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Regulation of Immunoglobulin D Synthesis in Murine Neonatal B Lymphocytes

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We analyzed the regulatory basis for the lower expression of immunoglobulin D (IgD) in lymphocytes from neonatal mice of various ages. The results indicate that the relative transcriptional rate of RNA for δ chains is similar to adult levels even in cells which have not started to express IgD. These results suggest that very early after the initiation of μ gene transcription, a defined fraction of polymerases is programmed to progress through the termination site to the δ exons regardless of the developmental stage of the cell. Similar results were obtained from adult CBA/N mice whose spleens contain a large fraction of cells expressing low levels of IgD. On the other hand, the relative steady state level of mRNA in neonatal lymphocytes is approximately half of that in adults, suggesting that there may be differences in the processing or stability of the nascent transcript. In addition, measurements of the in vivo translation rate show that an inefficient δ polypeptide chain processing machinery in neonatal lymphocytes is also an important factor contributing to the reduced expression of IgD.

The appearance of the two major cell surface immunoglobulin isotypes during ontogeny follows a highly regulated pattern. Immunoglobulin M (IgM) is the first and only isotype to be expressed on immature B lymphocytes, while IgD is gradually acquired during the first weeks of life (16, 19, 27). In the mouse, surface IgD does not approximate adult levels until 3 weeks after birth (27). These changes are probably not mediated by alterations at the genomic level since no further DNA rearrangements other than the initial VDJ joining need to occur for the expression of the Cδ gene (6, 9). Previous studies investigating the regulation of IgD synthesis in resting, adult B lymphocytes have shown that the δ gene is transcribed at approximately one-third the rate of the μ gene (25). The relative abundance of intact δ_mmRNA in the cytoplasm is approximately 1/10 of that of μ_mmRNA (24) suggesting that posttranscriptional events regulate the final expression of δ_mmRNA. Despite the lower translational rate of the δ polypeptide, which reflects the relative abundance of the mRNA, the density of cell surface IgD is much higher than that of IgM, due to a longer half life (21). In addition, it was found that the decrease in expression of cell surface IgD after B cell activation results from posttranscriptional events which downregulate the abundance of δ_mmRNA in the cytoplasm (24, 25). We have now investigated the transcriptional and posttranscriptional regulation of δ_mRNA synthesis in the neonatal mouse. The results, which are presented below, show that as in activated lymphocytes the decreased expression of IgD in neonatal lymphocytes does not result from alterations at the transcriptional level. However, posttranscriptional regulation of δ polypeptide synthesis appears to differ for the two differentiative stages. Activated B cells synthesize decreased levels of δ polypeptides due to lower mRNA abundance, while neonatal lymphocytes are less efficient in their ability to process nascent δ polypeptide chains.

CBA/N mice carrying the X-linked immune deficiency gene (XID) has been shown to have a number of defects which are manifested in the absence of a specific B cell subpopulation (1) as well as in the over preponderance of a subpopulation which has a high IgM but low IgD density (4). The molecular basis for the reduced expression of IgD is not known. For this reason, we have also examined the role of transcription in the regulation of this phenotype.

(A preliminary report of part of these findings was published in, Regulation of IgD synthesis in murine neonatal B lymphocytes, p. 353. *In* J. W. Streilein et al. [ed.], Advances in gene technology: molecular biology of the immune system, Cambridge University Press, Melbourne, Australia.)

MATERIALS AND METHODS

Cells. Spleen cells of neonatal mice obtained from matings between BALB/c mice (Cumberland Farms, Clinton, Tenn.) were teased and washed in balanced salt solution. CBA/N mice were originally obtained from the National Institute of Health but maintained in our own colony and used at 4 weeks of age.

Plate separation. Immunoglobulin-bearing or IgD-bearing cells were isolated by the method described by Wysocki and Sato (20) and modified as described previously (25). For purification of immunoglobulin-bearing cells, affinity-purified (AP) rabbit anti-mouse immunoglobulin (RAM immunoglobulin) was used, while a monoclonal anti-δ antibody, H10.4.22 (13), was used for isolation or depletion of IgD-bearing cells. In some experiments, the purified cells were cultured overnight in RPMI (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal calf serum (K.C. Biologicals, Kansas City, Kans.) and 2 mM glutamine.

Immunofluorescence analysis and cell sorting. Cells were stained with biotin-conjugated AP goat anti-mouse immunoglobulin antibodies (GAM immunoglobulin), AP goat anti-δ (GAδ), or AP goat antiovalbumin (GAOva) [all AP antibodies were prepared as described for GAM immunoglobulin (23)], followed by fluoresceinated avidin as previously described (25). Stained cells were analyzed with an Ortho 50HH Cytoflurograph (Ortho Diagnostic Systems, Westwood, Mass.) coupled to a Data General 2150 computer system as described previously (25). Sorting was performed on the FACS III (B-D FACS Systems, Sunnyvale, Calif.) as described (25).

