Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 554–558, January 1988
Immunology

Enhancement of antibody responses by IgD-binding factors induced by anti-IgD treatment of spleen cells

(Fc_δ receptor/rosette formation/surface IgD/T cell–B cell interaction)

MITSUNOBU ADACHI AND KIMISHIGE ISHIZAKA

Subdepartment of Immunology, The Johns Hopkins University School of Medicine, Good Samaritan Hospital, Baltimore, MD 21239

Contributed by Kimishige Ishizaka, September 11, 1987

ABSTRACT **Treatment of normal BALB/c splenocytes with anti-mouse IgD antibodies at 0°C followed by incubation of the cells at 37°C resulted in the formation of soluble factors that selectively inhibit rosette formation of lymphocytes bearing Fc_δ receptors with IgD-coated erythrocytes (i.e., IgD-binding factors). Treatment of the same cells with anti-IgM antibodies failed to induce the formation of the factors. Analysis of cellular mechanisms indicated that polymerized surface IgD on B cells, as well as surface IgD–anti-IgD complexes shed from the B cells, induced T cells bearing Fc_δ receptors to form IgD-binding factors. The factors formed by the anti-IgD treatment of splenic lymphocytes are composed of molecular mass species of 70 and 34 kDa, as estimated by gel filtration. Both the 70- and 34-kDa IgD-binding factors enhanced IgM and IgG1 plaque-forming cell responses of sheep erythrocyte-primed mouse spleen cells to the antigen.**

We have previously shown that incubation of normal mouse spleen cells with dimeric mouse IgD results in the formation of soluble factors that have affinity for IgD (1). The factors are derived from Lyt-1^+ T cells bearing Fc_δ receptors ($Fc_\delta R$) and could be demonstrated by their ability to inhibit rosette formation of $Fc_\delta R^+$ lymphocytes with IgD-coated fixed ox erythrocytes (E′-IgD). Since the majority of B lymphocytes bear IgD on their surface (2) and a substantial portion of Lyt-1^+, L3T4$^+$ T cells in normal spleen bear $Fc_\delta R$ (1, 3), we wondered whether the interaction between B cells bearing surface IgD (sIgD) and $Fc_\delta R^+$ T cells might induce the formation of IgD-binding factors (IgD-BF). The present experiments were undertaken to test this possibility. The results show that cross-linking of sIgD on B cells by anti-δ-chain antibodies, followed by incubation of the B cells with normal T cells, results in the formation of IgD-BF. In view of regulatory effects of immunoglobulin-binding factors, such as IgE-binding factors (IgE-BF) (4), IgG-binding factors (IgG-BF) (5), and IgA-binding factors (IgA-BF) (6) on the antibody response of the respective isotype, we anticipated that IgD-BF might regulate the antibody response. The present experiments show that mouse IgD-BF formed by anti-IgD-treated splenocytes enhance the secondary plaque-forming cell (PFC) response of mouse spleen cells primed with sheep erythrocytes (SRBC).

MATERIALS AND METHODS

Immunoglobulins and Antibodies. Monoclonal mouse IgD was obtained from ascitic fluids of BALB/c mice that had been injected with plasmacytoma TEPC 1017 and was isolated by the method of Finkelman *et al.* (7) with slight modifications (1). The purified IgD was in the form of a dimer and contained no other immunoglobulins. Purified mouse IgE from hybridoma H-1-DNP-ε-26 (8) and normal rabbit IgG were the same preparations as those previously described (9). Mouse IgD and IgE were coupled to Sepharose CL-4B; 5–8 mg of protein was coupled to 1 ml of Sepharose.

Specifically purified goat anti-mouse IgD antibodies were kindly supplied by F. D. Finkelman (Uniformed Service University of the Health Sciences, Bethesda, MD). Goat antiserum specific for mouse IgM was purchased from Cappel Laboratories (Cochranville, PA). Anti-μ-chain antibodies in the antisera were purified by using Sepharose that had been immunosorbent coated with IgM from MOPC 104E. Specifically purified anti-mouse immunoglobulin antibodies (polyvalent anti-mouse IgG) were the same preparation as that previously described (10). The IgG fraction of rabbit antiserum monospecific for mouse IgG1 was described in a previous article (11). Monoclonal anti-Thy-1 antibody was purchased from New England Nuclear. A rabbit antiserum against mouse brain (anti-brain θ) was described previously (12). Fluorescein-conjugated goat anti-mouse immunoglobulin and anti-goat IgG were purchased from Meloy Laboratories (Springfield, VA).

