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Interleukin-5 induces maturation but not class switching of surface IgA-positive B cells into IgA-secreting cells

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SUMMARY

There is a body of evidence suggesting that IgA production is regulated by helper T cells or their products. To elucidate molecular mechanisms of IgA production, the role of lymphokines in the *in vitro* antigen-specific and polyclonal IgA responses was examined. Supernatants from antigen-stimulated T cells or mitogen-stimulated T-cell clones can enhance 2,4-dinitro phenyl (DNP)-specific IgM, IgG1 and IgA production in cultures of DNP-ovalbumin (OVA)-stimulated T-cell-depleted spleen cells from DNP-keyhole limpet haemocyanin-primed mice. The IgA enhancement was inhibited by anti-IL-5 monoclonal antibody. Purified recombinant IL-5 could also enhance anti-DNP IgA production in a dose-dependent manner. This enhancing effect was not substituted by IL-1, IL-2, IL-3 or IL-4. Polyclonal IgA secretion of lipopolysaccharide-stimulated normal B cells was augmented preferentially by IL-5, but not by IL-4. Surface IgA-positive (sIgA$^+$) B cells, but not surface IgA-negative B cells, responded to IL-5 for the development of IgA-secreting cells. Limiting-dilution analysis revealed that IL-5 increases the frequency of IgA-secreting cells in sIgA$^+$ B-cell populations. These results indicate that IL-5 plays an essential role in the antigen-specific and polyclonal IgA formation as a maturation-inducing factor rather than class-switching factor.

INTRODUCTION

The molecular mechanism by which T cells co-operate with B cells for antibody responses has been the subject of extensive investigation. It has been shown that there are two pathways existing wtih regard to B-cell activation by T cells. In one of them, B cells can be triggered by interaction with helper T cells, cognate interaction. In this T–B-cell interaction, T cells recognize carrier determinants in association with I-region-encoded molecules on B cells. In the other pathway, B and T cells can interact in a non-cognate manner in which helper T cells act through the release of antigen non-specific factors (lymphokines) that facilitate the growth and differentiation of B cells in response to particular antigens in a MHC-unrestricted manner (Howard *et al.*, 1982). T-cell-derived factors have been shown to induce differentiation of activated B cells to high-rate Ig secretion (Howard & Paul, 1983: Kishimoto, 1985).

There has been considerable controversy regarding the role of T cells in the regulation of immunoglobulin (Ig) class switching (Cebra, Komisar & Schweltzer, 1984). Kishimoto & Ishizaka (1973, 1975) clearly demonstrated that T-cell factors involved in IgE synthesis are different from those involved in IgG synthesis in a rabbit system. Since then evidence has been accumulating suggesting that T cells play an important role in the regulation of Ig class switching. The studies by Isakson *et al.* (1982) and Vitetta *et al.* (1984), followed by others (Bergstedt-Lindqvist *et al.*, 1984; Lee *et al.*, 1986; Noma *et al.*, 1986), provide evidence regarding the existence of T-cell factors (BCDFγ) involved in IgG1 class 'switch'. It has also been demonstrated that IL-4 (BCDFγ, BSF-1) induces lipopolysaccharide (LPS)-stimulated B cells to secrete IgG_1 and IgE (Noma *et al.*, 1986; Coffman *et al.*, 1986; Paul & Ohara, 1987), and IFN-γ increases IgG2a secretion of LPS-stimulated B cells (Snapper & Paul, 1987).

Interleukin-5 (IL-5) is a T-cell-derived, antigen non-specific B-cell differentiation factor which we called T-cell replacing factor (TRF) (Takatsu, Tominaga & Hamaoka, 1980b; Takatsu *et al.*, 1980a; Takatsu *et al.*, 1985). Primary IL-5 molecule consists of a single polypeptide chain of 113 amino acids (MW 12,400), which is strongly hydrophobic and heavily glycosylated (Takatsu *et al.*, 1988; Kinashi *et al.*, 1986; Tominaga *et al.*,

