# TRANSGENIC MICE WITH $\mu$ AND $\kappa$ GENES ENCODING ANTIPHOSPHORYLCHOLINE ANTIBODIES

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Analysis of the antibody response on the cellular and molecular level is complicated by the fact that B lymphocytes are an enormously heterogenous population with respect to the immunoglobulin genes they express. It has been possible to alleviate this obstacle by studying monoclonal populations of myeloma cells or by immortalizing individual B cells in hybridomas. However, these cells are generally arrested in a particular stage of differentiation and do not permit the study of the dynamics of cell development and interaction. The introduction of rearranged Ig genes into the germline of mice has been a method to study a monoclonal response on the level of the whole animal (1–3). Transgenic mice have provided a unique and powerful tool to analyze the expression of Ig genes. Transgenic mice are produced by microinjection of cloned genes into the male pronucleus of fertilized eggs, and implantation of the embryos into the uterus of a foster female (4). We have previously produced transgenic mice with the functional $\kappa$ gene from the myeloma MOPC-21 (1). We found that the expression of this rearranged $\kappa$ transgene is restricted to B lymphocytes (5, 6), and that coexistence in a B cell of transgenic $\kappa$ and endogenous H chains prevents rearrangement of endogenous $\kappa$ genes (7, 8). Apparently, allelic exclusion of $\kappa$ genes is regulated by a feedback from a complete Ig molecule, not by free L chains. It was important to check these findings with another $\kappa$ gene that contains a different V region and 5′ upstream sequences. Also, the MOPC-21 $\kappa$ chain previously used cannot be secreted alone. The possibility of feedback by $\kappa$ chains that can be secreted on their own needs to be evaluated. Furthermore, it has been reported (3, 9) that heavy chain transgenes cause feedback inhibition of H gene rearrangement. It will be important to determine whether this finding can be generalized by using H transgenes with a different V region. Beyond simply establishing the fact that H genes, or their products, and H plus L chains cause feedback inhibition of H and $\kappa$ gene rearrangement, the molecular mechanism will have to be addressed. It appears possible that insertion of the H chain into

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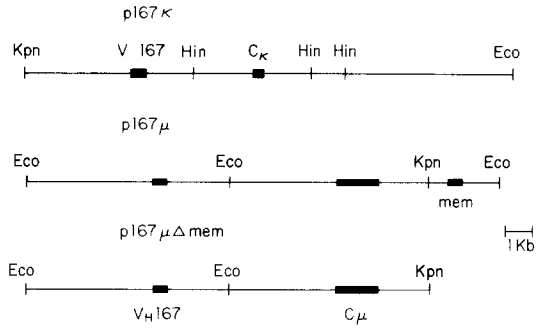


FIGURE 1. Maps of M167 $\kappa$ and $\mu$ transgenes (see Materials and Methods for details). Kpn I, Hind III, and Eco RI restriction enzyme sites are indicated. Exons are shown by thick lines.

the surface or intracytoplasmic membranes of B cells is required for such feedback. We therefore wished to compare the expression of a complete $\mu$ gene and of a stunted $\mu$ gene lacking the membrane terminus ($\mu$ $\Delta$ mem), and compare the relative influence on endogenous H and L gene expression. The genes we chose to address these questions contain the V regions of the functional H and L genes of the myeloma MOPC-167. Transgenic mice were produced, which had introduced into their germline functionally rearranged $V_\kappa$-167-C$\kappa$ genes and/or $V_H$-167-C$\mu$ genes. The $\mu$ gene is either complete or it lacks the portion that encodes the transmembrane sequence of $\mu$ chains. The antibodies encoded by these genes react with phosphorylcholine (PC). The normal anti-PC response has been extensively studied by other laboratories (reviewed in 10), and these mice will be valuable in the analysis of the regulation of this response on the cellular and molecular level.

## Materials and Methods

*Enzymes.* Restriction endonucleases, ligases, etc. were obtained from New England Biolabs, Beverly, MA.

*Transgenes.* The transgenes are the functional $\kappa$ gene of the myeloma MOPC-167, and $\mu$ genes that were constructed from the V region of the functional $\alpha$ H gene of the myeloma MOPC-167 and a germline C$\mu$ region (Fig. 1). The VJ and upstream region of the $\kappa$ gene was a gift from P. Gearhart, The Johns Hopkins University, Baltimore, MD (11). It was joined at the Hind III site in the J-C$\kappa$ intron to the 3′ portion of the MOPC-21 $\kappa$ gene, which we had previously expressed in transgenic mice (1). The Eco-Eco 5′ portions of the H genes were obtained from the MOPC-167 $\alpha$ gene (a gift of R. Perlmutter and L. Hood, California Institute of Technology, Pasadena, CA [12]) and ligated to the Eco-Eco 3′ portion of a germline C$\mu$ gene (a gift of S. Cory and J. Adams, Walter and Eliza Hall Institute for Medical Research, Victoria, Australia). To produce the p167 $\mu$ $\Delta$ mem gene, the $\mu$ gene was cut at the Kpn I site. The $\kappa$, $\mu$, and $\mu$ $\Delta$ mem genes were inserted into the plasmids pUC18, pUC13, and pUC19, respectively (13, 14). To produce transgenic mice, either the total plasmid was injected (linearized by cutting with Kpn I or Eco RI in case of the $\kappa$ gene, or with Sal I in case of the $\mu$ genes); or the inserts shown in Fig. 1 were excised with Eco RI and Pvu I in the case of $\kappa$, and Sal I and Pvu I in case of the $\mu$ genes; this leaves ~30 bp from the pUC polylinkers and ~120 bp of the *lac* gene from pUC to help in the identification of positive mice. The restriction sites used to linearize or trim the plasmids, except for Kpn I, are located within the pUC vectors, and are not shown in Fig. 1.

