Immunology 1988 **63** 277–283

Role of recombinant interleukin-1 compared to recombinant T-cell replacing factor/interleukin-5 in B-cell differentiation

N. KOYAMA,*† N. HARADA,* T. TAKAHASHI,* S. MITA,* H. OKAMURA,† A. TOMINAGA* & K. TAKATSU* * *Department of Biology, Institute for Medical Immunology and † Department of Obstetrics and Gynecology, Kumamoto University Medical School, Kumamoto, Japan*

Accepted for publication 22 September 1987

SUMMARY

The B-cell differentiation-inducing activity of interleukin-1 (IL-1) was compared with that of T-cell replacing factor (TRF)/interleukin-5 (IL-5), which was originally described as a late-acting B-cell differentiation-inducing factor. Human recombinant IL-1 and murine recombinant TRF/IL-5 were used in this study. Purified B cells from non-primed or antigen-primed mice, LPS-stimulated B-cell blasts, and chronic B-cell leukaemia (BCL_1) cells were used as the responding B-cell population. Addition of IL-1 to the culture of normal B-cells and sheep red blood cells (SRBC) induced a dose-dependent anti-SRBC IgM response, with maximal response at 100 U/ml, whereas the response induced by TRF/IL-5 was less than that induced by IL-1 and did not reach the maximum even at 100 U/ml. Addition of anti-IL-1 antibody, but not anti-TRF/IL-5 antibody or anti-IL-2 receptor antibody, inhibited IL-1-induced anti-SRBC responses. Depletion of cells adherent to Sephadex beads from splenic B cells showed no significant effect on the magnitude of the total responses. IL-1 could induce little, if any, differentiation in antigen-primed B cells, LPS-stimulated B-cell blasts, or BCL_1 cells into antibody-secreting cells, whereas differentiation could be induced by low doses of TRF/IL-5 (1–2 U/ml). Of great interest is that suboptimal doses of IL-1 (10 U/ml) could synergize with TRF in the primary anti-SRBC PFC responses. Kinetic studies revealed that IL-1 acts on B cells for the first 2 days and TRF/IL-5 for the last 3 days in 5-day cultures of B cells. These results suggest that IL-1 acts primarily on resting B cells as a differentiation-inducing factor in the presence of antigen, and also acts as a 'priming' factor for TRF/IL-5.

INTRODUCTION

It has been postulated that generally B-cell proliferation and differentiation into immunoglobulin (Ig)-secreting cells is regulated by several soluble factors derived from macrophages and T cells. Furthermore, there is evidence to support the idea that different stages of the B-cell response to antigenic stimulation are regulated by distinct soluble factors (Howard & Paul, 1983).

Since the description of macrophage-derived IL-1 as a lymphocyte-activating factor (Gery, Gershon & Waksman, 1972; Mizel, Oppenheim & Rosenstreich, 1978), its biological activity has been assumed to relate primarily to early events in activation of resting T cells at the Go phase (Larson, Iscove & Coutinho, 1980). IL-1 has also been implicated in activation of B lymphocytes (Howard & Paul, 1983; Booth & Watson, 1984; Marrack *et al.*, 1982; Durum, Schmidt & Oppenheim, 1985). There is an early effect on the maturation of pre-B cells. The observed maturational response by pre-B cells consists of kappa light chain synthesis, followed by membrane expression of assembled Ig molecules (Giri, Kincade & Mizel, 1984). Later in B-cell development, IL-1 has been implicated in promoting the clonal expansion of B cells after antigen stimulation (Wood *et al.*, 1976; Hoffmann, 1980). Since recombinant IL-1 (rIL-1) is available (Lomedico *et al.*, 1984; Auron *et al.*, 1984), it has been strongly suggested that IL-1 is not primarily an activating cytokine for B cells but is rather a growth and differentiation factor for B cells (Pike & Nossal, 1985; Chiplunkar, Langhorne & Kaufmann, 1986).

T-cell replacing factor (TRF) is a T-cell derived lymphokine that originally was shown to induce terminal differentiation of late-developing B cells to Ig-producing cells, rather than to augment B-cell proliferation (Schimpl & Wecker, 1972). We have studied extensively molecular and functional properties of monoclonal TRF dervied from a T-cell hybridoma B151K12

Abbreviations: B151, TRF-producing T-cell hybridoma B151K12; IL-1, interleukin-1; IL-5, interleukin-5; PFC, plaque-forming cells; SRBC, sheep red blood cells; TRF, T-cell replacing factor.

Correspondence: Dr K. Takatsu, Dept. of Biology, Institute for Medical Immunology, Kumamoto University Medical School, Kumamoto 860, Japan.

(B151), which was originally detected because of its capacity to induce secondary anti-2,4-dinitrophenyl (anti-DNP) IgG antibody responses in DNP-primed B cells (Takatsu *et al.*, 1980a). It was also found that B151 also produces B-cell growth factor II (BCGF II) (Harada *et al.*, 1985), which was originally detected because of its capacity to induce proliferation of murine chronic B-cell leukaemia BCL_1 *in vitro* (Swain & Dutton, 1982). The TRF and BCGF II produced by B151 cells were co-purified in every fractionation procedure employed, suggesting that the two activities were associated with the same molecule. This was substantiated further by the fact that oocyte translation products of cDNA encoding for murine TRF displayed both TRF and BCGF II activity (Kinashi *et al.*, 1986). Based on the diverse activity on different target cells, we propose that TRF be called interleukin-5 (IL-5) (Kinashi *et al.*, 1986). To avoid confusion, at any description in which recombinant TRF is used in this study, we refer to TRF as TRF/IL-5.