Mouse Spleen Cells and Cell Fractionation. BALB/c mice were purchased from Charles River Breeding Laboratories. Normal splenic lymphocytes were obtained by passing a spleen cell suspension through a Sephadex G-10 column. T cells in the splenic lymphocytes were depleted by using monoclonal anti-Thy-1 antibody together with rabbit complement (Accurate Chemicals, Westbury, NY) (9). A T-cell-enriched population was obtained from splenic lymphocytes by depleting B cells by means of tissue culture dishes coated with the $F(ab')_2$ fragments of anti-mouse IgG (13). Nonadherent cells recovered after two successive passages were used as a T-cell-enriched fraction. $Fc_\delta R^+$ cells in normal splenocytes were depleted by using IgD-coated tissue culture dishes (1).

Cell Cultures. Culture medium was RPMI 1640 medium (M. A. Bioproducts, Walkersville, MD) supplemented with 5% fetal calf serum, 3 mM L-glutamine, 50 μM 2-mercaptoethanol, and antibiotics. Normal splenic lymphocytes were suspended in the culture medium at a concentration of 5×10^6 or 10^7 nucleated cells per ml and incubated with either goat anti-IgD or anti-IgM antibodies (5 μg/ml) for 30 min at 0°C. The cells were washed twice with Hanks' balanced salt solution, resuspended in fresh culture medium at 10^7 nucleated cells per ml, and cultured for 24 hr at 37°C to recover culture supernatants. In some experiments, spleen cells treated with anti-IgD were fixed with glutaraldehyde by the method described by Shimonkevitz *et al.* (14).

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Abbreviations: PFC, plaque-forming cell(s); SRBC, sheep erythrocytes; RFC, rosette-forming cell(s); sIgD, surface IgD; FcR, Fc receptor; IgG-, IgA-, IgE-, and IgD-BF, IgG-, IgA-, IgE-, and IgD-binding factor(s); E′-IgD, fixed ox erythrocytes coated with IgD.

Rosette Technique. Fixed ox erythrocytes coated with mouse IgD (E'-IgD) or mouse IgE (E'-IgE) were used as indicator cells for the detection of lymphocytes bearing $Fc_\delta R$ and $Fc_\varepsilon R$, respectively. Sensitization of the fixed ox erythrocytes with mouse IgD, IgE, or human serum albumin (HSA) was carried out in 0.1 M acetate buffer (pH 5.0) according to published procedures (15); mouse IgE or HSA at 0.25 mg/ml (16) or IgD at 1.25 mg/ml (1) was employed to sensitize the cells. Lymphocytes bearing $Fc_\gamma R$ were detected by using ox erythrocytes coated with rabbit IgG antibody (16). The rosetting technique was exactly the same as that previously described (1); 600–1000 cells were examined for the determination of the percentage of rosette-forming cells (RFC). For the determination of IgD-RFC and IgE-RFC, the number of nonspecific RFC formed with E'-HSA was subtracted from the number of RFC formed with either E'-IgD or E'-IgE. Experimental errors for determination of the proportion of RFC were less than 10% of the average values.

Immunoglobulin-binding factors were detected by rosette inhibition (1). Culture supernatants were filtered through Diaflo YM 100 membranes (Amicon), and the filtrates were concentrated 3- to 5-fold. Thirty microliters of the filtrate was added to 15 μl of a 2% suspension of indicator cells, and the mixture was incubated for 30 min to 1 hr at 4°C. Fifteen microliters of a suspension (10^7 cells per ml) of normal splenic lymphocytes was then added to the mixture, and rosette formation was examined after overnight incubation at 0°C. The experimental variation between duplicate tubes was less than 10% of the average values.

Fractionation of IgD-BF. The factors were purified using mouse IgD-Sepharose by the procedures previously described (1). Proteins bound to the beads were eluted with 0.1 M glycine hydrochloride (pH 3.0).

The molecular mass of IgD-BF was estimated by gel filtration through a TSK G3000 SWG column (Beckman) that had been calibrated with lactate dehydrogenase, bovine serum albumin, ovalbumin, chymotrypsinogen, and cytochrome *c*. The procedures were exactly the same as that previously described (1).

