IgD-binding factors from mouse T lymphocytes

(Fc_ε receptor/rosette formation)

MITSUNOBU ADACHI AND KIMISHIGE ISHIZAKA

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

Contributed by Kimishige Ishizaka, June 5, 1986

ABSTRACT Incubation of normal mouse splenic lymphocytes with dimeric mouse IgD results in the formation of T-cell factors that selectively inhibit rosette formation of Fc_ε receptor-positive lymphocytes with IgD-coated erythrocytes. The rosette-inhibiting factors bound to IgD-Sepharose and were recovered by elution at acid pH. The results indicate that the rosette-inhibiting factors are IgD-binding factors. Neither IgE-binding factors nor IgG-binding factors inhibited IgD rosettes. The IgD-binding factors induced by IgD consist of two species of molecular weight 78,000 and 37,000, respectively. The source of IgD-binding factors is Lyt-1⁺ T cells bearing Fc_ε receptors. It appears that the binding of IgD to the receptors on the T cells induces the formation of IgD-binding factors.

Previous studies by several investigators established the presence of T-cell factors having affinity for one of the immunoglobulin isotypes, such as IgG, IgE, or IgA (1-3). The formation of the immunoglobulin-binding factors (Ig-BF) can be induced by incubation of lymphocytes with immunoglobulin of the respective isotype (2-4), and the factors are believed to be related to Fc receptors (FcR) for the respective isotype (5). Indeed, a monoclonal antibody against rat IgE-BF crossreacted with Fc_εR on both T and B cells (6). Coico *et al.* (7) reported that a subset of mouse T cells expressed FcR specific for IgD (Fc_δR) and that the proportion of Fc_δR⁺ cells in a normal spleen cell suspension increased upon incubation of the cells with IgD. In view of these findings, the present experiments were undertaken to determine whether mouse T cells produce soluble factors having affinity for IgD (i.e., IgD-BF). Since IgE-BF and IgG-BF could be detected by their ability to inhibit rosette formation of Fc_εR⁺ cells and Fc_δR⁺ cells, respectively (2, 8), we assessed the presence of IgD-BF by the ability of culture filtrates to inhibit rosette formation of Fc_δR⁺ lymphocytes with IgD-coated ox erythrocytes. The results show that mouse Lyt-1⁺ T cells form IgD-BF upon incubation with dimeric mouse IgD.

MATERIALS AND METHODS

Immunoglobulins and Antibodies. Monoclonal mouse IgD was obtained from ascitic fluid of BALB/c mice that had been injected with plasmacytoma TEPC-1017 (9) and was isolated by the method of Finkelman *et al.* (9) with slight modifications. Briefly, proteins were precipitated with 50% saturated ammonium sulfate, and the precipitate fraction was further fractionated by chromatography on a DE-52 column (Whatman). Proteins eluted with 0.1 M Tris/HCl buffer (pH 8.2) were then applied to an Ultrogel Aca 34 (LKB) column. IgD was eluted as a single peak shortly after the void volume, and the purified IgD contained no other immunoglobulin. Purified mouse IgE from hybridoma H1-DNP-ε-26 (10), monoclonal rat IgE from immunocytoma IR 162, and normal rabbit IgG were the same preparations as those previously

described (8). Normal mouse IgG2 was obtained by DEAE-cellulose column chromatography. MOPC-21 IgG1 myeloma protein was obtained from Bionetics (Charleston, SC). Each immunoglobulin was coupled to Sepharose CL-4B: 5-8 mg of protein was coupled to 1 ml of Sepharose.

Goat anti-mouse IgD antibodies were kindly supplied by F. D. Finkelman (Uniformed Services University of the Health Sciences, Bethesda, MD). Specifically purified anti-mouse immunoglobulin antibodies was the same preparation as that previously described (8). Monoclonal anti-Thy-1, anti-Lyt-1.2, and anti-Lyt-2.2 antibodies were purchased from New England Nuclear. Fluorescein-conjugated rat monoclonal anti-Lyt-1 (53-7.3) and anti-Lyt-2 (53-6.7) antibodies were purchased from Becton Dickinson (Mountain View, CA). A rabbit antiserum against mouse brain (anti-brain θ) was described previously (11). Fluorescein-conjugated goat anti-mouse immunoglobulins and anti-rabbit IgG were purchased from Meloy Laboratories (Springfield, VA).

