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Immunology

Functional differences between immunoglobulins M and D expressed on the surface of an immature B-cell line

(murine WEHI-231 cells/gene transfer/lymphoid proliferation/gene expression/B-cell tolerance)

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ABSTRACT **Crosslinked IgM molecules expressed on the surface of immature B cells mediate responses that inhibit further development, in contrast to the activational and proliferative events that follow crosslinking of the μ heavy chain in mature B cells. Concomitant with this change in IgM signaling capacity is the appearance of surface IgD, which has been proposed to modulate the response elicited by the μ heavy chain. In an attempt to gain insight into the mechanism(s) by which surface IgM is able to generate such disparate responses, δ heavy chain gene transfectants of the murine B-cell lymphoma line WEHI-231 were established. WEHI-231 cells resemble phenotypically immature B cells, in addition to being highly susceptible to the growth-inhibitory effect of surface IgM crosslinking. Endogenous μ and exogenous δ heavy chains expressed on the surface of the transfectants were compared for their role in cell proliferation and on gene expression. Our results indicate that the growth-inhibitory response is associated only with the μ heavy chain and that surface IgD does not mediate such a response. Furthermore, in contrast to IgM, IgD molecules appear to have an inductive effect on the expression of *Myc* and the endogenous μ and exogenous δ Ig heavy chain genes but not on the expression of the housekeeping gene encoding β_2-microglobulin. These findings suggest that IgM and IgD are functionally distinct when expressed on the surface of an immature B cell.**

One consequence of antigen binding by surface immunoglobulin (sIg) molecules is the triggering of intracellular signals that render the B lymphocyte receptive to subsequent immunoregulatory stimuli (1, 2). The nature of the response generated is dependent on the stage of differentiation of the stimulated B cell. Immature B cells characterized by the expression of sIgM typically undergo an abortive process of differentiation after μ heavy chain crosslinking (3–5). The sIgM molecules transduce signals that ultimately establish a state of unresponsiveness or tolerance to immunoregulatory stimuli needed for the generation of an immune response. This process has been suggested as reflecting an important mechanism for the elimination of autoreactive B cells (5). In contrast, mature resting B cells characterized by the coexpression of sIgM and sIgD undergo an activational event after sIg crosslinking. Both sIgM and sIgD mediate a response that enables the mature B cell to become receptive to stimuli required for further differentiation (6–9). In addition, this activational process is associated with the induction of certain genes such as the murine protooncogene *Myc* which is believed to be associated with the regulation of lymphoid cell proliferation (10).

At the present time, little is known about the biochemical and molecular mechanisms by which sIgM elicits such apparently contrasting responses in murine B lymphocytes. The biochemical events induced by sIgM crosslinking, such as phosphatidylinositol turnover and Ca^{2+} mobilization, appear similar in immature and mature B cells (11–13). Work by Vitetta *et al.* (14) and Scott *et al.* (15) suggested that sIgD expressed by a mature B cell may block potentially "tolerizing" responses (sensitivity to the induction of tolerance) mediated by sIgM. They have demonstrated that the selective removal of IgD from the surface made mature B cells more sensitive to the induction of tolerance *in vitro*. Cambier and Monroe (9) however, have shown that crosslinking of sIgM alone can drive mature resting B cells into cell cycle. Interestingly, these investigators also found that sIgM and sIgD differed somewhat in their capacity to induce cell cycling. Further elucidation of functional differences between these two heavy chain isotypes may provide insight into the mechanism(s) utilized by sIgM to mediate tolerizing or activational responses.