Determination of Enhancing Effects of IgD-BF on Secondary PFC Responses. BALB/c mice were immunized by an intravenous injection of 0.2 ml of 1% suspension of SRBC (Baltimore Biological Laboratory). Their spleen cells were obtained 11 days after priming and were resuspended in RPMI 1640 medium supplemented with 5% fetal calf serum, 15 mM Hepes (pH 7.2), 3 mM L-glutamine, 0.1 mM minimum essential medium (MEM) nonessential amino acids (GIBCO), 1× MEM vitamins (GIBCO), 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol, penicillin (100 units/ml), and streptomycin (100 μg/ml). Cultures were set up in triplicate in a 96-well culture plate (Corning) in the presence or absence of a sample to be tested. Each well contained 8×10^5 spleen cells and 6×10^5 SRBC in a total volume of 0.2 ml. Unless otherwise described, 20 μl of a test sample was added to each well. After culture for 4 days, the number of direct and indirect PFC in the cell suspensions were enumerated by the method of Cunningham and Szenberg (17). To develop indirect PFC, rabbit anti-mouse IgG1 antibodies were added to a mixture of cultured cells and guinea pig complement. The rabbit antibodies had no inhibitory effect on IgM plaques.

RESULTS

Induction of IgD-BF Formation by Anti-IgD. Normal mouse splenocytes were treated with either anti-IgD or anti-IgM antibodies at 0°C for 30 min. After washing, the cells were resuspended in fresh culture medium and incubated overnight at 37°C to recover culture filtrates. As shown in Table 1, culture filtrates of anti-IgD-treated spleen cells inhibited rosette formation of $Fc_\delta R^+$ cells with E'-IgD, whereas the filtrates of anti-IgM-treated cells failed to do so. Fractionation of culture filtrates on IgD-Sepharose indicated that the rosette-inhibiting activity bound to IgD-Sepharose and was recovered by elution at acid pH. It was also found that neither the effluent fraction nor the eluate fraction from IgD-Sepharose (in Table 1) inhibited IgE- or IgG-RFC rosettes, indicating that the treatment of normal splenocytes with anti-IgD failed to induce the formation of IgE-BF or IgG-BF.

Table 1. Inhibition of IgD-RFC by culture filtrates of anti-IgD-treated splenic lymphocytes

Splenocyte treatment	Filtrate fraction	IgD-BF in culture filtrates	
		% IgD-RFC	% inhibition
None	Total	22.8	0
Anti-IgD	Total	16.5	28
	Effluent	23.0	0
	Eluate	13.7	40
Anti-IgM	Total	22.0	4

Splenic lymphocytes were treated with anti-IgD or anti-IgM antibodies, and their culture filtrates were prepared. Culture filtrates from anti-IgD-treated splenocytes were fractionated on IgD-Sepharose. IgD-BF in filtrates or their fractions were measured as the percentage of inhibition of rosette formation of normal splenic lymphocytes with E'-IgD. The percentage of IgD-RFC in splenocytes assayed in the absence of culture filtrates was 22.8 ± 0.8% (mean ± SEM).

In order to determine cellular mechanisms for the formation of IgD-BF, each of the populations—unfractionated normal splenocytes, the T-cell-enriched population, the B-cell-enriched population, and the $Fc_\delta R^+$-cell-depleted population—was treated with anti-IgD antibodies at 4°C, and the cells were cultured for 24 hr after washings. As shown in Table 2, neither the B-cell fraction alone, the T-cell fraction alone, nor the $Fc_\delta R^+$-cell-depleted population formed IgD-BF. However, if the B-cell-enriched population was treated with anti-IgD and the antibody-treated B cells were cultured together with untreated T cells, culture filtrates of the mixed cell populations inhibited IgD-RFC (Table 2). It was confirmed that the rosette-inhibiting activity in the culture filtrate bound to IgD-Sepharose and was recovered in the acid eluate.

