PHYSIOLOGY OF IgD

VI. Transfer of the Immunoaugmenting Effect of IgD With Tδ-containing Helper Cell Populations

By RICHARD F. COICO,* BIN XUE,* DAVID WALLACE,[‡]
GREGORY W. SISKIND,[‡] AND G. JEANETTE THORBECKE*

*From the *Department of Pathology, New York University School of Medicine, New York 10016;
and the ‡Department of Medicine, Cornell University Medical College, New York 10021*

Injections of IgD into mice cause an enhancement of the primary immune response to T-dependent antigens administered ~1 wk thereafter (1). In addition, the 7S antibody response to relatively T-independent antigens is augmented in mice bearing IgD-secreting myelomas (1). This enhancing effect is not obtained in athymic mice and we have suggested, therefore, that it might be mediated by T cells. In further studies we demonstrated that T cells with receptors for IgD are induced by exposure to IgD either in vivo or in vitro (2). The kinetics of the appearance of these "Tδ" cells coincides with that of the in vivo enhancement of the immune response to trinitrophenylated hemocyanin (TNP-KLH),[1] suggesting that the Tδ cells mediate the immunoenhancing effect.

The results to be presented here indicate that T cells from IgD-pretreated mice can transfer the IgD-induced enhancement of immune responsiveness to normal recipients. The presence of an increased incidence of Lyt-1$^+$2$^-$ Tδ cells in the donor cell population correlates with the ability of the cells to augment the immune response in recipients. The data strongly suggest that T cells with receptors for IgD augment immune responses in an antigen-dependent but antigen-nonspecific manner.

Materials and Methods

Mice and Immunization. BALB/c and CBA/J mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. TNP-conjugated KLH (Sigma Chemical Co., St. Louis, MO) was prepared as described by Little and Eisen (3). Mice were injected intravenously at 6–8 wks of age (day 0) with 100 µg TNP-KLH.

Plasmacytomas. The IgD-secreting plasmacytoma, TEPC-1017, was maintained in vivo as previously described (1, 4). Ascites fluid was examined for the presence of IgD by double diffusion in agar using goat anti–mouse IgD (1). Ascites fluid from mice bearing the IgA-secreting MOPC-167 plasmacytoma was similarly collected. Double diffusion assays in agar confirmed the presence of IgA and the absence of detectable IgD in this ascites fluid.

[1] *Abbreviations used in this paper:* C, complement; PBS, phosphate-buffered saline; PFC, plaque-forming cell; RFC, rosette-forming cell; SE, sheep erythrocyte; TNP-KLH, trinitrophenylated hemocyanin.

Purification of IgD. TEPC-1017 ascites was passed over a column of rabbit anti-mouse IgD–coupled Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ). After extensive washing with phosphate-buffered saline (PBS), the IgD was eluted with 4.0 M $MgCl_2$. The eluate was dialyzed and reconstituted to the same volume as the original ascites fluid.

Spleen Cell Fractionation. Adherent cells were removed by incubation of spleen cells in tissue culture grade petri dishes (1400-1; Nunclon, Roskilde, Denmark) at 37°C for 1 h. T cells were eliminated by complement (C)-mediated cytolysis using a monoclonal antibody to Thy-1.2 (6.80) provided by Dr. U. Hammerling (Sloan-Kettering Institute, New York). L3T4+ cells were eliminated by treatment of spleen cells with C and the monoclonal antibody GK1.5 (5), provided by Dr. F. Fitch, (University of Chicago, IL). Splenic T cells were purified by negative selection with the use of goat anti-mouse Ig–coated petri dishes at 4°C as described (6). Lyt-1+2− T cells were prepared from isolated T cells by C-mediated cytolysis using the monoclonal antibody 19/178 (7) provided by Dr. U. Hammerling (Sloan-Kettering Institute).

