Immunology 1989 **67** 244–250

Analysis of the B-cell growth-promoting activity of human IL-4, the co-stimulatory assay with anti-immunoglobulin antibodies. Comparison with the B-cell growth-promoting activity of other lymphokines

F. ALMERIGOGNA, M. G. GIUDIZI, R. BIAGIOTTI, A. ALESSI, T. DEFRANCE, J. BANCHEREAU, M. RICCI & S. ROMAGNANI *Division of Allergology and Clinical Immunology and of Internal Medicine, University of Florence, Italy and the Laboratory of Immunology, Unicet, Dardilly, France*

Accepted for publication 5 January 1989

SUMMARY

Human recombinant interleukin-4 (rIL-4) was assessed for its ability to promote the proliferative response of purified human B cells co-stimulated with submitogenic concentrations of soluble $F(ab')_2$ fragments of anti-immunoglobulin (Ig) antibodies. The growth-promoting activity of rIL-4 was usually as potent as, or even more potent than, that of recombinant interleukin-2 (rIL-2), and more potent than that of recombinant interferon-gamma (rIFN-γ). Preincubation with rIL-4 did not cause enhancement of the proliferative response of B cells to the subsequent addition of rIL-4 and anti-IgM antibody. In contrast, the proliferative response of B cells preincubated with anti-IgM antibody and rIL-4 was potentiated by the subsequent addition of rIL-4. The simultaneous addition of rIFN-γ and rIL-2 or rIFN-γ and rIL-4 had an additive effect in comparison with the response induced by rIL-2 or rIL-4 alone, respectively, whereas simultaneous addition of rIL-2 and rIL-4 induced a response equal or lower than that stimulated by rIL-2 or rIL-4 alone. The addition of rIFN-γ at the beginning of culture or preincubation of B cells with rIFN-γ and anti-IgM antibody potentiated the proliferative response of B cells to the subsequent addition of either rIL-2 or rIL-4. Taken together, these data suggest that rIL-4 acts as a growth factor for activated human B cells and displays on such cells a growth-promoting activity similar to that of rIL-2.

INTRODUCTION

The large scale availability of molecules obtained by recombinant DNA technology is allowing the use of highly purified factors in functional studies on human B lymphocytes. Evidence on the use of recombinant interleukin-2 (rIL-2) and recombinant interferon-gamma (rIFN-γ) has suggested that both these interleukins can act as growth factors for activated human B cells (Tsudo, Uchiyama & Uchino, 1984; Mingari *et al.*, 1984; Muraguchi *et al.*, 1985; Romagnani *et al.*, 1985; Almerigogna *et al.*, 1985; Romagnani *et al.*, 1986b; Defrance *et al.*, 1986). More recently, two other human T-cell derived factors showing growth-promoting effect on human B cells have been prepared by recombinant DNA technology: the low molecular weight B-cell growth factor (12,000 MW; BCGF) (Sharma *et al.*, 1987) and the interleukin-4 (IL-4) (Yokota *et al.*, 1986). In contrast to mouse IL-4, human recombinant IL-4 (rIL-4) has been shown to be poorly mitogenic for splenic B cells in the co-stimulatory assay with soluble F(ab')2 fragments of anti-IgM antibody, while it appears very effective in promoting the proliferation of B cells preactivated with insolubilized anti-IgM antibody or *Staphylococcus aureus* Cowan I (SAC) bacteria (Defrance *et al.*, 1987b).

In this paper, we report that rIL-4 displays convincing growth-promoting effect on human tonsillar B cells co-stimulated with either anti-IgM or anti-IgD antibodies. We also provide evidence that the B-cell growth-promoting activity of rIL-4 is potentiated by IFN-γ and is very similar to, but independent of, the B-cell growth-promoting activity of IL-2.

Correspondence: Dr F. Almerigogna, Istituto Clinica Medica, Policlinico di Careggi, 50134-Firenze, Italy.

MATERIALS AND METHODS

Reagents

rIL-2 and rIFN-γ were kindly provided by Biogen (Geneva, Switzerland). Human rIL-4 was obtained as supernatants (SUPs) from COS-7 cells transfected with the pcD or pEBT vector containing the human IL-4 cDNA clone (Yokota *et al.*, 1986). One unit of IL-4 is defined as the amount providing a half maximal [^{3}H] thymidine uptake on activated phytohaemagglutinin (PHA) blasts (Yokota *et al.*, 1986).