*Transgenic Mice.* Transgenic mice were produced as described from (C57BL/6 ×

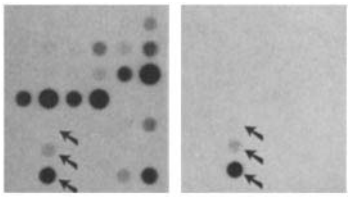


FIGURE 2. RNase treatment of RNA and DNA dots. Spleen and thymus RNAs (0.4 and 2 μg) of normal and transgenic mice. The arrows point to dots of p167-κ plasmid DNA. Hybridization with $C_\kappa$ probe. After exposure to x-ray film (*left*) the filter was treated with RNase and reexposed (*right*).

SJL)$F_2$ zygotes (4, 15). Positive mice were identified by dot hybridization of tail DNA with pUC DNA and $V_\kappa$-167 and/or $V_H$-167 probes, as previously described (1). The following groups of transgenic mice were produced: κ mice (contain only the 167-κ gene); μ mice (with the complete μ gene); μ Δ mem mice (with the membrane terminus–deleted μ gene); κ, μ, and κ μ Δ mem mice (these are derived from ova coinjected with the κ and complete μ genes or the κ and μ Δ mem genes; thus, both genes are inserted at the same integration site); κ × μ mice (derived by breeding κ mice with μ mice and selection of offspring positive for both genes; in these mice, the κ and μ insertion sites are in different chromosomes). Offspring of the other groups were produced by mating of a transgenic mouse with a nontransgenic C57BL/6 or (C57BL/6 × 5JL)$F_1$ hybrid mouse. We have not attempted to make homozygous mice with any of the transgenes.

*$V_\kappa$-167 and $V_H$-167 $C_\mu$ and $C_\kappa$ probes.* The plasmid pSVk167 contains a 310 bp Eco RI–Hinc II insert (the leader-$V_\kappa$ region) isolated from the cDNA clone p167κ RI (16) and cloned into pSP65 (Promega Biotech, Madison, WI). The plasmid pSPVH167 contains a 189 bp Alu I, $V_H$-167-specific fragment subcloned from ChM167α10.1 (12) into pSP65. From these plasmids, 324- and 210-bp-long fragments, respectively, were excised by Eco RI–BamHI, which cut inside the polylinkers.

The $C_\mu$ probe is an ~400 bp Pst I fragment isolated from pAB μ-1, which is specific for $C_\mu$ exons 3 and 4 (a gift of A. Bothwell, Yale University, New Haven, CT [17]).

The $C_\kappa$ probe is a $C_\kappa$ exon–specific fragment of ~500 bp isolated from pES201 (18).

The isolated fragments were ligated with T4 DNA ligase before nick translation.

*RNA Dot Hybridization and Northern Blots.* RNA was prepared and Northern blots were performed with total organ RNAs as described (5). For dot hybridization, RNA was diluted in 15 × SSC and 10% formaldehyde, heated to 60°C for 15 min and applied to nitrocellulose filters prewashed in 15× SSC using the Schleicher and Schuell (Keene, NH) minifold apparatus. Dots were washed with 15× SSC. For DNA control dots on RNA dot filters, the DNAs were denatured in 0.1 M NaOH, 2 M NaCl by boiling for 1 min; for application to nitrocellulose, the denatured DNAs were diluted in 15× SSC.

To assure that the RNA dot hybridizations were not due to contaminating DNA, all RNA preparations were extensively digested with RNase-free DNase I (Worthington Biochemical Corp., Freehold, NJ), and all RNA dots were also hybridized in parallel with vector DNA. In the case of κ transgenic mice, this generally did not give a signal. However, often with RNA from the μ or μ Δ mem mice carrying transgenes with vector attached, some hybridization was seen with vector DNA. This was not due to DNA contamination (see below), but apparently represented transcripts from vector DNA. Perhaps this is due to the fact that, in case of the μ and μ Δ mem genes, very little mouse DNA was present downstream of the poly(A) addition sites (in contrast to the κ gene); RNA polymerase seems to continue transcribing for a certain distance into the flanking vector sequences. The limits have not been defined. Finally, RNA dots were treated with RNase after hybridization. This eliminated RNA signals, but left DNA signals on the same filters intact (Fig. 2). For treatment with RNase, the dot filters were soaked in 0.2× SSC for 5 min at 37°C. Then pancreatic RNase was added to 10 μg/ml, and the incubation at 37°C was

continued for 30 min. The filters were extensively washed in 0.2× SSC, 0.1% SDS, 1 mM EDTA at 65°C before reexposure to x-ray film.

*DNA Quantitative Slot Hybridization.* DNA was denatured as above, diluted in 15× SSC, applied to nitrocellulose using a Schleicher and Schuell slot blotter, and hybridized with $C_\kappa$ or $C_\mu$ probes without vector in probe excess. Relative quantities of DNA were determined by scanning the slot hybridizations with a Helena Quick Scan (Helena Laboratories, Beaumont, TX). DNA from normal mice was included, and their $C_\kappa$ and $C_\mu$ signals represented two copies per genome.

*Transfection of DNA.* The $\mu$ and $\mu$ Δ mem genes with their vectors were cotransfected together with the pSV2-gpt gene (19) into J558L myeloma cells (20) by electroporation (21).