Abbreviations: BSA, bovine serum albumin; DNP, 2,4-dinitrophenyl; D9-Sup, medium conditioned by concanavalin A-stimulated D9 cells; EL4-Sup, medium conditioned by PMA-stimulated EL4 cells; Ig, immunoglobulin; IL-5, interleukin-5 prepared by recombinant DNA technology; LPS, lipopolysaccharide; OVA, ovalbumin; PFC, plaque-forming cells; PPD-Sup, medium conditioned by PPD (purified protein derivatives)-stimulated Tbc-primed cells; sIgA, surface IgA; Tbc, *Mycobacterium tuberculosis.*

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1988). IL-5 can promote DNA synthesis of activated B cells and murine chronic leukaemia cells (BCGFII activity) (Kinashi *et al.*, 1986; Tominaga *et al.*, 1988; Takatsu *et al.*, 1988; Harada *et al.*, 1985; Swain, 1985) and causes differentiation of 2,4-dinitrophenyl (DNP)-primed splenic B cells into anti-DNP IgM- and IgG-secreting cells (TRF activity) (Takatsu *et al.*, 1985; Kinashi *et al.*, 1986; Tominaga *et al.*, 1988; Harada *et al.*, 1985). It also induces an increase in the steady-state μ-mRNA expression for the secreted forms of IgM in normal as well as LPS-activated B cells (Matsumoto *et al.*, 1987).

IgA is the dominant Ig class present in mucosal secretions and plays a major role in influencing on a wide variety of pathogenic and non-pathogenic antigens (McNabb & Tomasi, 1981). The molecular events associated with IgA-specific B-cell switching are not completely understood. Kawanishi, Saltzman & Strober (1983a, b) demonstrated that T cells in Peyer's patches, but not in spleen, induce LPS-stimulated IgM-bearing B cells to switch to surface IgA-positive (sIgA$^+$) B cells. Mayer, Fu & Kunkel (1982) reported that malignant T cells from a patient with mycosis fungoides/Sezary's syndrome promoted Ig class switching from IgM to IgA and IgG.

Recently, Coffman and his associates (Bond *et al.*, 1987; Coffman *et al.*, 1987; Yokota *et al.*, 1987) and others (McKenzie *et al.*, 1987; Murray *et al.*, 1987; Harriman *et al.*, 1988) showed that IL-5 enhances a three to six-fold increase in IgA formation by LPS-stimulated B cells, together with less than a two-fold increase in other isotypes. It is not clear whether IL-5 acts on precommitted B cells as a switch factor for IgA and on post-switch sIgA$^+$ B cells and promotes their proliferation (BCGFII), or induces terminal differentiation into IgA-secreting cells (TRF activity). In this study, we determined whether IL-5 preferentially induces DNP-specific IgA responses in DNP-primed B cells and whether IL-5 acts as a class-switching factor or maturation factor. The results revealed that IL-5 can stimulate sIgA$^+$ B cells, but not sIgA$^-$ B cells, then giving rise into IgA-producing cells.

MATERIALS AND METHODS

Mice

Inbred, pathogen-free female BALB/cCrSlc mice were obtained from Shizuoka Animal Center, Hamamatsu. They were immunized, at 6-8 weeks of age, with DNP-keyhole limpet haemocyanin (KLH; Calbiochem, San Diego, CA) (Takatsu *et al.*, 1980a).

Culture Medium

RPMI-1640 medium (Sigma, St Louis, MO) was supplemented with 2-mercaptoethanol (50 μM), penicillin (100 U/ml), streptomycin (100 μg/ml) and 10% fetal calf serum (FCS) (Flow Laboratories, Inglwood CA). For IL-5 assay, we also used serum-free medium (ASF-101; Ajinomoto, Tokyo) supplemented with 2-mercaptoethanol (50 μM), penicillin, streptomycin and bovine serum albumin (BSA) (0·5%).

Cell lines

A TRF-producing T-cell hybridoma B151K12 was maintained *in vitro* as described elsewhere (Takatsu *et al.*, 1980a). EL4 (thymoma cells) and C1.Ly 1$^+$2$^-$/9 (D9) cell lines were kindly provided by Dr T. Hirano (Institute for Molecular and Cellular Biology, Osaka University) and Dr R. Coffman (DNAX Research Institute, Palo Alto), respectively, and were maintained *in vitro*. The plasmacytoma MOPC104E ($\mu,\lambda 1$), MOPC70A ($\gamma 1$,k), and MOPC315 ($\alpha,\lambda 2$) were maintained by intraperitoneal passage. Ascitic fluid from each myeloma-bearing mice were used for purification of each myeloma protein.

