Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 6919–6923, September 1988
Immunology

Immunoglobulins D and M mediate signals that are qualitatively different in B cells with an immature phenotype

(surface immunoglobulin receptors/B-cell lymphomas)

JOSÉ E. ALÉS-MARTÍNEZ*, GARVIN L. WARNER, AND DAVID W. SCOTT

Immunology Unit, Cancer Center, and Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

Communicated by William E. Paul, June 7, 1988

ABSTRACT **The CH family of murine B-cell lymphomas includes several members that are sensitive to growth inhibition when their membrane IgM (mIgM) receptors are crosslinked by anti-μ chain, anti-κ chain, or anti-idiotypic antibodies. These lymphomas are IgM$^+$, Ia$^+$, and IgD$^\pm$ and resemble neonatal B cells in terms of their exquisite sensitivity to anti-IgM-mediated negative signaling as a model for tolerance induction. In this report, we describe the properties of one of these lymphomas, CH33, which had been transfected with a construct containing an allotypically different δ chain constant region and the heavy chain variable region fragment from S107 (T15 idiotype positive). This transfected cell line allowed us to investigate the possibility that membrane IgD (mIgD) and mIgM can mediate different signals. Our results show that the transfected cells retained their exquisite sensitivity to anti-IgM-mediated growth inhibition; however, crosslinking of IgD with anti-δ chain antibody did not inhibit their growth. Furthermore, even prolonged pretreatment with anti-IgD antibodies did not affect cell growth nor did it modulate the inhibitory effects of anti-IgM antibody. Moreover, identical results were obtained with clones of CH33 that express significant amounts of endogenous IgD. Thus, the failure of mIgD to deliver a negative signal does not reflect a defect in the transfected IgD but appears to be a general property of IgD in these cells. The mIgD was shown to mediate transmembrane signals because anti-δ chain treatment resulted in Ca^{2+} mobilization in transfected CH33 cells and capping of those receptors. We conclude that mIgD can mediate qualitatively different signals than mIgM can and that mIgD expression *per se* is not sufficient to change the functional phenotype of immature B cells.**

The physiological role of the expression of membrane IgD (mIgD) during a discrete stage in B-cell maturation remains unclear. It is also still controversial to what extent mIgD and membrane IgM (mIgM) receptors deliver similar or different signals to B cells. Neonatal B lymphocytes, which do not express mIgD (1), are easily rendered unresponsive to specific antigens (2, 3). In addition, these immature B cells are extremely sensitive to functional inhibition by anti-IgM antibodies (4, 5). In contrast, adult B cells coexpressing mIgM and mIgD are more difficult to render sensitive to induction of tolerance and can be stimulated to enter the S phase of the cell cycle by either anti-IgM or anti-IgD antibodies. These data suggested that there is a correlation between the expression of IgD and B-cell development and function.

The CH33 cell line has been used as a model for immature B cells because it expresses mIgM but only low levels of mIgD (6). These cells, like other immature B-cell lymphomas (6), are blocked at the G_1/S interface when exposed to low concentrations of anti-IgM antibodies. We have used a CH33 cell line transfected with an exogenous, allotypically different, δ chain gene construct containing a heavy chain variable region (V_H) of known specificity to study IgD-mediated signaling. In addition, we have examined signaling via IgD in selected spontaneous derivatives of CH33 showing higher expression of endogenous mIgD than in the parental line. Our data suggest that mIgD and mIgM deliver qualitatively different signals to immature B cells and that IgD alone is not sufficient to change the functional phenotype of these cells.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. The characteristics of CH33 cells (donated by Geoffrey Haughton, University of North Carolina, Chapel Hill) have been described (6, 7). Briefly, the lymphoma was induced by a hyperimmunization protocol in B10.*H-2^{a}H-4^b*p/Wts mice and adapted to continuous growth in RPMI 1640 medium (GIBCO) supplemented with 5% (vol/vol) fetal bovine serum (HyClone, Logan, UT), 50 μM 2-mercaptoethanol, 2 mM L-glutamine, 1 mM sodium pyruvate, nonessential amino acids, 10 mM Hepes, and 100 units of penicillin and 100 μg of streptomycin per ml in humidified 7% CO_2/93% air at 37°C. ECH408-1 is a CH33 clone transfected with a δ chain gene construct as described below (provided by C. Webb, W. Born, and P. Tucker, University of Texas Health Science Center, Dallas). Proliferation of all cells was measured by culturing 10^4 cells per well in 96-well flat-bottom microtiter plates (Corning Glass Works, Corning, NY) for 44 hr at 37°C in 0.2 ml of medium containing the appropriate reagents, followed by a 6-hr pulse with 1 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine (Amersham). Cells were then harvested onto glass-fiber filters and assayed in a liquid scintillation counter (Packard Instrument, Downers Grove, IL).