Since the experiments described above suggested that sIgD on B cells is involved in the induction of IgD-BF

Table 2. Requirement of both B cells and T cells for the formation of IgD-BF

Cells treated with anti-IgD	Untreated cells added	IgD-BF in culture filtrates	
		% IgD-RFC	% inhibition
Splenocytes	None	17.0	33
$Fc_\delta R^+$-depleted	None	23.6	7
T-cell fraction	None	26.3	0
B-cell fraction	None	25.3	0
	T-cell fraction*	16.0	37

The percentages of $Fc_\delta R^+$ cells in the $Fc_\delta R^+$-depleted fraction and unfractionated splenocytes were 3.1% and 20.6%, respectively. The T-cell-enriched population was 1.0% sIg^+ cells and 88% Thy-1^+ cells, whereas the B-cell-enriched population was 91% sIg^+ cells and 1.6% Thy-1^+ cells, as determined by immunofluorescence. The culture filtrates obtained from the cells after anti-IgD treatment was assayed for IgD-BF as described in Table 1. The percentage of IgD-RFC in normal spleen cells incubated with E'-IgD in the absence of culture filtrate was 25.5 ± 0.5% (mean ± SEM).

*The B-cell fraction was treated with anti-IgD antibodies; then these cells were cultured with untreated T cells. The filtrate of the mixed cell population was then assayed for IgD-BF.

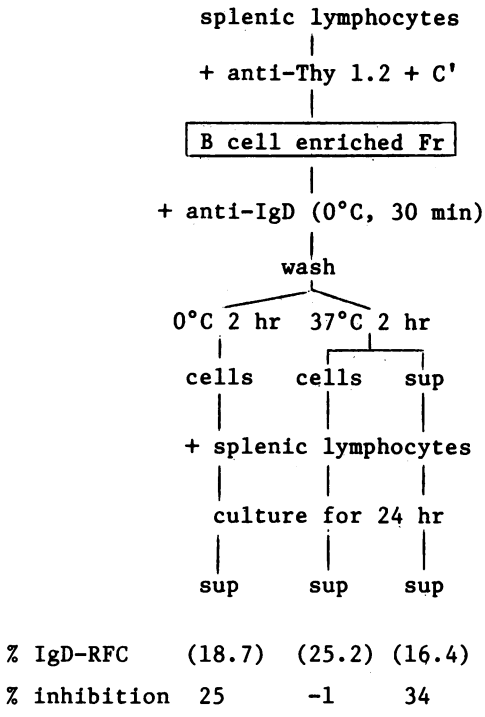

FIG. 1. Experimental design to show the requirement of sIgD on B cells to induce the formation of IgD-BF. Numbers in parentheses indicate the proportion of IgD-RFC in the presence of culture filtrate. The proportion of IgD-RFC in control tubes was 25.0 ± 0.8% (mean ± SEM). C', complement; Fr, fraction.

formation, experiments were carried out to determine whether the loss of sIgD on B cells is accompanied by the loss of the ability of anti-IgD-treated cells to induce the factor formation. The B-cell-enriched fraction of normal spleen cells was treated with anti-IgD. Half of the suspension of the antibody-treated cells was kept at 0°C for 2 hr, and the remainder was incubated at 37°C for 2 hr.

The latter cell suspension was centrifuged to recover both cell pellets and supernatants. Cell surface staining of an aliquot of the cells with anti-IgD and fluoresceinated anti-goat immunoglobulin antibodies confirmed that sIgD was lost by shedding during the 2-hr incubation of the anti-IgD-treated cells at 37°C. Aliquots of normal splenic lymphocytes were then cultured with the anti-IgD-treated B cells or supernatant of the antibody-treated B cells incubated at 37°C, and IgD-BF in culture filtrates were assessed by rosette inhibition. The results shown in Fig. 1 indicated that the anti-IgD-treated B cells, which were kept at 0°C, induced the formation of IgD-BF, whereas the same anti-IgD-treated cells incubated at 37°C failed to do so. The results also showed that culture supernatants of the anti-IgD-treated B cells, which should contain shedding materials, induced normal splenocytes to form IgD-BF.