Hoffmann & Watson (1979) reported that primary antibody responses of resting B cells to sheep red blood cells (SRBC) are effectively stimulated by macrophage-derived factor and T cells, and that T-cell function is replaced by T-cell derived products (TRF). It is also reported that IL-1 and IL-2 or IL-2 and TRF synergistically induce primary anti-SRBC PFC responses (Leibson, Marrack & Kappler, 1982; Swain *et al.*, 1981; Hoffmann *et al.*, 1987). During the course of our studies on the differentiation-inducing activity of TRF/IL-5, we found the activities of IL-1 and TRF/IL-5 clearly distinguishable in resting and activated B cells. Here we report that rIL-1 induces differentiation of resting B cells in the presence of antigen, and that TRF/IL-5 preferentially acts on pre-activated B cells.

MATERIALS AND METHODS

Mice

BALB/cCrS1c, C57BL/6CrS1c, CBA/N and (CBA/N × BALB/c)(NB)F_1 mice, 6–8 weeks of age, were obtained from the Shizuoka Animal Center, Hamamatsu.

Antigens

Sheep red blood cells (SRBC) were obtained from the Chemo-Sero-Therapeutic Research Institute, Kumamoto. DNP-coupled keyhole limpet haemocyanin (DNP_3–KLH) was prepared as described elsewhere (Takatsu, Tominaga & Hamaoka, 1980b).

Lymphokine

Human rIL-1α and rIL-1β were prepared as described elsewhere (Nishida *et al.*, 1987) and were kindly provided by Dr Y. Hirai (Cellular Engineering Laboratories, Ohtsuka Pharmacentical Co. Ltd, Tokushima). The IL-1 activity was determined by a thymocyte proliferation-inducing activity, as described elsewhere (Mizel *et al.*, 1978) and was expressed as U/ml. Murine TRF/IL-5 was prepared as described elsewhere (Kinashi *et al.*, 1986). In brief, the cDNA for murine TRF (pSP6K-mTRF23) was cleaved with *Sal*I to linearize plasmid DNA, and mRNAs were synthesized using SP6 RNA polymerase. The synthesized RNAs were injected into *Xenopus* oocytes. Incubation media were collected after 36 hr and used as a source of TRF/IL-5.

Antibodies

A monoclonal anti-Thy-1·2 antibody (clone F7D5) was obtained from Serotec (London, U.K.). Ascites containing a monoclonal rat IgG1 anti-TRF/IL-5 antibody were obtained from BALB/c nu/nu mice into which had been transplanted B-cell hybridoma cells (NC17), according to procedures as described elsewhere (Harada *et al.*, 1987b). The ascites used in this study can inhibit TRF/IL-5 activity (8 U/ml) at 1:10,000 dilution without showing any suppressive effect on IL-1, IL-2, IL-3, or BSF-1/IL-4 activity. Rat monoclonal anti-IL-2 receptor antibody was purified from cell-free supernatant of PC61 (Lowenthal *et al.*, 1985), kindly provided by Dr Marcus Nabholz (Swiss Cancer Institute, Epalinges/Lausanne, Switzerland). PC61 antibody can inhibit IL-2 activity (4 U/ml) at a concentration of 1 μg/ml. Polyclonal rabbit anti-human IL-1β antiserum (Tanaka *et al.*, 1987) was kindly provided by Dr Y. Hirai. The antiserum can inhibit human rIL-1β activity (10 U/ml) at 1:1,000 dilution.

B-cell preparation

Spleens were taken under ether anaesthesia from normal, DNP–KLH-primed or BCL_1-bearing mice that had received i.p. injection of 0·1 ml rabbit anti-mouse thymocyte serum 2 days before killing. Spleen cells were treated twice with anti-Thy-1·2 antibody and rabbit complement, to severely deplete T cells. To enrich the B-cell population, T-cell depleted spleen cells were incubated for 1 hr at room temperature on petri-dishes that had been precoated with purified rabbit anti-mouse IgG antibody, and the adherent cells were recovered according to methods described by Mage, McHugh & Rothstein (1977). B cells thus obtained were >87% surface Ig-positive and <2% Thy-1-positive. In certain experiments, accessory cells were removed from T-cell depleted spleen cell suspensions by passage through a Sephadex G-10 column (Pharmacia Fine Chemicals, Uppsala, Sweden), as originally described by Ly & Mishell (1974). The cells thus obtained are <2% alkaline phosphatase-positive.

Cell culture

All cultures were performed in a volume of 200 μl per well of a microplate (No. 25860; Corning Laboratory Sciences Company, Corning, NY). Culture medium consisted of RPMI-1640 medium supplemented with 5×10^{-5}M 2)-mercaptoethanol, 1 mM sodium pyruvate, 1 mM non-essential amino acid, 2 mM glutamine, penicillin (50 U/ml), streptomycin (50 μg/ml), and 15% fetal calf serum (FCS)(Flow Laboratories, McLean, VA).

Induction of primary in vitro *anti-SRBC antibody response*

Normal splenic B cells were cultured (1×10^6 in 0·2 ml per well) with 0·05% SRBC in the presence of lymphokines for 5 days. After culture, each well was assayed for direct SRBC-specific plaque-forming cells (PFC) as described elsewhere (Takatsu *et al.*, 1980a).

Assessment of secondary anti-DNP antibody responses

DNP–KLH-primed splenic B cells (5×10^5 in 0·2 ml per well) were cultured in the presence or absence of lymphokines for 5 days. All the cultures were stimulated with DNP–ovalbumin (12 ng in 20 μl) on Day 0. After culture, the anti-DNP IgG PFC were enumerated as described elsewhere (Takatsu *et al.*, 1980a).