Antibody

Monoclonal rat IgG1 anti-IL-5 (TRF) antibody was obtained from a B-cell hybridoma, TB13, which was conducted by fusion between murine myeloma cells (P3-X63-Ag8.653) and rat spleen cells that had been immunized with HPLC-purified B151-TRF as described elsewhere (Harada *et al.*, 1987). Ascitic fluid of mice injected with the hybridoma was purified using protein A-coupled Sepharose CL-4B beads (Pharmacia, Uppsala, Sweden) and was used as a source of anti-IL-5 antibody. The purified TB13 antibody (3 mg) was coupled to formylcellurofine beads (Seikagaku-Kogyo, Tokyo) (Harada *et al.*, 1987). Monoclonal rat anti-IL-2 receptor antibody, PC61, and anti-IL-4 antibody, 11B11, were kindly provided by Dr M. Nabholz (Swiss Cancer Research Center, Epalinges), and Drs Junichi Ohara and William E. Paul (NIH, Bethesda), respectively. Polyclonal rabbit antibody against IgM, IgG1, or IgA was prepared as previously described (Matsumoto *et al.*, 1987).

Lymphokines

Supernatants (Sup) of PPD-stimulated *Mycobacterium tuberculosis* (Tbc)-primed cells (PPD-Sup) was prepared by procedures as described elsewhere (Takatsu *et al.*, 1980b). PPD-Sup contains at least IL-1, IL-2, IL-3, IFN-γ and IL-5. IL-5-containing Sup produced by phorbol 12-myristate-13-acetate stimulated EL4 cells (EL4-Sup) and concanavalin A-stimulated D9 cells (D9-Sup) were prepared as described previously (Tominaga *et al.*, 1988). Naturally produced IL-5 by B151K12 was purified by using a TB13-coupled immunoaffinity column (Harada *et al.*, 1987). Recombinant murine IL-5 was also purified from Sup of HeLa cells that had been transfected with murine IL-5 cDNA (pkCR-Neo^r-m TRF) (Kinashi *et al.*, 1986), by the same procedures (Harada *et al.*, 1987). Recombinant human IL-1 was a kind gift from Dr Y. Hirai (Cellular Engineering Laboratories, Ohtsuka Pharmaceutical Co. Ltd, Tokushima); human recombinant IL-2 and murine recombinant IL-4 were provided by Dr M. Takaoki (Takeda Pharmaceutical Co. Ltd, Osaka) and Dr T. Honjo (Kyoto University), respectively.

Preparation of purified B cells

Purified B cells were prepared from cell suspensions of spleen from normal or DNP-KLH-primed BALB/c mice according to described methods (Takatsu *et al.*, 1985). In brief, spleen cells from mice which had received 0·2 ml of anti-thymocytes antiserum 2 days before the experiments were treated twice with anti-Thy-1.2 antibody (Serotec, London, U.K.) and non-cytotoxic rabbit complement. The cells thus obtained were used as purified B cells. To enrich sIgA$^+$ B cells, T-cell depleted splenic B cells were incubated for 1 hr at room temperature on petri-dishes that had been precoated with purified F(ab')$_2$ fragments of rabbit anti-mouse IgA antibody, and the adherent cells were recovered according to methods described by Mage, McHugh & Rothestein (1977) with minor modifications (Koyama *et al.*, 1988).

Table 1. Effects of anti-IL-5 antibody on the anti-DNP antibody response by spleen cells from DNP-KLH-primed mice

Antigen *in vitro*	Antibody	Anti-DNP antibody response		
		IgM	IgG1	IgA
None	None	24 (1·02)	26 (1·25)	16 (1·01)
DNP-KLH	None	330 (1·13)	1260 (1·05)	1458 (1·11)
DNP-KLH	TB13	94 (1·16)	628 (1·10)	706 (1·06)
DNP-KLH	11B11	269 (1·14)	1652 (1·08)	1604 (1·02)
DNP-KLH	PC61	284 (1·08)	1492 (1·11)	1560 (1·10)
KLH+DNP-OVA	None	98 (1·06)	399 (1·10)	299 (1·21)
KLH+DNP-OVA	TB13	36 (1·29)	98 (1·21)	95 (1·45)