The murine B-cell lymphoma WEHI-231, which resembles phenotypically an immature B cell as it expresses sIgM and low amounts of Ia but no detectable sIgD or Fc receptor (16), has been shown to be exquisitely sensitive to the specific growth-arresting action of anti-μ chain antibody treatment independent of Fc interaction (17, 18). This inhibition of cell proliferation may be similar to the inactivation process that occurs in normal immature B cells after anti-Ig antibody stimulation. Using established recombinant DNA and gene transfer techniques (19), we have transferred a δ heavy chain gene into the WEHI-231 cell line. These transfectants, expressing endogenous sIgM and exogenous sIgD provide an excellent model system to determine whether the growth inhibitory process associated with sIg crosslinking is an unique property of the μ heavy chain or is governed by other properties intrinsic to the immature B-cell stage independent of heavy chain isotype. In turn, potential functional differences between sIgM and sIgD can be assessed. Furthermore, whether the acquisition of sIgD alone is sufficient to overcome the growth-arresting capacity mediated by sIgM or whether additional events are needed to circumvent this response can now be unambiguously ascertained. We now report our findings in which endogenous sIgM and exogenous sIgD expressed in WEHI-231 transfectants were compared with regard to their effect on cellular proliferation and on gene expression after sIg isotype-specific crosslinking. We show here that the μ and δ heavy chains are functionally distinct by the above criteria.

MATERIALS AND METHODS

Cell Line. The murine B-cell lymphoma line WEHI-231 expressing sIgM was obtained from the American Type Culture Collection. Cells were maintained in Dulbecco's

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Abbreviations: $[Ca^{2+}]_i$, cytosolic Ca^{2+}; LPS, lipopolysaccharide; sIg, surface immunoglobulin; PMA, phorbol 12-myristate 13-acetate; TNP, 2,4,6-trinitrophenyl; V, variable; V_H, V region of heavy chain.

modified Eagle's medium (DME medium) containing 10% fetal bovine serum.

Antibodies. Affinity purified $F(ab')_2$ goat anti-mouse μ chain antibody (Cappel Laboratories, West Chester, PA), rabbit anti-mouse δ chain antibody (Nordic Immunological Laboratories, El Toro, CA), and goat anti-mouse γ-chain antibody (Tago, Burlingame, CA), either fluorescein-conjugated or nonconjugated, were utilized for these studies. The B-cell hybridoma anti-Sp6, producing antibody (anti-Sp6) against the Ig variable (V) heavy chain region (V_H) specific for 2,4,6-trinitrophenyl (TNP) hapten (V_{HTNP}), was a gift from G. Köhler (Max-Planck-Institute, Freiburg, F.R.G.).

Vector and Gene Transfer. The vector $pRC\delta\kappa_{TNP}$ was utilized for establishment of sIgD-expressing transfectants. In brief, the rearranged δ_{TNP} chain and κ_{TNP} chain genes specific for TNP (19) were inserted into the vector pSV2-neo that confers resistance to the antibiotic G418. Electroporation with a Bio-Rad Gene Pulsor was the method of choice to transfect WEHI-231 cells with the δ chain-carrying construct.

Flow Cytometric Analysis. Cells (2×10^5) were incubated for 30 min with fluorescein-conjugated anti-μ or anti-δ chain or biotinylated anti-idiotypic (anti-Sp6) antibodies. After being washed in phosphate-buffered saline containing 0.5% fetal bovine serum, cells that had been treated with the biotinylated antibody were incubated for 30 min with fluorescein-conjugated avidin (Sigma). Cells were then washed and analyzed with an EPICS-C Coulter flow microfluorimeter on a logarithmically amplified scale.

Assay for Cellular Proliferation. B-cell transfectants (2×10^4) were cultured for 24 hr in 96-well plates with various amounts of anti-μ or anti-δ chain or anti-idiotypic (anti-Sp6) antibodies in 0.2 ml of medium. Proliferation was determined by incorporation of [*methyl*-^{3}H]thymidine (1 μCi per well, 25 Ci/mmol, Amersham; 1 Ci = 37 GBq). After 4 hr of incubation, cells were harvested, and levels of incorporated [^{3}H]thymidine were measured.

