Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 7189–7193, November 1984
Immunology

Antibodies of the secondary response can be expressed without switch recombination in normal mouse B cells

(isotype switch/RNA processing/cell sorting/DNA rearrangement)

AARON P. PERLMUTTER AND WALTER GILBERT*

Department of Biochemistry and Molecular Biology, 16 Divinity Avenue, Harvard University, Cambridge, MA 02138

Contributed by Walter Gilbert, July 12, 1984

ABSTRACT **A few percent of mouse splenocytes express isotypes characteristic of the secondary response together with IgM, and some cells express these isotypes alone. We isolated populations of small memory cells that express (*i*) IgM but not IgG1, (*ii*) IgM but not IgA, (*iii*) IgM and IgG1, (*iv*) IgM and IgA, and (*v*) IgG1 but not IgM. We have analyzed their DNA to show that there has been no switch recombination or deletion in the Ig constant region (*C*) genes. Using sandwich RNA hybridizations, we have found that cells expressing IgG1 contain nuclear RNAs that have both C_μ and $C_{\gamma 1}$ sequences, and that cells expressing IgA contain nuclear RNAs that have both C_μ and C_α sequences. We propose that the expression of an isotype characteristic of the secondary response in memory cells is accomplished by alternative RNA processing of large (up to 180 kilobases) nuclear RNA transcripts that span the heavy chain gene locus.**

During the immune response, the antibody populations shift from IgM to IgG, IgA, and IgE. After the primary, IgM response, memory cells that express surface IgG, IgE, or IgA appear in the spleen (1). This surface Ig is indicative of the isotype and allotype of the antibody that the progeny of these memory cells will secrete upon antigenic restimulation.

The initial μ heavy chain is encoded by a gene with a heavy chain (H) variable-region exon, assembled by DNA recombination that joins heavy chain variable (V_H), diversity (D), and joining (J_H) segments (2, 3), that is attached by a long intron to the exons of the constant region C_μ. The expression of membrane-bound and secreted forms of IgM is regulated by RNA splicing, which adds alternative exons to the COOH-terminal sequence (4, 5). How do Ig molecules of the same antigen specificity but with one of the other heavy chain constant regions arise? In plasma cells, a second type of DNA rearrangement, which deletes the DNA between S_μ, the switch region between V_H-D-J_H and C_μ, and the switch region 5′ to the expressed heavy chain constant region gene, enables heavy chain class switching to occur (6, 7). The assembled V_H gene is thus brought near the C_H gene of the class to be expressed, and protein expression occurs by the splicing of V_H-D-J_H RNA to the proximal C_H RNA exons. Each switch region begins 1–4 kilobases (kb) 5′ to C_H, and the short repetitive sequences extend another 1 kb (S_ε) to 10 kb ($S_\gamma 1$) 5′ (8). This gene deletion model explains the mechanism of Ig expression in the secreting plasma cell (9, 10). Does it hold for memory cells?

We used fluorescence-activated cell sorting to purify memory cells of the secondary response. To ensure that all surface Igs were endogenously synthesized, washed spleen cells were trypsinized to remove surface proteins and incubated in cell culture to allow protein reexpression. Then we sorted the cells for a first isotype and repeated the trypsinization and cell culture before staining and sorting on the basis of a second isotype. To avoid direct binding of typing antibodies to cellular Fc receptors, we used purified $F(ab')_2$ fragments. Using first the $F(ab')_2$ fragment from fluorescein isothiocyanate (FITC)-conjugated anti-IgG1 and then the $F(ab')_2$ fragment from FITC-conjugated anti-IgM, we purified $IgG1^+IgM^+$, $IgG1^+IgM^-$, and $IgG1^-IgM^+$ cell populations. We found that 1–3% of the resting splenocytes express IgG1 and that ≈50% of those cells also express IgM. Similarly, using anti-IgA and anti-IgM, we isolated IgA^+IgM^+ and IgA^-IgM^+ cell populations. Approximately 2–3% of the splenocytes express IgA, and almost all of those also express IgM. These cell populations are 95% pure upon immediate reanalysis; 85% reexpress the isotype after tissue culture. They incorporate metabolic labels and are a good source of high molecular weight DNA and RNA.

If allelic exclusion is obeyed, the gene deletion model cannot explain this simultaneous expression since it proposes that C_μ has been deleted on the expressing chromosome. We have analyzed the heavy chain genes in these purified populations and show here that isotypes of the secondary response can be expressed without DNA switch recombination.