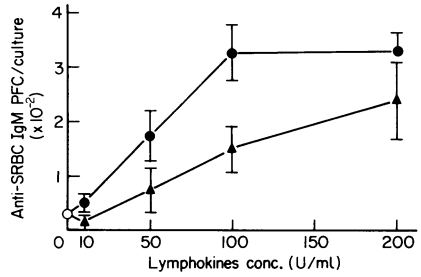

Figure 1. Dose response of IL-1 and TRF/IL-5 on anti-SRBC IgM responses. Purified normal splenic B cells (1×10^6/0·2 ml/well) were cultured with 0·05% SRBC in the presence of various concentrations of IL-1α (●) or TRF/IL-5 (▲). After 5 days of the culture, the anti-SRBC IgM PFC were counted. Results are expressed as geometric means of anti-SRBC IgM PFC and SE. (○) Denotes the background anti-SRBC IgM PFC.

Polyclonal IgM PFC assay

BCL_1 cells in spleen from BCL_1 tumour-bearing BALB/c mice were purified with the use of Percoll, as described elsewhere (Takatsu *et al.*, 1985), and were cultured at $1·5 \times 10^5$ in 0·2 ml per well in the presence or absence of lymphokines for 2 days. After culture, the IgM-producing cells were counted by a reverse PFC assay using protein A-SRBC and rabbit anti-mouse IgM, as described elsewhere (Takatsu *et al.*, 1985). Normal splenic B cells were precultured for 2 days with 50 μg/ml of LPS. The blastoid B cells were harvested and recultured for 3 days at 5×10^4/0·2 ml/well in the presence or absence of lymphokines. The IgM-producing cells were counted as described elsewhere (Takatsu *et al.*, 1985).

Statistical analysis

Each assay was performed in triplicate cultures. The numbers of PFC were logarithmically transformed, and geometric means and SE were calculated.

RESULTS

Enhancing effect of IL-1 on the primary *in vitro* anti-SRBC PFC responses

To evaluate the role of IL-1 in primary immune responses, purified splenic B cells from C57BL/6 mice were cultured with human rIL-1α and were stimulated with 0·05% SRBC. Murine TRF/IL-5 was also added to other cultures in place of IL-1. After 5 days of culture, the anti-SRBC IgM PFC were counted. IL-1 induced significant anti-SRBC PFC responses in a dose-dependent manner. The PFC responses induced were observed in the culture that received more than 10 U/ml IL-1 and reached the maximum at 100 U/ml (Fig. 1). No significant difference of PFC-inducible activity was observed between IL-1α and IL-1β (Table 1). Conversely, TRF/IL-5 increased the PFC responses from a level of 50 U/ml, and did not induce maximal response at 200 U/ml (Fig. 1). Although the data are not shown here, IL-2 itself was ineffective below 50 U/ml.

It has been reported that accessory cells play a role in the primary antibody response (Corbel & Melchers, 1984). To examine whether accessory cells are essential for the primary anti-SRBC PFC responses augmented by IL-1, we used two different approaches. First, splenic B-cells were passed through a Sephadex G-10 column and non-adherent cells (G-10-passed cells) were used as responding cells. Second, we enriched surface Ig-positive cells by panning procedures using rabbit anti-mouse Ig antibody-coated petri-dishes. As controls, unfractionated splenic B cells were also used as responding cells. A representative result from a series of three different experiments is shown in Table 1. Unfractionated B cells responded quite nicely to SRBC in the presence of IL-1. When G-10-passed B cells were stimulated with SRBC in the presence of IL-1, augmented primary anti-SRBC PFC response was observed, although the total response was lower than that observed in the unfractionated B cells. When the B cells that had adhered to the anti-Ig-coated petri-dishes were stimulated with SRBC and IL-1, a clear anti-SRBC IgM PFC response was also observed. These results suggest that accessory cells may not be essential for the augmented primary anti-SRBC PFC response induced by IL-1.

Table 1. Effect of the depletion of accessory cells on the primary anti-SRBC PFC responses mediated by IL-1

Lymphokines (U/ml)	Anti-SRBC IgM PFC/culture* Unfractionated†	Sephadex G-10 passed†	Anti-Ig absorbed†
None	53 (1·16)	9 (1·41)	2 (1·00)
IL-1α 20	142 (1·08)	69 (1·18)	282 (1·15)
100	362 (1·21)	109 (1.21)	728 (1·03)
IL-1β 20	96 (1·25)	68 (1·25)	238 (1·15)
100	212 (1·11)	126 (1·18)	693 (1·19)

* Results are expressed as geometric means and SE.

† Purified splenic B cells were prepared as described in the Materials and Methods. B cells thus prepared were further fractionated by using a Sephadex G-10 column or anti-Ig coated petri-dishes. Unfractionated B-cells, Sephadex G-10-passed B cells, or B cells adherent to anti-Ig-coated petri-dishes at 1×10^6/0·2 ml/well were cultured with 0·05% SRBC in the presence of IL-1 for 5 days. One of the representative results of a series of three different experiments is shown.

Another important question to be answered is the role of IL-2 or TRF/IL-5 in the IL-1-mediated anti-SRBC antibody response. Effects of monoclonal anti-IL-2 receptor and monoclonal anti-TRF/IL-5 antibody on the IL-1-dependent anti-SRBC response were tested. As a control, rabbit polyclonal anti-IL-1β antibody was also used. As shown in Table 2, anti-IL-1β antibody could inhibit the anti-SRBC antibody response induced by IL-1β but not that induced by IL-1α, clearly demonstrating that these effects are due to IL-1 itself and not to some possible contaminant in the preparation of rIL-1. Neither anti-IL-2 receptor antibody nor anti-TRF/IL-5 antibody showed striking suppressive effects under the same conditions. Furthermore, neither IL-2 nor TRF activity was detected in the culture supernatant of SRBC-stimulated B cells in the presence of IL-1α (100 U/ml) (data not shown).