Cell surface iodination, biosynthetic labeling, immunoprecipitation, and SDS-PAGE. Cell surface iodination and biosynthetic labeling of lymphocytes, immunoprecipitation, antibodies used for precipitation, and subsequent sodium

dodecyl sulfate-polyacrylamide gel electrophoretic analysis (SDS-PAGE) of the immunoprecipitates were performed as described previously (21). Before precipitation with specific antibodies, each lysate was first precleared by binding to GAOva and *Staphylococcus aureus*. IgM was then isolated by binding to goat anti-μ (GAμ) and *S. aureus*. Subsequently, the supernatant was incubated with a control antibody (rabbit anti-θX), and the immune complexes were removed with protein A-Sepharose as described previously (26). SDS-PAGE analysis of control antibody complexes revealed no radioactive species migrating in the region of δ or μ polypeptides.

Nuclear run-on transcription. The method used for labeling of nascent RNA chains in vitro was modified from that described by Hofer and Darnell (5) and has been described (25).

RNA extraction. Nuclear RNA was prepared by hot phenol extraction as described (25), while total RNA from lymphocytes was prepared by the LiCl-urea method and fractionated on oligodeoxythymidylate as described (24).

End labeling of RNA. A 2-μg amount of poly(A)$^+$ RNA was hydrolyzed in 0.1 M $NaBO_4$ (pH 9.2) at 65°C for 90 min and subsequently end labeled with [γ^{32}P]ATP (10).

Hybridization and quantitation of hybridization. Labeled RNA was hybridized to DNA probes immobilized on nitrocellulose in Denhardt solution containing 50% formamide, 0.1 mg of herring sperm DNA per ml, 30 μg of polyadenylic acid and polycytidylic acid per ml (Sigma Chemical Co., St. Louis, Mo.) in 5× SSC (17) (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 42°C for 3 to 4 days. Filters were first washed with 0.1× SSC for 2 h at 42°C and then treated with pancreatic RNase at 20 μg/ml and T1 RNase at 20 U/ml (both from Sigma Chemical Co.) in 2× SSC for 1 h at 37°C. Filters were then exposed to autoradiographic film for 3 to 10 days. Relative hybridization to each probe was evaluated by densitometric tracings. Scintillation counting of the most intense bands and trichloroacetic acid precipitation of a sample of the total labeled RNA permitted calculation of the absolute hybridization per base per input counts. Hybridization per base (parts per million) = counts per minute in specific band − counts per minute in control pBR band/size of insert × 1/total input counts per minute.

DNA clones. To avoid potential artifacts due to fragment contamination, all DNA segments used for hybridization analysis were individually cloned. Purified DNA from these plasmids was loaded onto nitrocellulose (Schleicher & Schuell, Keene, N.H.) by using a slot blot apparatus as described previously (25). See figure legends for detailed description of the clones.

RESULTS

Transcriptional level of RNA for δ chains in neonatal lymphocytes. The proportion of IgD-bearing B lymphocytes as well as the density of IgD per cell increases during murine neonatal development (16). This increase is reflected in the amount of radioiodinatable cell surface IgD (Fig. 1). δ chains increase from nondetectable levels to approximately 20% of total cell surface immunoglobulin between day 2 and day 12 of age (16, 19), while the normal adult ratio of IgD to IgM is as high as 2:1. On the other hand, the density of cell surface IgM per cell does not change significantly during this period (16), although the percent of IgM-bearing cells in the spleen does increase rapidly. This accounts for the increasing density of the radiolabeled μ band. To determine whether the progressive increase in cell surface IgD is regulated at the transcriptional level, the relative degree of polymerase loading on the Cμ and Cδ genes was examined by preparing nuclei from splenocytes obtained from neonatal mice of various ages and pulse-labeling in vitro with [^{32}P]UTP. RNA extracted from the labeled nuclei was hybridized to a panel of probes immobilized on nitrocellulose. The relative level of hybridization of the Cδ probes is always 1/4 to 1/2 of that to the Cμ probe regardless of the age of the animal (Fig. 2). This same relative level of hybridization has previously been shown in adult B lymphocytes (25). Therefore, the gradual increase in IgD expression during neonatal development is not a result of increased δ gene transcription.

To ascertain whether the δ gene is transcribed even in cells not expressing IgD, an IgD-negative population from neonatal splenocytes was prepared by sorting on the fluorescence-activated cell sorter. IgD-bearing cells were labeled with biotin-conjugated goat anti-δ, followed by fluoresceinated avidin. The insert in Fig. 3 shows the extent of deletion of IgD$^+$ cells. Post-sort analysis of the negative cells revealed a maximum contamination of 1.5% by IgD$^+$ cells. Nuclei were prepared from the negative cells and labeled in vitro with [^{32}P]UTP, and the hybridization profile of the RNA was compared to that derived from the same number of nuclei obtained from nonsorted cells. Removal of the IgD-bearing cells from day 15 neonates resulted in an 80% decrease in overall transcription of the μ-δ gene complex. These results are consistent with staining analysis (data not shown) which showed that 30% of the total splenocytes stained with an anti-L chain reagent, while only 25% stained with anti-δ. The difference in percent positive cells indicates that at this stage of development only ca. 20% of the total immunoglobulin-bearing lymphocytes express IgM alone. However, despite the lower level of transcription across the μ-δ gene complex, significant above-background hybridization to the δ probes can be detected by densitometer tracing. Therefore, in cells not expressing IgD, approximately the same proportion of polymerases continues through to the Cδ gene without termination 3′ to Cμ.