Measurement of Phosphoinositol Production. Accumulation of inositolphospholipids was determined by incubating cells at 37°C in inositol-free medium supplemented with *myo*-[2-^{3}H]inositol (15 Ci/mmol, Amersham). After 16–18 hr of culture, cells were washed and preincubated at 37°C with medium containing 10 mM LiCl for 15 min and then stimulated with 10 μg of the appropriate anti-Ig antibody or with 50 μg of lipopolysaccharide (LPS) (*Escherichia coli* 055:B5W, Difco) per ml or with 10 nM phorbol 12-myristate 13-acetate (PMA; Sigma). Cell extracts were prepared by treatment with chloroform/methanol, 1:2 (vol/vol). The resulting aqueous phase was applied onto a Dowex IX-8 column (100–200 mesh) (Sigma) in the formate form. After the columns were washed, a stepwise elution of inositol 1-phosphate, inositol 1,4-bisphosphate, and inositol 1,3,5-trisphosphate with formate was carried out as described (20).

Measurement of Cytosolic Ca^{2+} ($[Ca^{2+}]_i$). Changes in $[Ca^{2+}]_i$ were assessed by loading $2–3 \times 10^7$ cells with Indo-1. After an incubation with 2 μM of the acetoxymethyl ester for 30 min at 37°C, cells were washed and suspended in Ca^{2+}-containing buffer (140 mM NaCl/3 mM KCl/1.8 mM $CaCl_2$/1 mM $MgCl_2$/10 mM glucose/10 mM Tris-Hepes, pH 7.4). Once a baseline for $[Ca^{2+}]_i$ had been established, anti-Ig antibodies were added and Ca^{2+} concentration was determined as described (20).

Assessment of Gene Expression. Cells (10^7) were stimulated with 5 μg of anti-μ chain or anti-idiotypic (anti-Sp6) antibodies per ml for the indicated times. Total cytoplasmic RNA was isolated as described (21). RNA was either fractionated on 1% agarose gels (20 μg) and transferred to GeneScreen-*Plus* membranes (DuPont) or directly blotted (10 μg) onto the support membrane. Hybridization with the appropriate radiolabeled DNA probes were performed as described (22). The same RNA blot was utilized for each probe.

RESULTS

Establishment of δ Heavy Chain-Expressing Transformants. WEHI-231 cells were transfected with a δ heavy chain gene- and *neo*-carrying construct $pRC\delta\kappa_{TNP}$ by electroporation. After selection with the antibiotic G418, resistant clones were screened for sIgD expression by flow cytometric analysis with fluorescein-conjugated rabbit anti-mouse δ chain antibody or a biotinylated anti-idiotypic antibody, anti-Sp6. Anti-Sp6 recognizes a determinant specific for TNP found in the V_H region, independent of a TNP-specific light chain. Fig. 1 illustrates the reactivity of a representative sIgD clone WEHIδ with the anti-δ chain and anti-idiotypic antibodies. sIgM expression on the WEHIδ transfectant was equivalent to levels found on the parental WEHI-231 cells as assessed by the binding of fluorescein-conjugated goat anti-mouse μ heavy chain antibody (Fig. 1). Immunoprecipitation of intracellular [^{35}S]methionine-labeled lysates by an anti-κ light chain antibody showed that equivalent amounts of endogenous μ (76 kDa) and δ_{TNP} (61 kDa) chains were expressed by the WEHIδ transfectant that comigrated with Ig chains expressed by the Sp603 hybridoma (μ_{TNP}, κ_{TNP}) and B18δ.1 hybridoma (δ, λ) respectively.

Growth Arrest Seen in WEHIδ Cells After sIgM but Not After sIgD Crosslinking. WEHI-231 cells have been shown (17) to be highly susceptible to the growth inhibition induced by sIgM crosslinking. We wanted to ascertain whether crosslinking of the transferred δ heavy chain molecules could induce a similar growth-arrest process in the WEHIδ transfectants. After 24 hr of anti-μ chain antibody treatment, a marked inhibition in proliferation as measured by [^{3}H]thymidine uptake was observed in the WEHIδ cells (Fig. 2). In contrast, when the WEHIδ transfectants were stimulated with anti-Sp6 antibody, cell proliferation was not significantly affected, even at antibody concentrations as high as 40 μg/ml (Fig. 2). Proliferation of the WEHIδ cells was similarly unaffected after the addition of anti-δ chain antibody. In one representative experiment, untreated WEHIδ cells exhibited a [^{3}H]thymidine uptake of 74,500 ± 2500 cpm after 24 hr,