MATERIALS AND METHODS

Cell Sorting. Female BALB/c mice (Charles River Breeding Laboratories) were used when 6–12 months old. Splenocytes were sorted according to surface Ig heavy chain isotype. The typing antibodies were extensively positively and negatively purified using columns of immobilized myeloma proteins. The purified IgM^+IgG1^+ and IgM^-IgG1^+ populations incorporated radioactive label into both IgM and IgG1 or into IgG1 alone, respectively. Immunofluorescence microscopy verified that cells of these populations could be appropriately doubly or singly stained. The specificity of the typing antibodies was confirmed by blocking experiments with specific myeloma proteins, examining as targets both the cell-surface fluorescence and the precipitation of the newly synthesized radioactive antibodies. In a typical IgG1/IgM "sort," we purified 1–2 × 10^6 IgM^+IgG1^+, 1–2 × 10^6 IgM^-IgG1^+, and 4–6 × 10^7 IgM^+IgG1^- cells from 2 × 10^8 splenocytes (from 2 or 3 pooled spleens). Starting with the same number of cells, the IgA/IgM sort resulted in 4–5 × 10^6 IgM^+IgA^+ cells and 4–6 × 10^7 IgM^+IgA^- cells.

DNA Isolation and Southern Hybridizations. DNA from whole frozen 17-day BALB/c embryos was prepared by the method of Cate *et al.* (12). Fresh sorted splenocytes were washed, resuspended in phosphate-buffered saline, and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: V_H, heavy chain variable segment(s); D, diversity segment(s); J_H, heavy chain joining segment(s); C, constant region; S, switch region; kb, kilobase(s).

*To whom reprint requests should be addressed at: Biogen, 14 Cambridge Center, Cambridge, MA 02142.

lysed with 1 volume of 2× lysis buffer (12). We recovered 1–2 μg of DNA per 10^6 cells. Purified DNA (0.75 μg) was added to carrier salmon testis DNA (Sigma, type III) to give a total of 5 μg, digested with a 2-fold excess of restriction enzyme, electrophoresed through an agarose gel, and transferred to nitrocellulose by the method of Southern (13).

Purified inserts from cDNA or genomic clones were subcloned into the *Sma* I site of the M13 mp8 vector. ^{32}P-labeled single-stranded probes were synthesized and purified by the method of Church and Gilbert (14). Probes of specific activity of 1–2 × 10^9 cpm/μg were used at 1.5 × 10^6 cpm/ml of hybridization solution. Hybridization conditions and washes were described by Wu (15). After washing, filters were air-dried and exposed to preflashed Kodak X-Omat AR film at −70°C with an intensifying screen (16). Autoradiograms were scanned three times with an Ortec 4310 densitometer and the relative average signal intensities were determined by weighing the area under each peak. Filters were washed for 30 min at 25°C in 20 mM NaOH to remove the probe before being used for another hybridization.

RNA Isolation. Crude nuclei for RNA extraction were prepared by resuspending cells at a concentration of 10^6 per ml in cold 0.15 M KCl/4 mM $Mg(CH_3COO)_2$/0.05% Triton X-100/10 mM vanadyl ribonucleoside complex/10 mM Tris Cl (pH 7.4) and incubating them on ice for 3 min (17). The nuclei were collected by centrifugation and immediately lysed at the same concentration in 0.2 M Tris Cl (pH 8.8)/25 mM EDTA/0.5 M LiCl/1% $NaDodSO_4$. RNA was isolated by the method of Lomedico and Saunders (18). This nuclear RNA preparation is contaminated with DNA. Approximately 20 μg of nucleic acid was recovered from 10^6 cells, as quantitated by A_{260}. The RNA was then resuspended in 6× NaCl/Cit (1× NaCl/Cit, 0.15 M NaCl/0.015 sodium citrate)/0.5% $NaDodSO_4$/2 mM $EDTA_2$1 × Denhardt's solution (19) and used directly for hybridization.