*Immunofluorescence.* Thymuses were removed from the transgenic mice or normal littermate mice. A small piece was cut from the thymus for preparation of single-cell suspensions. The majority of the thymus was used for preparation of RNA. Each small thymus piece was gently rubbed over a 60-gauge stainless steel mesh. The cells were washed once in PBS plus 1% BSA (Sigma Chemical Corp., St. Louis, MO) and resuspended in PBS-BSA at a concentration of $10^6$ cells/ml. The cells were then spun into ethanol-cleaned glass microscope slides $10^5$ cells/slides, with a Shandon Southern Instruments, Inc. Cytospin (Sewickley, PA). The air-dried slides were fixed for 20 min at −20°C in 95% ethanol/5% acetic acid, then put through three 10-min washes of PBS. The antisera, tetramethylrhodamine isothyocyanate–conjugated goat anti–mouse $\mu$ (Cappel Laboratories, Cochranville, PA) and tetramethylrhodamine isothyocyanate–conjugated goat anti–mouse $\kappa$ (Southern Biotechnology Associates Inc., Birmingham, AL), were diluted 1:200 in PBS-BSA and added directly to the fixed cells. The slides were incubated for 30 min at 37°C in a humidified chamber, then washed three times, once in PBS, once in PBS-BSA, and a final time in PBS. The cells were covered with PBS-glycerol and viewed with a Zeiss fluorescence microscope with transmitted light, a dark field condenser, and a 200 W high-pressure mercury bulb equipped with KP-546 and KP-500 excitation filters coupled to appropriate barriers to detect the fluorescence of rhodamine. The photographs were taken on Kodak Ektachrome daylight slide film, ASA 400.

## Results

*Transgene Copy Number.* The number of DNA molecules integrated in the transgenic mice varied between 1 and 87 (Table I). Most often the multiple copies were inserted at a single site, i.e., all positive offspring had the same copy number as the parent. In several cases, however, two integration sites were found that segregated in the offspring (indicated by A and B offspring in Table I; see also 1201 vs. 1202 and 1205 in Fig. 3). Transgenic mice that had been coinjected with $\mu$ (or $\mu$ Δ mem) and $\kappa$ genes had similar copy numbers of both genes integrated (one exception, 217-7 A, has only $\mu$ and no $\kappa$ genes) (Table I).

There was no good correlation between the transgene copy number and the level of RNA (Table I). This finding had also been made with MOPC-21 $\kappa$ transgenic mice (5). It is different from the relatively good correlation found in B cells transfected with Ig genes (22).

*Tissue-specific Expression of Transgenes.* We had previously (5) found that, in mice with a $\kappa$ transgene from the myeloma MOPC-21, only B lymphocytes expressed the transgenic $\kappa$ RNA. The question of tissue specificity was analyzed with many of the transgenic mice presented in this paper, examples of which are shown in Fig. 4. Liver, kidney, and heart do not contain transgenic $\kappa$ or $\mu$ RNAs beyond a low level expected from B cell contamination. Thus we have not found evidence for $\mu$ expression in heart, as was found by Grosschedl et al. (2). Almost all the mice express the $\mu$ and/or $\kappa$ transgenes at a high level in spleen RNA

TABLE I

*DNA and RNA Quantitations of Some of the MOPC-167 Transgenic Mice*

| Transgene | Mouse number | Transgene copy number* | | | Spleen RNA‡ | |
|---|---|---|---|---|---|---|
| | | κ | μ | μ/κ | κ | μ |
| κ vector Δ | 229-1 | 4 | | | 146 | 5 |
| | 230-3 | 1 | | | 47 | 3 |
| | 231-8 | 2 | | | 1§ | 3 |
| | 233-4 | 41 | | | 117 | 25 |
| | 233-8 | 13 | | | 58 | 5 |
| | 234-3 | 20 | | | 9 | 2 |
| | 234-4 A | 11 | | | 233 | 1 |
| | B | 18 | | | | |
| κ | 189-4 | 11 | | | 47 | 1 |
| | 194-2 | 2 | | | 47 | 1 |
| μ | 199-9 | | 70 | | <9 | 20 |
| | 200-3 | | 3 | | 50 | 60 |
| | 200-6 | | 66 | | 2 | 40 |
| μ vector Δ | 243-2 | | 1 | | 2 | 3 |
| | 243-4 | | 6 | | 3 | 12 |
| μ Δ mem | 250-1 | | 12 | | 1 | 8 |
| | 250-2 | | 2 | | 1 | 8 |
| | 254-3 | | 2 | | 1 | 60 |
| | 254-3-11§ | | 2 | | 1 | 60 |
| | 254-3-12§ | | 2 | | 1 | 60 |
| κμ | 207-4 | 15 | 60 | 4 | 39 | 200 |
| | 210-3 | 3 | 4 | 1.3 | 20 | 30 |
| | 210-4 | 13 | 8 | 0.62 | 1.5 | 30 |
| | 212-2 | 10 | 19 | 1.9 | 3 | 25 |
| | 212-3 | 2 | 10 | 5 | 3 | 25 |
| | 212-5 | 37 | 23 | 0.62 | 10 | 100 |
| κμ Δ mem | 216-1 A | 1 | 2 | 2 | 12 | 100 |
| | B | 20 | 37 | 1.9 | | |
| | 216-2 | 18 | 6 | 0.33 | 1 | 6 |
| | 216-7 | 1 | 2 | 2 | 12 | 20 |
| | 217-1 | 29 | 87 | 3 | 3 | 25 |
| | 217-4 | 8 | 17 | 2.1 | 6 | 25 |
| | 217-6 | 17 | 56 | 3.3 | 300 | 300 |
| | 217-7 A | 0 | 2 | >2 | 6 | 25 |
| | B | 4 | 24 | 6 | | |

The mice shown contain the transgenes with the complete pUC vectors (see Fig. 1), except the κ vector del, the μ vector del, and the μ Δ mem groups, which contained only ~150 bp of the vector (see Materials and Methods).

* Transgene copy number was determined by quantitative slot hybridization (see Fig. 3) on tail DNA of offspring from the original set of transgenic mice. A and B represent offspring with different copy numbers indicating that the parent had two independently segregating transgene insertion sites.

‡ Quantities of spleen 167κ or 167μ RNA expressed as multiples of the levels in nontransgenic control mice. These determinations were made on the spleen RNA of the original transgenic mice, except in two offspring of mouse 254-3 (§).