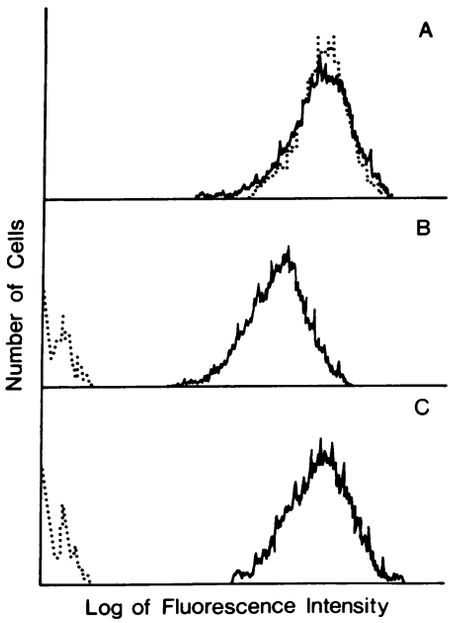

FIG. 1. Detection of surface μ and δ_{TNP} heavy chains on WEHIδ cells by flow cytometric analysis. WEHIδ transfectant (solid line) and WEHI-231 parental (broken line) cells were incubated with fluorescent anti-μ chain antibody (*A*), fluorescent anti-δ chain antibody (*B*), and biotinylated anti-idiotypic Sp6 antibody and fluorescent avidin (*C*).

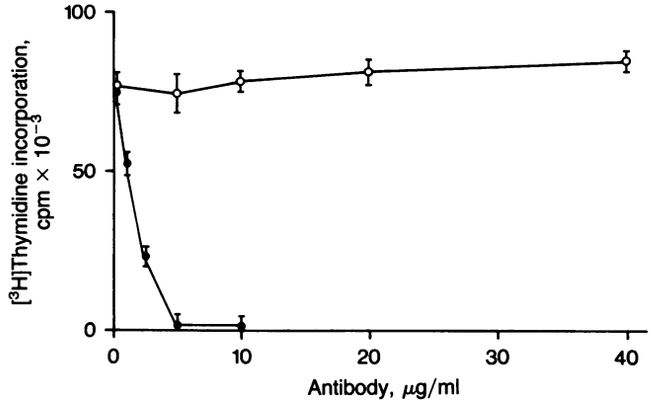

FIG. 2. Proliferation of WEHIδ cells after anti-μ chain or anti-idiotypic Sp6 antibody treatment. WEHIδ cells (2×10^4) were cultured for 24 hr with either anti-μ chain (●) or anti-idiotypic Sp6 (○) antibody. Cells were harvested after a 4-hr incubation with [^{3}H]thymidine. Each data point represents an average of triplicate cultures.

whereas cells treated with 50 μg of anti-δ chain antibody per ml demonstrated an uptake of 82,700 ± 3900 cpm. To determine whether crosslinking of exogenous sIgD could block the growth arrest mediated by sIgM receptors, WEHIδ cells were treated simultaneously with 40 μg of anti-Sp6 per ml and various amounts of anti-μ chain antibodies. The growth-arresting capacity of sIgM was not altered by concomitant crosslinking of sIgD (Table 1). Similar results were obtained when WEHIδ cells were treated with an anti-κ chain antibody. The above results suggest that unlike the endogenous sIgM molecules expressed by WEHIδ, sIgD cannot mediate a growth-arresting response upon crosslinking. In addition, crosslinking of sIgD is unable to reverse the growth arrest induced by anti-μ chain antibody treatment.