Antibodies. T68.3, a rat IgG_κ monoclonal antibody recognizing the T15 antigen–V_H idiotype was the gift of Matthew Scharff (Albert Einstein College of Medicine, Bronx, NY). AMS 28.1.3 (mouse $IgG2b_\kappa$), an anti-δ chain antibody (allotype *a*); AF3.33 (mouse $IgG2a_\kappa$), an anti-δ chain antibody (allotype *b,e*); AF6.78.25 (mouse $IgG1_\kappa$), an anti-μ chain antibody (allotype *b,d,n*); and AMS 15.1.5 (mouse $IgG2a_\kappa$), an anti-δ chain antibody (allotype *a,e*) were provided by Alan Stall (Stanford University) (8). Monoclonal Ig(5b)6.3 (formerly 11-6.3.1, a mouse $IgG1_\kappa$), an anti-δ chain antibody (allotype *b*); Ig(5a)7.2 (formerly 10-4-22, a mouse $IgG2a_\kappa$), an anti-δ chain antibody (allotype *a*); BET2 (rat $IgG1_\kappa$), an anti-μ chain antibody; and MAR 18.5 (mouse $IgG2a_\kappa$), an anti-rat κ light chain antibody, were obtained from the American Type Culture Collection. Hδ^a/1 (mouse $IgG2b_\kappa$), an anti-δ chain antibody (allotype *a*) in soluble or dextran-bound form, was

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: mIgD and mIgM, membrane IgD and IgM; V_H, heavy chain variable region; $[Ca^{2+}]_i$, cytosolic Ca^{2+}.

*To whom reprint requests should be addressed.

the gift of James Mond, Uniformed Services University, Bethesda, MD.

Flow Cytometry Analysis. B lymphoma cells were incubated for 20 min at 4°C in the presence of saturating amounts of antibody, washed, and stained with fluorescein-conjugated MAR 18.5 (for rat antibodies) or fluorescein-conjugated goat anti-mouse IgG reactive with both murine IgG1 and IgG2 (Southern Biotechnology Associates, Birmingham, AL). Irrelevant antibodies of the appropriate subclasses were always included as negative controls and are indicated. Typically, 10^4 viable cells were collected per histogram by using an EPICS PROFILE (Coulter) flow cytometer, and the data are presented as the relative cell number *versus* the logarithm of fluorescence intensity (256 channels). The mean fluorescence channel in each histogram is indicated in the figure legend.

Cytosolic Free Ca^{2+} ($[Ca^{2+}]_i$) Measurements. Lymphoma cells (5×10^7 cells per ml in Hanks' balanced salt solution, HBSS) were loaded with the acetomethoxy ester form of the Ca^{2+}-binding dye Indo-1 (2 μM, Molecular Probes, Junction City, OR) as described by Bijsterbosch *et al.* (9). Fluorescence was measured with a Perkin–Elmer 350-10S fluorimeter with a P-E 150 xenon power supply (excitation, 340 nm; emission, 390 nm). Individual assays were performed on 2.5×10^6 cells in 1 ml of HBSS (minus phenol red). Samples were allowed to equilibrate for at least 1 min at 37°C prior to the addition of anti-Ig and were monitored continuously for at least 3 min after the addition of anti-Ig (final concentration, 50 μg/ml). Maximal fluorescence was established for each sample by adding 50 μl of 1% Triton X-100; minimal fluorescence was determined by subsequently adding 100 μl of 0.1 M EGTA. Calculations of $[Ca^{2+}]_i$ were made by assuming a K_d of 250 nM for the Ca^{2+}–Indo complex. Typically, the basal, resting level of $[Ca^{2+}]_i$ for each of the cell lines examined was between 70 and 135 nM; peak $[Ca^{2+}]_i$ (241–402 nM) was achieved within the first 40 sec after stimulation with the appropriate anti-Ig (2.5- to 3-fold stimulation). Similar relative increases in $[Ca^{2+}]$ were stimulated with anti-μ and anti-δ chain antibodies (see Fig. 3).

