Exposure to crosslinked IgD induces receptors for IgD on T cells in vivo and in vitro

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Communicated by Michael Heidelberger, September 16, 1987

ABSTRACT IgD is a surface immunoglobulin, which is coexpressed with IgM on >90% of mature B cells, but its levels in serum are extremely low compared to those of IgM. Its role as a surface receptor has been reemphasized by our recent findings that IgD receptors are induced on helper T cells by exposure to IgD and that such cells have immunomodulating properties. The present study shows that crosslinking of soluble IgD or of monomeric cell surface IgD is required and sufficient for the induction of T cells bearing receptors for IgD, both in vivo and in vitro. Effective IgD crosslinking in this respect can be obtained with antigen or with heterologous and immunogenic as well as nonimmunogenic allotype-specific anti-IgD. These results reinforce the concept that the induction of T cells bearing receptors for IgD is an integral component of the normal immune response.

Recent studies in our laboratory have demonstrated the existence of helper T cells bearing receptors for IgD (T8) (1). This observation grew out of earlier studies in which mice that bore the IgD-secreting TEPC-1017 or TEPC-1033 myeloma were found to generate significantly increased antibody responses to various antigens (2). Investigation of the cellular basis for this immunomodulating effect of IgD revealed that the elevated serum IgD levels in such mice stimulated the appearance of a significantly increased number of T cells that were capable of forming rosettes with IgD-coated sheep erythrocytes (SRBC) (3). Results from cell transfer studies confirmed that T cells exposed to IgD myeloma protein either in vivo or in vitro have a significant immunomodulating effect on antibody production in recipients (3, 4).

Various methods of inducing T8 cells were investigated. It was found that IgD receptors can also be induced in vivo by injection of antigen (1) or lymphokines, including interleukin 2 (IL-2) and interferon γ (5). Exposure of helper T cells to such lymphokines in vitro also causes the expression of IgD receptors (5). In addition, resting T-cell clones of helper phenotype (L3T4+, Lyt-1+, Lyt-2–) grown in the absence of IL-2, responded with increased IgD-receptor expression following overnight exposure to IgD or recombinant IL-2 (5). This effect was also seen when T-cell clones were stimulated with antigen in the absence of exogenously supplied IgD or IL-2, presumably as a result of autocrine secretion of lymphokines in response to antigen (5). Our findings that both T-dependent and T-independent antigens cause a small but significant increase in the frequency of T8 cells within 5 days of antigen injection suggest that crosslinking of B-cell surface IgD by physiological stimuli induces T8 cells. The present studies were carried out to explore further the role of IgD crosslinking in IgD-receptor induction. The findings show that T cells are induced to express receptors for IgD following exposure to IgD aggregated by anti-IgD. In addition, taking advantage of the availability of an IgD-secreting B-cell hybridoma, B1-8.81, we show that soluble monomeric IgD is much less effective as a T8-inducing agent in its native than in an aggregated form, such as in antigen–IgD immune complexes. Furthermore, the results clearly demonstrate that crosslinking of IgD on the surface of B cells is necessary and sufficient for induction of T8 cells in vitro. The biological significance of these findings is discussed.

MATERIALS AND METHODS

Mice. BALB/c and CB6F1 mice were obtained from Charles River Breeding Laboratories. B10.A and C57BL/6 mice were purchased from The Jackson Laboratory.

Antigens. Keyhole limpet hemocyanin was purchased from Schwarz/Mann; Ficoll 400 (Mw ~ 40,000) was from Pharmacia; and trinitrobenzenesulfonic acid was from Sigma. Trinitrophenyl (TNP) conjugates were prepared as described by Little and Eisen (6). SRBC and horse erythrocytes (HRBC) were purchased from Colorado Serum (Denver).

Plaque-Forming Cell (PFC) Assay. Anti-TNP and anti-HRBC splenic PFC were assayed by the slide modification of the technique of Jerne et al. (7). IgG-producing cells were developed with rabbit anti-mouse immunoglobulin in the complement and goat anti-μ in the agar.