We wondered if cognate interaction between $sIgD^+$ B cells and T cells may induce the formation of IgD-BF. To test this possibility, both anti-IgD-treated normal splenocytes and untreated cells were fixed with glutaraldehyde, and the fixed cells were cultured for 24 hr with untreated normal splenocytes. As shown in Table 3, anti-IgD-treated, glutaraldehyde-fixed cells induced the formation of IgD-BF, whereas untreated, glutaraldehyde-fixed cells failed to do so. It was found that sIgD–anti-IgD complexes on the anti-IgD-treated, glutaraldehyde-fixed cells remained on their surface even after a 24-hr incubation, as determined by immunofluorescence. Thus, anti-IgD-treated, glutaraldehyde-fixed cells were incubated for 24 hr at 37°C, and the cell pellets or supernatants were cultured with normal splenocytes. As expected, the cell pellets, but not the supernatants, induced the formation of IgD-BF (results not shown). To confirm that IgD-BF is derived from $Fc_\delta R^+$ cells, the anti-IgD-treated, glutaraldehyde-fixed cells were incubated with either normal splenocytes or those depleted of $Fc_\delta R^+$ cells. The results included in Table 3 showed that the $Fc_\delta R^+$-cell-depleted population failed to form IgD-BF when they were incubated with the anti-IgD-treated cells. These findings collectively suggested that the interaction of cross-linked sIgD on B cells with $Fc_\delta R$ on T cells induced the formation of IgD-BF and excluded the possibility that the induction of IgD-BF formation is due to B-cell-derived lymphokine(s).

Enhancement of Secondary Antibody Response by IgD-BF. IgD-BF were obtained by treating normal splenocytes with anti-IgD at 0°C, followed by incubation of the cells at 37°C for 24 hr. The factors in culture filtrates were absorbed with IgD-Sepharose and were recovered by elution at acid pH. Since the affinity-purified IgD-BF may contain some lymphokines, the factors were refractionated on IgD-Sepharose. The effluent (unbound) fraction and the acid eluate (bound) fraction from the second IgD-Sepharose were adjusted to 1/10th the volume of the original culture supernatant, and the preparations were assessed for the abilities to affect the *in vitro* PFC response of SRBC-primed spleen cells. The results shown in Table 4 indicated that the eluate fraction, which contained IgD-BF, enhanced both the IgM- and IgG1-PFC responses, whereas the effluent fraction, which did not contain a detectable amount of IgD-BF, failed to do so. The possibility was considered that IgD-BF itself might induce the PFC responses of SRBC-primed spleen cells in the absence of antigen. However, when the SRBC-primed spleen cells were cultured with the purified IgD-BF (the eluate fraction from the second IgD-Sepharose column), the numbers of IgM- and IgG1-PFC in the cultures were comparable to those in control cultures, which contained the spleen cells alone (results not shown).

Table 3. Induction of IgD-BF formation by anti-IgD-treated, glutaraldehyde-fixed lymphocytes

Splenic lymphocytes			IgD-BF in culture filtrate	
Treatment	Fixation	Cells added	% IgD-RFC	% inhibition
None	Glutaraldehyde	Splenocytes	22.0	0
Anti-IgD	Glutaraldehyde	None	20.5	7
		Splenocytes	13.9	37
		$Fc_\delta R^+$-depleted	22.1	0
	None	None	13.0	41
No lymphocytes		Splenocytes	22.2	0

Splenic lymphocytes were treated and fixed as shown above and where indicated were cultured with normal splenic lymphocytes (20.6% $Fc_\delta R^+$ cells) or an $Fc_\delta R^+$-depleted population (3.0% $Fc_\delta R^+$ cells). The culture filtrates were assayed for IgD-BF as described in Table 1. Anti-IgD-treated splenocytes that were not fixed with glutaraldehyde were used as a positive control. The percentage of IgD-RFC in normal spleen cells incubated with E'-IgD in the absence of culture filtrate was 22.0 ± 1.1% (mean ± SEM).

Table 4. Enhancement of PFC responses of SRBC-primed spleen cells by IgD-binding factors

Exp.	Fraction of affinity-purified IgD-BF added	IgD-BF activity	SRBC added	PFC per culture IgM	PFC per culture IgG1
1	None		−	70 ± 10	360 ± 40
	None		+	3,200 ± 80	3,920 ± 40
	Effluent from IgD-Sepharose	0	+	3,360 ± 120	3,880 ± 80
	Eluate from IgD-Sepharose	32	+	7,980 ± 20	10,540 ± 40
2	None		−	120 ± 40	100 ± 50
	None		+	1,760 ± 40	1,720 ± 80
	Eluate from IgE-Sepharose	0	+	1,910 ± 90	2,060 ± 60
	Effluent from IgD-Sepharose	4	+	1,860 ± 20	1,960 ± 160
	Eluate from IgD-Sepharose	36	+	3,460 ± 30	3,620 ± 60
3	None		−	120 ± 40	100 ± 50
	None		+	1,760 ± 40	1,720 ± 80
	70-kDa fraction	42	+	4,580 ± 160	4,680 ± 120
	34-kDa fraction	40	+	4,500 ± 20	4,320 ± 80