Plasmids and Electroporation. A construct containing the membrane form of a δ chain constant (C) region (allotype *a*) and the T15–V_H idiotypic region from plasmacytoma S107 inserted into a pSV2-gpt plasmid was prepared in the laboratory of Phil Tucker and transfected by electroporation (C. Webb, W. Born, and P. Tucker, personal communications). Several clones expressing T15 antigen/δ chain allotype were isolated by selection in medium containing mycophenolic acid. Since all exhibited similar phenotypic and functional profiles (data not shown), only one (ECH408-1) was selected for subsequent studies. Two additional cell lines were obtained by transfecting CH33 with a [δ chain (*a* allotype) + κ chain] Sp6 (anti-TNP idiotype) construct (10) in N. Hozumi's laboratory (Toronto) and analyzed under the same experimental conditions.

Capping. For capping experiments, 10^6 cells were incubated with saturating doses of anti-IgD (rabbit anti-δ chain antibody from E. Vitetta, University of Texas Health Science Center, Dallas, and from Nordic, El Toro, CA) and kept in culture at 37°C for appropriate periods of time to allow capping before addition of a fluorescein-conjugated secondary antibody (goat anti-rabbit IgG, Southern Biotechnology Associates). Capping was evaluated by counting at least 200 cells per group with a Leitz Diavert inverted UV-light microscope.

RESULTS AND DISCUSSION

Because of its high sensitivity to mIgM-mediated growth inhibition, the CH33 lymphoma provides an excellent model to test whether increased IgD expression changes the func-

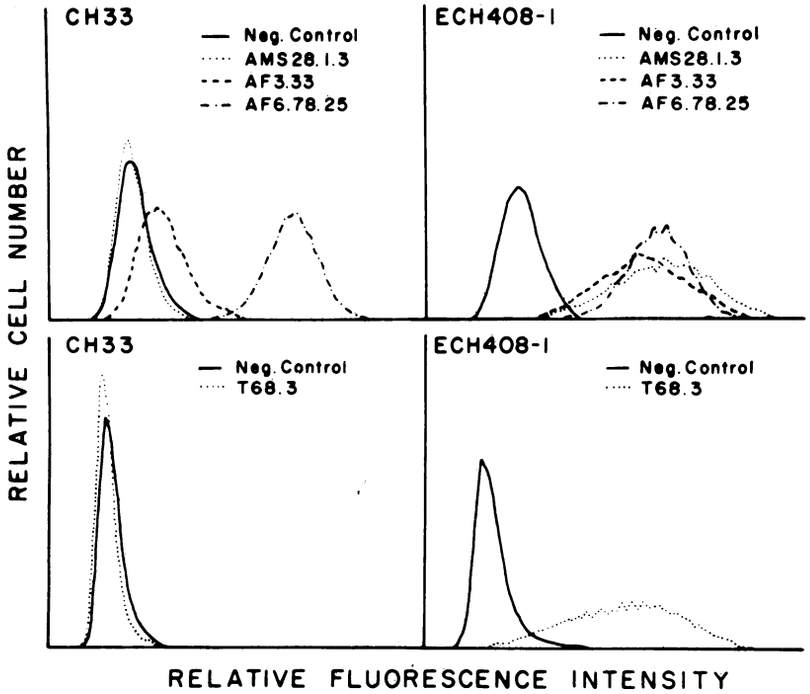

FIG. 1. To evaluate the expression of transfected DNA at the membrane level, 10^6 parental CH33 cells (*Left*) or δ chain gene-transfected ECH408-1 cells (*Right*) were stained with antibodies directed against the transfected δ chain (allotype *a*, AMS 28.1.3), endogenous IgD (allotype *b*, AF3.33), membrane IgM (AF6.78.25), or V_H idiotypic region of the exogenous Ig (T68.3) (*Lower*). Polyclonal mouse IgG (Jackson ImmunoResearch, Avondale, PA) and 7D4 anti-interleukin-2 receptor monoclonal antibody were the negative controls for mouse or rat antibodies, respectively. The mean fluorescence channels for each cell with the indicated antibodies were as follows: CH33 (*Upper Left*), negative control = 40, AMS 28.1.3 = 35, AF3.33 = 55, AF6.78.25 = 114; CH33 (*Lower Left*), negative control = 30, T68.3 = 27; ECH408-1 (*Upper Right*), negative control = 45, AMS 28.1.3 = 117, AF3.33 = 108, AF6.78.25 = 111; ECH408-1 (*Lower Right*), negative control = 33, T68.3 = 102.