Mouse Spleen Cells and Cell Fractionation. BALB/c mice were purchased from Charles River Breeding Laboratories (Wilmington, MA). Normal splenic lymphocytes were obtained by passing a spleen cell suspension through a Sephadex G-10 column. T cells or their subpopulations in splenic lymphocytes were depleted by using monoclonal anti-Thy-1, anti-Lyt-1.2, and anti-Lyt-2.2 antibodies together with rabbit complement (8). A T-cell-enriched fraction 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 immunoglobulin (12). Nonadherent cells recovered after two successive passages were used as a T-cell-enriched fraction. Fc_δR⁺ cells were depleted by a rosetting technique using ox erythrocytes coated with rabbit IgG antibodies (13). Fc_εR⁺ cells were depleted using IgD-coated tissue culture dishes. Polystyrene tissue culture dishes (Falcon, 60 × 15 mm) were coated with IgD (30 μg/ml) overnight. After washing, the dishes were quenched by 0.5% bovine serum albumin and then treated with anti-IgD (10 μg/ml). After washing, the dishes were treated again with IgD (30 μg/ml). Thirty million lymphocytes were suspended in 3 ml of Hanks' balanced salts solution containing 5% fetal bovine serum and were placed in an IgD-coated dish. After incubation at 4°C for 60 min, nonadherent cells were gently aspirated. Spleen cells and their fractions were cultured in RPMI 1640 medium supplemented with 5% fetal bovine serum, 3 mM glutamine, 50 μM 2-mercaptoethanol, and antibiotics.

Rosetting Technique. Ox erythrocytes coated with mouse IgD or mouse IgE were used as indicator cells for the detection of lymphocytes bearing Fc_εR and Fc_δR, respectively. Erythrocytes were fixed by exactly the same method as previously described (14). Sensitization of the fixed erythrocytes (E') with mouse IgD, IgE, or human serum

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: Ig-BF, immunoglobulin binding factor(s); FcR, Fc receptor(s); E'-IgD, fixed ox erythrocytes coated with mouse IgD; EA₁, ox erythrocytes coated with rabbit IgG antibodies; HSA, human serum albumin; DPBS, Dulbecco's phosphate-buffered saline; RFC, rosette-forming cell.

albumin (HSA) was carried out in 0.1 M acetate buffer (pH 5.0) according to published procedures (15). Unless otherwise specified, rat IgE or HSA at 0.25 mg/ml or IgD at 1.25 mg/ml was employed to sensitize the cells. Lymphocytes bearing Fc_γR were detected by using ox erythrocytes coated with rabbit IgG antibody (EA_γ) (15).

For the formation of rosettes, lymphoid cells were suspended (5×10^6 per ml) in Dulbecco's phosphate-buffered saline (DPBS) containing 20% heat-inactivated fetal bovine serum, and 20 μ l of the suspension was mixed with an equal volume of a 1% (vol/vol) suspension of indicator cells. After incubation for 10 min at 37°C, the mixture was centrifuged at $90 \times g$ for 5 min and kept at 0°C overnight. Usually, 600–1000 cells were counted for determination of the percentage of rosette-forming cells (RFCs). A positive RFC was defined as a cell having at least three erythrocytes adherent to the surface. For the determination of Fc_γR⁺ and Fc_εR⁺ cells, the number of nonspecific RFCs with E'-HSA was subtracted from the number of RFCs with either E'-IgD or E'-IgE. Experimental error for determination of the proportion of RFCs was less than 10% of the average.