Sandwich Hybridizations. Sandwich hybridizations were performed by adapting the method of Dunn and Sambrook (20) to hybridizations of small discs. Five micrograms of denatured $C_{\gamma 1}$ or C_α probes suspended in 1.0 M Tris Cl (pH 7.4)/0.6 M NaCl was applied to each 6-mm nitrocellulose disc. The discs were air dried, labeled with a permanent laboratory marker (VWR), baked in a vacuum oven for 2 hr at 70°C, and presoaked for 6 hr at 65°C in 6× NaCl/Cit/1× Denhardt's solution. Hybridization with 5 μg of nuclear RNA (from 2.5 × 10^5 nuclei) was carried out in a 1.5 ml Eppendorf tube in a total volume of 200 μl for 10–18 hr at 65°C. The discs were washed six times for 45 min per wash in 2× NaCl/Cit/0.5% $NaDodSO_4$. ^{32}P-Labeled single-stranded C_μ probe (2 × 10^6 cpm) was used for the second hybridization in 1 ml of 6× NaCl/Cit/1× Denhardt's solution/0.5% $NaDodSO_4$ at 65°C for 10–18 hrs. Discs were washed in 2× NaCl/Cit/0.5% $NaDodSO_4$ four to six times for 45 min at 65°C, air-dried, and exposed to preflashed Kodak XR-5 film with an intensifying screen at −70°C (16). The intensities of the hybridization signals corresponded to the probe hybridizing to about one molecule per 10 cells.

RESULTS

μ/γ1 DNA Hybridizations. The deletion model predicts that, in cells expressing only IgG1, one or both of the C_μ genes should be deleted, and that, in cells coexpressing IgM and IgG1, one copy of C_μ should be deleted. In the latter example, this model assumes that both chromosomes must be expressing. We chose a series of restriction enzymes that would allow us to assess not only rearrangements of the heavy chain genes but also the presence of the genes without regard to rearrangement. We examined the genomic arrangement of C_μ and $C_{\gamma 1}$ in the BALB/c embryo and in IgG1$^-$IgM$^+$, IgG1$^+$IgM$^+$, and IgG1$^+$IgM$^-$ purified-cell populations by Southern hybridization. Fig. 1 shows the probes and the restriction enzyme sites around C_μ and $C_{\gamma 1}$ (21). For C_μ, *Kpn* I cuts on the 5′ side of the four J_H segments; thus any rearrangements involving V_H-*D*-J_H joining will alter the size of the 13.5-kb *Kpn* I embryonic band. The *Kpn* I digest (Fig. 2*A*, lanes 1–4) shows the 13.5-kb C_μ germ-line band in the embryonic DNA (lane 1), but this band is replaced by a smear in the IgM$^+$IgG1$^-$ (lane 2), IgM$^-$IgG1$^+$ (lane 3), and IgM$^+$IgG1$^+$ (lane 4) sorted-cell populations. The smearing of the embryonic band (lanes 2–4) results from the variety of DNA rearrangements that have occurred 5′ to C_μ on both chromosomes, a result also reported by Nottenburg and Weissman (22). Allelic exclusion implies that only one of the rearrangements in each cell results in an expressed μ heavy chain. For $C_{\gamma 1}$, *Kpn* I is not sensitive to any rearrangement in $S_{\gamma 1}$, and this band is unaltered in the different populations.

We investigated directly the genomic context of $S_{\gamma 1}$ and $C_{\gamma 1}$. For $C_{\gamma 1}$, Fig. 1 shows that the 17-kb *Hin*dIII fragment is sensitive to DNA recombination in $S_{\gamma 1}$; any rearrangements should produce a smaller, smeared-out band. *Hin*dIII digestion (Fig. 2*B*) does not show any rearrangement 5′ to $C_{\gamma 1}$ either in the cells that synthesize IgG1 and IgM or in the cells that synthesize only IgG1. $S_{\gamma 1}$ spans almost 10 kb; a smeared band might be too spread out to be detected. However, we do not see any decrease in intensity of the 17-kb $C_{\gamma 1}$ band, which would accompany S_μ-$S_{\gamma 1}$ joining.