tional phenotype of immature B cells and whether mIgD mediates the same signals as those mediated by mIgM. Therefore, we have analyzed a CH33 clone transfected with a δ chain gene construct that expresses idiotypically distinct IgM and IgD receptors. The surface phenotype of clone ECH408-1 is shown in Fig. 1. ECH408-1 cells displayed similar expression of both the transfected (and endogenous) IgD and the endogenous mIgM (Fig. 1 *Upper Right*), whereas the original parental CH33 cells displayed little surface IgD (Fig. 1 *Upper Left*). Increased expression of endogenous IgD has been observed in other transfectants, including recipients of μ chain gene as well as δ chain gene constructs (J.E.A.-M., unpublished data). Whether this enhanced expression of endogenous IgD is the result of selection of high IgD-expressing cells or an effect of the transfected construct is not known. The presence of the T15 idiotype was detected only on those cells expressing the transfected allotype (Fig. 1 *Lower*).

Low concentrations of polyclonal or monoclonal antibodies reacting with different domains of the IgM receptor readily inhibit the growth of CH33 cells (6). Therefore, we asked whether the transfected cells would exhibit the same sensitivity to negative signaling by anti-μ chain antibody. δ chain-transfected cells showed the same dose–response (in terms of growth inhibition) by a monoclonal anti-μ chain antibody, BET-2 (Fig. 2*A*). This result excludes nonspecific alterations in anti-IgM sensitivity caused by transfection maneuvers and excludes as well a "trans" effect of higher levels of IgD on the functional phenotype of the cells.

Next, we tested the ability of mIgD *per se* to deliver an inhibitory signal. The experiment shown in Fig. 2*B* illustrates the lack of inhibitory activity of anti-δ chain antibodies on clone ECH408-1. Identical results were obtained in replicate experiments with several monoclonal antibodies (see *Materials and Methods*) that varied in affinity for IgD, crosslinking ability, and intrinsic stimulatory activity for normal B cells (8); with polyclonal rabbit anti-mouse δ chain antibody (which can crosslink endogenous and transfected IgD); and with an anti-T15–V_H antibody recognizing the idiotype on transfected δ chain (Fig. 1 and unpublished data). No significant reduction in thymidine uptake was detectable with concentrations of these antibodies ranging from 0.01 to 100 μg/ml. Coupling of anti-δ chain antibodies to dextran to cause more extensive cross-linking also did not result in growth inhibition (data not shown). We have recently confirmed these observations in two CH33 derivatives transfected with a different construct (carrying the V_H region of Sp6, an anti-TNP idiotype).

To determine whether cross-linking of IgD (while not delivering a negative signal *per se*) might alter subsequent responsiveness to anti-IgM, we pretreated ECH408-1 cells with anti-δ chain reagents that could react with either the transfected or the endogenous IgD allotype. ECH408-1 cells were incubated for 24 hr with either monoclonal anti-δ chain antibody allotype (or a mixture of both antibodies), washed, and then exposed to anti-μ chain antibody in the absence or presence of additional anti-δ chain antibody. Pretreatment with anti-δ chain antibody did not alter functional signaling via IgM because the dose–response curves in the presence or absence of anti-δ chain antibody were virtually identical whether the antibodies used recognized transfected or endogenous IgD allotype (or both) (Fig. 2 *C* and *D*). This experi-

