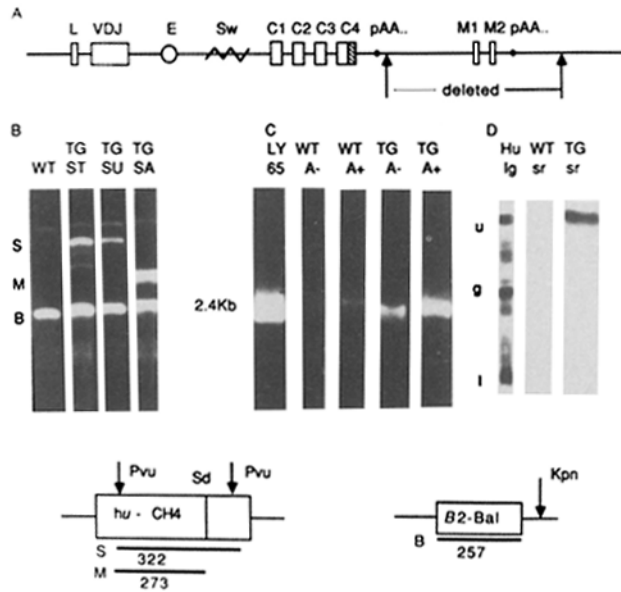


FIGURE 1. Expression of secreted Ig μ and Ig μ mRNA in transgenic mice. (A) IgM transgene with membrane exons deleted by Sal I digestion. (B) Human μ and β_2 -microglobulin mRNA expression in spleens of transgenic mice. mRNA was assayed by RNase protection using the probes diagrammed. The sizes of protected fragments given are in nucleotides (C) Hybridization analysis of RNA from TG.ST and wild-type mouse spleen. The Ly-65 Burkitt lymphoma cell line produces authentic human μ and was used as a control (20). The samples were either 2 μ g of poly(A)⁺ or 20 μ g of poly(A)⁻ RNA. (D) Immunoblots of authentic human Ig μ , transgenic and wild-type mouse serum samples. L, leader exon; VDJ, variable region exon; E, enhancer; Sw, switch; C1-4, constant region exons; pAA..., polyadenylation signal; M1-2, membrane exons; WT, wild type; TG.ST and TG.SU, transgenic strains that carry the secreted Ig μ gene; TG.SA, transgenic strain that carries the membrane-bound Ig μ gene; Ly-65, Ly-65 cell line; A+, oligo(dT)-selected RNA; A-, RNA that did not bind to oligo(dT); Hu Ig, purified human Ig μ standard; WT sr, wild-type mouse serum; TG sr, transgenic mouse serum; hu-CH4, human μ fourth constant region; β_2 -Bal, β_2 -microglobulin probe; Sd, splice donor; S, secreted; M, membrane; B, β_2 -microglobulin; u, position of Ig μ 75 kD; g, position of Ig γ , 50 kD; l, position of light chains, 20 kD; Pvu, Pvu I; Kpn, Kpn I.

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Figs. 1 and 2 were cloned into pGEMTM-3 or 4 vectors (Promega Biotec, Madison, WI) so that SP6 transcription was antisense in all cases (6). The β_2 -microglobulin Bal I probe was produced by Bal-31 deletion of 28 nucleotides from the 5' end of a previously described β_2 -microglobulin probe (6, 13).

Immunoblots. Human μ protein was concentrated from pooled transgenic mouse sera by affinity chromatography on columns of goat anti-human IgM (CooperBiomedical, Inc., Malvern, PA) coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ). The equivalent of 10 μ g of immunoreactive material was electrophoresed in 8% polyacrylamide, SDS gels and transferred to nitrocellulose (14). Biotin-labeled monoclonal anti-human IgM (15), or biotin-labeled goat anti-human IgM (Southern Biotechnology Associates, Inc., Birmingham, AL) and HRP-Avidin (Vector Laboratories, Inc., Burlingame, CA) were used to detect human μ protein. 10 μ g of purified human IgM protein (Cappel Laboratories, Malvern, PA) was used as a standard in all experiments.