Ig-BF were detected by rosette inhibition. Culture supernatants were filtered through Diaflo YM100 membranes (Amicon), and the filtrates were concentrated 3- to 5-fold. Thirty microliters of the filtrate was added to 15 μ l of a 2% (vol/vol) suspension of indicator cells and the mixture was incubated for 30 min at 4°C. Fifteen microliters of a lymphocyte suspension (10^7 cells per ml) was then added to the mixture and rosette formation was examined as described above.

Source of IgE-BF and IgG-BF. The factors were obtained from a mouse T-cell hybridoma, 231F1 (8). The hybridoma cells were incubated for 24 hr with syngeneic macrophages that had been exposed to ovalbumin, and culture supernatants were filtered through YM100 membranes.

Fractionation of Ig-BF. IgD-BF, IgE-BF, and IgG-BF were purified using mouse IgD-Sepharose, rat IgE-Sepharose, and rabbit IgG-Sepharose, respectively, by the procedures previously described (16). Briefly, culture filtrates were mixed with Ig-coupled Sepharose for 90 min, and the suspension then was packed into a small column. The beads were washed with DPBS, and bound material was eluted with glycine/HCl buffer (pH 3.0).

The molecular weight of IgD-BF was estimated by gel filtration through a TSK G3000 SWG column that had been calibrated with lactate dehydrogenase, bovine serum albumin, ovalbumin, chymotrypsinogen, and cytochrome c. A one-milliliter sample was applied to the column, and proteins were eluted with DPBS, using a high-performance liquid chromatography system (Waters Associates).

RESULTS

Detection of IgD-BF. In preliminary experiments, fixed ox erythrocytes (E') were treated with IgD at 0.25–1.25 mg/ml to prepare indicator cells, and the proportion of RFCs in normal splenic lymphocytes was determined. Repeated experiments showed that E' treated with IgD at 1.25 mg/ml gave the most consistent results. With these indicator cells, 20–27% of normal splenic lymphocytes formed rosettes. To determine the specificity of rosette formation, normal lymphocytes were suspended with mouse immunoglobulins of various isotypes and then were fixed with the indicator cells (Table 1). IgD at 50 μ g/ml markedly inhibited rosette formation, and IgD at 1 mg/ml inhibited the rosettes almost completely. In contrast, IgG1, IgG2, or IgE at 1 mg/ml failed to affect the proportion of RFCs. These results showed that rosetting of lymphocytes with E'-IgD is specific for IgD.

Since Coico *et al.* (7) reported that the proportion of IgD-RFCs in normal lymphocytes increased after exposure of

Table 1. Specificity of rosette formation of normal splenic lymphocytes with E'-IgD

Concentration, μ g/ml	% IgD-RFCs			
	IgD	IgG1	IgG2	IgE
50	12.3	22.0	22.2	23.6
100	11.7	22.7	22.4	23.1
500	6.9	22.7	22.6	23.9
1000	4.0	23.3	23.0	23.3

Rosette-formation assay mixtures contained various concentrations of mouse immunoglobulins as indicated. The proportion of IgD-RFCs in the absence of immunoglobulin was 24.0%.

the cells to IgD, we cultured normal splenic lymphocytes with 0.1–10 μ g of IgD, IgG1, or IgE per ml overnight and then determined the proportion of IgD-RFCs. In all experiments, the proportion of IgD-RFCs was 24.7–26.3%. These values were comparable to the proportion of IgD-RFCs (25.7%) in control cells that were cultured overnight in the absence of mouse immunoglobulin. Thus, incubation with IgD failed to increase the number of Fc_γR⁺ cells.