In some experiments, isolated T cells were exposed to IgD- or IgA-containing ascites fluid using either TEPC-1017- or MOPC-167-coated petri dishes, respectively. Briefly, bacteriological grade, 100-mm petri dishes (Fisher Scientific Co., Pittsburgh, PA) were coated for 1 h at room temperature with 10 ml Tris buffer, pH 9.5, containing 6 mg of ascites protein from either the TEPC-1017 or MOPC-167 plasmacytoma. After three washes with PBS containing 2% fetal calf serum, 3×10^7 T cells suspended in 4 ml of PBS were added and the plates were further incubated at 37°C for 1 h. After this incubation procedure, cells were vigorously triturated to remove them from the dish and the harvested cells were washed three times with PBS.

Cell Transfer. Unless otherwise indicated, 10^7 spleen cells, suspended in saline, were injected intravenously into recipients that had received 100 rad whole-body γ irradiation 1 d earlier from a ^{137}Cs source (Isomedix, Inc., Parsippany, NJ).

Plaque-forming Cell (PFC) Assay. Anti-TNP PFC were assayed by the slide modification of the technique of Jerne et al. (8). TNP sheep erythrocytes (SE) were prepared using the method described by Rittenberg and Pratt (9). IgG-producing cells were developed with rabbit anti-mouse Ig in the complement and goat anti-μ in the agar (10). Background anti-SE PFC were routinely determined. Mean anti-SE responses in all control and experimental groups were always <1,000 PFC/spleen.

Rosette-forming Cell (RFC) Assay. IgD-coated indicator cells were prepared by coupling affinity-purified IgD to SE using the $CrCl_3$ coupling method of Poston (11). Coupling of IgD to SE was confirmed both by positive hemagglutination and indirect immunofluorescence assays using a rabbit anti–mouse IgD antiserum. Enumeration of IgD-RFC was performed as previously described (2). Lymphocytes surrounded by more than three indicator cells were scored as rosettes and the results expressed as percent RFC. In each experiment, the percentage of cells forming rosettes with SE was determined and was always <2%.

Results

Transfer of the Augmentation of the Response to TNP-KLH by T Cells Exposed to IgD In Vivo. The results in experiments 1, 2, and 3 in Table I show that transfer of spleen cells from IgD-pretreated donors, simultaneously injected, intravenously, with TNP-KLH, led to enhanced responses in γ-irradiated (100 rad) recipients. Similar results were obtained using unirradiated recipients (Table I, Exp. 4). Normal spleen cells did not significantly raise the response above that of mice receiving antigen alone (Exp. 1). Spleen cells, depleted of Ig-bearing cells by panning on anti-Ig–coated petri dishes, transferred the IgD-induced augmentation of the immune response, whereas anti-Thy-1.2 and C–treated spleen cells from IgD-pretreated donors did not transfer augmentation (Exp. 2, Table I). Similarly, donor spleen cells from IgD-pretreated mice failed to transfer

TABLE I

Ability of Splenic T Cells to Transfer the Immunoaugmenting Effect to IgD

Donor cells transferred[*]	Pre-injection of donors with IgD[‡]	Geometric mean ($\overset{\times}{÷}$SE) of anti-TNP PFC/spleen[§]			
		Exp. 1	Exp. 2	Exp. 3	Exp. 4
None		2,230 (1.4)		2,480 (1.1)f,g	9,720 (1.1)
Spleen cells	−	3,950 (1.3)a[§§]	3,400 (1.4)b,c		9,840 (1.2)h
	+	12,320 (1.2)a	14,760 (1.1)b	6,950 (1.1)f	19,380 (1.1)h
	+[¶]		11,060 (1.1)c		
Splenic T cells[∥]	−		3,980 (1.2)d		
	+		13,380 (1.2)d		
Splenic B cells[**]	−		3,840 (1.2)e		
	+		4,370 (1.1)e		
L3T4⁻ spleen cells[‡‡]	+			3,240 (1.2)g	

* Recipients were irradiated on day −1 with 100 rad except in experiment 4. All recipients were injected intravenously on day 0 with 10^7 donor cells together with 100 µg TNP-KLH. Anti-TNP PFC responses were determined on day 5 ($n = 5{-}8$).