§ Because the presence of two adjacent, strongly hybridizing dots obscures the interpretation of the autoradiograph, the κ level for mouse 231-8 may actually be above normal.

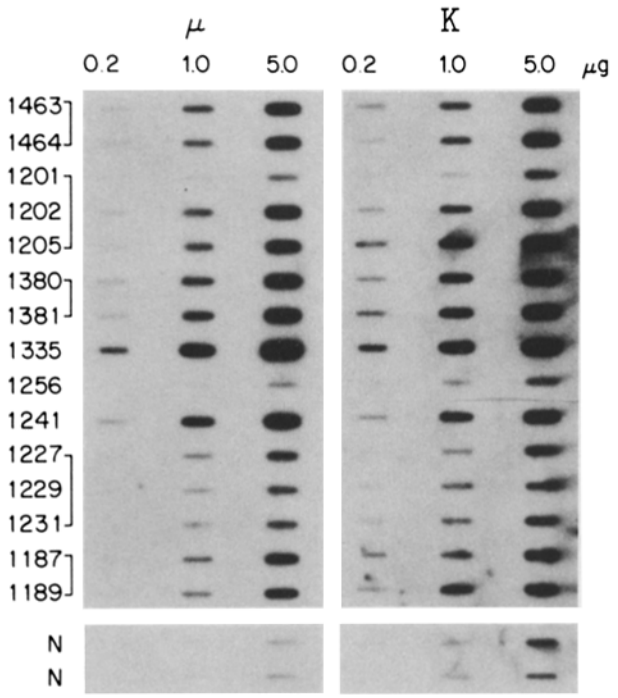


FIGURE 3. Quantitative slot hybridization of tail DNA samples. Shown are the DNAs from 15 offspring of κμ or κμ Δ mem transgenic mice produced by coinjection of the 167-κ and 167-μ or μ Δ mem genes, and of two nontransgenic (*N*) mice. Offspring from the same transgenic parent mated with a nontransgenic mouse are bracketed; from top to bottom the following transgenic lines are shown: 217-4, 216-1, 217-6, 217-1, 216-7, 207-4, 212-3, 212-2. The blots were hybridized with probes for $C_\mu$ (μ) or $C_\kappa$ (κ), as indicated.

(Table I). In nontransgenic littermates, no or very low amounts of the $V_\kappa$-167- and $V_H$-167-positive RNAs are found in the spleen (N in Figs. 4, 5, and 7). In the thymuses of mice carying μ, κμ, κ × μ, or κμ Δ mem transgenes, μ RNA with $V_H$-167 sequence is found at about one-fifth to one-half the level of $V_H$-167 RNA in the spleen (Figs. 4 and 5, and data not shown). This confirms, with a different $V_H$ gene, the finding of Grosschedl et al. (2) that rearranged μ transgenes are expressed in T cells. Interestingly, in mice that carry both μ and κ transgenes, only μ, and not κ transcripts are seen in thymus (Fig. 4, κ × μ, and data not shown).

κ transgenes are generally not expressed in the thymus, both in the case of the mice carrying the MOPC-21 κ transgene previously analyzed (5), and in the κ-167 mice reported here. However, the two κ mouse lines shown in Fig. 4 consistently had significant quantities of κ transgenic RNA in the thymus. Reduction of plasma cells to ~0.4% from thymus cell preparations by anti-Ia serum and complement did not eliminate the κ RNA (data not shown). No κ protein could be detected by immunofluorescence (not shown). These mice had a 58- and 117-fold increases of κ-167 RNA in spleen, compared with normal mice (Table I, 233-8 and 233-4). It appears possible that the κ RNA in the thymus is entirely due to B cells. However, we have not ruled out the possibility

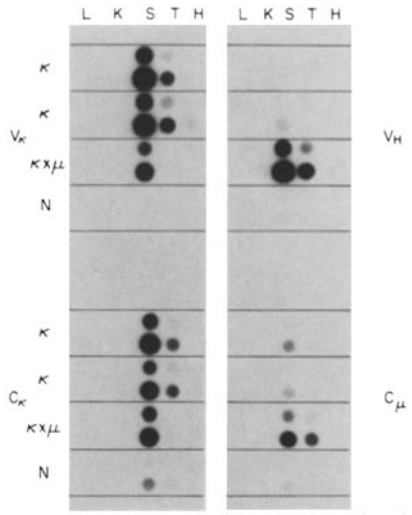


FIGURE 4. RNA dots of mouse organs. Each RNA was dotted as 2 and 10 μg for the $V_\kappa$ and $V_H$ hybridizations, and 0.5 and 2.5 μg for the $C_\kappa$ and $C_\mu$ hybridizations. *L*, liver; *K*, kidney; *S*, spleen; *T*, thymus; *H*, heart. On the left side, from top to bottom, the mice are indicated: κ: 233-8-3 and 233-4-2 are offspring of transgenic mice with the κ-167 gene without vector; κ × μ: an offspring from a mating between κ transgenic mouse 189-4 and μ transgenic mouse 199-9, the complete vector is present in these transgenes; *N*: nontransgenic littermate of the κ × μ mouse. $V_\kappa$, $C_\kappa$, $V_H$, and $C_\mu$ indicate the hybridization probes.

that the κ transgenes are actually expressed in T cells of these mouse lines, perhaps as a result of the relatively high copy number (13 and 41 copies) or chromosomal position. In the case of T cells transfected in vitro with κ genes, 5% of the transfectants expressed κ (22).

*κ and μ RNAs in Spleens of κ and/or μ Transgenic Mice.* In mice that carry both the 167-κ and μ or μ Δ mem transgenes, there is generally a good correlation between the relative levels of H and κ RNAs with the V167-H or κ sequences (Fig. 5). In most cases, the levels of transgenic H RNA are somewhat higher than those of transgenic κ RNA. This is not an artifact of the specific activities of the hybridization probes; in fact, the signal with the $V_\kappa$-167 probe is higher than the $V_H$-167 signal when the probes are hybridized with equal amounts of the respective plasmid DNAs on the same blot (Fig. 5, *bottom*).