Cell Lines. The IgD-secreting plasmacytoma TEPC 1017 (8) was maintained i.p. in pristane-primed BALB/c mice. The IgD-secreting B-cell hybridoma B1-8.81 was the kind gift of Klaus Rajewsky (Institute for Genetics, University of Cologne, Cologne, F.R.G.) (9). The B1-8.81 cell line secretes IgD that is specific for the hapten 4-hydroxy-3-nitrophenyl-acetyl (NP). The B1-8.81 cells were maintained i.p. in CB6F1 mice. The murine IgM*, IgD- B-cell lymphoma CH15 was obtained from G. Haughton (University of North Carolina, Chapel Hill) and was derived from B10.H-2b*H-4 np/ + Wts mice (10) and was maintained i.p. passage in B10.A mice.

Purification of IgD. TEPC-1017-derived IgD and monoclonal B1-8.81 IgD were purified by affinity chromatography on a rabbit anti-IgD-coupled Sepharose 4B column (1, 2).

Antibodies. The following antibodies were generated and purified as previously described: goat anti-mouse IgD, goat anti-mouse IgM (11), H&h/1 (12) (a monoclonal IgG2a derived from a mouse of the b allotype, which is specific for IgD of the a allotype), and rabbit anti-mouse immunoglobulin prepared by immunization with mouse IgG in complete Freund’s adjuvant. The univalent Fab/Fc fragment of H&h/1 was prepared by digestion with elastase (Sigma; enzyme-to-

Abbreviations: T8, helper T cells bearing receptors for IgD; SRBC, sheep erythrocytes; HRBC, horse erythrocytes; RFC, rosette-forming cell(s); PFC, plaque-forming cell(s); NP, 4-hydroxy-3-nitrophenylacetyl; BSA, bovine serum albumin; TNP, trinitrophenyl; IL-2, -4, -12, interleukin 2, 4.
substrate ratio of 1:100) as described (ref. 13 and D. K. Goroff and F. F., unpublished data). The monoclonal rat Ig2a antibody (2.4G2) to mouse B cell and monocyte Fcγ receptor (14) was isolated from ascites fluid by sequential (NH₄)₂SO₄ precipitation, DEAE-cellulose ion-exchange chromatography, and Sephadex G-200 gel filtration (13).

**Purification of Splenic T Cells.** Splenic T cells were depleted of adherent cells by incubation in tissue-culture-grade Petri dishes (1400-1, Nunclon, Roskilde, Denmark) at 37°C. Splenic T cells were then purified by panning adherent-cell-depleted spleen cells on Petri dishes coated with rabbit anti-mouse conjugated goat antibody (2.4G2) (14).

Goroff and completed of adherent cells by incubation in tissue-culture-grade rat CrCl3-coupling method (16). Two-tenths milliliter SRBC (1). Briefly, IgD-coated indicator cells were used in experiment (i.e., two) with combination of IgD molecules, since the failure of the native IgD receptors, in the experiment increases in the IgM and IgG anti-HRBC PFC responses were observed (Table 2, experiment 1). Similarly, when mice were immunized with TNP-keyhole limpet hemocyanin after treatment with TEPC-1017 IgD, their primary antibody response to this antigen was greatly enhanced (Table 2, experiment 3). In contrast, mice pretreated with the B1-8.81-derived IgD, subsequently primed with HRBC, and then challenged with this antigen 10 days later, significant increases in the IgM and IgG anti-HRBC PFC responses were observed (Table 2, experiment 1). Similarly, when mice were immunized with TNP-keyhole limpet hemocyanin after treatment with TEPC-1017 IgD, their primary antibody response to this antigen was greatly enhanced (Table 2, experiment 3). In contrast, mice pretreated with the B1-8.81-derived IgD mounted primary and secondary antibody responses that were not significantly different from those of saline-injected control mice (Table 2). This lack of immunoaugmenting effect was not due to a failure of mice of the a allotype to be affected by IgD of the b allotype, because (a × b)F1 allotype mice were also unaffected by B1-8.81 IgD. Thus, the failure of the native form of B1-8.81 IgD to induce T8 cells correlates with the lack of immunoaugmenting effects of this monoclonal IgD.