Transcription of Cδ in CBA/N mice. The cell surface density of IgD on a subpopulation of spleen cells from adult CBA/N mice is relatively low and resembles that of spleen cells from normal neonatal mice (4; Fig. 1, lane D). To determine whether this defect in IgD expression is regulated at the transcriptional level, the relative polymerase loading on the Cμ and Cδ genes in these cells was also examined. As in normal BALB/c mice, Cδ is transcribed at 1/3 the level of Cμ (Fig. 4).

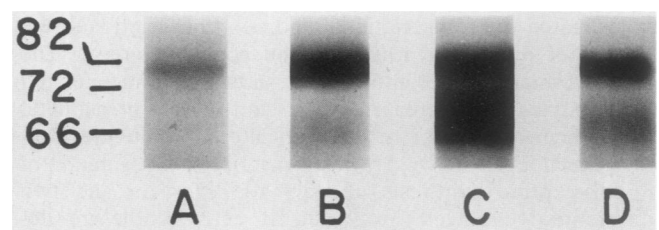

FIG. 1. Comparison of cell surface immunoglobulin between neonatal and adult BALB/c mice and CBA/N mice. Splenocytes from (A) 2 day, (B) 12 day, or (C) 4-week-old BALB/c mice or (D) 4-week-old CBA/N mice were surface labeled with Na[^{125}I] as described in the text, lysed, and immunoprecipitated with RAM immunoglobulin. The immune complexes isolated by adsorption to *S. aureus* were analyzed by SDS-PAGE under reducing conditions. Gels were dried and exposed to x-ray film for 1 day. Only the top part of the developed films showing heavy chains of membrane IgM (82 KDa) and membrane IgD (66 to 72 KDa) are shown.

As in neonatal BALB/c cells, it was important to determine whether this same relative level of transcription occurs in CBA/N splenocytes not expressing IgD. To compare the transcriptional profile of equal numbers of immunoglobulin-bearing cells, a portion of splenocytes were first depleted of

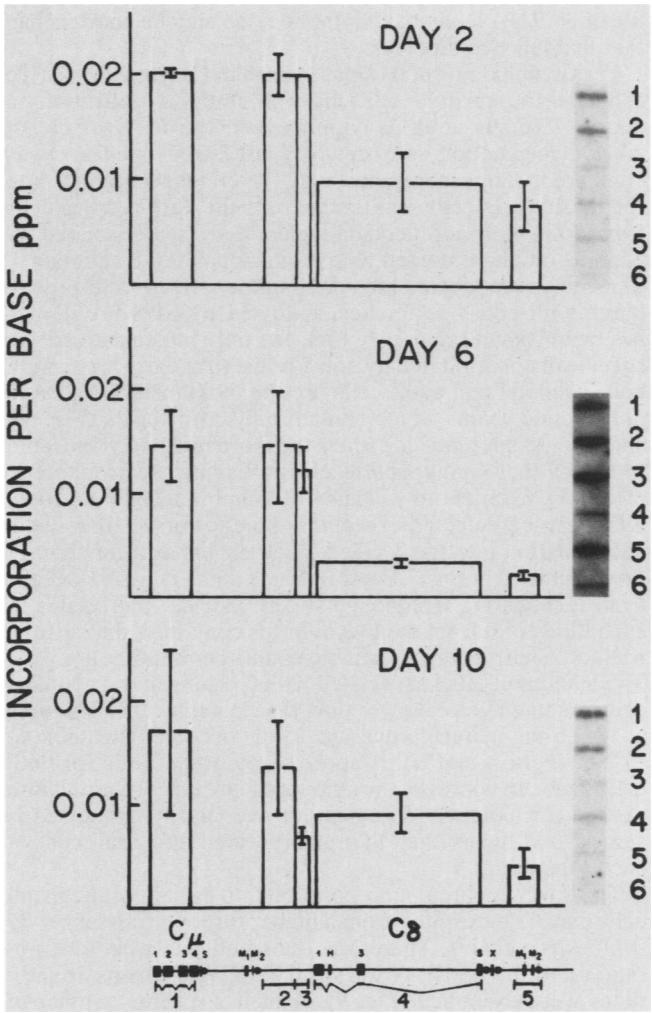

FIG. 2. Transcription of the μ-δ gene complex in neonatal B lymphocytes. Nuclei from 3 × 10^7 to 4 × 10^7 splenocytes from 2-day, 6-day, or 10-day-old BALB/c mice were labeled in vitro with [^{32}P]UTP for 12 min. RNA was extracted and hybridized to the DNA probes shown at the bottom of the figure. Probe 1, A 950-base-pair (bp) cDNA constructed in pMB9 by A-T tailing into the *Eco*RI site. This plasmid, pμ(3741)[9] contains most of the constant region sequences of the μ chain (Tucker and Marcu, unpublished data). Probe 2, A 1,084-bp *Hin*dIII-*Eco*RI genomic fragment comprised of the intervening sequences between Cμ and Cδ genes, subcloned from CH28-257.3 (8) into pBR322. Probe 3, A 447-bp *Eco*RI-*Bgl*II genomic fragment immediately 3′ to probe 7, subcloned from CH28-257.3 into pAT153. Probe 4, A 861-bp cDNA pδ54J (12) tailed into the *Pst*I site of pBR322. This plasmid contains most of the constant region sequence of Cδ, including the carboxyl terminus of the secreted form and 3′-untranslated region. Probe 5, A 1,288-kb *Bam*HI-*Mbo*I genomic fragment, containing the carboxyl terminal δ membrane exons, δM1 and δM2, subcloned from CH28-257.3 into pBR322. Probe 6, pBR322 plasmid with no insert. Relative incorporation per base was determined as described in the text. The results presented represent the average of two experiments for each age.