Antibodies

The anti-human IFN-γ monoclonal antibody (mAb) BGl was kindly provided by Dr C.M. Liang (Biogen, Cambridge, MA) MAb BGl against human rIFN-γ was produced by fusing the spleen cells of rIFN-γ-immune mice with P3-X63Ag8.653 in the presence of 50% polyethylene glycol 3350. To produce ascites fluids the mice were injected i.p. with 0·5 ml of pristan (Aldrich Chemical Co., Milwaukee, WI) and 10–14 days later inoculated with 1×10^6 hybridoma cells/mouse. The ascites fluids which occurred 14–21 days after inoculation of the cells were collected at 2–3 day intervals until the animals was killed. Anti-viral assay showed that the neutralization titres of the ascites fluids against rIFN-γ were 3×10^5. Titre was defined as the reciprocal of the highest dilution of ascites fluids which decreased the anti-viral activity of IFN from 10 U/ml to 1 U/ml.

$F(ab')_2$ fragments of rabbit antibodies specific for human IgM and human IgD were prepared and affinity-purified as reported elsewhere (Romagnani *et al.*, 1980). The anti-DR mAb PTF 29.12 was a generous gift of Dr G. Damiani (Istituto di Biochimica, Genva, Italy). The mAb was purified by immunoabsorption on Staphylococcal protein A (SpA)–Sepharose (Pharmacia, Uppsala, Sweden). The anti-TAC was a generous gift of Dr L. Moretta (Institute for Cancer, Genva, Italy). The anti-CD3 (OKT3), anti-CD4 (OKT4) and anti-CD8 (OKT8) mAbs were purchased from Ortho Diagnostics (Raritan, NJ); the anti-CD20 (B1) mAb was purchased from Kontron (Zurich, Switzerland); the anti-CD14 (anti-LeuM3) mAb was purchased from Becton-Dickinson (Mountain View, CA).

The rabbit anti-human IL-4 antibody was raised in the UNICET laboratory. The immunoglobulin fraction was purified on protein A columns (Biorad, Richmond, CA). This antiserum is specific for IL-4; it binds with IL-4 and blocks IL-4 induced T- and B-cell proliferation and IL-4-induced expression of CD23 on the Burkitt lymphoma cell line Jijoye (I. Chrètien, A. van Kimmemede, M. K. Pearce, J. Banchereau and J. S. Abrams, manuscript submitted for publication).

Cell preparation and characterization

B cells were isolated either from tonsils obtained from children with chronic tonsillitis, from traumatic spleens or peripheral blood (PB) of normal volunteers. Mononuclear cells (MNC) were separated by the standard Ficoll–Hypaque gradient method. Monocyes were removed by treatment of cell suspensions with carbonyl iron, followed by magnetism (Romagnani *et al.*, 1980). T cells were removed from MNC by rosetting twice with neuraminidase-treated sheep and red blood cells, followed by complement lysis of the remaining T cells with the use of anti-CD3, anti-CD4 and anti-CD8 mAbs (Romagnani *et al.*, 1986a). The B-cell-enriched populations obtained from tonsils were typically 95% CD20 and contained less than 1% CD3 and CD14 cells, as assessed by the indirect immunofluorescence technique using a fluoresceinated anti-mouse Ig rabbit antibody (Romagnani *et al.*, 1985; Almerigogna *et al.*, 1985). The B-cell-enriched populations obtained from spleen or PB contained 50–70% CD20 cells, less than 3% CD3 cells and less than 5% CD14 cells.

Low- and high-density cells were separated from tonsillar B-cell suspensions by centrifugation on Percoll (Pharmacia) density gradients, according to the technique described by Kurnick *et al.* (1979), as reported elsewhere (Romagnani *et al.*, 1986a). Briefly, highly purified B cells were layered onto Percoll-step gradients composed of 40%, 50%, 55% and 100% Percoll layers. Cells banding between 40% and 50% Percoll and between 55% and 100% Percoll were chosen for the study.