**RESULTS AND DISCUSSION**

**Requirement for Antigen Crosslinking of B1-8.61 IgD in the Induction of T8 Cells.** The results in Table 1 show that, in contrast with TEPC-1017 IgD, NP-specific B1-8.81 IgD used at similar concentrations does not induce T8 cells. This is not due to allotype differences in the IgD molecules, since TEPC-1017 induces IgD receptors on T cells from both BALB/c and C57BL/6 mice (i.e., from both a and b allotype strains). Moreover, B1-8.81 IgD is equally inefficient in inducing IgD receptors on cells from both strains. In combination with antigen [NP-bovine serum albumin (BSA)], however, B1-8.81 IgD is as effective as TEPC-1017 IgD in inducing T8 cells. Control monoclonal antibodies of the same specificity as those from B1-8.81 cells but of different isotype (IgM and IgG1), whether used with or without antigen, fail to induce IgD receptors (Table 1, experiments 1 and 2). In additional experiments (not shown), it was found that goat anti-IgD could also be used to crosslink B1-8.81 IgD (in antigen excess) with similar results. By itself, goat anti-IgD did not induce IgD-RFC in purified T cells (2%), whereas in combination with B1-8.61 IgD, 19% IgD-RFC were obtained.

TEPC-1017 IgD has been reported to be secreted as a 82x2 dimer (8), whereas B1-8.61 is probably secreted as a 82A2 monomer (9). This may explain why TEPC-1017 IgD, but not B1-8.81 IgD, induces T8 cells without any further aggregation. It is not surprising that dimeric or oligomeric forms of IgD should be needed for the induction of IgD receptors, since a similar requirement has been described for the induction of Fc receptors for IgE (17) and IgA (18).

**Effects of B1-8.61 IgD on Primary and Secondary Immune Responses.** Since T8 cells mediate the immunoaugmenting effects of IgD from TEPC-1017, we speculated that *in vivo* treatment of mice with B1-8.81 IgD, which fails to induce T8 cells in vitro unless it is crosslinked by antigen, might also fail to cause augmentation of humoral immune responses. The results in Table 2 demonstrate that this is indeed the case. When BALB/c mice were pretreated with TEPC-1017-derived IgD, subsequently primed with HRBC, and then challenged with this antigen 10 days later, significant increases in the IgM and IgG anti-HRBC PFC responses were observed (Table 2, experiment 1). Similarly, when mice were immunized with TNP-keyhole limpet hemocyanin after treatment with TEPC-1017 IgD, their primary antibody response to this antigen was greatly enhanced (Table 2, experiment 3). In contrast, mice pretreated with the B1-8.81-derived IgD mounted primary and secondary antibody responses that were not significantly different from those of saline-injected control mice (Table 2). This lack of immunoaugmenting effect was not due to a failure of mice of the a allotype to be affected by IgD of the b allotype, because (a × b)F1 allotype mice were also unaffected by B1-8.81 IgD. Thus, the failure of the native form of B1-8.81 IgD to induce T8 cells correlates with the lack of immunoaugmenting effects of this monoclonal IgD.