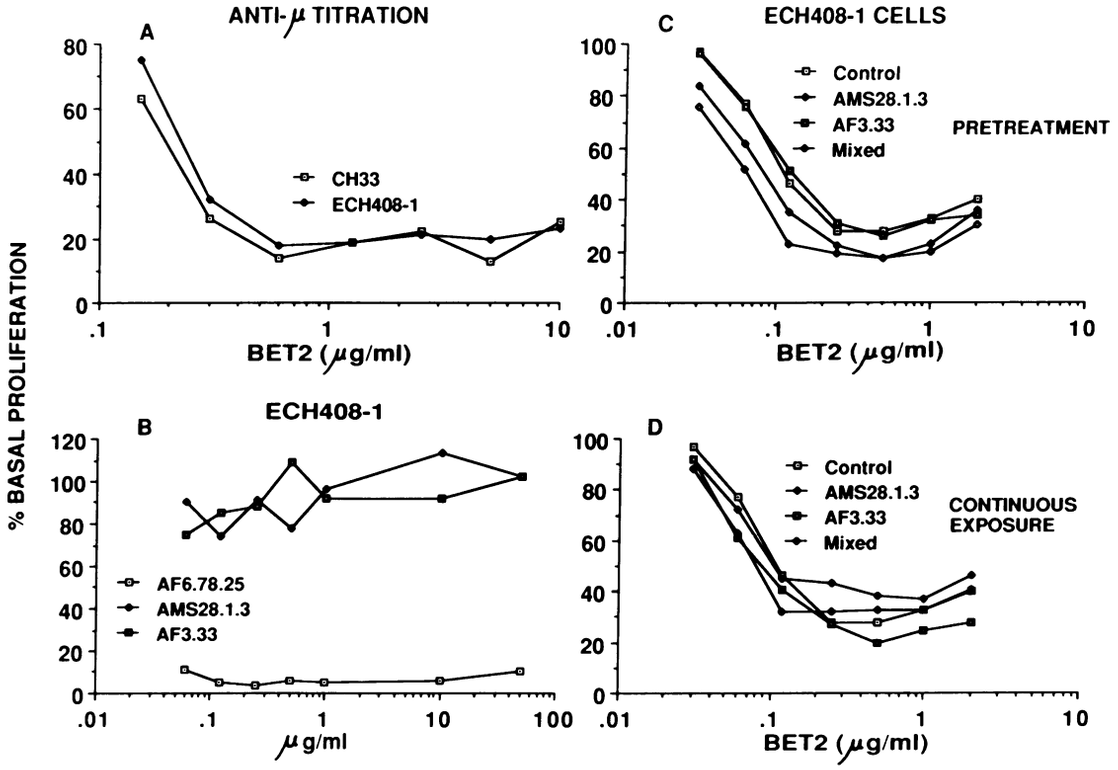

FIG. 2. (*A*) ECH408-1 or parental cells (10^4) per well were incubated in the presence of indicated amounts of a rat anti-mouse IgM (BET2). Results are expressed as the percentage of basal proliferation in the absence of inhibitory antibody (333,783 cpm for CH33 and 307,172 cpm for ECH408-1). (*B*) ECH408-1 cells become inhibited when exposed to an anti-μ chain antibody (AF6.78.25) but not to antibodies directed against endogenous (*b* allotype, AF3.33 antibody) or transfected δ chain (*a* allotype, AMS28.1.3 antibody). (*C* and *D*) Cells were preincubated 24 hr at a density of 10^5 cells per ml in medium containing 10 μg of anti-δ chain antibody against each allotype per ml or a mixture of them (10 + 10 μg/ml). After extensive washes, they were plated at 10^4 cells per well and titrated against different concentrations of BET2 with (*D*) or without (*C*) further presence of anti-δ chain antibodies. IgD^+ CH33 cells treated as in *C* and *D* showed identical dose–response curves but are not shown for simplicity.

ment has been repeated with recently derived parental CH33 subclones that possess as much mIgD as mIgM with identical results: anti-δ chain treatment did not alter the growth inhibition caused by anti-μ chain (data not shown). Thus, the failure to negatively signal with anti-δ chain antibody does not reflect a defect in the transfected isotype but rather a general property of IgD in these cells.

Interestingly, simultaneous binding or crosslinking of both Ig receptors by using anti-κ chain or anti-F(ab')$_2$ antibodies results in effective growth inhibition of transfected or parental CH33 cells (J.E.A.-M., unpublished data), a result that also suggests that signals delivered by IgD are not able to overcome or prevent growth inhibition by crosslinking of IgM. That is, inhibition is dominant.