‡ Donor mice were preinjected intraperitoneally with 0.5 ml of IgD-containing ascites fluid on days −8, −7, −6, and −5 relative to the day of sacrifice (day 0).

§ 19S anti-TNP PFC responses, geometric mean $\overset{\times}{÷}$ SE.

∥ T cells were prepared by panning on anti-Ig–coated dishes at 4°C after removal of adherent cells at 37°C. Control cells were simultaneously incubated on uncoated dishes.

¶ Donors were injected intraperitoneally with 250 µg affinity-purified IgD on days −8, −7, −6, and −5.

** B cells were prepared by anti-Thy-1.2 + C treatment; control cells were treated with C alone.

‡‡ L3T4⁻ spleen cells were prepared by cytotoxic elimination using GK1.5 + C.

§§ P values: (a) 0.007, (b) 0.005, (c) 0.01, (d) 0.005, (e) not significant, (f) 0.05, (g) not significant, (h) 0.04.

augmentation of the immune response when the L3T4⁺ T cells were eliminated from the donor cell population (Table I, Exp. 3). These results together indicate that the augmentation was mediated by L3T4⁺ T cells. Transfer of the helper T cell subpopulation from IgD-pretreated donors showed that 5×10^6 Lyt-1⁺2⁻ cells caused as much augmentation of the anti-TNP response as did 10^7 unfractionated T cells (Fig. 1).

The incidence of IgD-RFC in these cell populations from IgD-pretreated mice was also determined (Fig. 2). The background IgD-RFC was <5% for all cell populations studied. Splenic B cells from IgD pretreated donors showed an IgD-RFC frequency barely above this background. We have recently reported (2) that L3T4⁻ T cells from IgD-pretreated mice also show only background levels of IgD-RFC. In contrast, both unfractionated and Lyt-1⁺2⁻ T cells demonstrated a significantly increased percentage of IgD-RFC, ranging from 20 to 30% (Fig. 2).

Transfer of the Augmentation of the Response to TNP-KLH by T Cells Exposed to IgD In Vitro. In previous studies (2) it was shown that overnight incubation at
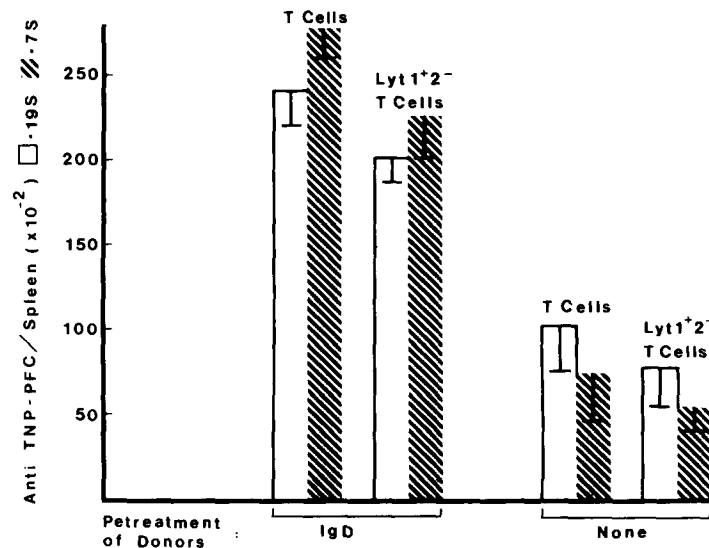
FIGURE 1. Augmenting effect on the immune response by transfer of Lyt-1$^+$2$^-$ T cells from IgD-pretreated donors. Groups of five BALB/c recipient mice received 100 rad γ irradiation on day -1. On day 0 they were injected intravenously with either 10^7 T cells or 5 × 10^6 Lyt-1$^+$2$^-$ T cells from IgD-pretreated or normal BALB/c mice, simultaneously with 100 μg TNP-KLH. The augmenting effect of cells from IgD-pretreated as compared with normal donors was statistically significant: T cells, $P = 0.01$ (19S) and $P = 0.002$ (7S); Lyt-1$^+$2$^-$ cells, $P = 0.03$ (19S) and $P = 0.01$ (7S).