We then determined whether IgD might induce the formation of IgD-BF. Aliquots of a suspension of normal splenic lymphocytes (10^7 nucleated cells per ml) were incubated overnight with 0.1–0.4 μ g of mouse IgD per ml or in the absence of the protein. Culture filtrates then were concentrated 5-fold, and an aliquot of each sample was assessed for the ability to inhibit rosette formation of normal lymphocytes with E'-IgD (Table 2). Culture filtrate of IgD(+) culture markedly inhibited the formation of IgD rosettes, while that of IgD(−) culture failed to do so. We wondered whether the rosette-inhibiting factor might have affinity for IgD. When an aliquot of the culture filtrates was fractionated on IgD-Sepharose, the rosette-inhibiting factor was adsorbed to IgD-Sepharose and recovered by elution of the beads at acid pH. When the same culture filtrate was fractionated on IgG-Sepharose or IgE-Sepharose, the rosette-inhibiting factor was detected in the effluent fractions (Table 2). The results indicate that the factors have affinity for IgD but not for IgG or IgE.

Experiments were carried out to confirm that the eluate from IgD-Sepharose does not affect IgG rosettes or IgE rosettes. Normal splenic lymphocytes were incubated with either EA_γ or E'-IgE in the presence or absence of the eluate fraction from IgD-Sepharose. The proportion of IgE-RFCs in the presence or absence of the fraction was 22.9% and 21.5%, respectively. The proportion of IgG-RFCs in the presence or absence of the fraction was 24.1% and 23.8%.

Finally, we determined whether IgE-BF or IgG-BF might inhibit IgD rosettes. Both Ig-BF were obtained by stimulation of 231F1 cells with macrophages that had been exposed to ovalbumin. Culture filtrates were incubated with IgG-Sepharose or IgE-Sepharose, and elution at acid pH was used to

Table 2. Inhibition of IgD-RFCs by culture filtrates of IgD-stimulated splenic lymphocytes

Culture	Fractionation	% IgD-RFCs		
		Filtrate	Effluent	Eluate
IgD(−)	IgD-Sepharose	23.5	22.9	21.4
IgD(+)*	IgD-Sepharose	16.7	23.2	16.2
	IgG-Sepharose		15.4	23.0
	IgE-Sepharose		15.5	23.4

IgD-rosette assays were set up in the presence of unfractionated culture filtrate or of the effluent (unbound) or eluate fraction of the filtrate. The proportion of IgD-RFCs in normal spleen cells was 23.3%.

*Splenic lymphocytes were incubated overnight with IgD (0.4 μ g/ml).

recover IgG-BF or IgE-BF. Each of the preparations was added to EA_γ, E'-IgE, or E'-IgD, and normal splenic lymphocytes were added to the mixtures for rosette formation. The proportions of RFCs in the presence or absence of IgE-BF or IgG-BF are summarized in Table 3. It is apparent that both IgE-BF and IgG-BF failed to inhibit IgD rosettes.

The molecular weights of IgD-BF were estimated by gel filtration. Splenic lymphocytes were incubated with IgD (0.4 μg/ml) for 24 hr, and IgD-BF in culture filtrates were purified by using IgD-Sepharose. The eluates from the beads were concentrated to 1/20th the volume of the original culture supernatants and applied to a TSK G3000 SWG column (Fig. 1). It appears that IgD-BF consist of two species of molecular weights approximately 78,000 and 37,000, respectively.

Cell Sources of IgD-BF. Normal spleen cells were depleted of Thy-1⁺ cells, Lyt-1⁺ cells, or Lyt-2⁺ cells, and each cell population was incubated overnight with IgD (0.4 μg/ml). Culture filtrates were concentrated 4-fold and then incubated with IgD-Sepharose, and the eluate fraction from the beads were assayed for the presence of IgD-BF (Table 4). The cell sources of IgD-BF were completely removed by the depletion of Thy-1⁺ cells or Lyt-1⁺ cells but not by the depletion of Lyt-2⁺ cells. The results indicate that Lyt-1⁺ T cells are required for the formation of IgD-BF. In order to determine the distribution of Fc_γR⁺ cells among subsets of T cells and B cells, normal splenic lymphocytes were depleted of Thy-1⁺ cells, Lyt-1⁺ cells, or Lyt-2⁺ cells, and Fc_γR⁺ cells in each population were enumerated by rosette formation. The proportion of IgD-RFCs was diminished by depletion of either Thy-1⁺ cells or Lyt-1⁺ T cells but did not change after depletion of Lyt-2⁺ cells (Table 4). In order to determine whether Lyt-2⁺ T cells bear Fc_γR, normal T cells were enriched and the T-cell enriched population was depleted of either Lyt-1⁺ cells or Lyt-2⁺ cells. The Lyt-2⁺-depleted fraction contained a high proportion of Fc_γR⁺ cells, whereas the Lyt-1⁺-depleted fraction contained a very low proportion of Fc_γR⁺ cells (Table 4). The results indicate that a substantial portion of Lyt-1⁺ T cells, but very few, if any, Lyt-2⁺ T cells, bear Fc_γR.