<table>
<thead>
<tr>
<th>Addition to T cells</th>
<th>% IgD-RFC, mean ± SD</th>
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<tbody>
<tr>
<td></td>
<td>Exp. 1; BALB/c</td>
</tr>
<tr>
<td>TEPC-1017 ascites (10%)</td>
<td>30.5 ± 1.4</td>
</tr>
<tr>
<td>TEPC-1017 IgD* (100 μg/ml)</td>
<td>ND</td>
</tr>
<tr>
<td>B1-8.81 ascites (10%)</td>
<td>0</td>
</tr>
<tr>
<td>B1-8.81 IgD* (100 μg/ml)</td>
<td>ND</td>
</tr>
<tr>
<td>S8.15 IgM* (100 μg/ml)</td>
<td>0</td>
</tr>
<tr>
<td>B6.55.1 IgGl* (100 μg/ml)</td>
<td>0</td>
</tr>
<tr>
<td>NP-BSA (50–100 μg/ml)</td>
<td>0</td>
</tr>
<tr>
<td>NP-BSA (500 μg/ml)</td>
<td>ND</td>
</tr>
<tr>
<td>B1-8.61 IgD* and NP-BSA (50–100 μg/ml)</td>
<td>18.5 ± 1.5</td>
</tr>
<tr>
<td>B1-8.61 IgD* and NP-BSA (500 μg/ml)</td>
<td>ND</td>
</tr>
<tr>
<td>S8.15 IgD* and NP-BSA (100 μg/ml)</td>
<td>0</td>
</tr>
<tr>
<td>B6.55.1* and NP-BSA (100 μg/ml)</td>
<td>0</td>
</tr>
</tbody>
</table>

Splenic T cells (2.5 × 10⁶/ml) were incubated with the appropriate additions for 18 hr at 37°C in 5% CO₂, and the percentages of IgD-RFC were determined. The values given represent the percentage of IgD-RFC after exposure to indicated substances minus the percentage of IgD-RFC after exposure to medium only. Subtracted background IgD-RFC varied from 3–7%. ND, not determined.

*Affinity purified, 100 μg/ml. S8.15 and B6.55.1 are monoclonal antibodies to NP of IgM and IgG1 isotypes, respectively.

**Ascites (10% in Eagle’s minimal essential medium) were used in experiments 1 and 2; the final concentration of IgD was 300 μg/ml.**

**Ascites (10%) were used in experiment 2; the final concentration of IgD was 300 μg/ml. Affinity-purified IgD (100 μg/ml) was used in experiment 3.**

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*Immunology: Coico et al.*

In Vivo Induction of Tß Cells by Goat Anti-IgD Antiserum. Our finding that crosslinking of IgD is necessary for induction of Tß cells in vitro suggested that a similar stimulus might also be capable of inducing such cells in vivo. Preliminary support for this hypothesis was provided in previously reported studies (1), which showed that following injection with antigen a small but significant increase in the incidence of Tß cells is observed. In order to confirm if such crosslinking in vivo could also induce Tß cells, we injected mice with affinity-purified goat anti-IgD and followed the kinetics of induction of Tß cells in their spleens. The results show that, 24 hr following injection with goat anti-IgD, there is a significant increase in the frequency of splenic IgD-RFC (Fig. 1). As expected, cell fractionation studies confirmed that the splenic T cells were responsible for rosette formation with the IgD-coated indicator cells. It has been reported that treatment with goat anti-IgD results in polyclonal activation of the B-cell population (11, 19–21). Analysis of the cellular basis for this effect established that such activation is largely due to B-cell stimulation by the combination of anti-IgD and lymphokines produced by T cells, which are responding to the goat immunoglobulin presented to them by the anti-IgD-coated B cells (22). Under such optimal conditions of “antigen” presentation, T cells produce high quantities of lymphokines, including interferon γ and IL-4 (23). This lymphokine response is first detected three days after the injection of the goat anti-IgD (22–24) and is therefore unlikely to be responsible for the early phase of Tß-cell induction, which is maximal within 24 hr after treatment (Fig. 1). However, since previous studies have established that certain lymphokines such as IL-2, interferon γ (5), and IL-4 (25) can also induce Tß cells, it is possible that the continued presence of increased numbers of Tß cells observed beyond day 3 is attributable to the stimulatory effect of such factors.