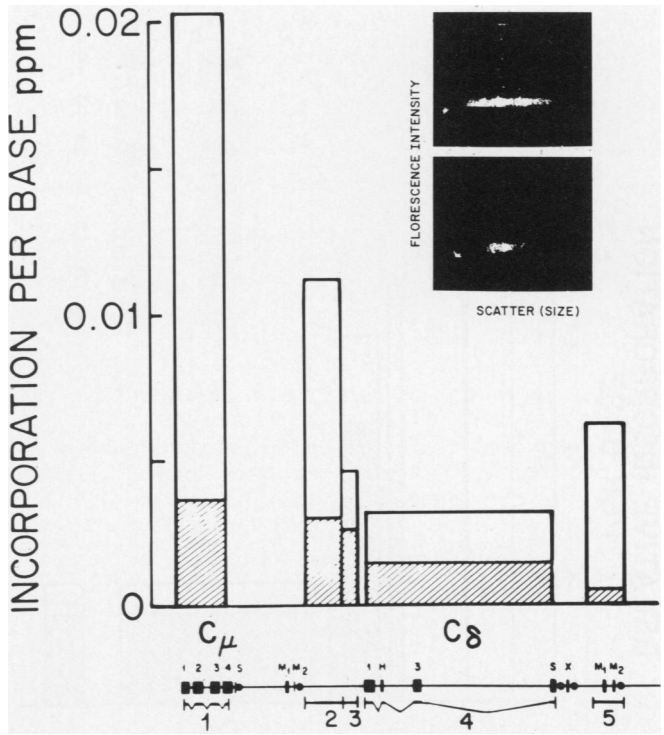

FIG. 3. Transcription of the μ-δ gene complex in neonatal cells depleted of IgD-bearing cells. Neonatal (15-day-old) BALB/C splenocytes were stained with GAδ and sorted on the FACS II as described. The profiles of fluoresceinated cells before (top) and after (bottom) depletion of IgD-positive cells are shown in the insert. Nuclei of 2 × 10^6 cells from the negative cell fraction (shaded bars) and the same number of unfractionated cells (open bars) were prepared and labeled with [^{32}P]UTP, and the RNA was processed for hybridization as described in the legend to Fig. 2. Relative incorporation per base was determined as described in the text.

IgD-bearing cells by panning on anti-δ plates. Immunoglobulin-positive cells (bearing only surface IgM) in the nonbound population were then isolated by binding to plates coated with RAM immunoglobulin. A second population of immunoglobulin-positive cells was prepared by binding to the RAM immunoglobulin-coated plates without previous removal of the IgD-bearing cells. A portion of each of these two populations were surface labeled with Na[^{125}I], lysed, and then immunoprecipitated. The SDS-PAGE analysis of the cell surface immunoglobulin (data not shown) confirms the depletion of cells bearing surface IgD, although cell surface IgM also appears to be decreased in the remaining cells. The amount of radioactivity associated with δ chains was depleted by 80%, while that associated with μ chains was depleted by 50%. The transcriptional profiles of these two populations were, however, nearly identical (Fig. 4), suggesting that the relative density of surface IgD expression is independent of the relative transcriptional level of δRNA.

Relative steady state level of μ and δ mRNA. To determine whether the lower level of IgD expression in neonatal B lymphocytes is due to differences in posttranscriptional events which regulates the final expression of mRNA for δ chains, the steady state abundance of δmRNA and μmRNA in adult and neonatal lymphocytes was compared. This was accomplished by end labeling total poly(A)$^+$ RNA extracted from adult and neonatal cells in vitro with [^{32}P]ATP and hybridizing the RNA to an excess of DNA immobilized on