Biochemical Signaling Capacity of the Exogenous sIgD Molecules. The lack of a noticeable effect on the proliferation of WEHIδ cells after sIgD crosslinking may reflect an inability of the transferred δ heavy chain to properly integrate into the cytoskeleton and thereby disrupt the signaling capacity of the receptor. To rule out this possibility, we assessed the capacity of the δ heavy chain to induce phosphatidylinositol turnover and changes in $[Ca^{2+}]_i$, events known to be associated with sIg signal transduction (23). The addition of 10 μg of anti-Sp6 or anti-δ chain antibody per ml to the culture resulted in a marked increase in the accumulation of inositol phosphates after a 20-min incubation. The increases were similar to those seen when WEHIδ cells were stimulated with 10 μg of anti-μ chain antibody per ml (Table 2). No significant differences were observed in inositol phosphate accumulation between sIgM and sIgD crosslinking at earlier time intervals measured. Inositol phosphate production was not increased when the WEHIδ transformants were treated with an anti-γ chain antibody or with the polyclonal stimulators LPS and PMA (Table 2). As expected from the observed inositol phosphate accumulation, a distinct increase in $[Ca^{2+}]_i$ was observed after anti-Sp6 antibody stimulation of the WEHIδ cells (Fig. 3). The extent of $[Ca^{2+}]_i$ rise differed only marginally from that exhibited when anti-μ chain antibody was used as a stimulant, although slight differences appeared to exist in the kinetics and the final concentration of $[Ca^{2+}]_i$ (Fig. 3) of the two responses. Whether these differences reflect true kinetic properties of the two sIg receptors or are attributable to differing affinities of the antibodies used is unknown. When WEHIδ cells were treated with 10 μg of anti-δ chain antibody per ml, a peak concentration of 750 nM Ca^{2+} was observed after subtracting the resting cell Ca^{2+} concentration. Accumulation of inositol phosphates or changes in $[Ca^{2+}]_i$ were not observed when the parental WEHI-231 cells were treated with the anti-Sp6 or anti-δ chain antibodies (data not shown). These data indicate that the transferred sIgD expressed by WEHIδ cells can transduce signals associated with sIg molecule crosslinking and cell activation.

Table 1. IgD crosslinking does not block sIgM-induced growth arrest

Antibody treatment, μg/ml		
Anti-μ chain	Anti-Sp6	[^{3}H]Thymidine incorporated, cpm
0	0	85,200 ± 4450
	40	83,770 ± 3180
0.5	0	63,449 ± 2407
	40	66,081 ± 2899
1.0	0	48,292 ± 1320
	40	46,956 ± 2726
2.5	0	19,227 ± 1460
	40	18,746 ± 1146
5.0	0	930 ± 263
	40	1047 ± 304
10.0	0	753 ± 202
	40	889 ± 364

WEHIδ cells (2×10^4) were cultured for 24 hr with the indicated amounts of anti-μ chain or anti-Sp6 antibody. Stimulated cells were incubated with [^{3}H]thymidine for 4 hr and then harvested. Each data point represents an average of triplicate cultures.

Table 2. Inositol phosphate accumulation in WEHIδ cells after sIgM and sIgD crosslinking

Additions	[^{3}H]Inositol phosphates, cpm
Medium	870 ± 211
Anti-μ chain	4608 ± 606
Anti-Sp6	5447 ± 500
Anti-δ chain	4500 ± 390
Anti-γ chain	775 ± 105
LPS	910 ± 27
PMA	875 ± 82

Total inositol phosphate accumulation was measured after 20 min of initial treatment. Anti-Ig antibodies at 10 μg/ml, LPS at 50 μg/ml, and 10 nM PMA were used to stimulate WEHI cells.

sIgM and sIgD Have Differing Effects on Gene Expression. Crosslinking of sIg has previously been shown to induce the expression of various genes, such as the protooncogene *Myc* (10, 24). In view of the differential effects on cell growth after sIgM or sIgD crosslinking and the association of this protooncogene with proliferation, the expression of *Myc* was assessed after WEHIδ cells were stimulated with either