Flow Cytometry. Single cell suspensions from spleens were stained with a monoclonal anti-mouse IgM (16) directly labeled with fluorescein (Becton Dickinson & Co., Mountain View, CA). The labeled cells were analyzed or sorted by standard flow cytometry on a Cytofluorograf IIS (Ortho Diagnostic Systems Inc., Westwood, MA).

Results and Discussion

Secreted Human μ mRNA and Protein are Produced in Transgenic Mice. Spleen cells from transgenic mice that carry the gene that encodes secreted μ (strains TG.ST and TG.SU) express only the secreted form of human μ mRNA as assayed in RNase protection experiments (Fig. 1 B). As shown by analysis of poly(A)⁺ and poly(A)⁻

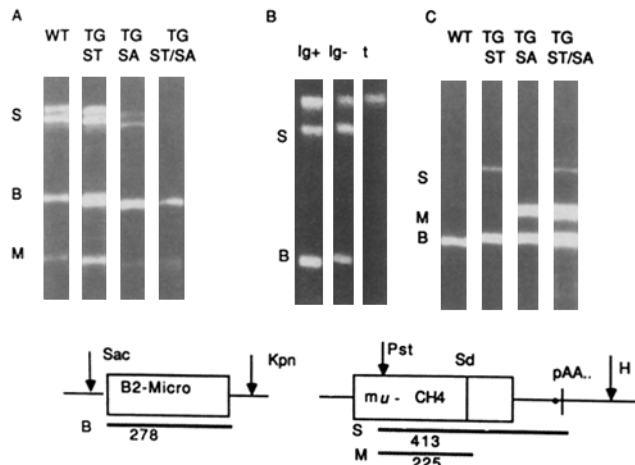


FIGURE 2. Expression of endogenous mouse μ and transgenic human μ mRNA in transgenic mice and their hybrid progeny. (A) Mouse μ and β_2 -microglobulin mRNA expression in transgenic, hybrid, and wild-type spleen RNA samples were measured by RNase protection. RNase protection probes and the size of the fragments expected from protection in nucleotides are diagrammed. Duplication of the sequence ATAAAAA found at the 3' end of the murine secreted μ mRNA could result in a 23-nucleotide loop that would produce the doublet observed. (B) Human Ig μ mRNA in spleen

cells of TG.ST mice that had been selected for the presence or absence of cell surface mouse IgM. (C) Human Ig μ and β_2 -microglobulin mRNA expression in transgenic, hybrid, and wild-type spleens. RNAs were assayed by RNase protection in the same spleen RNA samples shown in A. Abbreviations are the same as in Fig. 1 with the addition of: TG.ST/SA, F1 hybrid animals that carry both secreted and membrane μ transgenes; Ig $^+$, sorted TG.ST spleen cells that have mouse IgM on their surface; Ig $^-$, sorted spleen cells that do not have IgM on their surface; t, tRNA; β_2 -micro, β_2 -microglobulin probe; m μ -CH4, fourth constant region of mouse Ig μ ; Sac, Sac I; Pst, Pst I; H, Hind III.

fractions of spleen RNA, transgenic mRNA is of the expected size (2.4 kb), and is polyadenylated (Fig. 1 C). In addition, this mRNA is induced by LPS in vitro, and human IgM is present in transgenic mouse serum at a level of 10 μ g/ml as radioimmunoassayed with both polyclonal and monoclonal antibodies (not shown). The immunoreactivity can be accounted for by a protein of the size of human μ in Western blots (Fig. 1 D).

The number of mouse IgM-bearing splenic B cells is reduced in transgenic mice carrying the gene for the membrane-bound form of human μ (6). By contrast, the number of mouse IgM $^+$ splenic B cells was not significantly different in the secreted μ transgenics and wild types. Furthermore, the expression of other B cell, T cell, and macrophage surface markers was also similar (not shown). Thus, expression of secreted human μ does not seem to affect the number of T cells, macrophages, or mouse IgM-bearing B cells in the spleen.