Induction of Tß by an Allotype-Specific Monoclonal Anti-IgD Antibody. To distinguish further between IgD crosslinking and lymphokine induction as mechanisms of Tß-cell induction by anti-IgD antibodies, the effectiveness of polyclonal goat anti-IgD antibody and a monoclonal antibody specific for IgD of the a allotype, Hßa/1, at inducing Tß cells were compared. Mice of three different strains representing IgD allotypes a (BALB/c), b (C57BL/6) and a × b (CB6F1) were used to examine this question. Goat anti-IgD antibody crosslinks IgD and induces T-cell help, lymphokine production, and polyclonal immunoglobulin production in all three strains used. In contrast, Hßa/1 crosslinks IgD in BALB/c and CB6F1 but not in C57BL/6 mice and induces T-cell activation and polyclonal immunoglobulin production only in the BALB/c mice, presumably because T cells in the CB6F1 (a × b allotype hybrid) mice fail to recognize the b allotypic determinants expressed on the Hßa/1 antibody as foreign (ref. 19; and F.F., unpublished data). As expected, i.v. injection of goat anti-IgD resulted in the appearance of increased numbers of Tß cells in all strains tested (Table 3). However, Hßa/1 induced Tß cells in both the a allotype and (a × b)F1, allotype strains but not in the homozygous b allotype strain. Thus, IgD crosslinking, rather than induction of T-cell activation, is sufficient for Tß induction by anti-IgD antibody.

**Requirements for Crosslinking of IgD in the Induction of Tß Cells in Vivo.** Having established that crosslinking of IgD with Hßa/1 is capable of inducing Tß cells in vivo, we then asked whether a univalent anti-IgD antibody would fail to induce Tß cells. To address the question, we investigated whether a monovalent Fab/Fc fragment of Hßa/1, which consists of a single Fab fragment linked to the Fc fragment by the hinge region (13), could induce Tß in the absence of 2.4G2, an antibody that blocks the B-cell and macrophage IgG2b Fc receptor. This approach was taken because Hßa/1 Fab/Fc has recently been shown to be capable of inducing B cells to increase their surface ßa antigen expression by crosslinking their surface IgD through an FcR, receptor (Fc-R)-dependent mechanism. Antibody 2.4G2, by blocking the IgG2b FcR, blocks Hßa/1 Fab/Fc-induced B-cell surface IgD crosslinking and activation (13). The results in Table 4 show that whereas the Hßa/1 Fab/Fc was active in inducing Tß cells when injected by itself into BALB/c mice, simultaneous injection of Hßa/1 Fab/Fc and 2.4G2 failed to induce Tß cells. The 2.4G2 antibody, which does not prevent IgD crosslinking or B-cell activation by intact Hßa/1, also had no effect on the ability of the intact Hßa/1 antibody to induce Tß cells in vivo. We conclude that induction of IgD receptors using the Fab/Fc fragment of Hßa/1 is mediated by binding of the Fc portion of this univalent antibody to FcR, R* cells, which either crosslinks the Hßa/1 Fab/Fc-bound B-cell surface IgD or mimics the effect of crosslinking IgD on the B cell surface.

**Crosslinking of Surface IgD but not Surface IgM Induces Tß Cells.** Although these studies suggested that direct crosslinking of B-cell surface IgD could induce Tß cells, we could not exclude the possibility that soluble IgD-anti-IgD immune complexes generated in the serum or released from the surface of the B cells might actually mediate the induction of Tß cells. To determine whether crosslinking of IgD on the surface of the B cell is sufficient for optimal induction of IgD
by injection from whole mice and the approach to CH15 anti-goat immunoglobulin anti-IgD Hα/1 (100 μg), Hαa/Fab/Fc (100 μg), 2.4G2 (500 μg), or a combination of the above. Splenic T cells were prepared on day 0, and the IgD-RFC assay was performed. Also, T cells were cultured for 24 hr with either anti-immunoglobulin (heavy and light chain) or anti-IgD-treated, paraformaldehyde-fixed CH15 cells. In contrast, CH15 cells pretreated with an anti-IgM had no effect on the expression of IgD receptors on T cells and neither did CH15 cells treated with paraformaldehyde alone. These results indicate that crosslinking of surface IgD, but not surface IgM, under conditions that prevent the shedding of these ligands from the surface of the B cells is sufficient for induction of Tδ cells.