37°C of BALB/c T or spleen cells with IgD induced Lyt-1$^+$2$^-$ T cells from normal BALB/c mice to express detectable IgD receptors. It was of interest to examine the kinetics of induction of IgD receptor-positive T cells in vitro and to determine whether such T cells could augment antibody formation in vivo. The results in Fig. 3 show that incubation for 1 h in IgD-coated petri dishes, induced the appearance of IgD receptors on a large percentage of T cells, whereas cells incubated in IgA-coated or uncoated petri dishes did not.

Whole spleen or splenic T cells were examined for their ability to augment immune responses in 100-rad–γ-irradiated recipients after 1 h preincubation in vitro in petri dishes coated with IgD- or with IgA-containing ascites fluid or left uncoated (Table II). Cells from normal donors incubated in IgD-coated dishes enhanced the immune response of recipients as compared with the responses of mice that received either antigen alone or antigen and cells incubated in uncoated dishes. It should be noted that the TNP-KLH preparation used in Exp. 2, Table II induced much higher responses than the preparation used in the other experiments. However, the enhancement obtained with IgD-exposed T cells was still 56% for 19S ($P = 0.02$) and 79% for 7S ($P < 0.01$) as compared with 37% for 19S ($P = 0.02$) and 0% for 7S with cells incubated on uncoated dishes. Cells incubated in IgA-coated petri dishes, on the other hand, appeared to cause a suppression of the immune response in recipients. To rule out the possibility that the enhancement by cells incubated with IgD was actually due to the injection of IgD which leached off the dish during incubation, thymocytes were similarly exposed to IgD-coated dishes and transferred. The responses in recipients of
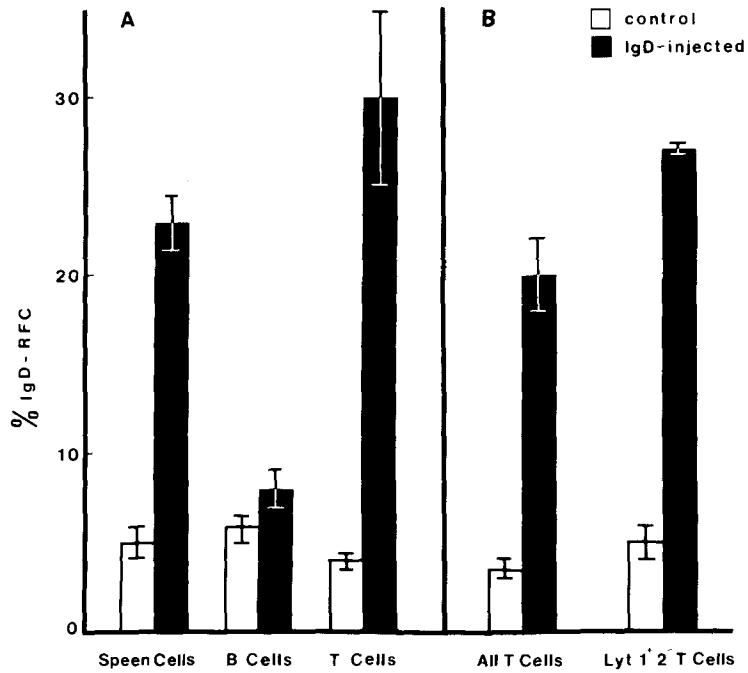
FIGURE 2.   Incidence of IgD-RFC in spleen cells, and in isolated B and T cells of IgD-pretreated and control BALB/c mice.
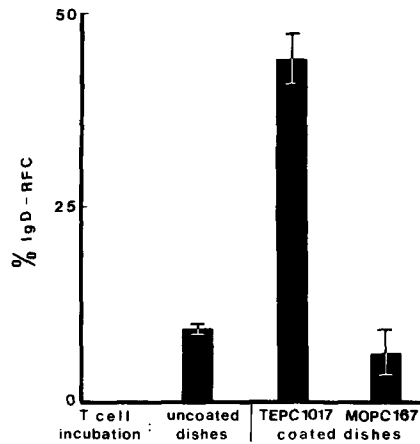