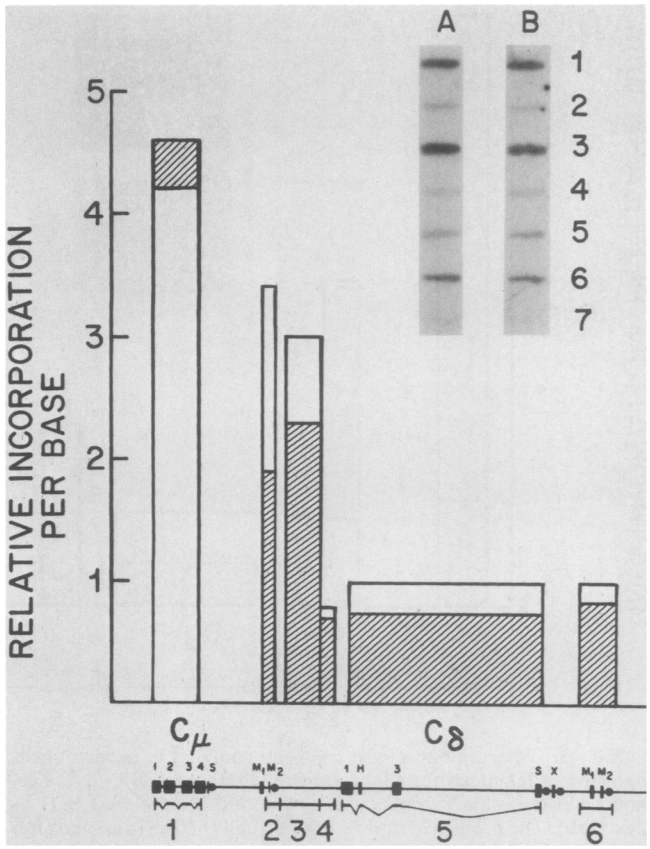

FIG. 4. Comparison of transcriptional levels of IgD$^+$ and IgD$^-$ cells in CBA/N mice. Splenocytes from 3-week-old CBA/N mice were separated into two fractions. Cells were first depleted of IgD-bearing lymphocytes by panning on plates coated with the MA H10.4.22. The remaining immunoglobulin-bearing cells in the nonbound fraction were then isolated by panning on plates coated with RAM immunoglobulin. (A, depicted by open bars) Total immunoglobulin-bearing cells were prepared by directly panning on plates coated with RAM immunoglobulin. (B, depicted by hatched bars). Nuclei from equal numbers of cells of each of the two populations were labeled and assessed for relative polymerase loading of the μ-δ gene complex as described in the legend to Fig. 2. Probes used in this experiment are as follows. Probe 1, A 950-bp cDNA constructed in pMB9 by A-T tailing into the *Eco*RI site. This plasmid, pμ(3741)[9] contains most of the constant region sequences of the μ chain (P. W. Tucker and K. Marcu, unpublished data). Probe 2, A 200-bp *Pst-Hin*cII genomic fragment from BALB/c which contains μ_mII sequences and 3′-untranslated region of the μ_mII subcloned from CH28-257.3 (8) into pUC8. Probe 3, A 1,084-bp *Hin*dIII-*Eco*RI genomic fragment comprised of the intervening sequences between Cμ and Cδ genes, subcloned from CH28-257.3 into pBR322. Probe 4, A 447-bp *Eco*RI-*Bgl*II genomic fragment immediately 3′ to probe 7, subcloned from CH28-257.3 into pAT153. Probe 5, A 861-bp cDNA pδ54J (12) tailed into the *Pst*I site of pBR322. This plasmid contains most of the constant region sequence of Cδ including the carboxyl terminus of the secreted form and 3′ untranslated region. Probe 6, A 1,288-kb *Bam*HI-*Mbo*I genomic fragment, containing the carboxyl terminal δ membrane exons, δM1 and δM2, subcloned from CH28-257.3 into pBR322. Probe 7, pBR322 plasmid with no insert. Relative hybridization was determined by densitometric screening of the autoradiograph.

nitrocellulose filters. This method allows the assessment of the relative steady state abundance of μmRNA versus δmRNA without relying on the use of different probes which may not be labeled to exactly the same specific activities. The relative hybridization to the μm and δm probes is approximately equal for RNA from adult cells, whereas in RNA from neonatal cells, the level of hybridization to the δm probe is only half of that to the μm probe (Fig. 5). These results, which are representative of three independent experiments, suggest that the two messages may not be processed at the same rate in the two cell stages despite the similarity in transcriptional profiles. Alternatively, the half life of δmRNA in neonatal lymphocytes may be shorter than that in adult lymphocytes.

Translational rate of δ chains in neonatal B lymphocytes. To estimate the relative efficiency of δmRNA utilization in neonatal versus adult B lymphocytes, the in vivo rate of label incorporation into newly synthesized δ chains was compared. To ensure that only label incorporation into IgD-positive B cells was compared, the IgD-bearing cells from both adult and neonatal splenocytes were isolated by panning on plates coated with monoclonal anti-δ antibodies. The cells were labeled for various times, lysed, and precipitated with anti-δ antibodies, followed by SDS-PAGE. As has been shown previously (21), the only molecular species specifically precipitated by anti-δ antisera at early times from both neonatal and adult cells are the 59-kilodalton (KDa) δ polypeptide chains which contain only core sugars (Fig. 6A and 6B). At later labeling times the more highly glycosylated forms of the δ polypeptide chains having electrophoretic mobilities identical to δ chains of membrane IgD (66 to 72 KDa) can be detected. To obtain kinetics of labeling, label accumulation into the δ polypeptide chains as a function of time is plotted in Fig. 7A and B. Since equal numbers of cells from IgD-bearing lymphocytes were labeled, the lysates at each time point from the two cultures contained similar total trichloroacetic acid-precipitable counts per minute (Fig. 7C). The amount of label associated with δ chains in the neonatal cells is some fivefold lower than that in adult cells. The time at which the mature processed forms become detectable (1 to 2 h, Fig. 6A and B), is approximately the same for both cell stages. In contrast, the amount of label incorporated into mature μm polypeptide chains of neonatal lymphocytes is some 2-fold higher than that incorporated into adult cells at each time point.