Culture conditions and assay

Purified B cells were cultured at a density of $0{\cdot}5 \times 10^6$ per ml in 96-well U-bottom microtitre trays (Falcon Plastic, Oxnard, CA) in the presence of $F(ab')_2$ fragments of rabbit antibodies specific for human IgM or human IgD (5 μg/ml) in a final culture volume of 200 μl of RPMI-1640 medium (Flow Laboratories, Rockville, MD) containing 10% heat-inactivated fetal calf serum (FCS; Gibco, Grand Island, NY) (Romagnani *et al.*, 1985). Recombinant interleukins or SUPs were added either at the beginning of culture or at different culture time periods, as detailed. Cells were pulsed with 0·5 μCi of methyl [^{3}H] thymidine during the last 16 h of a 3-day culture period. [^{3}H] Thymidine uptake was measured by standard liquid scintillation counting techniques after harvesting, using a Packard scintillation counter.

RESULTS

Recombinant IL-4 acts as a B-cell growth factor (BCGF) in the co-stimulation assay with soluble anti-Ig antibodies

Human rIL-4 has recently been described for its ability to promote the proliferative response of human B cells preactivated with insolubilized anti-IgM antibody or SAC bacteria, while it is found to be less active on human splenic B lymphocytes in the co-stimulatory assay with suboptimal concentrations of soluble anti-IgM antibody (Defrance *et al.*, 1987b). The data displayed in Table 1 confirm that human rIL-4 is poorly mitogenic for human splenic, as well as PB, B cells co-stimulated with submitogenic concentrations of soluble $F(ab')_2$ fragments of anti-IgM antibody; the proliferative response of splenic and PB B cells was not improved using rIl-4 concentrations ranging between 20 and 500 U/ml (data not shown). However, in the same assay system, tonsillar B lymphocytes displayed convincing proliferation in response to human rIL-4; the proliferative response was inhibited by addition to the cultures of rabbit antibodies against human IL-4 (data not shown). In general, human rIL-4 appeared to act as a BCGF at least as potent as, or more potent than, rIL-2, and consistently

Table 1. Proliferative response of human B lymphocytes in the co-stimulatory assay with anti-IgM antibody and rIL-4

B-cell Population	DNA synthesis (c.p.m./well) with:			
	Medium	Anti-IgM*	rIL-4†	anti-IgM + rIL-4
Tonsils	1050 ± 42‡	1609 ± 382	2263 ± 433	14,807 ± 2081
PB	408 ± 98	2971 ± 269	603 ± 127	5960 ± 1477
Spleens	4680 ± 581	5804 ± 1204	5889 ± 944	6400 ± 903

* Anti-IgM was added at the final concentration of 5 μg/ml.

† Final concentration for rIL-4 was 200 U/ml.

‡ Results represent the mean values (± SE) of c.p.m. obtained in eight separate experiments with tonsillar B cells in five experiments with PB B cells and in three experiments with splenic B cells. 1×10^5 purified B cells/well were cultured for 3 days.

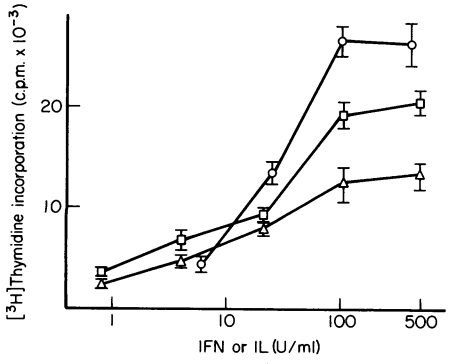

Figure 1. Proliferative response of human tonsillar B lymphocytes in the 3-day co-stimulatory assay with anti-IgM antibody in the presence of different concentrations of rIL-4 (○), rIL-2(□) or rIFN-γ (△). Results represent the mean values (± SE) of c.p.m. obtained in triplicate cultures of a representative experiment.