In two of the mice, the 167-κ RNA levels were almost as low as in normal mice (fourth and sixth columns in the κ μ and κ μ Δ mem mice of Fig. 5, respectively). Both these mice had relatively high copy numbers (13 and 18 copies) of the κ transgenes. We have no explanation for the minimal κ transgene expression. Both mice produced normal levels of total κ RNA in the spleen (not shown).

In general, there is a wide variation in the expression of κ and/or μ RNA between individual mice. This does not seem to be related to the gene copy number (Table I). If this variability were dependent upon the insertion site of the transgenes, offspring of high expressors should also be high expressors, and vice versa with low expressors. In one particular strain of MOPC-167 κ-transgenic mice, we found high expression over two generations, but low expression in the second offspring generation (R. L. O'Brien and U. Storb, unpublished data).

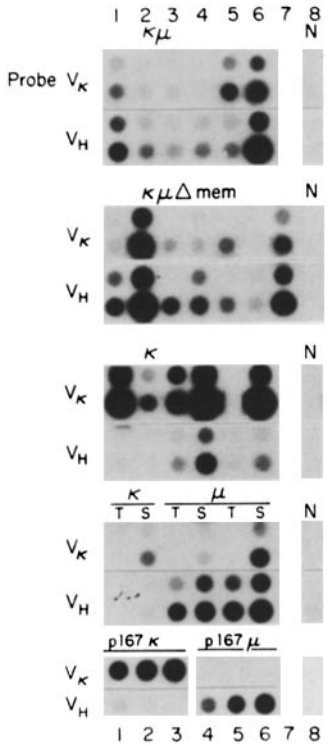


FIGURE 5. RNA dots of spleen and thymus. In each row the top dot is 5 μg, the bottom dot 25 μg of total RNA. Each group of transgenic mice is indicated on the top of the panels: κμ, eggs coinjected with the κ-167 and μ-167 genes with the vector (212-5, 212-3, 212-2, 210-4, 210-3, and 207-4); κμ Δ mem, eggs coinjected with the κ-167 and μ-167 Δ mem genes with the vector (217-7, 217-6, 217-4, 217-1, 216-7, 216-2, and 216-1); κ, eggs injected with the κ-167 gene without the vector (third panel) (234-4, 234-3, 233-8, 233-4, 231-8, and 229-1) or with the vector (fourth panel) (189-4); μ, eggs injected with the μ 167 gene with the vector (200-6 and 200-3). *N*, spleen of normal littermates of mice in the same panel, except in the fourth panel where *N* is (SJL × C57BL/6)$F_1$ spleen. The three top panels show spleen RNA, the fourth panel shows thymus (*T*) and spleen (*S*) RNAs. The probes were $V_κ$-167 or $V_H$-167, as indicated. The probes were excised from the vector and contain essentially no vector DNA, as shown on DNA dots (*bottom*): p167-κ and p167-μ are dots of the plasmid DNAs, including the vector.

Thus, perhaps the environmental levels of PC vary in our mouse colony (see Discussion). The variability in RNA levels will be further investigated.

*Immunoglobulin Protein in T Cells.* Thymocytes from a mouse carrying the 167-μ and 167-κ transgenes were analyzed for μ and κ proteins by immunofluorescence (Fig. 6). ~60% of the transgenic thymocytes contained μ (Fig. 6*D*). In normal thymus, only ~0.1% of the cells were $\mu^+$ (Fig. 6*B*); these appear to have plasma cell morphology. When stained with anti-κ, only a few plasma cells, but not the thymocytes of the κ μ–transgenic mouse were stained (Fig. 6*F*). The $\mu^+$ cells are indeed T lymphocytes, because 98% of the thymocytes were Thy-$1^+$ (not shown). Thymocytes from the same κ μ mouse were also stained for surface immunofluorescence with anti-μ, and analyzed by FACS (not shown). The thymocytes were found to be negative for surface-bound immunoglobulin.