Effects of IL-1α on the differentiation of BCL_1, LPS-stimulated B-cell blasts or DNP-primed B cells

To compare B-cell differentiation-inducing activity of IL-1 with TRF/IL-5, we tested whether IL-1α is also effective in inducing differentiation of activated B cells that respond to TRF/IL-5. As activated B cells, we used BCL_1, LPS-stimulated B-cell blasts, and DNP-primed B cells. As can be seen in Table 3, TRF/IL-5

Table 2. Effects of anti-lymphokine antibodies on the primary anti-SRBC PFC responses mediated by IL-1*

	Lymphokine (100 U/ml)	Antibody*	Anti-SRBC IgM PFC/culture†
Exp. 1	None	—	7 (1·18)
	IL-1α	—	227 (1·06)
	IL-1β	—	203 (1·10)
	IL-1α	Anti-IL-1β	302 (1·22)
	IL-1β	Anti-IL-1β	6 (1·25)
Exp. 2	None	—	22 (1·16)
	IL-1α	—	365 (1·19)
	—	PC61	7 (1·18)
	IL-1α	PC61	288 (1·21)
	—	NC17	19 (1·22)
	IL-1α	NC17	362 (1·12)

* Antibodies were added to the culture of purified B cells and SRBC at Day 0. Antibodies used were rabbit anti-IL-1β antiserum at 1:100 dilution, anti-IL-2 receptor antibody (PC61)(1 μg/ml), and rat monoclonal anti-TRF/IL-5 (NC17) ascites at 1:3,000 dilution.

† Results are expressed as geometric means and SE.

Table 3. Failure of IL-1 to induce differentiation of activated B cells into antibody-secreting cells

Lymphokines (U/ml)		B-cell source: BCL_1*	LPS-blasts*	DNP-primed B cells†
None		38 (1·38)	17 (1·14)	42 (1·06)
IL-1α	20	14 (1·18)	20 (1·18)	38 (1·11)
	100	15 (1·38)	21 (1·20)	76 (1·35)
	200	10 (1·22)	31 (1·06)	58 (1·09)
TRF/IL-5	1	624 (1·03)	217 (1·13)	96 (1·11)
	2	1236 (1·07)	881 (1·08)	108 (1·08)
	10	1382 (1·04)	806 (1·04)	328 (1·05)

* BCL_1 cells or LPS-stimulated B-cell blasts were cultured with IL-1 or TRF/IL-5 as described in the Materials and Methods. After culture, polyclonal IgM PFC responses were determined by reverse PFC assay. The results were represented by geometric means of IgM PFC responses/culture and SE.

† DNP-primed B cells were cultured with DNP–ovalbumin (12 ng/well) for 5 days. Lymphokines were added on Day 0. Results were expressed as the geometric means of anti-DNP IgG PFC/culture and SE.

induced a notable polyclonal IgM PFC response of BCL_1 as well as of LPS-stimulated B-cell blasts at 1–2 U/ml. It is also clear that TRF/IL-5 induced remarkable anti-DNP IgG PFC responses. In contrast, IL-1 induced few, if any, PFC responses in all assay systems tested, even at 200 U/ml.

Synergistic effect of IL-1 and TRF/IL-5 on primary anti-SRBC PFC responses

On the basis of our results shown in Fig. 1 and Tables 1 and 2, we suggest that IL-1 preferentially triggers resting B cells to induce differentiation to antibody-forming cells. It was considered worthwhile to determine whether IL-1α synergizes with TRF/IL-5 to induce the antibody responses. To evaluate this possibility, suboptimal doses of IL-1α (10 U/ml) and various doses of TRF/IL-5 were added to the assay system (Fig. 2a). It was clear that IL-1 could synergistically induce anti-SRBC responses with TRF/IL-5 and that the magnitude of the total response was dependent on the amount of TRF/IL-5 added. Next, the constant doses of TRF/IL-5 (10 U/ml) were added to the culture with different doses of IL-1α. The increase in PFC response was also observed in the presence of IL-1α and TRF/IL-5, and was dependent on the amount of IL-1α (Fig. 2b).

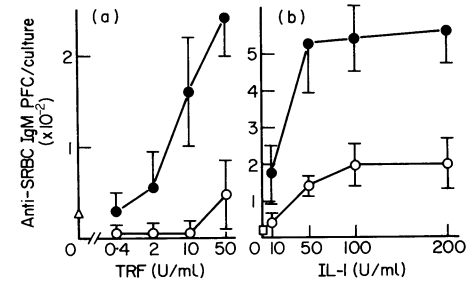

Figure 2. Synergistic effect of IL-1 and TRF/IL-5 on the primary anti-SRBC PFC responses. Purified splenic B cells were cultured with 0·05% SRBC for 5 days. (a) Various concentrations of TRF/IL-5 in the presence (●) or absence of (○) 10 U/ml of IL-1α were added to the culture on Day 0. (b) Various concentrations of IL-1α in the presence (●) or absence of (○) 10 U/ml of TRF/IL-5 were added to the culture on Day 0. Background anti-SRBC IgM PFC/culture is denoted with (△), (□), respectively.