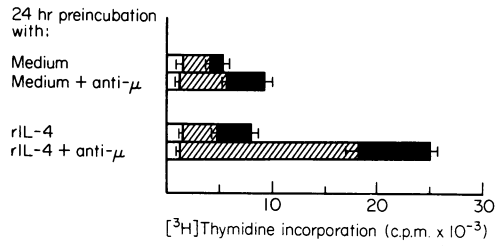

Figure 2. Effect of preincubation with anti-IgM antibody or rIL-4 on the proliferative response of B cells to the subsequent addition of anti-IgM antibody and/or rIL-4. B cells were incubated for 24 hr with medium alone, anti-IgM antibody (5 μg/ml, rIL-4 (100 U/ml) or anti-IgM antibody (5 μg/ml) + rIL-4 (100 U/ml), washed and then cultured for additional 48 hr with anti-IgM alone (5 μg/ml) (□), rIL-4 (100 U/ml) (■) or rIL-4 (100 U/ml) plus anti-IgM antibody (5 μg/ml) (■). Results represent the mean values (± SE) of c.p.m. obtained in three separate experiments.

more potent than rIFN-γ. Figure 1 shows the response obtained in a typical experiment in which B cells were stimulated with anti-IgM antibody in the presence of different concentrations of rIFN-γ, rIL-2 or rIL-4.

Mouse IL-4 (ex-B-cell stimulatory factor 1 or BSF-1), which was initially regarded as a BCGF as well (Howard *et al.*, 1982), has subsequently been shown to function as an activation factor on resting B cells, preparing them to enter S phase more promptly in response to anti-IgM antibody (Rabin *et al.*, 1986a). To establish whether human IL-4 acted on human B cells in a similar way, tonsillar B lymphocytes were precultured for 24 hr in medium alone, rIL-4, anti-IgM antibody or rIL-4 and anti-IgM antibody. At the end of the pre-culture period, the cells were washed and recultured at 1×10^5 per well in either rIL-4 or anti-IgM alone or rIL-4 and anti-IgM antibody. The results of these experiments are summarized in Fig. 2. Preincubation with rIL-4 alone did not significantly enhance the proliferative response of B cells to the subsequent addition of anti-IgM antibody, rIL-4 or rIL-4 and anti-IgM antibody in comparison with the response of B cells preincubated with medium alone. Preincubation with anti-IgM antibody induced a slight increase of the proliferative response to the subsequent addition of rIL-4. In contrast, preincubation of B cells with both rIL-4 and anti-IgM antibody resulted in a strong potention of the [^{3}H] thymidine uptake of B cells to the subsequent addition of rIL-4, suggesting that, unlike mouse Il-4, human Il-4 could act as a true growth factor for activated B cells.

Recombinant IL-4 was also able to promote the proliferative response of human tonsillar B cells activated with submitogenic concentrations of soluble F(ab')$_2$ fragments of anti-IgD antibody, and the B-cell response was still higher when submitogenic concentrations of both anti-IgM and anti-IgD antibodies were added together. Both rIFN-γ and rIL-2 behaved in a similar fashion (Table 2).

rIL-4 and rIL-2 interact with different receptors on at least partially overlapping B-cell subsets

The effect of some mAbs reactive with B-cell membrane components on the IL-4-mediated proliferation of human tonsillar B cells was then investigated. The proliferative response promoted by rIL-4 on anti-IgM-activated B cells was not significantly affected by the addition to B-cell cultures of anti-IFN-γ, anti-TAC or anti-DR mAbs, suggesting that the B-cell receptor for IL-4 is distinct from receptors for IL-2 or IFN-γ, and its interaction with IL-4 was not influenced by coating and/or capping of DR antigens. In contrast, the rIFN-γ-induced proliferation was abolished by the addition of anti-IFN-γ mAb and the rIL-2-mediated proliferation of the same anti-IgM-activated B cells was virtually abolished by the anti-TAC MAb and partially inhibited by the presence of anti-DR mAb (Table 3).

To investigate whether IL-4 and IL-2 acted on the same or on different B-cell subsets, purified human tonsillar B cells were further fractionated by centrifugation on Percoll density gradients. High- and low-density B-cell fractions were than assessed in comparison with unfractionated B cells for their ability to proliferate in the co-stimulatory assay with anti-IgM antibody in response to rIL-4, rIL-2, or rIFN-γ. The results of these experiments are summarized in Fig. 3. In agreement with previously reported data (Romagnani *et al.*, 1986b), we found that high-, but not low-density, B cells responded to IFN-γ, whereas both anti-IgM-activated B-cell fractions proliferated in response to rIL-2. rIL-4 displayed growth activity on both B-cell fractions as well. However, unlike rIL-2, which usually gave stronger proliferation on low- than high-density B cells, rIl-4 was consistently more active on these latter.