Recently, Tisch *et al.* (ref. 11) reported successful transfection of a complete IgD gene (expressing a unique idiotype) into another murine B-cell lymphoma line, WEHI-231. They also found that anti-μ chain antibody inhibited the growth of these transfected cells, but anti-idiotypic antibody reactive with the transfected IgD gene products did not. In addition, V. Udhayakumar and B. Subbarao (personal communication; also ref. 12) have described an IgD$^+$/IgM$^+$ B-cell lymphoma that is growth-inhibited by anti-μ chain but not by anti-δ chain antibody. However, Webb *et al.* (13) have reported that B-cell lymphomas transfected with IgD constructs may show partial sensitivity to anti-idiotypic reagents. At present, it is not possible to reconcile these differences, but it appears that differences in the quantitative expression of IgM and IgD in our respective clones are not responsible.

Lack of a biological response via the δ chain in our cell lines could result from defective cellular processing of the IgD formed by transfected δ chains; we excluded this possibility in two ways. First, similar percentages of ECH408-1 cells showed capping after treatment with monoclonal anti-δ chain antibody (against either allotype) or with anti-Ig (data not shown). Thus, the transfected gene product interacts with the cytoskeleton.

Second, antibodies against the transfected δ chain allotype generated Ca^{2+} fluxes measured by the Ca^{2+}-binding dye, Indo-1, to the same extent as did anti-μ chain antibodies (Fig. 3). This result is consistent with reports of Ca^{2+} fluxes generated in normal B cells by anti-δ chain antibody (ref. 14; G.L.W. and J.E.A.-M., unpublished data). These studies demonstrate that the transfected δ chain gene expressed by our CH33 cells forms a functionally active IgD molecule that can undergo capping and mediate "normal" Ca^{2+} mobilization. Previous studies have shown that Ca^{2+} mobilization stimulated with either anti-μ or anti-δ chain antibody would desensitize normal B cells for elicitation of a Ca^{2+} signal by subsequent stimulation with the other antibody (15). Virtually identical desensitization for an anti-μ chain-mediated Ca^{2+} signal can be duplicated in either ECH408-1 cells or parental CH33 variants (that are IgD$^+$) by short-term pretreatment with anti-δ chain allotypic monoclonal antibodies (this paper and ref. 16). This result provides further evidence that the transfected IgD is functional in ECH408-1 cells.

Nonetheless, it should be noted that crosslinking of mIg not only leads to Ca^{2+} mobilization via the production of inositol trisphosphate but also to the activation and translocation of protein kinase C through its natural ligand, diacylglycerol (reviewed in ref. 14). Monroe (17) and Page and deFranco (18) have suggested that protein kinase C activation as elicited by phorbol esters may play a critical role in growth inhibition of WEHI-231 cells because these tumor promoters can partially mimic growth inhibition of these lymphomas. Why then does the crosslinking of either mIgM or mIgD, resulting in similar increases in $[Ca^{2+}]_i$ (this paper) and the production of inositol phosphates (ref. 11), have such different consequences on B-cell lymphoma growth? A likely explanation is that phorbol esters evoke a much more