The present results show that at least certain preparations of IgD fail to induce IgD receptors on T cells except when crosslinked with antigen. The monoclonal B1-8.81 IgD at 100 μg/ml does not induce Tδ cells when incubated overnight with T cells at 37°C, in contrast to TEPC-1017 IgD, which invariably does so when added at concentrations >0.008 μg/ml (1). Addition of NP-BSA to the mixture of B1-8.81 IgD and T cells resulted in the induction of as high a percentage of IgD-RFC as did TEPC-1017 IgD alone. This finding suggested to us that crosslinking of IgD on the surface of B cells, by either antigen or by anti-IgD, might also be capable of inducing Tδ cells. Indeed, findings presented here are consistent with this prediction. Thus injection of anti-IgD induces Tδ cells in vivo. This could be the result of the exposure of T cells to crosslinked IgD present in immune complexes either on the surface of B cells, shed from B-cell surfaces, or formed through interaction of the anti-IgD with serum IgD. However, even B cells that were fixed after exposure to anti-IgD and that clearly bore the crosslinked IgD on their surface were capable of inducing IgD receptors on T cells upon coincubation. Therefore, it seems likely that our previous observation (1) of a small increase in Tδ cells following injection of a multivalent antigen is also

Table 4. Requirement for crosslinking of IgD in the induction of Tδ cells in vivo

<table>
<thead>
<tr>
<th>Injection</th>
<th>% IgD-RFC, mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>4.8 ± 2.7</td>
</tr>
<tr>
<td>2.4G2 (anti-Fcγ receptor)</td>
<td>6.4 ± 1.7</td>
</tr>
<tr>
<td>Hαa/1 (anti-IgDα)</td>
<td>32.5 ± 3.9</td>
</tr>
<tr>
<td>Hαa/1 + 2.4G2</td>
<td>29.6 ± 8.8</td>
</tr>
<tr>
<td>Hαa/1 Fab/Fc</td>
<td>17.1 ± 5.2</td>
</tr>
<tr>
<td>Hαa/1 Fab/Fc + 2.4G2</td>
<td>4.2 ± 0.7</td>
</tr>
</tbody>
</table>

Groups of three mice were injected i.v. on day -1 with Hαa/1 (100 μg), Hαa/1 Fab/Fc (100 μg), 2.4G2 (500 μg), or a combination of the above. Splenic T cells were prepared on day 0, and the IgD-RFC assay was performed.

Table 5. Crosslinking of surface IgD but not surface IgM on CH15 lymphoma cells induces IgD receptors on T cells

<table>
<thead>
<tr>
<th>Pretreatment of CH15 cells</th>
<th>IgD-RFC in T cells, mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3.6 ± 1.6</td>
</tr>
<tr>
<td>Anti-immunoglobulin</td>
<td>22.7 ± 2.3</td>
</tr>
<tr>
<td>Anti-IgD</td>
<td>39.3 ± 1.7</td>
</tr>
<tr>
<td>Anti-IgM</td>
<td>1.7 ± 0.6</td>
</tr>
</tbody>
</table>

CH15 cells (10⁶ cells per ml) were pretreated with anti-immunoglobulin (10 μg/ml) in Dulbecco’s phosphate-buffered saline (PBS) or with PBS alone (control) for 30 min at 0°C. Then the CH15 cells were fixed with 0.1% paraformaldehyde in PBS (26). Equal numbers (10⁶ cells per ml) of splenic T cells and CH15 cells were incubated for 18 hr at 37°C in Eagle’s minimal essential medium with 2% fetal calf serum, antibiotics, and 50 μM 2-mercaptoethanol.

receptors on T cells, we utilized the B-cell lymphoma CH15, which coexpresses IgD and IgM (10). Our experimental approach was to coculture T cells isolated from B10.A mice with CH15 cells that had been preincubated with antibodies to their surface immunoglobulin and then fixed with paraformaldehyde (26). Under these conditions, we showed by immunofluorescent staining methods (using fluorescein-labeled anti-goat immunoglobulin and indirect staining for surface IgD) that the anti-immunoglobulin antibodies re-