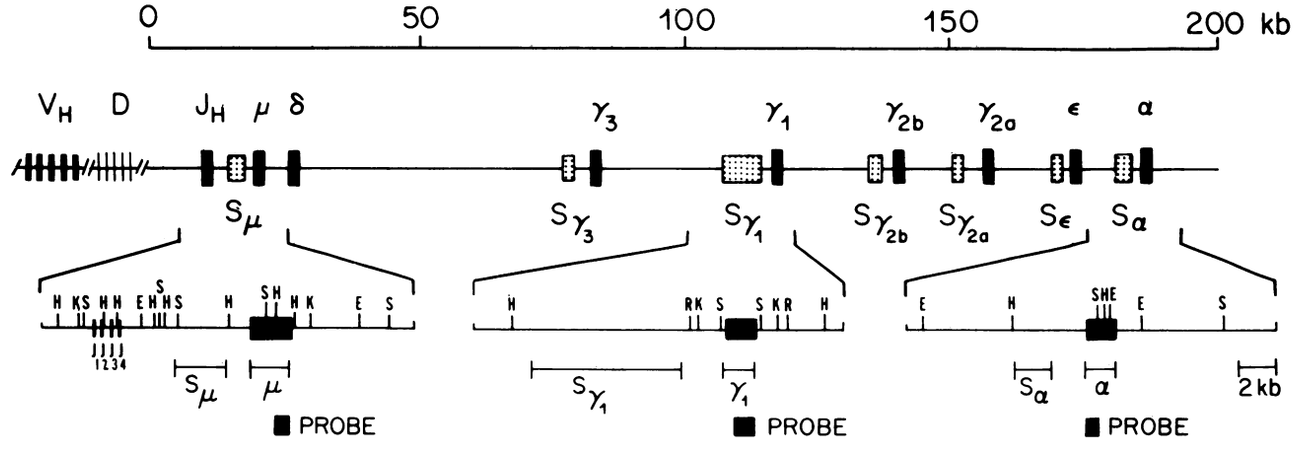

FIG. 1. Schematic representation of the mouse Ig heavy chain gene locus (21). Restriction sites and probes used for Southern hybridization analysis are shown in the expanded IgM, IgG1, and IgA regions. *Kpn* I, K; *Eco*RI, E or R; *Sac* I, S; *Hin*dIII, H. C_H regions are indicated by designations of heavy chain type.

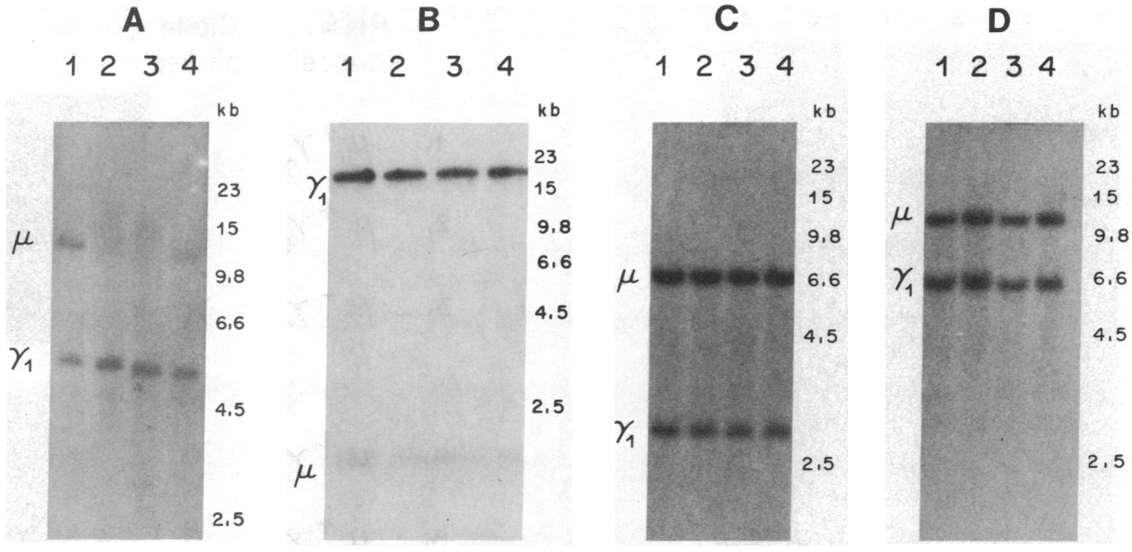

FIG. 2. Southern hybridization analysis of BALB/c DNA from embryos (lanes 1), and from IgM^+IgG1^- (lanes 2), IgM^+IgG1^+ (lanes 3), and IgM^-IgG1^+ (lanes 4) sorted-cell populations. The DNA was digested with *Kpn* I (*A*), *Hin*dIII (*B*), *Sac* I (*C*), and *Eco*RI (*D*). The nitrocellulose filters were hybridized with C_μ and $C_{\gamma 1}$ probes together.

We used two methods to undertake a careful evaluation of the presence of the C_μ gene in the purified cell populations. *Sac* I and *Eco*RI digests (Fig. 2 *C* and *D*) are not sensitive to any rearrangement in either S_μ or $S_{\gamma 1}$ using the probes shown in Fig. 1, so these enzymes serve to quantitate the genes. To obtain an independent comparison of the amount of DNA in each lane, the same nitrocellulose filters were reprobed with the unique and nonrearranging mouse serum albumin gene (*SA*). The ratios of intensity of the *Sac* I and *Eco*RI C_μ bands to the serum albumin gene band, as determined by scanning densitometry, allow a comparison of the presence of the C_μ gene in the different cell populations. Table 1 shows that, within 16%, the C_μ band has the same intensity for each cell type. If C_μ were deleted on the chromosome expressing $C_{\gamma 1}$, a 50% reduction in the C_μ band intensity would be expected. Therefore, we conclude that the C_μ gene is not deleted on either one or both chromosomes upon expression of IgG1.