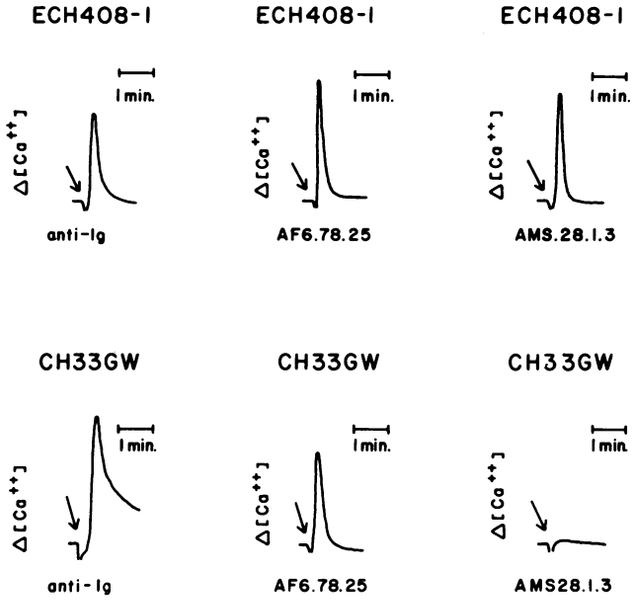

FIG. 3. Stimulation of increased intracellular Ca^{2+} by a 50-μg pulse of anti-μ chain (AF6.78.25) or anti-δ chain (AMS 28.1.3) antibody per ml on CH33 and ECH408-1 cells preloaded with Ca^{2+}-binding dye Indo-1. The abscissa represents time in minutes after addition of the indicated antibody (arrow). The ordinate is the increment in Ca^{2+} concentration in response to the stimulus. $[Ca^{2+}]_i$ rose to approximately 300 nM from a baseline of <100 nM in each case. This is one of three replicate experiments with similar results; note that the increase in $[Ca^{2+}]_i$ elicited by anti-μ or anti-δ chain antibody in ECH408-1 cells was virtually identical and reproducible.

extensive and prolonged protein kinase C activation than does anti-Ig and, furthermore, that the kinetics of phorbol-mediated growth inhibition is quite different from that caused by anti-Ig (19). These results emphasize that the roles of Ca^{2+} and protein kinase C activation in B-cell negative signaling are still unresolved and that the early Ca^{2+} increase elicited by anti-Ig antibodies may not be required for subsequent growth inhibition (20).

In B-cell ontogeny, after an initial δ chain negative stage (21), IgD becomes the predominant Ig receptor in mature resting lymphocytes (22–24). This membrane isotype disappears soon after the cell is activated (25). Such transitory expression implies a role for IgD in B-cell maturation and signaling. However, as noted above, studies in normal and malignant cells have shown that both mIgM and mIgD are similarly effective in inducing early and intermediate activation events such as membrane depolarization, Ca^{2+} fluxes, phosphatidylinositol hydrolysis, protein kinase C activation and translocation, increased Ia expression, blast transformation, transferrin receptor augmentation, and elevation of c-*myc* transcription (reviewed in ref. 14). With appropriate lymphokines, both receptors have been reported to drive B cells along proliferation and/or differentiation pathways and to function in capturing antigen for presentation to T cells with equal competence (8, 26–28). The major functional difference reported between these isotypes is that adult populations of IgD$^-$/IgM$^+$ B cells, obtained by negative or positive selection, can be more easily rendered tolerant than their double-positive counterparts (29–31). Our data suggest that immature B lymphoma cells remain sensitive to negative signaling by anti-μ chain antibodies after transfection of a functional δ chain and that they are not negatively signaled by crosslinking of mIgD. These data are consistent with those of two other laboratories and lead us to conclude that in our cell lines IgM can deliver a signal qualitatively different from IgD and that increased expression of IgD is not sufficient to

modify the functional phenotype of B lymphoma cells. Recent data of Tisch *et al.* (11) demonstrate down-regulation of c-*myc* transcription after anti-μ chain treatment but not after anti-δ chain treatment of their transfectants. This suggests that regulation of oncogene transcription may be a critical point of departure between mIgM and mIgD signaling. The events between initial Ca^{2+} mobilization, inositol phosphate production, and subsequent oncogene activation will be of crucial interest to determine.

We thank Drs. Carol Webb, Willi Born and Phil Tucker for the CH33 δ chain gene transfectants and construct, Dr. John Whitin for his expert advice on $[Ca^{2+}]_i$ measurements, Dr. Helen Quill for critically reading this manuscript, and Ms. Susan Davies for excellent technical assistance. We also thank Dr. N. Hozumi and R. Tisch for informing us of their similar data with IgD gene-transfected WEHI-231 cells (see accompanying article, pp. 6914–6918). This work was supported by U.S. Public Health Service Grant CA 41363 and grants from the Council for Tobacco Research (1840) and the American Cancer Society (IM-495). J.E.A.-M. was supported in part by a fellowship (Exp 86/1374) from Fondo de Investigaciones Sanitarias (FISS), Ministerio de Sanidad, Spain, and a Wilmot Foundation Fellowship; G.L.W. was supported by National Institutes of Health Training Grant T32 CA 09363. This is publication 36 from the Immunology Unit, University of Rochester Cancer Center.