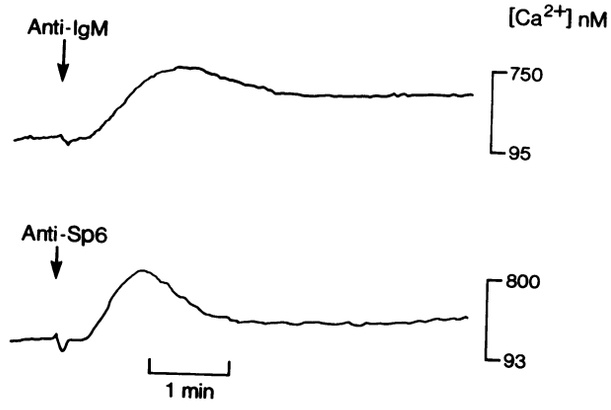

FIG. 3. Changes in $[Ca^{2+}]_i$ in WEHIδ cells after anti-μ chain or anti-idiotypic Sp6 antibody treatment. WEHIδ cells (2×10^7) loaded with the Ca^{2+} indicator Indo-1 were stimulated with 10 μg of either anti-μ chain (anti-IgM) or anti-idiotypic Sp6 antibody per ml.

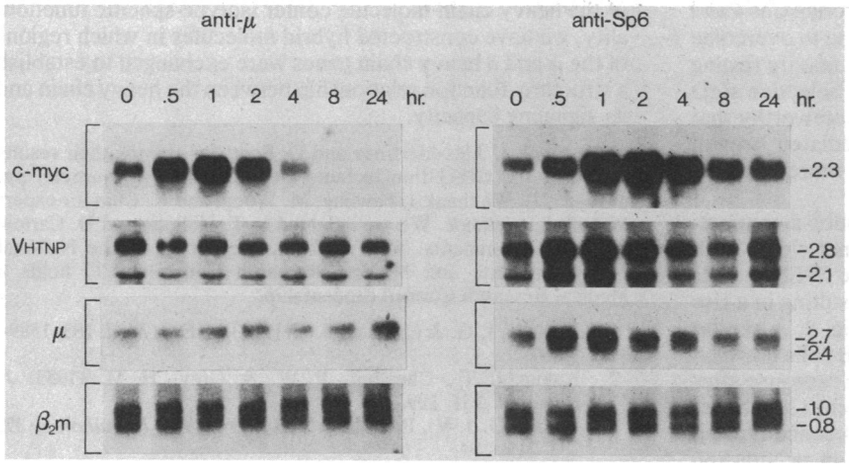

FIG. 4. Analysis of gene expression in WEHIδ cells after anti-μ chain or anti-idiotypic Sp6 antibody treatment. WEHIδ cells (10^7) were cultured with 5μg of either anti-μ chain or anti-idiotypic Sp6 antibody per ml for the indicated times. Size-fractionated (on agarose gel) total cytoplasmic RNA (20 μg per lane) was hybridized with the appropriate DNA probes. The size demarcations on the sides of the blots are in kilobases (kb). The same RNA blot was used for each probe. β_2m, β_2-microglobulin.

anti-μ chain or anti-Sp6 antibodies. The kinetics of expression of Myc mRNA in WEHIδ cells differed markedly between the two antibody treatments (Fig. 4). Crosslinking of endogenous sIgM induced a rapid increase (30 min) in Myc mRNA expression, with maximum levels attained after 1 hr followed by a decline in mRNA to levels lower than that seen in the unstimulated state (4 hr). After 8 hr Myc mRNA expression was almost undetectable. The viability of the cells after anti-μ chain antibody treatment was no less than 95% for each time interval tested, as determined by trypan blue exclusion. WEHIδ cells stimulated with anti-Sp6 antibody also demonstrated a rapid increase in Myc mRNA expression. However, in contrast to cells treated with the anti-μ chain antibody, the amounts of Myc mRNA after 8 and 24 hr were maintained at levels approximately 2-fold greater than that seen in the unstimulated cell, apparently reflecting the proliferative state of the WEHIδ transfectant.