A second method for assessing the presence of C_μ is to compare the $C_\mu/C_{\gamma 1}$ ratio of band intensities for the embryo to that for cells expressing IgG1. Since *Sac* I and *Eco*RI are blind to any DNA rearrangement, a decrease in this ratio would indicate a loss of C_μ sequences relative to $C_{\gamma 1}$ sequences. As Table 1 shows, the $C_\mu/C_{\gamma 1}$ ratio for *Sac* I or *Eco*RI digests remains relatively constant.

Table 1. Quantitation of C_μ and $C_{\gamma 1}$ genes by scanning densitometry of Southern-hybridized restriction digests

	Band intensity ratios			
	C_μ/SA		$C_\mu/C_{\gamma 1}$	
Cell class	*Eco*RI	*Sac* I	*Eco*RI	*Sac* I
IgM^+IgG1^-	1.12	0.98	1.08	0.89
	1.15	1.13	0.90	0.93
IgM^+IgG1^+	0.96	1.16	0.94	0.95
	1.17	1.04	1.11	0.85
IgM^-IgG1^+	1.15	0.90	0.91	1.08
	0.91	0.97	1.12	1.06
Embryo	1.00	1.00	1.00	1.00

The ratios of Southern hybridization band intensities are normalized to the embryonic value. The two sets of ratios represent two separate experiments. Each autoradiogram was scanned three times, and the standard deviation of these measurements in the final ratio is 0.04. *SA*, mouse serum albumin gene.

Although we could not detect any deletion of C_μ or rearrangement in $S_{\gamma 1}$, could $S_{\gamma 1}$ and $C_{\gamma 1}$ be moved close to the C_μ gene so that IgG1 is expressed by a mechanism and transcript size similar to those for IgD? We were able to investigate C_δ by probing *Bam*HI-digested DNA with C_μ because the 11.5-kb *Bam*HI fragment that contains the 3′ half of C_μ also contains C_δ. We also tested by hybridization for the presence of $C_{\gamma 3}$, since S_μ-$S_{\gamma 1}$ recombination should delete $C_{\gamma 3}$. A Southern blot showed no alteration in the size of the 11.5-kb C_δ band (result not shown). C_δ and $C_{\gamma 3}$ are present in the same amount in these expressing cells as they are in the embryo (Table 2). If IgG1 were coexpressed with IgM by DNA switch recombination on the chromosome not expressing C_μ (allelic inclusion), we would have detected the S-S recombination and the deletion of one copy of C_μ.

μ/α DNA Hybridizations. We completed a similar analysis for those cells that synthesize IgA. Since almost all of the memory cells that synthesize IgA coexpress IgM, the DNAs from IgM^+IgA^+ and IgM^+IgA^- purified cell populations were compared to embryonic DNA. We chose to use the restriction enzymes *Eco*RI and *Hin*dIII since they are both sensitive to switch recombination in the S_α region and are both blind to C_μ rearrangements (see Fig. 1). Fig. 3 shows that there is no rearrangement in S_α detected using *Eco*RI or *Hin*dIII in the IgM^+IgA^+ cells (lanes 3 and 6) when compared to the embryo (lanes 1 and 4) or IgM^+IgA^- cells (lanes 2 and 5). The C_μ hybridization does not decrease in intensity. Table 2 shows that the C_δ and $C_{\gamma 3}$ hybridization intensi-

Table 2. Quantitation of C_δ and $C_{\gamma 3}$ genes by scanning densitometry of Southern-hybridized *Bam*HI digests

	Relative band intensity	
Cell class	C_δ	$C_{\gamma 3}$
IgM^+IgG1^-	1.11	1.05
IgM^+IgG1^+	1.06	0.88
IgM^-IgG1^+	1.10	1.09
IgM^+IgA^-	1.03	0.91
IgM^+IgA^+	1.21	0.94
Embryo	1.00	1.00

Equal amounts of each digested DNA sample were loaded on the gels. Autoradiograms were scanned as described in the legend to Table 1. Results are normalized to the embryonic value.