The above findings prompted us to examine whether rIL-4 and rIL-2 would co-operate to induce the proliferation of co-stimulated B lymphocytes. To this end, B lymphocytes co-stimulated with anti-IgM or anti-IgD antibody were cultured for 3 days with rIL-2, rIL-4 or IFN-γ alone, and effect of the combined addition of rIL-4 and rIL-2 or of rIL-4 and rIFN-γ or of rIL-2 and rIFN-γ was also evaluated. [^{3}H]Thymidine incorporation is illustrated in Table 4. The [^{3}H]thymidine incorporation induced by the combination of rIL-4 and rIL-2 was usually equal to or even lower than that induced by rIL-4 or rIL-2 alone, whereas the combined addition of rIL-4 and rIFN-γ resulted in a proliferative response usually higher than the sum of responses obtained with single lymphokines. The absence of an additive effect using a combination of rIL-2 and rIL-4, together with the results obtained in fractionation experiments of B cells on a Percoll gradient, suggests that IL-4 act on at least partially

Table 2. B-cell growth-promoting activity of different lymphokines in the co-stimulatory assay with anti-Ig antibodies

Factor added	Final concen.	DNA synthesis (c.p.m. well)* with: Medium	Anti-IgM	Anti-IgD	Anti-IgM + anti-IgD
Medium		654 ± 137	1200 ± 253	2182 ± 645	2315 ± 780
rIL-4	(100 U/ml)	1061 ± 216	11,406 ± 2700	19,928 ± 3721	29,333 ± 2118
rIFNγ	(200 U/ml)	749 ± 155	4210 ± 528	5034 ± 1020	9645 ± 1278
rIL-2	(100 U/ml)	1647 ± 347	9042 ± 1519	13,268 ± 1095	20,236 ± 2561

* 1×10^5 purified tonsillar B cells are stimulated for 3 days with suboptimal concentrations of $F(ab')_2$ fragments of anti-IgM (5 μg/ml), anti-IgD antibody (5 μg/ml) or both (5 μg/ml of anti-μ + 5 μg/ml g/ml of anti-δ). Results represent the mean value ± SE of c.p.m. obtained in triplicate culture from five consecutive experiments.

Table 3. Effect of the addition of anti-TAC, anti-DR or anti-IFN-γ mAbs on the B-cell proliferation induced by rIL-4, rIFN-γ or rIL-2 in the co-stimulatory assay with anti-IgM antibody

MAb added*	DNA synthesis (c.p.m./well) with: Anti-IgM	Anti-IgM + rIL-4†	Anti-IgM + rIFN-γ†	Anti-IgM + rIL-2†
Control ascites	2734 ± 325‡	20,176 ± 1769	7360 ± 1208	22,198 ± 2345
Anti-TAC	2173 ± 437	20,748 ± 2265	7183 ± 1024	4021 ± 1834
Anti-DR	2362 ± 261	23,503 ± 1988	8279 ± 1111	14,001 ± 2501
Anti-IFN-γ	2544 ± 589	19,429 ± 654	1868 ± 437	21,798 ± 1586

* Anti-TAC mAb was added at the final dilution of 1:100; anti-DR mAb was added at the final concentration of 10 μg/ml; anti-IFN-γ was added at the final dilution of 1:100.

† Final concentration of lymphokines was 100 U/ml for IL-4, 200 U/ml for IFN-γ and 100 U/ml for IL-2.

‡ Results represent the mean value ± SE obtained in triplicate cultures from three consecutive experiments.