Since non-T cells contain Fc_γR⁺ cells, we determined whether non-T cells are required for the formation of IgD-BF. Normal spleen cells and a T-cell-enriched fraction, which contained less than 2% B cells, were incubated with IgD, and IgD-BF in culture filtrates were assessed. The T-cell-enriched fraction formed more IgD-BF than unfractionated spleen cells, when they were incubated with IgD. These results suggest that interaction of IgD with Fc_γR on Lyt-1⁺ T cells results in the formation of IgD-BF.

We confirmed that Fc_γR⁺ cells are required for the formation of IgD-BF. Splenic lymphocytes were depleted of Fc_γR⁺ cells by use of IgD-coated dishes. By this procedure, Fc_γR⁺ cells diminished from 29.7% to 8%. Unfractionated splenic lymphocytes and the Fc_γR⁺-depleted population were incubated with IgD, and culture filtrates were assessed for the

Table 3. Failure of IgG-BF and IgE-BF from 231F1 cells to inhibit IgD rosettes

Ig-BF added to indicator cells	% IgE-RFCs	% IgG-RFCs	% IgD-RFCs
None	30.8	31.1	20.9
IgG-BF	31.5	21.2	19.2
IgE-BF	22.9	32.3	20.4

Ig-BF formation was induced by incubation of 231F1 hybridoma cells with macrophages that had been exposed to ovalbumin. IgG-BF and IgE-BF in culture filtrates were purified by using IgG-Sepharose and IgE-Sepharose, respectively. Normal splenic lymphocytes were incubated, in the presence or absence of purified Ig-BF, with E'-IgE, EA_γ, or E'-IgD for assay of IgE-RFCs, IgG-RFCs, and IgD-RFCs, respectively.

FIG. 1. Gel filtration of IgD-binding factors through a TSK G-3000 column. One milliliter of affinity-purified IgD-BF was applied to the column, and proteins were eluted with DPBS. Distribution of IgD-BF in 3-ml fractions was determined by the ability of the fractions to inhibit formation of IgD rosettes. Arrows indicate elution volumes of bovine serum albumin (BSA), ovalbumin (OA), chymotrypsinogen (CH), and cytochrome c (CT). According to the manufacturer (Toyo Soda, Tokyo) of the column, these elution volumes correspond to molecular weights of 94,000, 54,000, 18,000, and 12,000, respectively.

presence of IgD-BF (Table 5). The Fc_γR⁺-depleted fraction failed to form IgD-BF. We also found that the depletion of Fc_γR⁺ cells was accompanied by a decrease of IgG-RFCs from 30% to 16.5%. Since the results suggested that Fc_γR⁺ cells may also bear Fc_γR, we depleted Fc_γR⁺ cells in splenic lymphocytes by rosetting with EA_γ and determined the proportion of IgG-RFCs and IgD-RFCs in an Fc_γR-depleted fraction. IgG-RFCs diminished from 24.9% to 1% by depletion of Fc_γR⁺ cells, and this change was accompanied by a decrease of Fc_γR⁺ cells from 22.7% to 4.7%. The results indicate that the majority of Fc_γR⁺ cells also bear Fc_γR.