Kinetic study of the IL-1 and TRF/IL-5 requirement

We analysed the time-course of IL-1α and TRF/IL-5 for their synergistic effects on primary anti-SRBC PFC responses. TRF/IL-5 (10 U/ml) was added on Day 0 or 2 to the culture in the presence of IL-1α (10 U/ml) for 5 days (Fig. 3, groups 3 and 4). In another group, IL-1α was added to the culture on Day 2 in the presence of TRF/IL-5 (group 5). As controls, either IL-1α or TRF/IL-5 alone was added to the culture (groups 1 and 2). When TRF/IL-5 was added either on Day 0 or Day 2 in the presence of IL-1α, significant anti-SRBC PFC response was observed in both cases, and addition of TRF/IL-5 on Day 2 induced maximal anti-SRBC PFC response (group 4). In contrast, when IL-1α was added later than Day 2, augmented anti-SRBC PFC responses were not maximally induced (group 5 versus group 4).

To substantiate the early effect of IL-1 on B-cell triggering, B cells were cultured with IL-1α for 2 days, after which IL-1α was removed by washing with medium and TRF/IL-5 was immediately added to the culture (group 6). In another group (group 7), B cells were cultured with TRF/IL-5 for the first 2 days, washed, and then cultured with IL-1α for the remainder of the culture period. The PFC response was determined on Day 5. These experimental designs enabled us to determine the amount of time required for IL-1α to convert immature B cells into TRF/IL-5-responsive B cells. As also shown in Fig. 3, maximal PFC responses were observed when B cells were cultured with IL-1α for the first 2 days (group 6). When TRF/IL-5 and IL-1α were

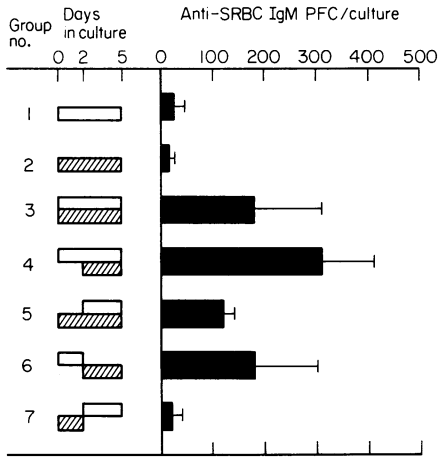

Figure 3. Kinetic analysis of synergy between IL-1 and TRF/IL-5. Purified splenic B cells were cultured for 5 days in the presence of 0·05% SRBC. IL-1 (10 U/ml) or TRF/IL-5 (10 U/ml) was added to the culture as indicated. (□) Time period during which IL-1 was present; (■) time period during which TRF/IL-5 was present.

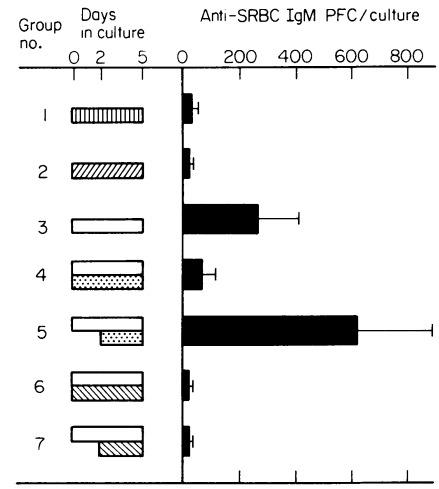

Figure 4. Effect of anti-lymphokine antibodies on the synergy between IL-1 and TRF/IL-5. Purified splenic B cells were cultured for 5 days under conditions described in Fig. 3. In some experiments, anti-IL-1 or anti-TRF/IL-5 antibody was added at indicated period of the culture. (■) Time period during which IL-1 (10 U/ml) was present; (■) time period during which TRF/IL-5 (10 U/ml) was present; (□) time period during which IL-1 and TRF/IL-5 were present; (▨) time period during which anti-IL-1 antibody (1:1,000) was present; (■) time period during which anti-TRF/IL-5 antibody (1:3,000) was present.

added in a reversed sequence to B-cell cultures, they failed to synergize each other in generation of antibody-forming cells (group 6).

We then tested the effect of anti-IL-1β or anti-TRF/IL-5 antibodies on IL-1β- and TRF/IL-5-induced anti-SRBC PFC responses. IL-1β and TRF/IL-5 were added to the culture on Day 0. When anti-IL-1β antibody was added on Day 0, the PFC response was inhibited to 75% of the level of the control response (Fig. 4, group 4 versus group 3). Of note is that the PFC response was not affected when anti-IL-1β antibody was added on Day 2 (group 5). On the other hand, when anti-TRF/IL-5 antibody was added to the culture on either Day 0 or Day 2, the PFC responses decreased to the level of that induced by IL-1β (groups 6 and 7 versus group 1). These results further support the notion that IL-1 is required for the first 2 days of the culture.

Non-responsiveness to IL-1 plus TRF/IL-5 of B cells from Xid mice

B cells from CBA/N mice are well-known non-responders to TNP–Ficoll and low responders to SRBC (Scher, 1982). It was considered worthwhile to test whether the above low responsiveness could be overcome by addition of IL-1 and TRF/IL-5. Purified B cells from BALB/c, CBA/N, NBF_1 mice were cultured with SRBC in the presence of IL-1 and/or TRF/IL-5. As can be seen in Table 4, B cells from CBA/N as well as NBF_1 male mice did not respond to SRBC, even in the presence of TRF/IL-5 and IL-1, whereas B cells from BALB/c as well as NBF_1 female mice did.