Table 3. Induction of Tδ cells by anti-IgD

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% IgD-RFC in splenic T cells, mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c (IgDα)</td>
<td>7.8 ± 0.7</td>
</tr>
<tr>
<td>C57BL6 (IgDβ)</td>
<td>3.6 ± 1.0</td>
</tr>
<tr>
<td>CB6F1 (IgDαβ)</td>
<td>2.8 ± 1.1</td>
</tr>
<tr>
<td>Goat anti-IgD (800 μg)</td>
<td>32.0 ± 4.2</td>
</tr>
<tr>
<td>Hαa/1 (100 μg)</td>
<td>24.2 ± 2.9</td>
</tr>
</tbody>
</table>

Groups of three mice were injected i.v. on day -1. Splenic T cells were obtained on day 0 and IgD-RFC assays were performed.
due to exposure of T cells to antigen-crosslinked IgD on B cells. In other words, the mechanism by which T cells come into play in the normal immune response is through their induction by antigen-crosslinked IgD on the surface of B cells. This is probably a locally occurring phenomenon that happens, early after the injection of antigen, in the surroundings of B cells that have specificity for that antigen. Thus, within a local microenvironment, nearby helper T cells acquire receptors for IgD, which facilitate their interaction with IgD+, antigen-bound B cells. Lymphokines, which readily promote the expression of these IgD-specific receptors (5), would help to maintain the presence of T cells during the emerging immune response. It is well established that following activation of IgD+ B cells with antigen IgD translation is rapidly down-regulated (27, 28). Such down-regulation results in the disappearance of IgD from the B-cell surface within a few days of activation with antigen (28). Indeed, these findings are consistent with the concept that IgD is primarily a receptor molecule, which, unlike other immunoglobulins, is not secreted following triggering of B cells expressing the δ heavy chain.

The relative role of induction of Tδ cells in the stimulation of polyclonal immunoglobulin production, which occurs after injection of anti-IgD antibody, appears to be minor as compared to the combination of (i) the direct activating effect of anti-IgD antibody on B cells and (ii) the presentation to T cells of the allo- or xenogeneic determinants on anti-IgD antibodies that are bound to and processed by B cells and that stimulate T-cell activation and lymphokine production (20, 23, 24). This is suggested by the comparison of our present results on the induction of Tδ cells with the results of Finkelman et al. (20). In all cases where the polyclonal activation of polyclonal immunoglobulin production seen when the allotype anti-IgD is antigenic for the mice into which it is injected (20). Thus, whereas enhancement of antibody production to a totally unrelated antigen is produced by simultaneous injection or pretreatment with syngeneic IgD (2), injection of anti-IgD primarily enhances the immune response to haptons bound to the anti-IgD or to epitopes of the anti-IgD itself if the anti-IgD antibody is foreign and thus itself immunogenic (20, 29). In view of the Tδ-mediated immunoaugmenting effect of TEPC-1017 IgD, it is not immediately clear why the response to simultaneously injected unrelated antigens is not always augmented by Tδ cells induced by allotypic or goat anti-IgD. It seems possible that the large excess of anti-IgD used in these experiments causes such a rapid down-regulation of surface IgD expression that the augmenting effect of Tδ cells on antibody production is prevented by the absence of IgD–antigen complexes. However, a less impressive but definite effect on antibody formation to unrelated antigens has been obtained in (α × b)F1 hybrids with nonimmunogenic allotypic anti-IgD (30). In the absence of additional lymphokine injections, this effect is limited to the allotype not affected by the anti-IgD (30) and may be a consequence of Tδ-cell induction. Although Tδ cells are clearly not allotype specific (1), they apparently augment the responses of those B cells that express surface IgD interacting with antigen more than the response by B cells that have had their surface IgD down-modulated by the anti-IgD.

The technical assistance of Marilyn Shapiro and Ariel Soriano is greatly appreciated. This work was supported by Public Health Service Grants AI-22645, AG-04860, AI-11694, and AI-21258, and by the Uniformed Services University of the Health Sciences Research Protocol No. R08308.