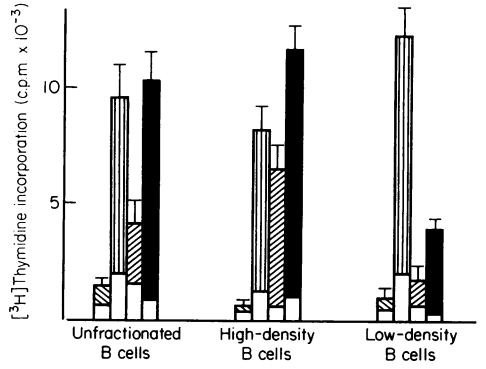

Figure 3. Proliferative response of high- and low-density tonsillar B cell subpopulations co-stimulatory assay with anti-IgM antibody and rIL-4. Purified tonsillar B cells were fractionated by centrifugation on Percoll gradients. Unfractionated B cells, as well as their high- ($P > 1080$ kgl) and low- ($P < 1073 > 1060$ kg/l density fractions, were cultured with anti-IgM antibody (5 μg/ml) in the absence (▨) or in the presence of rIL-2 (100 U/ml) (■), rIFN-γ (200 U/ml) (■), rIL-4 (100 U/ml) (■). White area at the bottom of the columns represents the mean value of c.p.m. obtained in the absence of anti-IgM antibody. Results represent the mean values (± SE) of c.p.m. obtained in three separate experiments.

overlapping B-cell subsets and that probably IL-4 exerts no or an inhibitory rather than a potentiating effect on the IL-2-mediated B-cell proliferation.

IFN-γ prepares activated B cells to respond to either IL-2 or IL-4

In previous papers, we demonstrated that rIFN-γ was able to potentiate the growth activity of rIL-2 on human B cells (Romagnani *et al.*, 1986a, b). In this study we have shown that IFN-γ has an additive effect on IL-4 response as well (Table 4). To establish whether rIFN-γ favoured the proliferative response of B cells to either rIL-2 or rIL-4 acting in a similar fashion, tonsillar B cells were cultured for 3 days in the presence of submitogenic concentrations of anti-IgM antibody and rIFN-γ, whereas rIL-2 or rIL-4 were added at different intervals from the beginning of culture. The results of these experiments are depicted in Fig. 4. The addition of either rIL-2 or rIL-4 after 24 or 48 hr to anti-IgM-stimulated B-cell cultures containing rIFN-γ from the beginning, resulted in a proliferative response greater than that obtained by adding the same interleukins, alone or in combination with rIFN-γ (data not shown), after 24 or 48 hr to B-cell cultures stimulated with anti-IgM antibody alone, whereas the reverse was not true, suggesting that IFN-γ

Table 4. Effect of the stimultaneous addition of different lymphokines on the proliferative response of anti-IgM co-stimulated B cells

Factor* added	DNA synthesis (c.p.m. well) with:		
	Medium	Anti-IgM†	Anti-IgD†
Medium	592±73‡	587±102	1140±111
rIL-4	1757±271	10,066±278	13,304±61
rIFN-γ	891±147	4944±1069	4466±924
rIL-2	2256±226	8926±330	13,758±113
rIL-4+rIFN-γ	1368±302	16,362±525	18,250±1305
rIL-4+rIL-2	1632±294	10,126±854	11,084±674
rIL-2+rIFN-γ	2541±173	13,076±1383	16,764±580

* Final concentration of lymphokines was 100 U/ml for IL-4, 200 U/ml for IFN-γ and 100 U/ml for IL-2 either when added alone or in mixing experiments.

† Anti-IgM or anti-IgD antibodies were added at the final concentration of 5 μg/ml.

‡ Results represent the mean value±SE obtained in triplicate cultures from three consecutive experiments.

has to be present in the early phases of activation in order to favour the proliferative response of B cells to both IL-4 and IL-2.

In a subsequent series of experiments, purified tonsillar B cells were preincubated for 24 hr with rIFN-γ or rIFN-γ plus anti-IgM antibody. At the end of the preculture period, the cells were washed and recultured at 1×10^5 cells per well in either rIL-4 or rIL-4 and anti-IgM antibody. As shown in Fig. 5, preincubation with rIFN-γ and anti-IgM antibody potentiated the proliferative response of B cells to the subsequent addition of either rIL-4 alone or rIL-4 and anti-IgM antibody. This suggests that rIFN-γ, in the presence of anti-IgM, prepares B cells to respond to both rIL-2 (Romagnani *et al.*, 1986a, b) and rIL-4 without needing to be present during the entire period of culture.