FIGURE 3.   Incidence of IgD-RFC in BALB/c T cells after incubation in dishes coated with IgD-containing ascites (TEPC-1017), IgA-containing ascites (MOPC-167), or in uncoated petri dishes. The incubation period was 1 h at 37°C followed by the RFC assay as described in Materials and Methods.

these cells were no higher than in recipients of antigen alone (data not shown). It was previously shown (2) that thymocytes cannot be induced to exhibit IgD-RFC by incubation with IgD.

The response in recipients of IgD-pretreated donor cells incubated in uncoated dishes (Exp. 1, Table II) was significantly higher than that in recipients of normal

TABLE II

Effect of In Vitro Exposure to IgD on the Immune Augmenting Ability of T Cells

Donors	Donor cells incubated in:	TNP-PFC/spleen in recipients of:*					
		Exp. 1 Whole spleen cells		Exp. 2 Splenic T cells		Exp. 3 Splenic T cells	
		19S	7S	19S	7S	19S	7S
Normal mice	Uncoated dishes	3,400 (1.4)a,b§	1,200 (1.3)d,e	45,100 (1.1)g	35,300 (1.1)i	—	—
	TEPC-1017 (IgD)–coated dishes	11,400 (1.4)a,c	6,700 (1.0)d,f	51,400 (1.1)h	62,900 (1.1)i-j	—	—
	MOPC-167A (IgA)–coated dishes	—	—	15,000 (1.1)g	23,400 (1.1)	—	—
	No cells transferred	—	—	33,000 (1.2)h	35,200 (1.1)j	—	—
IgD-preinjected mice‡	Uncoated dishes	14,800 (1.1)b	6,700 (1.3)e	—	—	11,900 (1.4)k	2,800 (1.3)l
	TEPC-1017 (IgD)–coated dishes	18,600 (1.1)c	10,700 (1.2)f	—	—	17,900 (1.1)k	8,000 (1.2)l
	MOPC-167A (IgA)–coated dishes	—	—	—	—	11,900 (1.1)	2,600 (1.2)

* Recipients were irradiated with 100 rad on day −1, then received 10^7 donor cells on day 0 together with 100 µg TNP-KLH. Their anti-TNP PFC responses were determined and results are expressed as the geometric mean $\overset{\times}{\div}$ SE ($n = 3–7$).

‡ Mice were injected intraperitoneally with 0.5 ml of IgD-containing ascites fluid on days −8, −7, −6, and −5 before the day of sacrifice (day 0).

§ P values: (a) 0.05, (b) 0.003, (c) 0.002, (d) 0.0005, (e) 0.002, (f) 0.035, (g) 0.002, (h) 0.02, (i) 0.003, (j) 0.006, (k) 0.03, (l) 0.01.

spleen cells similarly incubated ($P = 0.003$ for 19S and $P = 0.002$ for 7S PFC). It was of interest to determine whether this immunoaugmenting effect of spleen cells that had already been exposed to IgD in vivo could still be enhanced further by reexposure to IgD in vitro. The results of Exps. 1 and 3, Table II show that this was indeed possible. The responses of recipients receiving cells incubated in IgD-coated petri dishes (Exp. 1) showed a greater enhancement when cells from IgD-pretreated donors rather than normal cells were used ($P = 0.002$ for 19S and $P = 0.035$ for 7S PFC). Similarly, as shown in Exp. 3, Table II, when splenic T cells isolated from IgD-pretreated donors were reexposed to IgD in vitro, their ability to enhance the response in recipients was further enhanced as compared with T cells incubated either on uncoated or in IgA-coated petri dishes ($P = 0.03$ for 19S and $P = 0.01$ for 7S PFC).