DNP-KLH-primed spleen cells (5×10^5/0·2 ml/well) were stimulated with either DNP-KLH (12 ng/well) or KLH (25 μg/well) plus DNP-OVA (12 ng/well). Each antibody was added at the commencement of the culture: TB13, anti-IL-5 antibody (3 μg/ml); 11B11, anti-IL-4 antibody (3 μg/ml); PC61, anti-IL-2 receptor antibody (3 μg/ml). Results are expressed as mean anti-DNP PFC per culture and standard errors.

Table 2. Supernatants of T cells or T-cell lines support IgA production

T-cell Sup		TB13	Anti-DNP antibody response		
Source	%	(μg/ml)	IgM	IgG1	IgA
Medium	—	0	12 (1·58)	76 (1·29)	59 (1·89)
PPD-Sup	50	0	130 (1·02)	582 (1·04)	396 (1·10)
	12	0	68 (1·05)	291 (1·03)	254 (1·21)
	50	3	46 (1·30)	298 (1·05)	189 (1·13)
EL4-Sup	50	0	48 (1·21)	389 (1·06)	358 (1·08)
	12	0	26 (1·55)	183 (1·10)	124 (1·02)
	50	3	10 (1·01)	143 (1·21)	119 (1·25)
D9-Sup	50	0	118 (1·04)	368 (1·25)	388 (1·20)
	50	3	54 (1·01)	116 (1·06)	150 (1·02)

A total of 5×10^5 DNP-primed B cells in 0·2 ml per well was stimulated with DNP-OVA (12 ng/well) and T cell Sup indicated on Day 0. After 5-day culture, anti-DNP PFC assay was conducted. Results are expressed as mean anti-DNP PFC per culture and standard errors.

In vitro *cultures*

To examine anti-DNP antibody responses, purified DNP-primed B cells (5×10^5/0·2 ml/well) were cultured in Corning microplates (no. 25860; Corning, NY) for 5 days in the presence of various interleukins and stimulated with DNP-ovalbumin (OVA) (12 ng/well). After the culture the numbers of anti-DNP PFC were enumerated by Jerne's plaque assay using TNP-SRBC and rabbit anti-mouse isotype-specific antiserum (Takatsu *et al.*, 1980b). The numbers of IgM PFC were determined by direct PFC. The numbers of IgA- or IgG1-specific PFC were calculated as the difference between indirect PFC for IgA or for IgG1 and direct PFC. To assess polyclonal antibody response, purified normal splenic B cells (1×10^5/well) were cultured in microplates (no. 25860; Corning) in the presence of 10 μg/ml LPS (Difco Laboratory, Detroit, MI). Recombinant interleukins were added 24 hr later to the culture, as described elsewhere (Matsumoto *et al.*, 1987). The numbers of Ig-secreting cells were enumerated 5 days after the initiation of the culture by reversed PFC assays using protein A-coupled SRBC and isotype-specific rabbit anti-mouse serum (Gronowitz, Coutinho & Melchers, 1976).

Limiting-dilution analyses were performed on the various B-cell preparations in cultures containing 3×10^5 rat thymocytes as filler cells and purified recombinant IL-5 according to the methods described by Andersson, Lernhardt & Melchers, (1979). B-cell concentrations covered the range of 4×10^3 to 1 cell/culture in two-fold steps. The cultures for each dilution were set up in microplates.

The number of PFC per culture was logarithmically transformed, and geometric means and standard errors were calculated. Group comparisons were made by employing Student's *t*-test.