Table 4. Failure of IL-1 plus TRF/IL-5 to induce differentiation of B cells from Xid mice into antibody-secreting cells

Lymphokines (U/ml)		B-cell response to SRBC*			
		BALB/c	CBA/N	NBF_1♀	NBF_1♂
None		2 (1·15)	0 (1·00)	2 (1·15)	0 (1·00)
IL-1α	10	13 (1·37)	4 (1·49)	10 (1·18)	6 (1·03)
	50	31 (1·40)	3 (1·41)	55 (1·29)	4 (1·12)
	100	46 (1·15)	0 (1·00)	59 (1·21)	7 (1·39)
TRF/IL-5	10	12 (1·73)	0 (1·00)	10 (1·30)	0 (1·00)
	50	104 (1·89)	0 (1·00)	39 (1·18)	4 (1·20)
IL-1α + TRF/IL-5	10 + 10	92 (1·27)	0 (1·00)	81 (1·19)	3 (1·19)

* Results are expressed as geometric means of numbers of anti-SRBC IgM PFC and SE.

DISCUSSION

It has been reported that IL-1 acts on various target cells, including T cells, B cells, fibroblasts, synovial cells, endothelial cells and others, as activating factors (Durum *et al.*, 1985). It is, however, still controversial whether or not IL-1 acts directly on B cells as a differentiation-inducing factor. In this study, we attempted to clarify the functional differences between the effects of IL-1 and TRF/IL-5 on B cells for their differentiation in different phases. The major findings of this study are as follows: (i) Human rIL-1α and rIL-1β act on murine-resting B cells to augment the primary antibody response to SRBC more effectively than TRF/IL-5. (ii) IL-1 can rarely induce already activated B cells or the neoplastic B-cell line BCL_1 for further differentiation into Ig-producing cells, whereas TRF/IL-5 acts primarily on pre-activated B cells. (iii) Suboptimal doses of IL-1 can synergize with TRF/IL-5 for primary anti-SRBC antibody responses; IL-1 acts in the early phases of culture and TRF/IL-5 acts during later stages of culture.

Hoffman (1980) has presented evidence indicating that IL-1 can induce the expression of several B-cell surface markers and increase the frequency of reactive B cells. It is probable, however, that IL-1 acts, in part, via induction of IL-2 synthesis to increase the number of helper T cells. Actually, it has been reported that IL-1 and IL-2 synergize in induction of an anti-SRBC antibody response by T-cell depleted spleen cells (Leibson *et al.*, 1982). The point to be considered is that IL-1 might have stimulated B cells indirectly by acting on T cells contaminating in our B-cell preparations, which then secreted factors that may have affected B cells.

It is clear that IL-1 induces primary anti-SRBC IgM PFC responses in extensively T-cell depleted B-cell populations (Fig. 1 and Table 1). Rabbit polyclonal anti-IL-1-1β antibody completely inhibited IL-1β but not IL-1α-dependent PFC responses (Table 2), demonstrating that the augmented effect observed is a property of IL-1 itself. The addition of nylon-wool-purified T cells to the culture of extensively purified B cells did not lead to significant enhancement of the PFC response (data not shown). Furthermore, primary anti-SRBC PFC responses with the use of B cells purified using anti-Ig-coated petri-dishes were also remarkably augmented by IL-1. Despite the addition of anti-IL-2 receptor antibody, we still found that IL-1 stimulated anti-SRBC responses (Table 2). Therefore, IL-1 may contribute more to the antibody response than simple induction of IL-2 synthesis, although the possibility that IL-2 might play a partial role in this action still remains.

It was demonstrated recently that IL-1 stimulates fibroblasts to induce the production of interferon-β_2/BSF-2 (Damme *et al.*, 1987), which induces B-cell differentiation (Hirano *et al.*, 1986). To evaluate the possibility that BSF-2 produced by contaminated cells in the B-cell preparation after stimulation with IL-1 may be responsible for the effect of IL-1, we added human recombinant BSF-2 (kindly provided by Drs T. Hirano and T. Kishimoto, Division of Immunology, Institute of Molecular and Cellular Biology, Osaka University) to the culture for the assay system in place of IL-1. The results revealed that PFC responses were not induced by addition of 100 U/ml of BSF-2.

Concerning the B-cell differentiation-inducing activity of IL-1, we noted distinct differences from the activity of TRF/IL-5. TRF/IL-5 induces differentiation of activated B cells and memory B cells at lower doses (1–2 U/ml), whereas IL-1 triggers such responses less efficiently than TRF/IL-5 and does not induce maximal response at even higher doses (50–100 U/ml). In contrast, IL-1 effectively stimulated SRBC-specific resting B cells, whereas higher doses of TRF/IL-5 are required to induce a similar result. These results may be accounted for by two different mechanisms: (i) IL-1 and TRF/IL-5 may stimulate different B-cell subsets. (ii) IL-1 and TRF/IL-5 may act on B cells with different activation stages. We do not have a definitive answer at this point, and therefore cannot exclude either possibility.

Recently, two groups of investigators (Pike & Nossal, 1985; Chiplunkar *et al.*, 1986) independently reported that IL-1 is not primarily an activating cytokine for B cells, but is rather a B-cell growth and differentiation factor. Chiplunkar *et al.* (1986) showed that IL-1 by itself is capable of inducing antibody secretion in separated low-density B cells and shows clear synergy only with dextran sulphate (DXS). These authors concluded that IL-1 is a B-cell differentiation factor that acts at advanced stages of B-cell maturation or on a distinct B-cell subset. In our studies, however, IL-1 had little effect on activated B cells.