DISCUSSION

The data show that incubation of normal mouse splenic T cells with IgD results in the formation of soluble factors that inhibit rosette formation of Fc_γR⁺ lymphocytes with IgD-coated erythrocytes. The factors failed to inhibit either IgE rosettes or IgG rosettes, indicating that the factors are

Table 4. Cell sources of IgD-BF and the distribution of Fc_γR⁺ cells (IgD-RFCs) in splenic lymphocytes

Cell population	IgD-BF*	% IgD-RFCs	
		Splenic lymphocytes	T-cell-rich fraction†
Unfractionated	51	23.4	31.9
Thy-1 ⁺ -depleted	0	10.1	ND
Lyt-1 ⁺ -depleted	0	11.4	4.0
Lyt-2 ⁺ -depleted	41	22.0	33.2

ND, not done.

*Splenic lymphocytes were depleted of Thy-1⁺ cells, Lyt-1⁺ cells, or Lyt-2⁺ cells and each population was incubated with IgD. IgD-BF in culture filtrates was determined by rosette inhibition. Numbers represent % rosette inhibition. The proportion of IgD-RFCs in the absence of culture filtrate was 26.9%.

†Thy-1⁺ cells, Lyt-1⁺ cells, and Lyt-2⁺ cells comprised 89%, 79%, and 29%, respectively, of the T-cell-rich fraction, as determined by immunofluorescence. Only 3% of the Lyt-1⁺-depleted population was stained by anti-Lyt-1 antibody, and 5% of the Lyt-2⁺-depleted population was stained by anti-Lyt-2 antibody.

Table 5. $Fc\gamma R^+$ cell depletion abolishes formation of IgD-BF

Cell population	% IgD-RFCs	% IgG-RFCs	IgD-BF*
Unfractionated	22.9	29.7	23.4
$Fc\gamma R^+$ cell-depleted	7.0	16.5	0

*Cell preparation was incubated with IgD for the formation of IgD-BF. Numbers represent % rosette inhibition by culture filtrates. In the absence of culture filtrates, 23.1% of cells formed IgD rosettes.

specific for IgD. The rosette-inhibiting factor bound to IgD-Sepharose and was recovered by elution at acid pH, but this factor failed to bind to either IgE-Sepharose or IgG-Sepharose. On the other hand, mouse IgE-BF and IgG-BF from T-cell hybridoma 231F1 failed to inhibit IgD rosettes. The results indicate that the soluble factors induced by IgD are IgD-BF.

The cell source of IgD-BF was removed from normal spleen cells by depletion of either $Thy-1^+$ cells or $Lyt-1^+$ cells but not by depletion of B cells. Depletion of $Fc\gamma R^+$ cells also removed the cell source of IgD-BF. Since the majority of $Fc\gamma R^+$ cells appear to bear $Fc\gamma R$ (Table 5), it appears that $Lyt-1^+$, $Fc\gamma R^+$, $Fc\gamma R^+$ T cells are the source of IgD-BF.

Coico *et al.* (7) reported that 7–9% of normal spleen cells bear $Fc\gamma R$ and that the proportion of $Fc\gamma R^+$ cells in splenic lymphocytes increased upon incubation with IgD. In our experiments, however, 20–27% of normal splenic lymphocytes formed rosettes with E'-IgD, and the proportion of IgD-RFCs did not increase after incubation of the cells with IgD. Nevertheless, the rosette formation with our indicator cells was specific; IgD rosette formation was inhibited by IgD but not by either IgE or IgG. The quantitative difference between the results of Coico *et al.* and our findings is probably due to the sensitivity of the rosette technique used. It is quite possible that IgD enhanced the expression of $Fc\gamma R$ on $Fc\gamma R^+$ cells, and that only the cells with a high density of $Fc\gamma R$ were detected by Coico *et al.* A difference in the sensitivity of the rosette assay may also explain why they detected $Fc\gamma R$ only on $Lyt-1^+$ T cells (7), whereas we detected $Fc\gamma R$ on both $Lyt-1^+$ T cells and a subset of B cells. Similar discrepancies have been reported for $Fc\alpha R^+$ cells. Thus, Hoover *et al.* (17) reported that IgA could induce $Fc\alpha R$ on only $Lyt-2^+$ cells, whereas Yodoi *et al.* (18) found that not only a subpopulation of T cells but also B cells express $Fc\alpha R$ upon incubation with IgA.