RESULTS

Effect of anti-IL-5 antibody on T-cell-dependent IgA antibody response

To evaluate whether IL-5 is involved in the induction of antigen-specific T-cell-dependent IgA response, DNP-KLH-primed spleen cells were stimulated with either DNP-KLH or a mixture of KLH and DNP-OVA in the presence or absence of anti-IL-5, anti-IL-4, or anti-IL-2 receptor antibody. Stimulation of DNP-KLH-primed spleen cells with DNP-KLH gave anti-DNP IgM, IgG1 and IgA responses (Table 1). This was significantly inhibited by anti-IL-5 antibody, but not by either anti-IL-4 or anti-IL-2 receptor antibody. Significant anti-DNP IgA antibody production was also observed in culture of DNP-KLH-primed cells stimulated with KLH plus DNP-OVA. This anti-DNP IgA production was also profoundly inhibited by anti-IL-5 antibody (Table 1). These results strongly suggest that IL-5 plays an important role in the generation of antigen-specific secondary antibody responses induced by cognate as well as factor-mediated T–B-cell interaction.

Enhancement of DNP-specific IgA antibody response of DNP-primed B cells by IL-5

We stimulated purified DNP-KLH-primed splenic B cells with DNP-OVA in the presence of PPD-Sup, EL4-Sup, or D9-Sup. As shown in Table 2, each Sup enhanced anti-DNP antibody response of IgM, IgG1 and IgA isotype. The enhancement of IgA production ranged from five to seven-fold in three separate experiments, and was inhibited by the anti-IL-5 antibody. Thus we determined enhancement of anti-DNP IgA antibody response by natural and recombinant IL-5 and other interleukins. As shown in Fig. 1, not only naturally produced B151-IL-5, but also recombinant IL-5 induced anti-DNP IgA antibody responses, whereas the other lymphokines failed to do so.

Enhancement of polyclonal IgA response in LPS-stimulated B cells by IL-5

To compare the effect of IL-5 with IL-4 on isotype expression of LPS-stimulated B cells, purified normal B cells were stimulated with LPS. Either IL-5 or IL-4, in the presence or absence of IFN-γ was added to the culture on Day 1. Results revealed that IL-5 can induce polyclonal IgM, IgG1 and IgA production (Table 3). In contrast, IL-4 could induce only IgG1 production to higher extent than IL-5, and no significant IgA production. The IL-4 but not IL-5 activity of IgG1 induction was remarkably inhibited by IFN-γ. However, IL-5-dependent polyclonal IgG1 and IgA production was not affected by IFN-γ. IL-1, IL-2, IL-3 or GM-CSF did not induce polyclonal IgA production in LPS-stimulated B cells (data not shown).

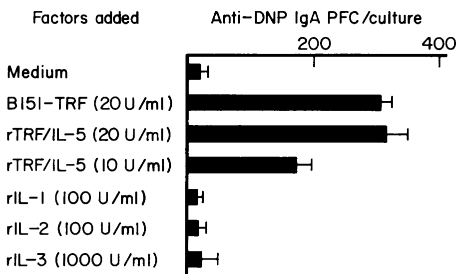

Figure 1. Effect of IL-5 on anti-DNP IgA antibody production by DNP-primed B cells. DNP-primed splenic B cells (5×10^5/0·2 ml/well) were cultured with purified lymphokines (units/ml) indicated for 5 days. All the culture was stimulated with DNP-OVA (12 ng/well). After the culture the numbers of anti-DNP IgA PFC were enumerated. Results are expressed as mean anti-DNP IgA PFC per culture and standard errors. B151-IL-5 was purified from B151K12-Sup using anti-IL-5 antibody coupled affinity column.

Table 3. Enhancement of IgA production by LPS-stimulated B cells by IL-5

Lymphokines*			Polyclonal Ig production		
IL-5	IL-4	IFN-γ	IgM	IgG1	IgA
—	—	—	1652 (1·02)	78 (1·23)	92 (1·19)
10	—	—	2809 (1·11)	166 (1·05)	308 (1·11)
10	—	100	2398 (1·11)	178 (1·13)	302 (1·36)
100	—	—	2451 (1·06)	240 (1·11)	356 (1·02)
—	10	—	1596 (1·19)	319 (1·12)	79 (1·35)
—	100	—	1136 (1·19)	494 (1·19)	49 (1·10)
—	100	10	1582 (1·11)	118 (1·22)	46 (1·50)

Splenic B cells (1×10^5/0·2 ml/well) were cultured with LPS (10 μg/ml) for 5 days. Lymphokines (units/ml) indicated were added on Day 1 of the culture. After the culture numbers of IgM, IgG1 or IgA-secreting cells were enumerated by a reversed phase PFC assay. Results are expressed as mean PFC per culture and standard errors.