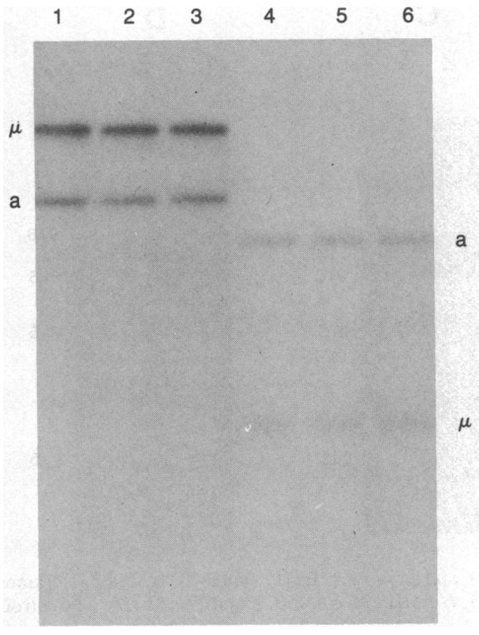

FIG. 3. Southern hybridization analysis of C_μ and C_α genes. Lanes 1–3: *Eco*RI-digested BALB/c DNA from embryos (lane 1) and from IgM^+IgA^- (lane 2) and IgM^+IgA^+ (lane 3) sorted-cell populations. Lanes 4–6: *Hin*dIII-digested BALB/c DNA from embryos (lane 4) and from IgM^+IgA^- (lane 5) and IgM^+IgA^+ (lane 6) sorted cell populations. The nitrocellulose filters were hybridized with C_μ and C_α probes together.

ties are the same in the IgM^+IgA^- and IgM^+IgA^+ purified-cell populations as in the embryo. The fact that C_μ is not deleted in either the IgG1- or IgA-producing cells suggests that isotype-class switching results from the processing of a long nuclear RNA spanning from V_H to the expressed C_H.

RNA Hybridizations. To search for a transcript that contains either both C_μ and $C_{\gamma 1}$ or both C_μ and C_α sequences, we devised a sandwich hybridization procedure to detect such an RNA in the cell nucleus. We bound C_α or $C_{\gamma 1}$ clones to nitrocellulose discs and sequentially hybridized the discs, first to nuclear RNA from a sorted-cell population and then to a ^{32}P-labeled single-stranded C_μ probe. If an RNA contains sequences that hybridize both to the clone immobilized on the disc and to the ^{32}P-labeled C_μ sequences, a positive signal should result. Fig. 4 shows a variety of hybridizations using $C_{\gamma 1}$ discs or C_α discs and nuclear RNAs from the IgG1/IgM and IgA/IgM sorts. The positive signal on disc 2 shows that cells that coexpress IgG1 and IgM contain RNA that possesses both C_μ and $C_{\gamma 1}$ sequences. Furthermore, disc 3 shows that even the cells expressing IgG1 alone transcribe through C_μ. The lack of signal on discs 5 and 6 indicates that the read-through transcription that allows expression of IgG1 does not continue to C_α. The signal on disc 8 shows that there is transcription through $C_{\gamma 1}$ in IgM^+IgA^+ cells, even though IgG1 protein is not expressed (11). The signal on disc 10 shows that the IgA^+IgM^+ cells contain RNA with both C_μ and C_α sequences. Discs 1, 4, 7, and 9 show that we do not detect transcription past C_μ in the bulk of IgM^+ cells. The vast majority of these cells synthesize IgM alone; the assay does not detect the read-through transcripts produced by the few percent of the cells that synthesize IgM together with an isotype of the secondary response (11). These experiments, however, do not demonstrate the existence of full length transcripts but do show that RNA molecules exist that contain both regions.

The negative controls, discs 11–13, show that the labeled probe does not bind nonspecifically to the filter. The nuclear