IL-1 was shown to act on resting (not naturally activated) B cells in synergy with TRF/IL-5 to induce the primary *in vitro* SRBC PFC responses. In 5-day cultures, IL-1 was required during the early phases (up to 2 days) and TRF/IL-5 was required for the late phases of culture (the last 3 days) (Figs 3 and 4). Hoffmann (1980) has reported previously essentially similar results. However, the TRF activity in his assay system may have been mediated by IL-2, since the lymphokines used were not purified. If his lymphokine preparations contained both IL-2 and TRF, he might have seen the results of synergy between IL-2 and IL-1, IL1 and TRF, and IL-2 and TRF, because we have demonstrated clearly that TRF/IL-5 and IL-2 synergistically trigger SRBC-specific B cells (Harada *et al.*, (1987a) for anti-SRBC PFC. On the basis of results obtained in this study, we conclude that IL-1 and TRF/IL-5 synergistically stimulate anti-SRBC responses. It is likely that IL-1 induces or increases expression of the TRF/IL-5 receptor in SRBC-stimulated B cells. In our preliminary experiments, culture of resting B cells with SRBC in the presence of IL-1 for 2 days induced increased levels of TRF/IL-5 receptor expression (data not shown). It was also suggested by Hoffman (1980) that IL-1 has a 'priming' effect on B cells for the T-cell signal that induces further differentiation.

In summary, we have demonstrated clearly that IL-1, alone or in conjunction with TRF/IL-5, acts on B cells at different activation stages. We have also presented evidence suggesting that IL-1 induces or increases the expression of TRF/IL-5 receptors on B cells. The experimental systems utilized in this study may give us more information on the mechanisms of B-cell differentiation mediated by IL-1, TRF/IL-5, or IL-1 and TRF/IL-5.

ACKNOWLEDGMENT

We are grateful to Drs Naoto Yamaguchi and Yoshikatsu Hirai for useful suggestions and for providing recombinant IL-1 and rabbit anti-IL-1 antibody, respectively. Ms Sayuri Tachimoto is also thanked for her excellent secretarial assistance.

This work was supported in part by a Grant-in-Aid from the Japanese Ministry of Education, Culture and Science, and by research grants from the Japan Research Foundation for Clinical Pharmacology and from the Mochida Memorial Medical and Pharmaceutical Foundation.

REFERENCES

AURON P.E., WEBB A.C., ROSENWASSER L.J., MUCCI S.F., RICH A., WOLFF S.M. & DINARELLO C.A. (1984) Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc. natl. Acad. Sci. U.S.A.* **81,** 7907.

BOOTH R. J. & WATSON J.D. (1984) Interleukin 1 induces proliferation in two distinct B cell subpopulations responsive to two different murine B cell growth factors. *J. Immunol.* **133,** 1346.

CHIPLUNKAR S., LANGHORNE J. & KAUFMANN S.H.E. (1986) Stimulation of B cell growth and differentiation by murine recombinant interleukin 1. *J. Immunol.* **137,** 3748.

CORBEL C. & MELCHERS F. (1984) The synergism of accessory cells and of soluble α-factors derived from them in the activation of B cells to proliferation. *Immunol. Rev.* **78,** 51.

DAMME J.V., OPDENAKKER G., SIMPSON R.J., RUBIRA M.R., CAYPHAS S., VINK A., BILLIAU A. & SNICK J.V. (1987) Identification of the human 26-kd protein, interferon β_2 (IFN-β_2), as a B cell hybridoma/

plasmacytoma growth factor induced by interleukin 1 and tumor necrosis factor. *J. exp. Med.* **165,** 914.

DURUM S.K., SCHMIDT J.A. & OPPENHEIM J.J. (1985) Interleukin 1: an immunological perspective. *Annu. Rev. Immunol.* **3,** 263.

GERY I., GERSHON R.K. & WAKSMAN B.H. (1972) Potentiation of the T-lymphocyte response to mitogens, I. The responding cell. *J. exp. Med.* **136,** 128.

GIRI J.G., KINCADE P.W. & MIZEL S.B. (1984) Interleukin l-mediated induction of κ-light chain synthesis and surface immunoglobulin expression on pre B cells. *J. Immunol.* **132,** 223.

HARADA N., KIKUCHI Y., TOMINAGA A., TAKAKI S. & TAKATSU K. (1985) BCGF II activity on activated B cells of a purified murine T cell-replacing factor (TRF) from a T cell hybridoma (B151K12). *J. Immunol.* **134,** 3944.

HARADA N., MATSUMOTO M., KOYOMA N., SHIMIZU A., HONJO T., TOMINAGA A. & TAKATSU K. (1987a) T cell-replacing factor/interleukin 5 induces not only B-cell growth and differentiation, but also increased expression of interleukin 2 receptor on activated B-cells. *Immunol. Lett.* **15,** 205.

HARADA N., TAKAHASHI T., MATSUMOTO M., KINASHI T., OHARA J., KIKUCHI Y. *et al.* (1987b) Production of a monoclonal antibody to and its use in the molecular characterization of murine T cell-replacing factor (TRF) and B cell growth factor II (BCGF II). *Proc. natl. Acad. Sci. U.S.A.* **84,** 4581.

HIRANO T., YASUKAWA K., HARADA H., TAGA T., WATANABE Y., MATSUDA T. *et al.* (1986) Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin *Nature (Lond.),* **324,** 73.

HOFFMANN M.K. (1980) Macrophages and T cells control distinct phases of B cell differentiation in the humoral immune response *in vitro*. *J. Immunol.* **125,** 2076.

HOFFMAN M.K., GILBERT K.M., HIRST J.A. & SCHEID M. (1987) An essential role for interleukin 1 and a dual function for interleukin 2 in the immune response of murine B lymphocytes to sheep erythrocytes. *J. Molec. Cell. Immunol.* **3,** 29.