Lack of Strain Specificity of the Effects of IgD. In previous experiments (2) it was shown that IgD-RFC can be induced by exposure to IgD of *a* allotype in spleen cells from *b* allotype congenic C.B20 mice. In addition, IgD-injected C.B20 mice showed significantly enhanced anti-TNP PFC responses (2). It was of interest to determine whether exposure of T cells from a mouse strain of different genetic background to TEPC-1017 protein had similar effects. Overnight incubation of CBA/J spleen cells at 37°C in IgD-containing medium caused the appearance of $29.4 \pm 1.9\%$ IgD-RFC ($n = 9$). The results in Fig. 4 show, in addition, that T cells from normal CBA/J spleen cells incubated in IgD-coated petri dishes significantly enhanced both the 19S and 7S PFC responses to TNP-KLH of 100-rad γ-irradiated syngeneic mice.

Discussion

The present results establish for the first time the existence of immunoaugmenting T cells that are induced, either in vivo or in vitro, by exposure to IgD. Pretreatment of donors with IgD induced the appearance of a population of Lyt-1^+2^- T cells with both receptors for IgD (Tδ) and the ability to transfer augmentation of the immune responses to lightly irradiated or unirradiated recipients. B cells from such IgD-pretreated donors or spleen cells from which only the L3T4$^+$ T cells had been removed did not transfer immunoaugmentation and did not show increased rosette formation with IgD-coated SE. These results agree with our previous observation (2) that IgD-RFC induced by treatment with IgD in vivo are predominantly L3T4$^+$, Lyt-1$^+$, Lyt-2$^-$. In addition, the kinetics of the appearance of these IgD-RFC after injection of IgD in vivo correlates with the enhancement of immune responses in IgD-injected mice (2).

The augmenting effect of spleen cells incubated in IgD-coated petri dishes was probably not due to cotransfer of IgD, since the petri dishes had been extensively rinsed to remove any weakly bound IgD, and thymus cells incubated on IgD-coated petri dishes did not cause immunoaugmentation. Previous experiments (2) have shown that IgD-RFC can be induced in spleen and lymph node cells, but not in thymus cells after in vivo or in vitro exposure to IgD. The observation that IgD also induces immune-augmenting properties and IgD-RFC in T cells from CBA mice, indicates that these effects are not strain specific. This is consistent with our previous finding (2) of augmentation of the immune response
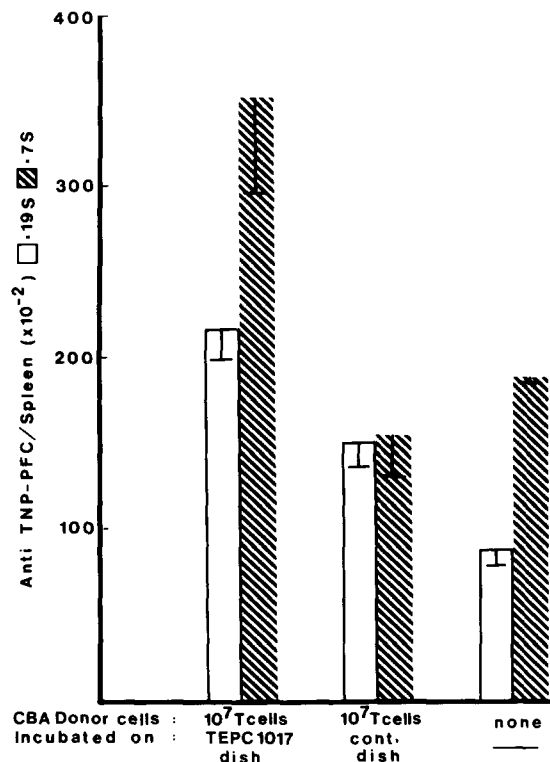
FIGURE 4.   Augmenting effect on the immune response of splenic T cells from CBA mice, after incubation for 1 h in TEPC-1017 ascites-coated petri dishes, in syngeneic, 100-rad γ-irradiated recipients. The augmentation by T cells exposed to IgD as compared with control cells, incubated in uncoated dishes, was statistically significant: $P = 0.02$ (19S) and $P = 0.008$ (7S).