This particular κ μ mouse (207-4) had very high levels of κ and μ RNAs in the

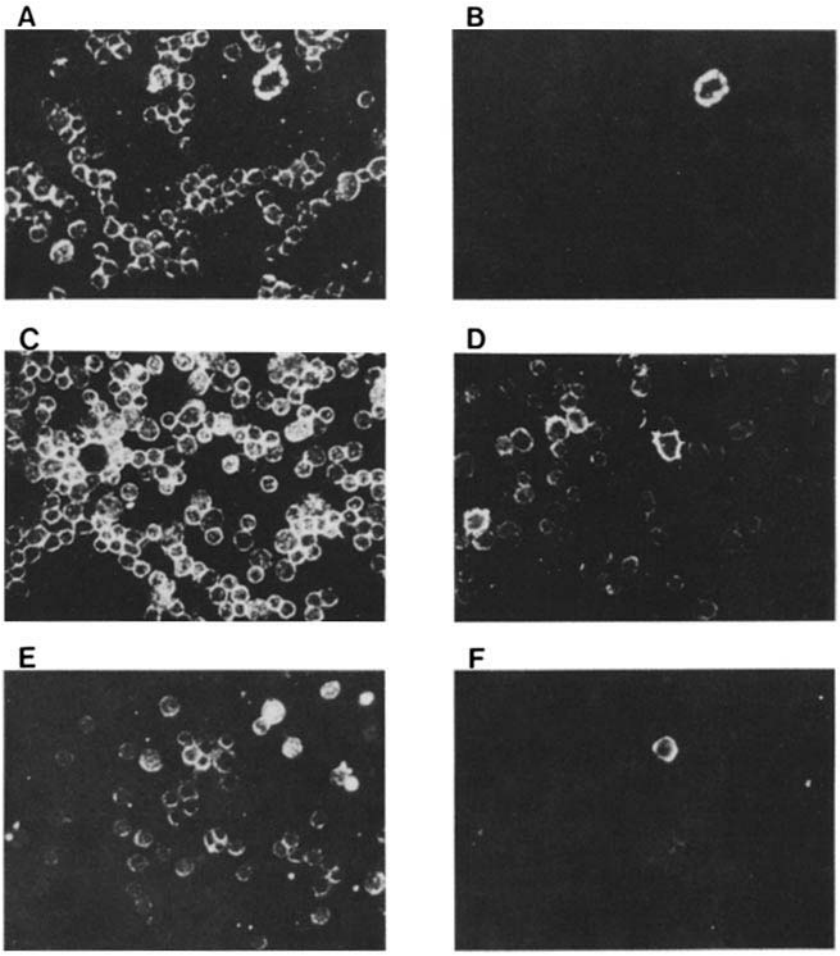


FIGURE 6. Immunofluorescence of thymocytes. Thymocytes of normal (*A* and *B*), and $\kappa\mu$ transgenic mice (*C–F*) (207-4) were stained with rhodamine-conjugated anti-$\mu$ (*A–D*) or anti-$\kappa$ (*E* and *F*) and photographed in darkfield (*A*, *C*, and *E*) or fluorescence microscopy (*B*, *D*, and *F*).

spleen (Fig. 5, $\kappa$ $\mu$ column *6*), and of $\mu$ RNA in the thymus (not shown). The thymuses of two other $\kappa$ $\mu$ mice (212-2 and 212-3) were also analyzed for cytoplasmic immunofluorescence and found to be weakly positive for $\mu$ protein in a high proportion of the thymocytes (not shown).

*Influence of Transgenes on Expression of Endogenous MOPC-167-like Genes.* Several of the mice that carry only $\kappa$-167 transgenes show, in addition to a large amount of $V_\kappa$-167 RNA, also a several-fold increased level of H RNA with the $V_H$-167 sequence compared with normal littermates (Fig. 5, $\kappa$; Table I, $\kappa$ vector $\Delta$). This RNA is encoded by endogenous H genes. There may be some correlation between the amount of transgenic $\kappa$-167 and endogenous H-167 RNA, since only the mice with very high levels of $\kappa$-167 RNA show an increase of H-167 RNA. However, some mice with very high levels of $\kappa$-167 do not have high levels

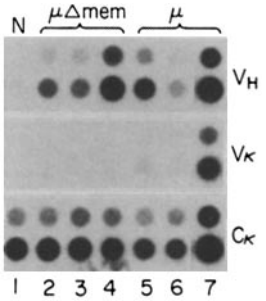


FIGURE 7. RNA dots of spleen from different $\mu$ transgenic mice. The dots contain 2 and 10 $\mu$g RNA for the $V_H$ and $V_\kappa$ probes, and 0.5 and 2.5 $\mu$g for the $C_\kappa$ probe. Mice from which the spleen RNAs were obtained are indicated on the top. *N*, nontransgenic littermate of the $\mu\Delta$ mem mice; *2–4* are 250-1, 250-2, and 254-3; *5–7* are 243-4, 243-2, and 200-3. The hybridization DNA probes are indicated on the right side.

of H-167 RNA (Fig. 4 $\kappa$ mice; Fig. 5, third panel, $\kappa$, column *1*; Table I mouse 234-4).

Likewise, mice with the complete 167-$\mu$ gene show relatively high levels of 167-$\kappa$ RNA in the spleen (Figs. 5 and 7, $\mu$). Of five total mice with the 167-$\mu$ transgene, all had increased expression of endogenous 167-$\kappa$-like genes. In contrast, no mice ($n$ = 5) with the 167 $\mu$ $\Delta$ mem gene showed an increased amount of 167-$\kappa$ RNA (Fig. 7 shows three of these; see also Table I), despite high levels of 167-H RNA and total $\kappa$ RNA (probed with $C_\kappa$). These results are significant with respect to B cell triggering (see Discussion).

*Types of 167 Heavy Chain RNAs.* Northern blots of spleen RNA probed with $V_H$-167 confirmed that normal mice (Fig. 8, *N*) do not produce detectable levels of $V_H$-167-containing RNA (Fig. 8*A*). As controls, the two $\mu$ genes were transfected into the myeloma J558L, and only the secreted form of the 167-$\mu$ mRNA was seen, even with the complete $\mu$ gene (Fig. 8*C*). This reflects RNA processing in favor of the secreted form in the plasma cell stage. However, transgenic mice with the complete $\mu$ gene show both the membrane and the secreted form of 167-$\mu$ RNA in spleen and thymus (Fig. 8, *A* and *C*). In the spleen, the secreted form is predominant, probably indicating that the majority of the 167-$\mu$ RNA is derived from plasma cells. In the thymus, an equal or greater amount of the $V_H$-167 RNA is in the membrane form. Thus, T cells appear to process $\mu$ RNA like pre-B and early B lymphocytes, with both polyadenylation sites of $\mu$ being used. We do not know how these steady-state levels of $\mu$ RNA in the transgenic thymus are influenced by differential stabilities versus transcription rates. Besides the defined bands of secreted and membrane 167-$\mu$ RNA (Fig. 8, *A* and *C*) two shorter-length transcripts are seen when Northern blots of thymus RNA are probed with $C_\mu$ (Fig. 8*B*). These incomplete transcripts do not contain $V_H$-167 sequences (compare, in Fig. 8, *A* with *B*). They are very prominent in the thymus of mice with or without $\mu$ transgenes (Fig. 8 *B* and other data not shown). In spleens of mice with or without a $\mu$ transgene, only the incomplete $\mu$ RNA of the higher molecular mass is observed (Fig. 8 *B*, and not shown). These short transcripts in spleen and thymus probably represent RNA transcripts from DJ rearrangements of endogenous H genes (23, 24).