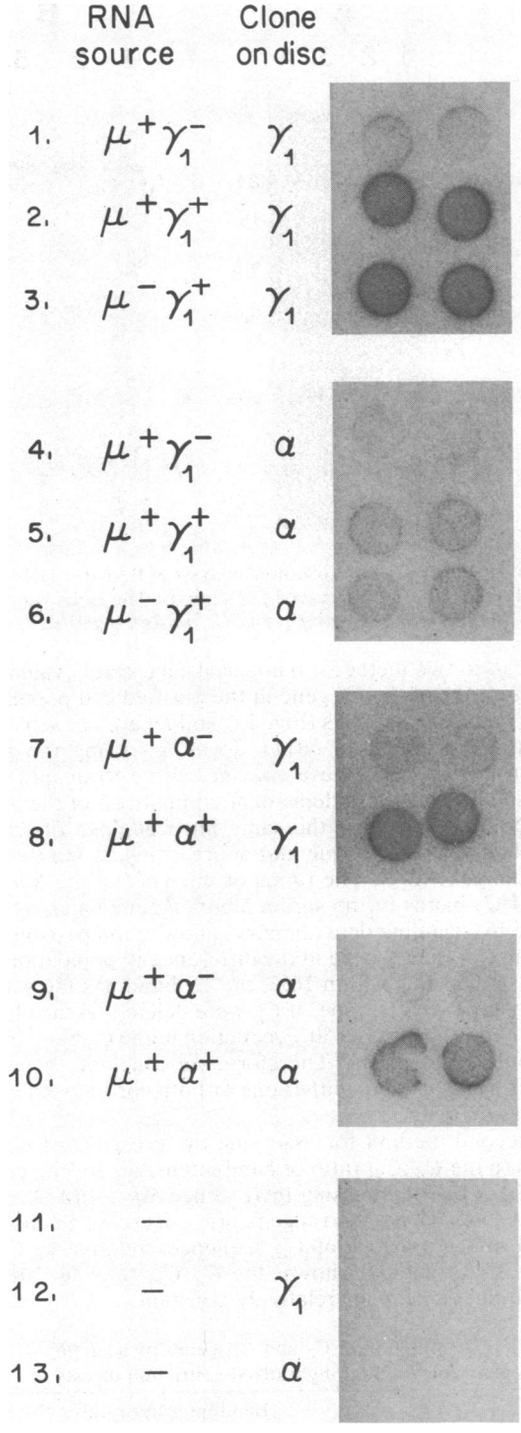

FIG. 4. RNA sandwich disc hybridizations. Nuclear RNA isolated from the sorted cell populations was hybridized to nitrocellulose discs containing either $C_{\gamma 1}$ or C_α. The discs were washed, hybridized to a ^{32}P-labeled single-stranded C_μ probe, washed again, and autoradiographed. The Ig heavy chains expressed in the sorted-cell populations and the types of C_H clone initially bound to the discs are given on the left.

RNA preparation contains DNA, but since no step denatured the double-stranded DNA it did not interfere with the assay. This is proved by the negative results from discs 1, 4, 5, 6, 7, and 9. Could these effects be due to complex formation between mature mRNAs? We think not, because the positive signals on discs 3 and 8 cannot be explained this way.

DISCUSSION

We conclude that the expression of isotypes of the secondary response in memory B cells is controlled entirely at the RNA level. We believe that these cells have altered their RNA termination patterns to generate long transcripts, 120-kb for $\gamma 1$ or 180-kb for α, rather than deleting their DNA in such a way as to bring V_H-D-J_H close to $C_{\gamma 1}$ or C_α.

We have studied the DNA structure in purified populations of memory cells that express either IgM alone, or IgM and IgG1, or IgM and IgA, or IgG1 alone. Of the 1–3% of splenocytes that express IgG1, half express both IgG1 and IgM, while the other half express only IgG1. Almost all the cells that express IgA also express IgM. At the DNA level, we find no evidence that DNA has been deleted or that $S_{\gamma 1}$ has been rearranged in memory cells synthesizing IgG1 either alone or together with IgM. Also, S_α has not been rearranged and DNA has not been deleted in cells expressing IgA together with IgM. To look for transcription through C_μ and into the genes of the secondary response, we used a sandwich hybridization assay to detect whether single RNA molecules have sequences from two heavy chain genes. We demonstrated the existence of single RNA molecules that have both C_μ and $C_{\gamma 1}$ sequences in the cell population that expresses IgG1 alone and in the cell population that coexpresses IgG1 with IgM. Furthermore, RNA molecules exist that have both C_μ and C_α sequences in cell populations that simultaneously express IgA and IgM. However, any RNA that contains the two domains will give a positive signal; we may be detecting smaller splicing intermediates rather than the primary transcript.

Thus, we conclude that, in a cell producing only IgM, termination of transcription occurs after C_μ, but that, in a cell producing both IgM and IgG1, some termination occurs after C_μ and some read-through transcription occurs through $C_{\gamma 1}$. Similarly, IgA would be expressed, in a cell also synthesizing IgM, from a 180-kb V_H–C_α transcript. However, in a cell producing IgG1 but not IgM, we believe that there is a long transcript passing over C_μ from which only $\gamma 1$ mRNA is spliced. The mechanism underlying the coexpression of IgM with an isotype characteristic of the secondary response may be analogous to the changes in RNA termination and splicing that modulate the coexpression of IgM and IgD (23, 24).