McCormack and associates have demonstrated that crosslinking of sIgM expressed by WEHI-231 cells has no effect on the expression of the μ heavy chain gene (24). Since anti-μ chain and anti-Sp6 antibody treatment elicit such different effects on the expression of Myc mRNA, exogenous and endogenous heavy chain gene expression was assessed in the WEHIδ transfectants after sIgM and sIgD crosslinking. Anti-μ chain antibody treatment resulted in no significant enhancement in the expression of the endogenous μ chain mRNA coding for membrane (2.7 kb)- and secretory (2.4 kb)-type transcripts or in the expression of the transferred V_{HTNP}-encoding δ heavy chain gene (Fig. 4). V_{HTNP}-specific transcripts of 2.8 and 2.1 kb are characteristically expressed by the pRCδκ construct (R.T., unpublished data). When WEHIδ cells were stimulated with anti-Sp6 antibody, a transient enhancement of mRNA encoding endogenous membrane and secretory μ chain and transferred δ heavy chains was observed. A similar kinetic profile was seen for the two heavy chain genes. These results were highly reproducible with either RNA-blot or dot-blot analysis. The expression of the large (1.0 kb) and small (0.8 kb) transcripts encoding β_2-microglobulin showed no marked change after anti-μ chain or anti-Sp6 antibody stimulation. When parental WEHI-231 cells were treated with anti-μ chain antibody, expression of *Myc* and endogenous μ chain-specific mRNA was comparable to that seen when the WEHIδ transfectant cells were similarly treated (Fig. 5). In contrast, WEHI-231 cells exhibited no significant response after anti-Sp6 treatment (Fig. 5). These results indicate that sIgD crosslinking can induce a transient enhancement of Ig heavy chain gene expression that is not seen after sIgM crosslinking.

DISCUSSION

Utilizing the murine B-cell lymphoma WEHI-231 as a recipient cell line for an exogenous δ heavy chain gene, we were able to compare the functional role of the resulting sIgD receptor with that of the endogenous sIgM molecule. Our results suggest that marked differences exist between these two heavy chain isotypes with regard to their effect on cellular proliferation. Unlike sIgM, sIgD upon crosslinking is unable to transduce growth-arresting signals in a representative WEHIδ transfectant. Furthermore, this inability to mediate inhibitory signals has also been observed in exogenous sIgD-expressing transfectants established from the murine B-cell lymphoma CH33 (R.T., unpublished data; Alés-Martínez *et al.*, ref. 25). The CH33 line exhibits similar growth-arresting characteristics upon sIgM crosslinking as do the WEHI-231 cells. Taken together these findings suggest that the differences observed between sIgM and sIgD in the WEHIδ transfectant reflect intrinsic disparities in function and are not attributable to cell line-specific characteristics. In turn, the capacity to mediate growth-inhibitory signals appears to be a specific property of the μ heavy chain. This notion is further supported by the finding that exogenous μ_{TNP} heavy chains expressed on a WEHIμ transfectant function analogously to the endogenous molecules (R.T., unpublished data).

The acquisition of sIgD alone was not sufficient to overcome the growth-arrest mediated by sIgM crosslinking (Table 1). It is possible that the addition of lymphokines associated

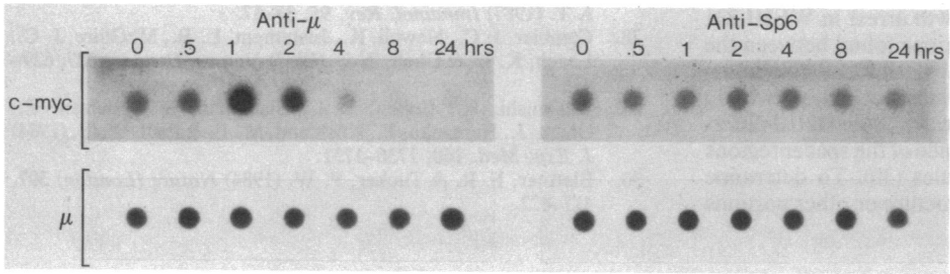

FIG. 5. Analysis of gene expression in parental WEHI-231 cells after anti-μ chain or anti-idiotypic Sp6 antibody treatment. WEHI-231 cells (10^7) were cultured with 5 μg of either anti-μ chain or anti-idiotypic Sp6 antibody per ml for the indicated times. Total cytoplasmic RNA (10 μg) was blotted and hybridized with the appropriate DNA probes. The same RNA blot was used for each probe.

with proliferation or differentiation such as interleukins 4 and 5 are required in concert with sIgD crosslinking to overcome such signals. Indeed, evidence suggests that a mature resting B cell requires helper T-cell interaction upon selective sIgD crosslinking to induce Ig secretion (9). It is noteworthy that LPS can prevent anti-μ chain antibody-mediated growth arrest at early times, possibly by inducing a more differentiated state in the cells (26).