In experiments 1 and 2, each fraction was adjusted to 1/10th the volume of the original culture supernatant. In experiment 3, the fraction was 1/20th the volume of the original culture supernatant. Twenty microliters of the fraction was added to SRBC-primed spleen cell cultures in a total volume of 0.2 ml. IgD-BF was detected by inhibition of rosette formation as described in Table 1. The numbers represent the percentage of rosette inhibition. The percentage of IgD-RFC detected in the absence of IgD-BF was 24.2 ± 1.0% (mean ± SEM).

To exclude the possibility that the factors responsible for the enhancement of the PFC response nonspecifically bound to Sepharose, another preparation of affinity-purified IgD-BF was absorbed with IgE-Sepharose, and proteins bound to the beads were eluted at acid pH. Proteins that did not bind to IgE-Sepharose were fractionated again on IgD-Sepharose to obtain the effluent (unbound) and eluate (bound) fractions. As shown in Table 4 (Exp. 2), only the eluate fraction from IgD-Sepharose, which contained IgD-BF, enhanced both IgM- and IgG1-PFC responses. The results confirm that IgD-BF are responsible for the enhancement of PFC responses.

Previous studies have shown that IgD-BF induced by IgD dimers consisted of two molecular mass species (1). Thus, we determined if anti-IgD induced the formation of the two species and which species was responsible for the enhancement of PFC responses. Normal spleen cells were treated with anti-IgD, and the antibody-treated lymphocytes were cultured overnight at 37°C. IgD-BF in 30 ml of the culture filtrates were purified by using IgD-Sepharose, and the purified IgD-BF were fractionated on a TSK G3000 column. Distribution of the factors in the eluate fractions was determined by the ability of the fractions to inhibit IgD rosettes. IgD-BF consisted of two molecular mass species of approximately 70 and 34 kDa, as shown in Fig. 2. The fractions containing IgD-BF were pooled separately, and each pooled fraction was assessed for its ability to enhance the PFC response. The results included in Table 4 (Exp. 3) indicated that both the 70- and 34-kDa IgD-BF enhanced the IgM- and IgG1-PFC responses.

DISCUSSION

Data presented in this paper show that sIgD cross-linked by anti-IgD induces T cells to form IgD-BF. Failure of the anti-IgD-treated B cells or T cells alone to form IgD-BF and the requirement of $Fc_\delta R^+$ cells for the formation of IgD-BF indicated that polymerized sIgD on B cells induced $Fc_\delta R^+$ T cells to form IgD-BF. The conclusion is supported by the facts that the majority of $Fc_\delta R^+$ lymphocytes are Lyt-1^+, L3T4$^+$ T cells (3) and that dimeric IgD induces these cells to form IgD-BF (1). Induction of IgD-BF formation by anti-IgD-treated, glutaraldehyde-fixed lymphocytes, which bear IgD–anti-IgD complexes fixed to the cell surface and do not release the complexes during the culture, suggests that cognate interaction between the polymerized sIgD on B cells and $Fc_\delta R^+$ T cells can induce the factor formation. However, the present experiments also indicated that sIgD–anti-IgD complexes shed from B cells could induce the factor formation. Indeed, the inducer of IgD-BF present in the culture supernatants of anti-IgD-treated B cells was removed by filtration of the culture supernatants through YM 100 membranes (results not shown).