* Units/ml.

Table 4. Induction of IgA production from B cells in Peyer's patches by IL-5

	Lymphokines*		Polyclonal antibody response		
	IL-5	IL-4	IgM	IgG1	IgA
Peyer's patch	—	—	2825 (1·18)	122 (1·21)	152 (1·13)
	10	—	4256 (1·10)	238 (1·09)	398 (1·18)
	100	—	5866 (1·05)	426 (1·02)	596 (1·09)
	—	10	2699 (1·16)	186 (1·11)	198 (1·17)
	—	100	1899 (1·31)	228 (1·09)	178 (1·21)
Spleen	—	—	1440 (1·02)	158 (1·05)	64 (1·19)
	100	—	3016 (1·09)	371 (1·24)	250 (1·04)
	—	100	1097 (1·02)	577 (1·09)	91 (1·08)

Purified B cells (1×10^5/well) either from spleen or Peyer's patches were stimulated with LPS (10 μg/ml). Lymphokines were added on Day 1. After 5-day culture, polyclonal Ig-secreting cells were enumerated by a reverse PFC assay. Results are expressed as mean PFC and standard errors.

* Units/ml.

Peyer's patch B cells have been shown to preferentially produce IgA antibody in the presence of T cells by appropriate stimuli (Elson, Heck & Strober, 1979). To evaluate whether IL-5 is able to enhance IgA production of Peyer's patch B cells, Peyer's patch B cells were stimulated with LPS in the presence of IL-5 or IL-4 (Table 4). Stimulation of Peyer's patch B cells with LPS could increase in the levels of background polyclonal IgA-secreting cells, and addition of IL-5 further augmented polyclonal IgA as well as IgM and IgG1 production. In contrast, LPS and IL-4 augmented only polyclonal IgG1 production.

IL-5 acts on sIgA-positive B cells to induce IgA production

Purified splenic DNP-primed B cells were used for panning to separate $sIgA^+$ cells from $sIgA^-$ cells, as described in the Materials and Methods. Initially, unfractionated B cells (85% $sIgM^+$) contained 53% $sIgA^+$ cells. After the panning, 88% of adherent cells were $sIgA^+$. Contamination of $sIgA^+$ B cells in the non-adherent population was less than 5%. The cells thus obtained were cultured with IL-5 in the presence of DNP-OVA. Anti-DNP IgA response was observed only when the $sIgA^+$ B cells were cultured with IL-5 (Table 5). Intriguingly, anti-DNP IgG1-producing cells were also observed in the culture of $sIgA^+$ B cells. Although we do not show the data, anti-DNP IgA-producing cells were observed in the culture of DNP-primed $sIgG1^+$ B cells in response to IL-5.

Capacity of IL-5 to support clone formation of IgA secreting cells from sIgA-positive B cells

Limiting dilution analysis was performed by using various numbers of either unfractionated or sIgA⁺ DNP-primed B cells. They were cultured in medium alone or in medium containing recombinant IL-5 (50 U/ml). All the cultures were stimulated with DNP-OVA. After 5 days, anti-DNP IgG1 and IgA antibody productions were assessed separately by an anti-DNP PFC assay. Figure 2 shows a typical experiment demonstrating that recombinant IL-5 can act to promote differentiation of DNP-specific B cells into anti-DNP IgG1 and IgA-secreting cells. In all cases, the logarithm of the percentages of negative wells was linearly related to the input B-cell number, showing that only one cell type was limiting. When sIgA⁺ B cells were stimulated with IL-5, higher anti-DNP PFC clone formation was observed than that observed in unfractionated DNP-primed B cells (1/1,300 vs. 1/2,000) (Fig. 2b). The frequency of anti-DNP IgA PFC clone formation from sIgA⁻ B cells was less than 1 out of 25,000. Similar findings have been noted when assay of accumulated antibody in the Sup was carried out by enzyme-linked immunoabsorbent assay (ELISA) (Matsumoto *et al.*, unpublished data). Although we do not present data, the degree of anti-DNP clone formation from sIgA⁺ DNP-primed B cells was observed over a wide range of IL-5 concentrations (1–200 U/ml). The response profile was flat, with 50 U/ml exerting near maximal effects.