The lower apparent incorporation into δ chains of neonatal cells may have resulted from a higher turnover rate of newly synthesized chains. Therefore, the kinetics of label incorporation into the newly synthesized 59 KDa δ_i chains at early times were examined (Fig. 8). A higher specific activity of labeling was achieved by incubating cells at higher concentrations in the labeling medium. Within the short labeling period, the cells remained viable. However, at these early time points it is difficult to compare absolute incorporation rates of neonatal versus adult cells, since the equilibration rate of the amino acid precursor pools may be different between the two cell types. Nevertheless, it should be valid to compare the shapes of the two kinetics profiles. The relatively high rate of incorporation into neonatal lymphocytes which levels off rapidly suggests that there is indeed rapid turnover of δ_i in these cells. There are two possible reasons for this rapid turnover. (i) Newly synthesized δ chain in neonatal cells are more rapidly processed, or (ii) part of the newly synthesized δ chains is rapidly degraded. The slow kinetics of conversion of newly synthesized molecules into mature δ_m shown in Fig. 7B already suggest that the higher turnover is not due to more rapid processing. This is confirmed by pulse-chase experiments in which neonatal B lymphocytes were labeled with a high concentration of [^{35}S]methionine for 40 min. Subsequently, the cells were

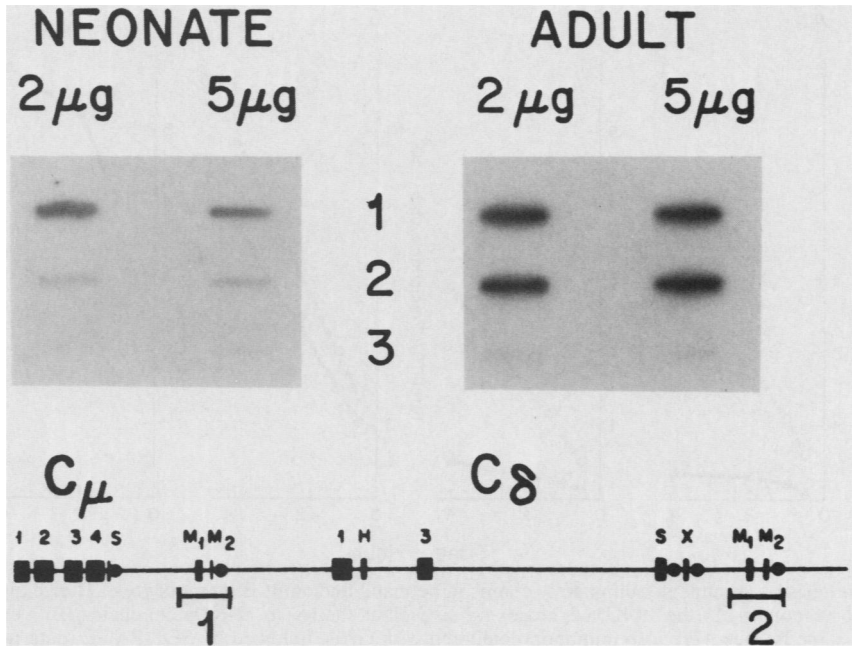

FIG. 5. Relative abundance of μ and δ mRNA in neonatal versus adult mice. A 2-μg amount of poly(A)$^+$ RNA from neonatal or adult splenocytes was end labeled with [γ-^{32}P]ATP as described in the text and hybridized to 2 and 5 μg of plasmid DNA immobilized on nitrocellulose. Probe 1, μm, 917-bp *Kpn-Hin*cII genomic fragment from CH28-257.3 (8) cloned into pUC8. Probe 2, δm, 1,288-bp *Bam*HI-*Mbo*I genomic fragment subcloned from CH28-257.3 (8) into pBR322. Probe 3, pBR322 plasmid with no insert. The blot was subsequently washed with 0.1× SSC at 42°C and exposed to x-ray film. The relative intensity of signals were determined by densitometry. Approximately equal hybridization to both 2 and 5 μg of DNA indicates that the DNA was in excess.

washed and resuspended in culture medium. Figure 6C shows the SDS-PAGE analysis of anti-δ precipitates obtained from cells lysed at various times after removal of label. It is apparent that while some of the nascent (59 KDa) δ_i chains can be chased into the mature 66- to 72-KDa forms, a substantial fraction of the label associated with δ_i at time 0 cannot be accounted for and may be degraded shortly after synthesis.