HOFFMANN M.K. & WATSON J. (1979) Helper T cell-replacing factors secreted by thymus-derived cells and macrophages: cellular requirements for B cell activation and synergistic properties. *J. Immunol.* **122,** 1371.

HOWARD M., MIZEL S.B., LACHMAN L., ANSEL J., JOHNSON B. & PAUL W.E. (1983) Role of interleukin 1 in anti-immunoglobulin-induced B cell proliferation. *J. exp. Med.* **157,** 1529.

HOWARD M. & PAUL W.E. (1983) Regulation of B-cell growth and differentiation by soluble factors. *Annu. Rev. Immunol.* **1,** 307.

KINASHI T., HARADA N., SEVERINSON E., TANABE T., SIDERAS P. KONISHI M. *et al.* (1986) Cloning of complementary DNA encoding T-cell replacing factor and identity with B-cell growth factor II. *Nature (Lond.),* **324,** 70.

LARSSON E.L., ISCOVE N.N. & COUTINHO A. (1980) Two distinct factors are required for induction of T-cell growth. *Nature (Lond.),* **283,** 664.

LEIBSON H.J., MARRACK P. & KAPPLER J. (1982) B cell helper factors. II. Synergy among three helper factors in the response of T cell- and macrophage-depleted B cells. *J. Immunol* **129,** 1398.

LOMEDICO P.T., GUBLER U., HELLMANN C.P., DUKOVICH M., GIRI J.G., PAN Y.E., COLLIER K., SEMIONOW R., CHUA A.O. & MIZEL S.B. (1984) Cloning and expression of murine interleukin-1 cDNA in *Escherichia coli. Nature (Lond.),* **312,** 458.

LOWENTHAL J.W., ZUBLER R.H., NABHOLZ M. & MACDONALD H.R. (1985) Similarities between interleukin-2 receptor number and affinity on activated B and T lymphocytes. *Nature (Lond.),* **315,** 669.

LY I.A. & MISHELL R.I. (1974) Separation of mouse spleen cells by passage through columns of Sephadex G-10. *J. immunol. Meth.* **5,** 239.

MAGE M.G., MCHUGH L.L. & ROTHSTEIN T.L. (1977) Mouse lymphocytes with and without surface Ig: preparative scale separation in polystyrene tissue culture dishes coated with specifically purified anti-immunoglobulin. *J. immunol. Meth.* **15,** 47.

MARRACK P., GRAHAM S.D., KUSHNIR E., LEIBSON H.J., ROEHM N. & KAPPLER J.W. (1982) Nonspecific factors in B cell responses. *Immunol. Rev.* **63,** 33.

MIZEL S.B., OPPENHEIM J.J. & ROSENSTREICH D.L. (1978) Characterization of lymphocyte-activating factor (LAF) produced by the macrophage cell line, P388D1 I. Enhancement of LAF production by activated T lymphocytes. *J. Immunol.* **120,** 1497.

NISHIDA T., NISHINO N., TAKANO M., KAWAI K., BANDO K., MASUI Y., NAKAI S. & HIRAI Y. (1987) cDNA cloning of IL-1α and IL-1β from mRNA of U937 cell line. *Biochem. Biopys. Res. Commun.* **143,** 345.

PIKE B.L. & NOSSAL G.J.V. (1985) Interleukin 1 can act as a B-cell growth and differentiation factor. *Proc. natl. Acad. Sci. U.S.A.* **82,** 8153.

SCHER I. (1982) The CBA/N mouse strain: an experimental model illustrating the influence of the X-chromosome on immunity. *Adv. Immunol.* **33,** 1.

SCHIMPL A. & WECHER E. (1972) Replacement of T-cell function by a T cell product. *Nature (Lond.),* **237,** 15.

SWAIN S.L., DENNERT G., WARNER J.F. & DUTTON R.W. (1981) Culture supernatants of a stimulated T-cell line have helper activity that acts synergistically with interleukin 2 in the response of B cells to antigen. *Proc. natl. Acad. Sci. U.S.A.* **78,** 2517.

SWAIN S.L. & DUTTON R.W. (1982) Production of a B cell growth-promoting activity, (DL)BCGF, from a cloned T cell line and its assay on the BCL_1 B cell tumor. *J. exp. Med.* **156,** 1821.

TAKATSU K., HARADA N., HARA Y., TAKAHAMA Y., YAMADA G., DOBASHI K. & HAMAOKA T. (1985) Purification and physicochemical characterization of murine T cell-replacing factor (TRF). *J. Immunol.* **134,** 382.

TAKATSU K., TANAKA K., TOMINAGA A., KUMAHARA Y. & HAMAOKA T. (1980a) Antigen-induced T cell-replacing factor (TRF). III. Establishment of T cell hybrid clone continuously producing TRF and functional analysis of released TRF. *J. Immunol.* **125,** 2646.

TAKATSU K., TOMINAGA A. & HAMAOKA T. (1980b) Antigen-induced T cell-replacing factor (TRF). I. Functional characterization of a TRF-producing helper T cell subset and genetic studies on TRF production. *J. Immunol.* **124,** 2414.

TANAKA K., ISHIKAWA E., OHMOTO Y. & HIRAI Y. (1987) Sandwich enzyme immunoassay for human interleukin-1β (hIL-1β) in urine. *Clinica Chemica Acta,* **166,** 237.

WOOD D.D., CAMERON P.M., POE M.T. & MORRIS C.A. (1976) Resolution of a factor that enhances the antibody response of T cell-depleted murine splenocytes from several other monocyte products. *Cell. Immunol.* **21,** 88.