As expected, only IgD induced the formation of IgD-BF. Incubation of normal splenic lymphocytes with either mouse IgE or IgG1 failed to induce the formation of the factors (results not shown). It is known that monomeric IgE induces the formation of IgE-BF and enhances the expression of $Fc\epsilon R$ (2). However, dimeric IgE is much more effective than monomeric IgE for the induction of IgE-BF formation (19). It is also known that dimeric IgA, but not monomeric IgA, induces the expression of $Fc\alpha R$ (18). The successful induction of IgD-BF formation in the present experiments may have been due to the fact that the monoclonal IgD employed was in the dimeric form (4). Indeed, the elution volume of this protein from an AcA 34 column corresponded to that of dimeric IgD. Marcelletti and Katz (20) reported that interaction of IgE with $Fc\epsilon R^+$ B cells resulted in the formation of a soluble factor, which they called EIR_T, and that this factor induced T cells to express $Fc\epsilon R$. According to their scheme, EIR_T is also involved in the formation of IgE-BF by T cells (21). The presence of $Fc\gamma R^+$ B cells suggests that the cells may be involved in the formation of IgD-BF by T cells. However, this possibility is unlikely because a T-cell-enriched fraction that contained very few B cells formed IgD-BF upon incubation with IgD. Since a subset of $Lyt-1^+$ T cells bear $Fc\gamma R$, it is more likely that direct binding of IgD to the receptors on the T cells results in the formation of

IgD-BF. Indeed, rodent T-cell hybridomas 23B6 and 231F1 form IgE-BF upon incubation with homologous IgE (8, 22). A mouse T-cell hybridoma, T₂D₄, produces IgG-BF upon incubation with IgG (5), whereas the same hybridoma produces IgA-BF when incubated with IgA (3).

The biologic role(s) of IgD-BF is unknown. However, injections of IgD into mice cause an enhancement of the primary immune response to T-cell-dependent antigens (23), but this enhancement is not obtained in athymic nude mice. Since an injection of IgD also induced an apparent increase in $Fc\gamma R^+$ T cells, Coico *et al.* (7, 24) suggested that these cells are responsible for the enhancement of the immune response. Indeed, they have shown that transfer of normal T cells exposed to IgD augmented the antibody response of the recipients. The formation of IgD-BF by $Lyt-1^+$ T cells exposed to IgD suggests strongly that the factors are involved in the augmentation of antibody responses. Previous studies on IgE-BF have shown that some of the factors selectively enhanced the IgE response, while the other type of IgE-BF suppressed the response (25). Evidence has been presented that the factors regulate the differentiation of surface-IgE⁺ B cells by binding to the cell surface IgE (26, 27). Similarly, IgG-BF selectively suppress the IgG response (5), whereas IgA-BF selectively suppress the IgA response (3). It is known that the majority of B cells bear surface IgD. If the target of IgD-BF is actually surface-IgD⁺ B cells, which are not committed for a certain isotype, it is conceivable that the factors may regulate the differentiation of these cells and affect the antibody response of all isotypes. It is known that both IgG-BF and IgE-BF are formed by antigenic stimulation of antigen-primed spleen cells (28, 29). If IgD-BF are formed during the immune response, these factors may play an important role in the regulation of the antibody response.

We express great appreciation to Dr. B. Pernis (Columbia University) for helpful advice and to Dr. F. Finkelman for monospecific anti-IgD antibodies. This work was supported by Grant AI22980 from the Department of Health and Human Services. This paper is publication no. 658 from the O'Neill Laboratories at the Good Samaritan Hospital.