Secreted Human μ Does Not Suppress Expression of Mouse IgM. To test the effect of the secreted form of human μ on the level of endogenous mouse μ , we measured endogenous μ mRNA in spleen cells by RNase protection (Fig. 2 A). β_2 -microglobulin was used as a control for the amount of mRNA in the sample. Unlike the membrane μ transgene, the presence of the secreted form of human μ has no significant effect on the level of endogenous mouse IgM mRNA (Fig. 2 A; see TG.SA vs. TG.ST vs. WT).

To confirm that the human secreted μ chains are expressed in the same cells that express mouse IgM, we purified mouse IgM $^+$ B cells from the spleens of transgenic mice using a cell sorter. RNA was extracted from the sorted cells that were >97% pure on reanalysis (not shown). In RNase protection assays, secreted human μ mRNA was detected in similar quantities in cells that were either positive or nega-

tive for cell surface mouse IgM (Fig. 2 *B*). Expression of human μ in the mouse IgM⁻ pool is consistent with the presence of both pre-B cells and T cells in this population. Both of these cell types would be expected to transcribe the human μ transgene (8, 17). However, expression of the secreted human μ mRNA in mouse IgM⁺ B cells seems to violate allelic exclusion since two types of IgM molecules, human and mouse, are being produced in the same cells. Production of more than one type of IgM molecule in a B cell could account for the relatively low level of human μ in serum if there is preferential association between murine light and heavy chains. Incomplete human IgM molecules or partial chimeras between human and mouse proteins might either fail to be secreted or, once secreted, may be cleared more rapidly from serum. Whether the transgenic animals are tolerant to human IgM has yet to be determined.

The most likely explanation for our results is that the secreted μ protein, unlike the membrane-bound, is unable to signal allelic exclusion. However, the secreted transgene differs from the membrane transgene in that the level of expression of the former is relatively lower (10–30%; Figs. 1 *A* and 2 *C*). In addition, the secreted human μ gene is missing the 3' nucleotide sequences that include and flank the two small transmembrane and cytoplasmic exons. It is unlikely that the level of expression influences allelic exclusion since we have observed allelic exclusion in a transgenic line that produces only 5% of the membrane mRNA found in TG.SA (TG.SD [6]) (data not shown). A definitive experiment to examine the effects of the 3' sequences would require a transgene with a mutation that prevents translation of the membrane exons.

Two Functional Ig Genes Can Be Coexpressed. While it is clear that allelic exclusion operates by preventing the rearrangement of Ig genes (3–5, 18, 19), the possibility that this control could operate at some further step in gene expression has not been tested. The availability of independent strains of transgenic mice that produce two different types of functional μ chains, membrane-bound and secreted, allows us to test this possibility in vivo. By crossing mice that carry the secreted transgene (TG.ST) with mice that carry the membrane μ transgene (TG.SA), we obtained mice that carry both types of productively rearranged human μ genes. Since both Ig transgenes are rearranged, expression of these two functional alleles can only be influenced by events that operate following VDJ joining.

RNAs from spleens of five independent litters of F₁ animals were analyzed. When both the membrane-bound and secreted μ transgenes are present in a single hybrid mouse, both genes are expressed at levels seen when each is present alone (Fig. 2 *C* [TG.SA and TG.ST vs. TG.SA/ST]). At the same time, mouse μ expression is decreased in these mice, presumably as a consequence of the expression of the membrane-bound human transgene (Fig. 2 *A* [TG.ST/SA]). Thus, the membrane μ transgene can exclude mouse μ production, but has no effect on the expression of a productively rearranged secreted human μ gene.

Summary

Expression of the membrane-bound version of the human μ chain in transgenic mice results in the allelic exclusion of endogenous mouse Ig heavy chain genes (6). The secreted version of the human Ig transgene has no such effect. F₁ hybrid animals that carry transgenes for both secreted and membrane-bound human μ

chains produce both forms of the human heavy chain while strongly suppressing endogenous mouse μ expression. The simultaneous expression of the two rearranged transgenes in primary B cells suggests that allelic exclusion operates before the formation of a second functionally rearranged heavy chain gene *in vivo*.

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