How signals transduced by sIgM mediate such a contrasting response from that observed by sIgD is not clear. Both heavy chain isotypes stimulate signals in the WEHIδ cells through the inositolphospholipid pathway, resulting in a rise in $[Ca^{2+}]_i$ as demonstrated in Table 2 and Fig. 3. A similar observation has been made in mature resting B cells in which sIgM and sIgD both elicit a growth-promoting response after anti-μ chain or anti-δ chain antibody treatment (27). Noteworthy is the difference observed in the final concentration of Ca^{2+} in transfectant cells after anti-μ chain or anti-Sp6 antibody treatment. This difference may reflect, for instance, disparate usage of intra- vs. extracellular Ca^{2+} by the μ and δ heavy chains for the respective induced responses. Furthermore, differences in the kinetics of the signals generated through the inositolphospholipid pathway or even the involvement of other signal transduction pathways (28) may confer the functional properties distinctive of sIgM and sIgD. Fahey and DeFranco have recently shown that WEHI-231 cells generate high levels of inositol 1,3,4,5-tetrakisphosphate, an intermediate in the hydrolysis of inositolphospholipid that has yet to be detected in normal mature B cells (13). Whether crosslinking of IgD on the surface of WEHIδ transfectants also leads to the production of inositol 1,3,4,5-tetrakisphosphate or whether this inositolphospholipid metabolite is unique to sIgM signaling is unknown.

Interestingly, sIgM and sIgD differ markedly with regard to their effect on gene expression. McCormack and associates (24) originally found that anti-μ chain antibody treatment of WEHI-231 cells led to a rapid induction of c-myc mRNA, which declined to levels below that seen prior to stimulation (Fig. 5), reflecting the nonproliferative state of the treated cells. Furthermore, anti-μ chain antibody treatment was shown to have no effect on the expression of μ chain mRNA. Stimulation of the WEHIδ transfectant with anti-μ chain antibody results in an identical response (Fig. 4). In contrast, exogenous sIgD crosslinking leads to enhanced expression of Myc mRNA and a transient induction of endogenous μ chain and transferred δ chain mRNA in the transfectant cells. Whether the elevated levels of Myc mRNA in anti-Sp6-treated WEHIδ cells results in the maintenance of growth or is the consequence of the proliferative state produced by sIgD crosslinking is not clear at the present time. The transient nature of the observed mRNA enhancement for the endogenous μ and exogenous δ chain genes may reflect a requirement for additional factors or events that are either: (*i*) induced by stimuli such as T cell-derived lymphokines that are required for the induction of μ chain-specific mRNA transcripts in normal mature B cells (29) or (*ii*) not expressed because of the immature stage of differentiation WEHIδ cells represent.

It is apparent from our findings that sIgM and sIgD generate qualitatively distinct signals after crosslinking. Whereas sIgD is seen to elicit a stimulatory response, sIgM mediates a response that ultimately leads to growth arrest in WEHI-231 cells. In view of the structural similarities found between the μ and δ heavy chains in the intracytoplasmic tail and transmembrane regions (30), it is not obvious how these two sIg isotypes could differ in function. However, substantial differences do exist in the amino acid sequence of the spacer regions for the μ and δ heavy chain molecules (30). To determine whether this divergence in protein structure or other portions of the heavy chain molecule confer isotype-specific functionality, we have constructed hybrid molecules in which regions of the μ and δ heavy chain genes were exchanged to establish a structure–function relationship between the heavy chain and its signaling capacity.

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