by IgD of a allotype in b allotype C.B20 mice, indicating a lack of allotype specificity.

Previous studies have shown that the injection of antigen (2) or allo anti-IgD (Coico, unpublished results) induces the appearance of significantly increased numbers of IgD-RFC (11 and 41%, respectively) by day 5 after injection in BALB/c mice. It is possible that the augmentation of immune responses previously shown to occur after injection of heterologous anti-IgD (12, 13) is, in part, the result of the induction of Tδ cells, although an enhanced response to the foreign Ig bound to B cells, as shown by Finkelman et al. (14), clearly plays an important part in this enhancement. It is probable that the augmenting effect of allotype-specific murine anti-IgD on the immune response to TNP-KLH of the alternate allotype in heterozygous mice (15) is due to induction of Tδ cells.

The immunoaugmenting effect of two injections of IgD 1 wk apart was previously shown to be greater than that of a single IgD injection (2). This is consistent with the present results showing that reexposure of T cells from IgD-pretreated mice to IgD in vitro further increases their immunoaugmenting capability. Since the frequency of IgD-RFC is already at maximal levels 7 d after a single injection of IgD, it seems possible that, as we suggested previously (2),

the reexposure to IgD increases the avidity of the Tδ cells for IgD either by increasing the number of receptors or their affinity.

IgD injection or transfer of Tδ cells did not cause an increase in the background PFC to SE. Thus, the immunoaugmentation appears to be antigen dependent. We propose that the augmenting effect of Tδ cells is mediated by their interaction with surface IgD on B cells that are in the process of reacting with antigen, and not by recognition of IgD on the surface of resting B cells. These findings provide a biological rationale for the presence of IgD on the B cell surface and its relatively low concentration in serum. It might be presumed that, after its reaction with antigen, B cell surface IgD receives a signal from Tδ cells. The Tδ cells may either be induced by increased serum IgD levels, or possibly by IgD-antigen immune complexes in serum or on the B cell surface itself. It is interesting to note that both IgD-secreting myelomas (TEPC-1017 and TEPC-1033) secrete IgD molecules which, on polyacrylamide gel electrophoresis, appear to be oligomers rather than monomers (4). Thus, we are proposing the existence of a regulatory circuit based upon interaction between Tδ cells, antigen, B cell surface IgD, and serum IgD. In addition, the possible role of passively acquired IgD-antigen complexes on the T cell surface in the induction of idiotype-antiidiotype circuits needs to be evaluated.

Summary

We show that the IgD-induced augmentation of the immune response to trinitrophenylated keyhole limpet hemocyanin can be transferred to syngeneic mice with spleen cells from IgD-injected donors. The augmenting activity is present in the Lyt-1^+2^-, L3T4$^+$ T cell population and is absent from B cells. The ability of transferred T cells to augment the immune response correlates with the presence of a high frequency of Lyt-1^+2^- T cells that form rosettes with IgD-coated sheep erythrocytes (Tδ cells). Such rosette-forming cells can also be induced by incubation of spleen cells from normal donors in IgD-coated petri dishes. Injection of normal spleen cells exposed to IgD-coated petri dishes together with antigen also augments the immune response of recipients. The existence of a regulatory circuit based upon interactions between Tδ cells, antigen, B cell surface IgD, and serum IgD, is proposed.

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