Mice with the $\mu$ $\Delta$ mem transgene have only the secreted form of the $V_H$-167-

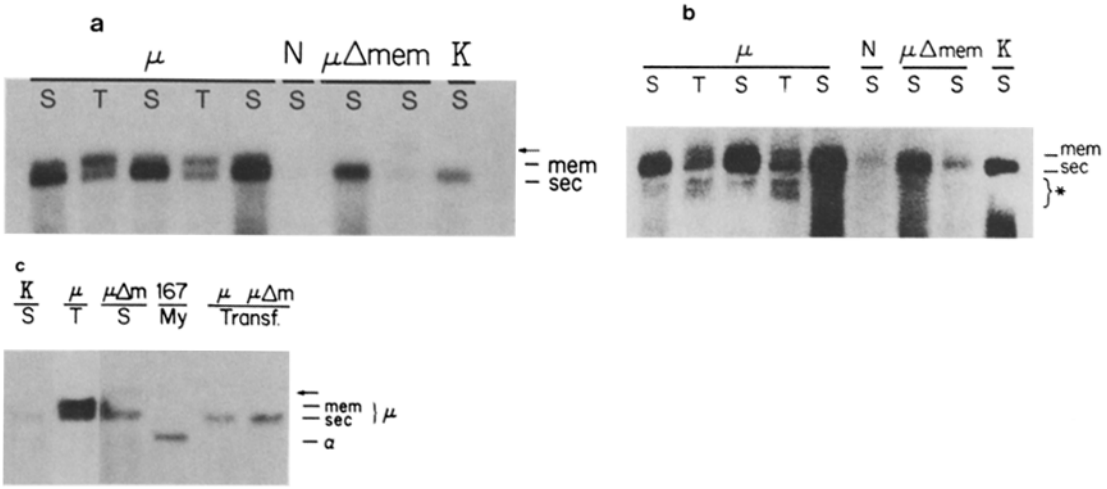


FIGURE 8. Northern blots. Spleen (*S*) and thymus (*T*) RNAs from transgenic and normal (*N*) mice; the transgenic mice contained the p167-μ gene (μ) (200-3, 200-6, and 207-4), the p167-μ Δ mem gene (μΔ mem) (217-6), or the p167-κ(K) gene (233-4); RNA from the myeloma MOPC 167 (*167/my*); and RNAs from the myeloma J 558L transfected with the p167μ and p167μ mem gene (μ, μΔ *mem/Transf.*). *A* and *B* are the same blots (20 μg RNA each lane) hybridized in *A* with the $V_H$-167 probe, and after decay of the signal, rehybridized with $C_\mu$. *C*, 30 μg RNA for S and T, 10 μg for the rest, hybridized with $V_H$-167. *Mem* and *sec*: membrane (2.7 kb) and secreted (2.4 kb) forms of μ mRNA. α, marker for 1.9 kb size of secreted form of α and γ mRNAs. Arrow indicates high-mol-wt form in spleen RNA of two different p167 μΔ mem transgenic mice. Asterisk indicates incomplete μ transcripts.

μ RNA in their spleen, as expected (Fig. 8, *A* and *C*). In two cases, the spleen RNAs contain a large transcript of ~3 kb in addition to the secreted form (Fig. 8, *A* and *C*). This is ~300 nucleotides larger than the membrane form. Interestingly, this transcript does not contain $C_\mu$ sequences (Fig. 8*B*). We have not yet investigated the origin of the long transcripts. Perhaps transcription into flanking sequences and aberrant splicing is involved.

The endogenous 167-μ RNA produced in the spleen of mice with the 167-κ transgene did not contain any detectable membrane form (Fig. 8, *A* and *C* κ/S). In addition to the secreted μ form, one of the κ mice also contained a detectable amount of $V_H$-167$^+$ RNA of the size of secreted γ or α RNA (Fig. 8*C*).

## Discussion

The MOPC-167 μ and κ genes are expressed at high levels in the transgenic mice reported here. This study represents the first attempt at expressing a μ gene with deleted membrane terminus, either in cell transfection or when propagated through the germline of mice. It clearly shows that the region just 5′, within, and 3′ of the μ membrane terminus is not required for correct expression and processing of secreted μ. This is apparently in contrast to the $C_\kappa$ gene, where we have preliminary evidence that a region 6 kb 3′ of $C_\kappa$ is required for high expression in transgenic mice (U. Storb, C. Pinkert, R. Brinster, and S. L. McKnight, unpublished).

The μ and κ transgenes are generally transcribed in a tissue-specific fashion. However, the μ transgenes are expressed in thymus, where normally correctly rearranged μ genes do not exist. Thus, T cells seem to have the capacity to

activate $\mu$ genes sufficiently for transcription, but they seem to lack the ability to correctly rearrange Ig genes. The $V_H$ 167-containing transcripts in the transgenic thymuses are of normal length for secreted and membrane $\mu$–encoding mRNAs. The mRNAs are apparently stable and translated into stable $\mu$ protein, which can be visualized in thymocyte cytoplasm. The $\mu$ protein cannot be detected on the cell surface by immunofluorescence, and one may conclude that $\mu$ does not associate with the $\alpha$ or $\beta$ chain of the T cell receptor, or at least not in a way that allows binding of anti-C$\mu$ antibodies. Only ~60% of the thymocytes showed intracytoplasmic $\mu$ protein (Fig. 6*D*). In normal thymus, ~50–70% of the thymocytes have T cell receptor protein on the surface (25). It is reasonable to postulate that these same cells would express $\mu$ genes, although we have not made the direct correlation. Presumably the same *trans* acting factors for gene expression that interact with T cell receptor genes can also activate $\mu$ genes.