DISCUSSION

The increase in cell surface density of IgD during B lymphocyte development offers an interesting system for investigating the factors which regulate immunoglobulin gene expression. We have examined the possibilities for transcriptional, posttranscriptional, translational, and post-translational regulation of δ polypeptide synthesis. The first major conclusion which can be derived from these experiments is that very early in B cell development, the relative transcriptional rate of the Cμ versus Cδ gene is fixed, so that even in early B lymphocytes expressing only IgM, the fraction of polymerases which read through the termination site 3′ to Cμ is the same as in adult B lymphocytes. Therefore, the gradual increase in IgD expression during neonatal development cannot be attributed to, for example, a change in efficiency of termination. On the other hand, it is possible that at an earlier stage of B cell development, when only intracellular μ chains are expressed, there is no transcription of the Cδ gene since it has been found that

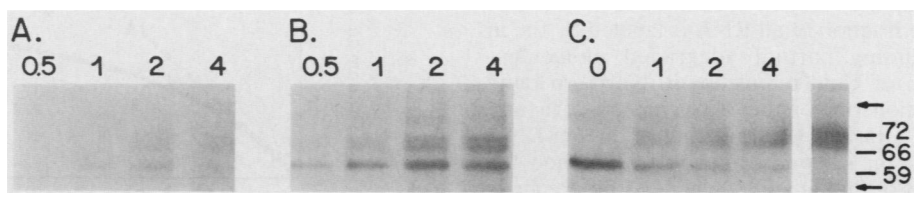

FIG. 6. SDS-PAGE profiles of newly synthesized δ polypeptide chains in neonatal and adult IgD-bearing B lymphocytes. IgD$^+$ cells from splenocytes obtained from 7- to 12-day-old neonatal (A) or adult mice (B) were prepared by panning on plates coated with the MA H10.4.22. The eluted cells were cultured overnight to ascertain that antibodies used for panning which may have remained on the cells are completely modulated. Equal numbers of recovered cells were labeled continuously with 1 mCi of [^{35}S]methionine per ml at 2 × 10^7 cells per ml. Samples were removed at the indicated times and lysed, and subsequent to preclearing, IgD was isolated by binding to RAS and protein A-Sepharose, eluted, and analyzed by SDS-PAGE under reducing conditions. In panel C, neonatal B lymphocytes prepared as above were labeled with 1 mCi of [^{35}S]methionine per ml at 10^8 cells per ml for 40 min. The cells were then washed once with RPMI and reincubated in culture media. Samples were removed at the indicated times as processed as above. Apparent molecular weights were determined by comparison with reduced IgM from iodinated adult B lymphocytes (μ chain = 82 KDa) and [^{35}S]methionine-labeled myeloma IgG (γ chain = 56 KDa) electrophoresed in adjacent lanes (positions indicated by horizontal arrows). δ chains from cell ^{125}I surface-labeled lymphocytes immunoprecipitated with RAδ are shown in the last lane for comparison.

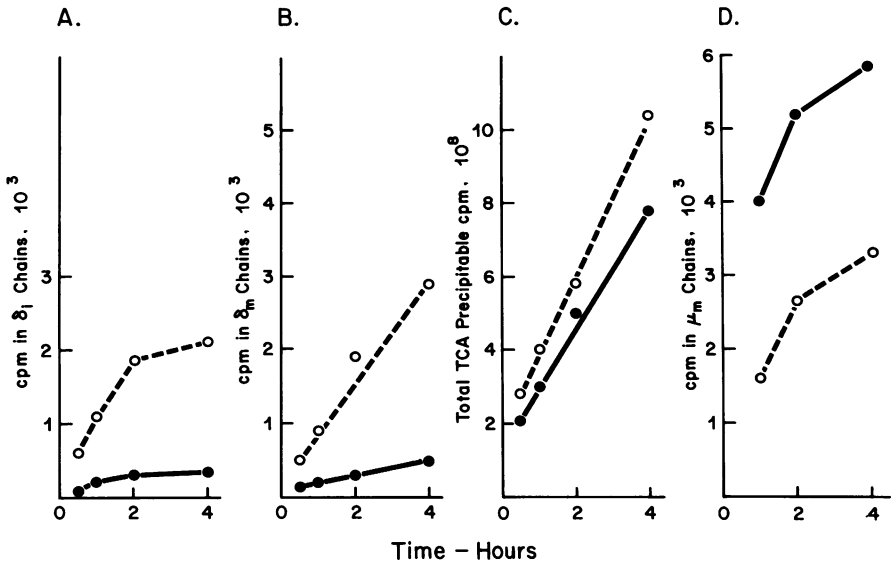

FIG. 7. Kinetics of synthesis of immunoglobulin heavy chains in neonatal and adult B lymphocytes. After exposure of the gels in Fig. 6 to x-ray film, the bands corresponding to the 59 KDa δ_i chains (A) as well as the 66- to 72-KDa δm chains (B) were excised and counted in scintillation cocktail. The same lysates were also immunoprecipitated with GAμ, followed by SDS-PAGE (data not shown) as described in the text. The bands corresponding to μm were excised and counted as above. (D) Closed symbols indicate values from neonatal cells, and open symbols indicate values from adult cells. (C) Total labeled protein contained in each lysate was determined by trichloroacetic acid precipitation of an aliquot at each time point.

polymerases appear to terminate 3′ to Cμ in 70/Z cells (22), a pre-B cell tumor line (14). It is interesting that whatever defects present in CBA/N mice which result in their expressing a lower amount of IgD on their cell surface are also not at the level of δ RNA transcription.