1. Kearney, J. F., Cooper, M. D., Klein, J., Abney, E. R., Parkerhouse, R. M. E. & Lawton, A. R. (1977) *J. Exp. Med.* **146,** 297–301.
2. Raff, M. C., Owen, J. J. T., Cooper, M. D., Lawton, A. R., Megson, M. & Gathings, W. E. (1975) *J. Exp. Med.* **142,** 1052–1064.
3. Lawton, A. R. & Cooper, M. D. (1973) *Contemp. Top. Immunobiol.* **3,** 193–226.
4. Metcalf, E. S., Schrater, A. F. & Klinman, N. R. (1979) *Immunol. Rev.* **43,** 143–183.
5. Nossal, G. J. V., Pike, B. L., Teale, J. M., Layton, J. E., Kay, T. W. & Battye, F. L. (1979) *Immunol. Rev.* **43,** 185–216.
6. Pennell, C. A. & Scott, D. W. (1986) *Eur. J. Immunol.* **16,** 1577–1581.
7. Pennell, C. A., Arnold, L. W., Lutz, P. M., LoCascio, N. J., Willoughby, P. J. & Haughton, G. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3799–3803.
8. Goroff, D. K., Stall, A., Mond, J. J. & Finkelman, F. D. (1986) *J. Immunol.* **136,** 2382–2392.
9. Bijsterbosch, M. K., Rigley, K. P. & Klaus, G. G. B. (1986) *Biochem. Biophys. Res. Commun.* **137,** 500–508.
10. Tisch, R., Watanabe, M., Letarte, M. & Hozumi, N. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3831–3835.
11. Tisch, R., Roifman, C. & Hozumi, N. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6914–6918.
12. Udhayakumar, V. & Subbarao, B. (1988) *J. Cell. Biochem.*, Suppl. 12B, 123 (abstr.).
13. Webb, C., Nikai, C. & Tucker, P. (1988) *J. Cell. Biochem.*, Suppl. 12B, 154 (abstr.).
14. Cambier, J. C. & Ransom, J. T. (1987) *Annu. Rev. Immunol.* **5,** 175–199.
15. Cambier, J. (1987) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **46,** 865 (abstr.).
16. Alés-Martínez, J., Warner, G. & Scott, D. W. (1988) *FASEB J.* **2,** 1467 (abstr.).
17. Monroe, J. (1988) *J. Immunol.* **140,** 1454–1460.
18. Page, D. & deFranco, A. (1988) *J. Immunol.* **140,** 3717–3726.
19. Warner, G. W. & Scott, D. W. (1988) *Cell. Immunol.* **114,** in press.
20. Scott, D. W., Livnat, D., Whitin, J., Dillon, S., Snyderman, R. & Pennell, C. A. (1987) *J. Mol. Cell. Immunol.* **3,** 109–120.
21. Vitetta, E. S., Melcher, U., McWilliams, M., Lamms, M. E., Phillips-Quagliata, J. M. & Uhr, J. W. (1975) *J. Exp. Med.* **141,** 206–215.
22. Hardy, R. R., Hayakawa, K., Haaijman, J. & Herzenberg, L. A. (1982) *Ann. N.Y. Acad. Sci.* **399,** 112–121.
23. Havran, W. L., DiGiusto, D. L. & Cambier, J. C. (1984) *J. Immunol.* **132,** 1712–1716.
24. Lafrenz, D., Teale, J. M. & Strober, S. (1982) in *Immunoglobulin D: Structure and Function*, eds. Thorbecke, G. J. & Leslie, G. A. (N.Y. Acad. Sci., New York), pp. 375–388.
25. Yuan, D. & Tucker, P. W. (1984) *J. Exp. Med.* **160,** 564–583.
26. Isakson, P. C., Krolick, K. A., Uhr, J. W. & Vitetta, E. S. (1980) *J. Immunol.* **125,** 886–892.
27. Pure, E. & Vitetta, E. S. (1980) *J. Immunol.* **125,** 1240–1242.
28. Mizuguchi, J., Tsang, W., Morrison, S. L., Beaven, M. A. & Paul, W. E. (1986) *J. Immunol.* **137,** 2162–2167.
29. Scott, D. W., Layton, J. E. & Nossal, G. J. V. (1977) *J. Exp. Med.* **146,** 1473–1483.
30. Cambier, J. C., Vitetta, E. S., Kettman, J. R., Wetzel, G. & Uhr, J. W. (1977) *J. Exp. Med.* **146,** 107–117.
31. Vitetta, E. S., Cambier, J. C., Ligler, F. S., Kettman, J. R. & Uhr, J. W. (1977) *J. Exp. Med.* **146,** 1804–1808.