Most of the transgenic mice express the $\mu$ and $\kappa$ genes at a high rate in the spleen. Furthermore, most of the transgenic mice produce 10–100-fold higher levels of anti-PC antibodies than normal mice (C. Pinkert, J. Manz, R. L. Brinster, and U. Storb, unpublished observations). For B cell activation to antibody secretion, antigen is probably required. Phosphorylcholine, the target antigen for MOPC-167 antibodies, is a fairly ubiquitous antigen, as it is a component of bacterial membranes, fungi, parasites, etc. (26, 27). The levels of anti-PC antibodies and of endogenous 167-$\kappa$ RNA are greatly increased in transgenic mice with the complete $\mu$ gene (and conversely, endogenous 167-$\mu$ RNA in $\kappa$ mice). Apparently, B cells that express the 167-$\mu$ gene and at the same time happen to express an endogenous 167-$\kappa$ transgene are triggered and expanded at a much higher rate than B cells that express other endogenous $\kappa$ genes.

Mice with the $\mu$ $\Delta$ mem transgene produce high levels of mRNA encoded by this gene. In contrast to the mice with the complete $\mu$ gene, however, they do not produce an increased amount of MOPC-167-like $\kappa$ RNA. This difference probably indicates that B cells expressing the $\mu$ $\Delta$ mem transgene are not activated by PC. In contrast to membrane Ig, the secreted molecule lacks a hydrophobic carboxy terminus required for stable anchorage in the membrane. Recent work (28, 29) has shown that the role of membrane Ig in B cells is to capture antigen for endocytosis and processing. Processed antigen is then presented to T cells in association with Ia, not in association with surface Ig. Dependent on the carrier, PC can be a T-dependent antigen (30). It appears that, in transgenic mice with the $\mu$ $\Delta$ mem gene, B cells that produce the transgenic secreted H chain together with endogenous M-167 $\kappa$ chains are not selected. Although secreted Ig molecules are released from the cell surface, they apparently do not dwell in the plasma membrane in a way that would permit cell activation. It is curious, then, that mice with the $\mu$ $\Delta$ mem transgene produce such high levels of the secreted M.167 $\mu$ RNA. The high transgenic and total endogenous $\mu$ mRNA levels in these mice (Fig. 8, *A* and *B*) suggest that most B cells express the $\mu$ $\Delta$ mem transgenes together with complete endogenous membrane immunoglobulins. This would mean that the $\mu$ $\Delta$ mem gene or its encoded secretory $\mu$ protein does not cause feedback inhibition of endogenous H and L gene rearrangement. As the transgenic $\mu$ $\Delta$ mem B cells with endogenous H and L chains become activated by any

of a large number of antigens, the transgenic mRNA probably becomes amplified together with the endogenous Ig mRNAs in the same cell.

While the $\mu$ $\Delta$ mem protein molecules would not interfere with efficient triggering of B cells that express endogenous Igs, they do apparently associate with endogenous L chains in secreted molecules (not shown). Thus, ~50% scrambled (transgenic $\mu$ $\Delta$ mem H plus endogenous H and L) antibody molecules and 25% pure $\mu$ $\Delta$ mem H, endogenous L molecules (in addition to 25% pure endogenous HL molecules), would be secreted by most individual plasma cells. Nevertheless, the mice, now ~1 yr old, appear to be healthy without special protection from pathogens. Apparently, the multilevel immune defense system contains enough safeguards that dilution of pure antibodies by a high proportion of scrambled and irrelevant molecules can be tolerated. With respect to this plasticity of the immune system, and in relation to allelic exclusion, it will be interesting to determine on the cellular level how the coexpression of endogenous Ig genes and transgenes is controlled.

## Summary

Transgenic mice were produced that carried in their germlines rearranged $\kappa$ and/or $\mu$ genes with $V_\kappa$ and $V_H$ regions from the myeloma MOPC-167 $\kappa$ and H genes, which encode anti-PC antibody. The $\mu$ genes contain either a complete gene, including the membrane terminus ($\mu$ genes), or genes in which this terminus is deleted and only the secreted terminus remains ($\mu$ $\Delta$ mem genes). The $\mu$ gene without membrane terminus is expressed at as high a level as the $\mu$ gene with the complete 3′ end, suggesting that this terminus is not required for chromatin activation of the $\mu$ locus or for stability of the mRNA. The transgenes are expressed only in lymphoid organs. In contrast to our previous studies with MOPC-21 $\kappa$ transgenic mice, the $\mu$ transgene is transcribed in T lymphocytes as well as B lymphocytes. Thymocytes from $\mu$ and $\kappa\mu$ transgenic mice display elevated levels of M-167 $\mu$ RNA and do not show elevated levels of $\kappa$ RNA, even though higher than normal levels of M-167 $\kappa$ RNA are detected in the spleen of these mice. ~60% of thymocytes of $\mu$ transgenic mice produce cytoplasmic $\mu$ protein. However, despite a large amount of $\mu$ RNA of the membrane form, $\mu$ protein cannot be detected on the surface of T cells, perhaps because it cannot associate with T cell receptor $\alpha$ or $\beta$ chains.

Mice with the complete $\mu$ transgene produce not only the $\mu$ transgenic mRNA but also considerably increased amounts of $\kappa$ RNA encoded by endogenous MOPC-167 like $\kappa$ genes. This suggests that B cells are selected by antigen (PC) if they coexpress the $\mu$ transgene and appropriate anti-PC endogenous $\kappa$ genes. Mice with the $\mu$ $\Delta$ mem gene, however, do not express detectable levels of the endogenous MOPC-167 $\kappa$ mRNA. Like the complete $\mu$ transgene, the M-167$\kappa$ transgene also causes amplification of endogenous MOPC-167 related immunoglobulins; mice with the $\kappa$ transgene have increased amounts of endogenous MOPC-167-like $\mu$ or $\alpha$ or $\gamma$ in the spleen, all of the secreted form. Implications for the regulation of immunoglobulin gene expression and B cell triggering are discussed.

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