Table 5. Enhancement of IgA production by sIgA⁺ B cells by IL-5

	IL-5	Unfractionated		sIgA⁺		sIgA⁻	
	(U/ml)	IgG1	IgA	IgG1	IgA	IgG1	IgA
Exp. 1	—	32	24	42	26	32	26
	10	89	78	158	142	53	39
	100	218	201	278	228	58	32
Exp. 2	—	38	14	60	31	21	32
	20	152	188	208	252	42	46

Surface IgA-positive (sIgA⁺) cells in DNP-primed splenic B cells (unfractionated) were separated from surface IgA-negative (sIgA⁻) B cells by panning method as described in the Materials and Methods. Cells of each population (5×10^5/0·2 ml/well) were cultured with IL-5 for 5 days. All the culture was stimulated with DNP-OVA (12 ng/well) on the commencement of the culture. Results represent mean anti-DNP PFC per culture.

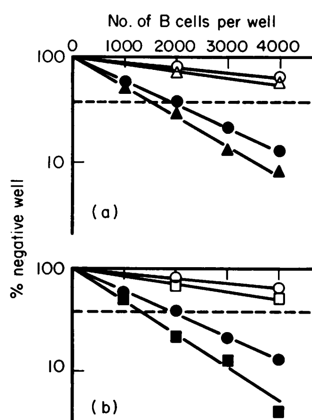

Figure 2. Limiting-dilution analyses of the response of DNP-specific B cells as assessed by PFC. All analyses were carried out in the presence of 3×10^5 rat thymocytes as filler cells. (a) Anti-DNP IgG1 (△,▲), and IgA (○,●) responses of unfractionated DNP-primed B cells. (○,△) denotes cultures in medium, and (●,▲) denotes cultures in IL-5 (50 units/ml). (b) Anti-DNP IgA response of unfractionated (○,●), or sIgA⁺ (□,■) DNP-primed B cells. (○,□) denotes cultures in medium, and (●,■) denotes cultured in IL-5 (50 units/ml).

DISCUSSION

This report demonstrates that (i) anti-IL-5 antibody inhibits IgA production induced by a cognate as well as non-cognate T–B-cell interaction (Table 1); (ii) recombinant IL-5 can induce differentiation of DNP-primed B cells into anti-DNP IgA-secreting cells (Fig. 1); (iii) IL-5 enhances polyclonal IgA formation of LPS-stimulated splenic B cells as well as Peyer's patch B cells (Tables 3 and 4); (iv) sIgA⁺ B cells can differentiate into IgA-secreting cells in response to IL-5 (Table 5); and (v) IL-5 increases the frequency of IgA-secreting cells from sIgA⁺ B cells (Fig. 2).

It is still controversial whether lymphokines are involved in a cognate-type T–B-cell interaction. As shown in Table 1, anti-IL-5 antibody significantly inhibited anti-DNP antibody responses in all isotypes so far examined, indicating that IL-5 is involved at least in part in B-cell triggering in a cognate T–B-cell interaction in the secondary antibody response. Similar finding was reported in the primary response by Rasmussen *et al.* (1988) in collaboration with us. It was also shown that IL-5 plays a major role in the polyclonal IgA production by DNP-primed as well as LPS-stimulated B cells.

It was shown that stimulation of resting B cells with LPS and IL-4 induces IgG1 secretion in sIgG1-negative precursors (Vitetta *et al.*, 1984). The resulting IgG1-secreting cells have been shown to undergo DNA rearrangements from $C\mu$ to $C\gamma 1$ on both the active and inactive IgH loci, which suggests strongly that IL-4 induces class-switch to IgG1 (Vitetta *et al.*, 1984). Similarly, it is possible that the enhancing of IL-5 on IgA production could be mediated by expanding the small percentages of sIgA⁻ cells to switch to sIgA⁺ cells. Alternatively, IL-5 may simply be expanding a specific subset of B cells or B cells at a particular stage of differentiation that is already programmed to switch to IgA. IL-5 was only effective in enhancing IgA formation in splenic sIgA⁺ B cells (Table 5), and increased frequency of IgA-secreting cells in sIgA⁺ B cells (Fig. 2). Taken collectively, these results suggest that the enhancement of IgA formation by IL-5 is caused by expansion of sIgA⁺, post-switched B cells at a particular stage of differentiation or differentiation of these cells to IgA-forming cells. Recently Harriman *et al.* (1988) reported similar observations using LPS-stimulated Peyer's patch B cells. They demonstrated that only sIgA⁺ B cells from Peyer's patches, when incubated with LPS and IL-5, show increases in the number of IgA-secreting cells.