The present experiments showed that IgD-BF obtained by stimulation of T cells with polymerized sIgD enhanced both the antigen-induced IgM and IgG1-PFC responses of SRBC-primed spleen cells. The factors present in the affinity-purified IgD-BF that are responsible for the enhancement of antibody responses failed to bind to IgE-Sepharose but bound to IgD-Sepharose and were recovered from IgD-Sepharose by elution at acid pH. It was also found that IgD-BF in the affinity-purified preparation consisted of two molecular mass species of 70 and 34 kDa and that both species enhanced the IgM- and IgG1-PFC responses. In our previous publication (1), it was found that Lyt-1^+ T cells treated with dimeric IgD formed the 78- and 37-kDa IgD-BF,

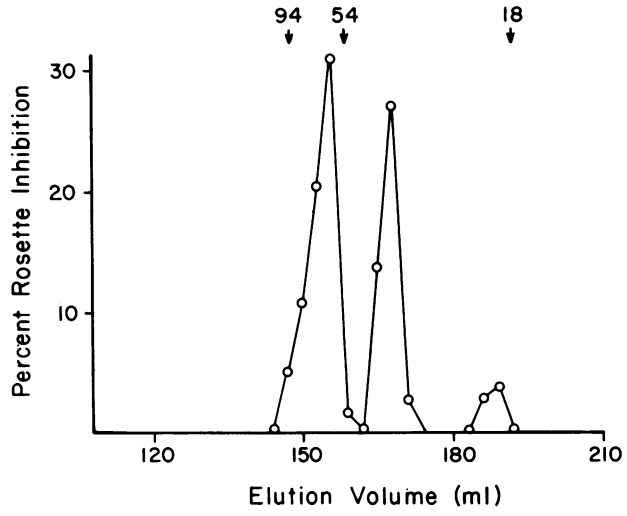

FIG. 2. Estimation of molecular mass of IgD-BF formed by anti-IgD-treated splenocytes. The factors in culture filtrates were affinity-purified and then applied to a TSK G3000 SWG column. The distribution of the factors was determined by rosette inhibition (ordinate). Elution volumes of reference proteins (94, 54, and 18 kDa) are shown by arrows.

as estimated by gel filtration. Considering experimental errors in elution volumes of a certain protein from a TSK G3000 SWG column, the two species of IgD-BF formed by anti-IgD treatment would be the same as those induced by dimeric IgD.

It has been reported that an injection of IgD enhanced the primary antibody response to T-cell-dependent antigens (18). Coico *et al.* (3) reported that an injection of IgD into mice induced an increase in $Fc_\delta R^+$, L3T4$^+$ T cells and that the transfer of these cells into irradiated or nonirradiated animals enhanced the antibody response of the recipients to trinitrophenylated keyhole limpet hemocyanin. Since IgD-BF are derived from $Fc_\delta R^+$, Lyt-1$^+$ T cells (1), it is reasonable to speculate that the factors formed by the cells are involved in the enhancement of antibody responses. It is also known that an intravenous injection of goat anti-IgD antibodies enhances polyclonal immunoglobulin synthesis and antibody responses to unrelated antigens (19). Finkelman *et al.* (20) reported that an intravenous injection of goat anti-IgD antibodies into mice induces the formation of interleukin 4 by their spleen cells. As the polyclonal immunoglobulin production by the anti-IgD treatment requires T cells, they interpreted that the goat antibodies were recognized as a foreign antigen for the polyclonal activation of B cells (20). Nevertheless, an injection of the monoclonal mouse antibody specific for IgD of the a allotype significantly enhanced both IgG1 and IgE production (21). We speculate that the formation of IgD-BF by anti-IgD treatment of spleen cells may partly explain the enhancement of the *in vivo* antibody formation by the injection of anti-IgD.

It is known that the target cells for IgE-BF and IgA-BF are sIgE$^+$ cells and sIgA$^+$ cells, respectively, and that these factors selectively regulate the antibody response of the homologous isotype (4, 6). In contrast, the effect of IgD-BF was not isotype-specific. Since the target of IgD-BF is probably sIgD$^+$ B cells, one may expect that IgD-BF affect the differentiation/proliferation of sIgD$^+$ B cells, which are precursors of antibody-forming cells of various isotypes (22). Our recent experiments indicated that antigen-primed spleen cells formed IgD-BF upon antigenic stimulation. The results suggest that the factors may be involved in the antibody response under physiological conditions.

The authors express their appreciation to Dr. F. D. Finkelman for supplying purified goat anti-IgD antibodies. This work was supported by Research Grant AI-22980 from the Department of Health and Human Services. The paper is publication no. 695 from the O'Neill Laboratories.

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