To detect possible posttranscriptional differences in the processing or half life of the δmRNA, the relative abundance of total RNA containing μm and δm sequences in cells from the two developmental stages was also compared. This was done by labeling isolated poly(A)$^+$ RNA of the two cell types in vitro and comparing their hybridization to an excess of μm and δm probes. It should be emphasized that while this method does not allow the comparison to relative mRNA abundance between two populations, it does provide a valid comparison of the ratio of μ/δ mRNA within the two developmental stages. The relative ratio of total (nuclear plus cytoplasmic) μm to δm mRNA in adult cells as determined by this method appears to be higher than the ratio previously determined by Northern blot analysis (δ_m/μ_m = 1/10 [24]). This discrepancy may be accounted for by the fact that a representative fraction of all RNA is labeled by the in vitro method, including partially degraded molecules, whereas comparison of band intensities in Northern blots take into consideration only intact RNA species. In any case, the amount of total RNA hybridizing to the δm DNA probe as compared to the μm probe in neonatal lymphocytes is approximately half of that in adult cells. This difference is not due to a relative increase in the amount of μm mRNA in neonatal lymphocytes as determined by Northern blot analysis (unpublished observations). These results suggest that posttranscriptional processing or stability of RNA for δ chains may be different in immature cells. However, a 2-fold difference in the δmRNA abundance still cannot account for the approximately 10-fold lower level of cell surface IgD expression in neonatal lymphocytes.

In previous experiments investigating the synthesis of μ and δ polypeptide chains in adult B lymphocytes, it was possible to correlate the relative translational efficiencies of the two chains with the abundance of the respective intact message as detected by Northern blot analysis (21). With the assumption that the amino acid precursor pool sizes in adult versus neonatal lymphocytes are not significantly different if time is permitted for equilibration (as evidenced by similar incorporation rates into total trichloroacetic acid-precipit-

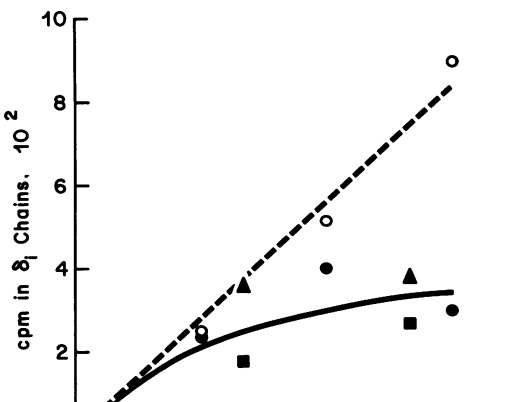

FIG. 8. Short-term kinetics of label incorporation into δ_i of neonatal and adult lymphocytes. Equal numbers of B lymphocytes from neonatal (closed symbols) and adult (open symbols) animals purified on anti-immunoglobulin plates were labeled with 1 mCi of [^{35}S]methionine per ml at 10^8 cells per ml. Samples were removed at the indicated times, lysed, immunoprecipitated, and analyzed by SDS-PAGE under reducing conditions. The 59 KDa δ_i bands were excised and counted as above. The different symbols represent independent experiments.

able material), the labeling kinetics of δ polypeptide chains show that the lower cell surface expression of IgD is due to a lower rate of δm polypeptide accumulation. We have shown by kinetic measurements of the rate of label incorporation into δ_i that the lower accumulation rate of δ_m is due to a lower net translational rate of nascent δ_i polypeptide chains. Thus, even though the relative abundance of δmRNA in neonatal cells is only twofold lower than in adult cells (Fig. 5), the net translation rate of δ_i is at least fivefold lower due to a higher turnover rate of nascent chains (Fig. 7 and 8). The inability to process all of the newly synthesized chains may be a reflection of a less mature glycopeptide processing machinery. It is interesting that processing of μ chains does not appear to be affected. (The significance of the twofold difference in μ_m synthesis rate is not clear since it was not consistently found in all experiments.) Previous experiments have shown that the transit time of IgD through the cell is slower than that of IgM (3, 21), probably due to the fact that the former is more extensively glycosylated (2, 18). In addition, O-linked sugars, postulated to be present on the δ polypeptide chain (18) may require different processing enzymes. It is also of interest to note that studies investigating the translation of IgD in the murine tumor cell line, B1-8, (2, 18) have shown that cell surface IgD cannot be biosynthetically labeled even after extensive labeling periods. An intracellular membrane species as well as the secreted forms are, however, readily labeled. The results imply that these cells may also be defective in processing enzymes for δ_m chains and, together with our findings suggest that the ability of B lymphocytes to process δ_m polypeptides may be regulated by the differentiative stage of the B lymphocytes.

Finally, these studies of neonatal B lymphocytes and CBA/N lymphocytes are completely in line with earlier conclusions derived from investigations of the regulation of membrane immunoglobulin expression in adult lymphocytes (21, 24, 25) which showed that the regulation of the expression of both membrane IgM and IgD occurs at posttranscriptional levels, while the regulation of secreted IgM expression is mediated mainly at the transcriptional level. Studies with other systems may prove that such a dichotomy in regulatory mechanisms is a general phenomenon for cells which synthesize both a mediator and the receptor for it. A case in point, for example, is whether regulation of the IL2 receptor (7, 11) expression occurs via posttranscriptional events, while induction of IL2 secretion (15) is mediated via transcriptional signals.

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