While this work was in progress, Yaoita *et al.* (25) reported that IgM^+IgE^+ lymphocytes purified from *Nippostrongylus brasiliensis*-infected SJA/9 mice have not undergone DNA switch recombination. These cells are unable to secrete IgE into the serum because of a T-cell defect in the SJA/9 mouse. They speculate that RNA splicing can modulate the coexpression of IgM together with an isotype characteristic of the secondary response (26).

Switch recombination, which is found in plasmacytoma cells, has been shown to occur in IgG3-producing blasts that form during lipopolysaccharide-stimulated splenocyte cell culture (11). Thus, DNA switch recombination occurs after the resting cells are stimulated. We hypothesize that, in the memory cell, the switch regions may not be accessible to the DNA recombining enzymes, but become so only after transcription through the region "opens" the chromosome. If switch recombination occurs between S_μ and the most 3′ S region that is accessible to the "recombinase," the RNA termination site determines not only the size of the read-through transcript, but also which switch region is used during the subsequent DNA recombination. This mechanism would guarantee that the isotype expressed by alternative RNA termination is the same isotype expressed after DNA switch recombination.

We thank Joan McDowell for cell-sorting expertise; Drs. A. Bothwell and P. Leder for generously providing clones; Vicki Sato for discussions; and George Church, Harry Nick, and Donald Straus for advice on various aspects of this project. This work was supported by National Institutes of Health Grant GM09541-22 and by Biogen.

1. Okumura, K., Julius, M. H., Tsu, T., Herzenberg, L. A. & Herzenberg, L. A. (1976) *Eur. J. Immunol.* **6**, 467–472.
2. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. (1980) *Nature (London)* **286**, 676–683.
3. Early, P., Huang, H., Davis, M., Calame, K. & Hood, L. (1980) *Cell* **19**, 981–992.
4. Alt, F., Bothwell, A. L. M., Knapp, M., Koshland, M. & Baltimore, D. (1980) *Cell* **20**, 293–301.
5. Rogers, J., Early, P., Calame, K., Bond, M., Hood, L. & Wall, R. (1980) *Cell* **20**, 303–312.
6. Kataoka, T., Kawakami, T., Takahashi, N. & Honjo, T. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 919–923.
7. Maki, R., Traunecker, A., Sakano, H., Roeder, W. & Tonegawa, S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2138–2142.
8. Nikaido, T., Yamawaki-Kataoka, Y. & Honjo, T. (1982) *J. Biol. Chem.* **257**, 7322–7329.
9. Rabbitts, T. H., Forster, A., Dunnick, W. & Bentley, D. L. (1980) *Nature (London)* **283**, 351–356.
10. Cory, S. & Adams, J. M. (1980) *Cell* **19**, 37–51.
11. Radbruch, A. & Sablitzky, F. (1983) *EMBO J.* **2**, 1929–1935.
12. Cate, R. L., Chick, W. & Gilbert, W. (1983) *J. Biol. Chem.* **258**, 6645–6652.
13. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
14. Church, G. M. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1991–1995.
15. Wu, C. (1980) *Nature (London)* **286**, 854–860.
16. Laskey, R. A. & Mills, A. P. (1977) *FEBS Lett.* **82**, 314–316.
17. Schibler, U., Marcu, K. B. & Perry, R. P. (1978) *Cell* **15**, 1495–1509.
18. Lomedico, P. T. & Saunders, G. F. (1976) *Nucleic Acids Res.* **3**, 381–391.
19. Denhardt, D. T. (1966) *Biochem. Biophys. Res. Commun.* **23**, 641–646.
20. Dunn, A. R. & Sambrook, J. A. (1980) *Methods Enzymol.* **65**, 468–478.
21. Shimuza, A., Takahashi, N., Yaoita, Y. & Honjo, T. (1982) *Cell* **28**, 499–506.
22. Nottenburg, C. & Weissman, I. L. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 484–488.
23. Maki, R., Roeder, W., Traunecker, A., Sidman, C., Wabl, M., Raschke, W. & Tonegawa, S. (1981) *Cell* **24**, 353–365.
24. Moore, K. W., Rogers, S., Hunkapiller, T., Early, P., Nottenburg, C., Weissman, I., Bazin, H., Wall, R. & Hood, L. E. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1800–1804.
25. Yaoita, Y., Kumagai, Y., Okumura, K. & Honjo, T. (1982) *Nature (London)* **297**, 697–699.
26. Shimizu, A. & Honjo, T. (1984) *Cell* **36**, 801–803.