Intriguingly, more than 85% of a sIgA⁺ B-cell population was sIgG1⁺ and was capable of differentiating into IgG1-producing cells in response to IL-5 (Table 5). We also observed

that most of purified sIgG1$^+$ B cells were also sIgA$^+$ and could differentiate into IgG1$^-$ or IgA-producng cells in response to IL-5 (data not shown). There is a body of evidence showing that a small percentage of non-primed B cells is sIgM$^+$ and/or sIgG$^+$ (Andersson, Coutinho & Melchers, 1978; Zan-Bar, Strober & Viteta, 1977; Cooper, Lawton & Kincade, 1972), and sIgA appears later in ontogeny after the appearance of IgG (Zan-Bar *et al.*, 1978). Expression of surface Ig on memory B cells, however, has not been well characterized. Teale *et al.* (1981) reported that some memory B cells which are sIgG1$^+$ as well as sIgA$^+$ are capable of differentiating into IgA-producing cells under the influence of T cells. Our result is in agreement with her result and inconsistent with the work of Okumura *et al.* (1976) who found that the sIgG$^+$ cells dictates the class and allotype commitment of the memory cell. Perhaps these discrepancies relate to differences utilized in the assays. It is not clear at this moment whether sIgA$^+$/sIgG1$^+$ B cells maturate to sIgA$^+$/sIgG1$^-$ in response to IL-5 before terminal differentiation into IgA-producing cells. It is also not clear whether sIgA$^+$ DNP-primed B cells are sIgE$^+$. These will be the subject of another communication.

Concerning T-cell regulation of IgA, expression has been demonstrated to operate at different stages of B-cell differentiation (Cebra *et al.*, 1984). Studies by Cebra *et al.* (1984) demonstrated that antigenic stimulation at mucosal sites is particularly effective at generating clones that make only IgA. These investigators proposed that B cells that are stimulated by environmental antigens undergo cell divisions that lead to a progressive deletion of heavy-chain constant (C)-region encoding genes. However, the commitment of B cells to IgA expression by a process dependent on cell division has not been formally proven. An alternative hypothesis suggests that IgA precursors are derived from IgM B-cell precursors after interaction with accessory cells and/or T-cell subsets. In this regard, we would like to point out that IgA-producing cells were observed in the culture of Peyer's patch B cells stimulated with LPS and IL-5 in a higher frequency compared with the culture of splenic B cells (Table 4).

Mossman *et al.* (1986) has described two types of helper T-cell (Th) clones that differ in the pattern of lymphokines secreted. IL-5 is secreted by Th2 which does not produce IL-2, IL-3, and IFN-γ. Activation of Th2 may related to a specific class of antigens or, alternatively, accessory cells in organs such as Peyer's patch may selectively activate Th2 cells. Sequential stimulation of Th2-type T cells by microbial antigen or helminthic infection may induce preferential production of IL-5, which in turn induces sIgA$^+$ B cells to differentiate into IgA-secreting cells. The class switch from IgM to other isotypes occurs in both small B cells and plasma cells (Cebra *et al.*, 1984; Zan-Bar *et al.*, 1978; Honjo, 1983). The small B cells are thought to be memory B cells that become the majority of IgG secreting cells or IgA-secreting cells in the secondary immune responses. It is postulated that DNA rearrangements for class switch occur in plasma cells, whereas RNA splicing, another postulated mechanism for class switching, occurs in memory B cells (Cebra *et al.*, 1984; Honjo, 1983; Yaoita *et al.*, 1982; Perlmutter & Gilbert, 1984). The experimental system described here using DNP-primed B cells and LPS-stimulated B cells in conjunction with IL-5 should prove to be very useful in further analysis of the steps involved in the T-cell-dependent IgA production of murine B cells.

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