1. Fridman, W. H. & Goldstein, P. (1974) *Cell. Immunol.* **11**, 442–455.
2. Yodoi, J. & Ishizaka, K. (1980) *J. Immunol.* **124**, 1322–1329.
3. Yodoi, J., Adachi, M., Teshigahara, K., Miyama-Inaba, M., Masuda, T. & Fridman, W. H. (1983) *J. Immunol.* **131**, 303–310.
4. Löwry, I., Brezin, C., Neauport-Sautes, C., Theze, J. & Fridman, W. H. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2323–2327.
5. Fridman, W. H., Rabourdin-Combe, C., Neauport-Sautes, C. & Gisler, R. H. (1981) *Immunol. Rev.* **56**, 51–88.
6. Huff, T. F., Yodoi, J., Uede, T. & Ishizaka, K. (1984) *J. Immunol.* **132**, 406–412.
7. Coico, R. F., Xue, B., Wallace, D., Pernis, B., Siskind, G. W. & Thorbecke, G. J. (1984) *Nature (London)* **316**, 744–746.
8. Jardieu, P., Uede, T. & Ishizaka, K. (1985) *J. Immunol.* **135**, 922–929.
9. Finkelman, F. D., Kessler, S. W., Mushinski, J. F. & Potter, M. (1981) *J. Immunol.* **126**, 680–687.
10. Liu, E.-T., Bohn, J. W., Ferry, E. L., Yamamoto, H., Molinaro, C. A., Sherman, L. A., Klinman, N. R. & Katz, D. H. (1980) *J. Immunol.* **124**, 2728–2737.
11. Okudaira, H. & Ishizaka, K. (1974) *J. Immunol.* **113**, 563–573.
12. Mage, M. G., McHugh, L. L. & Rothstein, T. (1977) *J. Immunol. Methods* **15**, 47–56.
13. Yodoi, J. & Ishizaka, K. (1979) *J. Immunol.* **123**, 2004–2010.
14. Gonzalez-Molina, A. & Spiegelberg, H. L. (1977) *J. Clin. Invest.* **57**, 616–624.
15. Yodoi, J. & Ishizaka, K. (1979) *J. Immunol.* **122**, 2577–2583.
16. Yodoi, J., Hirashima, M. & Ishizaka, K. (1980) *J. Immunol.* **125**, 1436–1441.

17. Hoover, R. G., Dieckgraefe, B. K., Lake, J., Kemp, J. D. & Lynch, R. G. (1982) *J. Immunol.* **129**, 2329–2331.
18. Yodoi, J., Adachi, M. & Masuda, T. (1982) *J. Immunol.* **128**, 888–894.
19. Huff, T. F. & Ishizaka, K. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1514–1518.
20. Marcelletti, J. F. & Katz, D. H. (1984) *J. Immunol.* **133**, 2821–2828.
21. Marcelletti, J. F. & Katz, D. H. (1984) *J. Immunol.* **133**, 2845–2851.
22. Huff, T. F., Uede, T. & Ishizaka, K. (1982) *J. Immunol.* **129**, 509–514.
23. Xue, B., Coico, R., Wallace, D., Siskind, G. V., Pernis, B. & Thorbecke, G. J. (1984) *J. Exp. Med.* **159**, 103–113.
24. Coico, R. F., Xue, B., Wallace, D., Siskind, G. W. & Thorbecke, J. (1985) *J. Exp. Med.* **162**, 1852–1861.
25. Ishizaka, K. (1984) *Annu. Rev. Immunol.* **2**, 159–182.
26. Suemura, M., Ishizaka, K., Kobatake, S., Sugimura, K., Maeda, K., Nakanishi, K., Kishimoto, S., Yamamura, Y. & Kishimoto, T. (1983) *J. Immunol.* **130**, 1056–1060.
27. Uede, T., Huff, T. F. & Ishizaka, K. (1984) *J. Immunol.* **133**, 803–808.
28. Fridman, W. H., Nelson, R. A., Jr., & Liabeuf, A. (1974) *J. Immunol.* **113**, 1008–1016.
29. Uede, T., Huff, T. F. & Ishizaka, K. (1982) *J. Immunol.* **129**, 1384–1390.