DISCUSSION

These studies were carried out to compare the B-cell growth-promoting activity of human rIL-4 with that of other lymphokines, such as mouse IL-4 (BSF-1) or human rIL-2 and human rIFN-γ. Mouse IL-4, initially identified for its ability to promote the proliferation of anti-IgM-co-stimulated B cells, has subsequently been shown to act as a competence factor or a competence co-factor for virtually all haematopoietic lineage cells (Lee *et al.*, 1986; Mosmann *et al.*, 1986; Grabstein *et al.*, 1986; Smith & Rennick, 1986; Crawford *et al.*, 1987; Ohara & Paul, 1987; Kishimoto, 1987). With regard to the B lineage, mouse IL-4 functions either as an activating factor for resting B cells (Kishimoto, 1987; Rabin, Ohara & Paul 1985) or as a differentiation factor for activated cells (Kishimoto, 1987; Vitetta *et al.*, 1985). The more recently cloned human IL-4 revealed several functional homologies with mouse IL-4, such as induction of CD23/FcεR2 (Defrance *et al.*, 1987a, b), but some activities on B cells were different. For example, human rIL-4 was able to stimulate the proliferation of human B cells preactivated with optimal concentrations of anti-IgM antibodies coupled to beads or SAC bacteria (Yokota *et al.*, 1986;

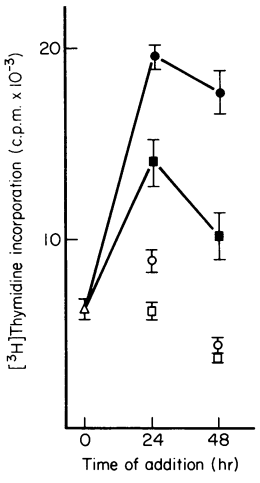

Figure 4. Effect of the late addition of rIL-2 or rIL-4 on the B-cell proliferation promoted by rIFN-γ in the co-stimulatory assay with anti-IgM antibody. Purified tonsillar B cells were cultured with anti-IgM antibody (5 μg/ml) and rIFN-γ (200 U/ml). After 24 or 48 hr rIL-2 (100 U/ml) (●) or rIL-4 (100 U/ml) (■) were added and cultures harvested on Day 3. Open symbols represent values of c.p.m. obtained in cultures stimulated with anti-IgM antibody (5 μg/ml) containing rIFN-γ alone (△) or in cultures to which rIL-2 (○) or rIL-4 (□) were added after 24 or 48 hr to anti-IgM-stimulated B cells in the absence of rIFN-γ. Results represent the mean values (±SE) of c.p.m. obtained in three separate experiments.

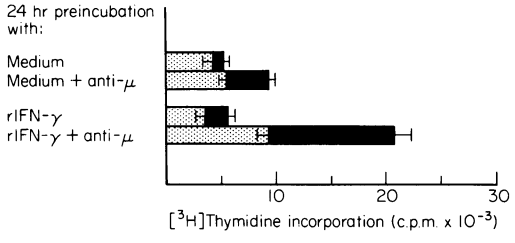

Figure 5. Effect of preincubation with anti-IgM antibody, rIFN-γ or both on the proliferative response of B cells to rIL-4 or rIL-4+anti-IgM antibody. Purified B cells were incubated for 24 hr with medium alone, anti-IgM antibody alone (5μg/ml), rIFN-γ alone (200 U/ml) or rIFN-γ+ anti-IgM antibody, washed and then cultured for additional 3 days in the presence of rIL-4 alone (100 U/ml) (□) or rIL-4 (100 U/ml)+anti-IgM antibody (5 μg/ml) (■). Results represent the mean values (±SE) of c.p.m. obtained in four separate experiments.

Defrance *et al.*, 1987a, b), but it was found to be poorly mitogenic for human splenic B cells in the co-stimulatory assay with submitogenic concentrations of soluble anti-IgM antibody. This study confirms that human rIL-4 is poorly or not mitogenic for splenic, as well as PB, anti-IgM-co-stimulated B cells, but it was found to display convincing growth-promoting activity in the co-stimulatory assay with both anti-IgM and anti-IgD antibodies on human tonsillar B cells. It is not clear whether this particular behaviour was due to differences in the reactivity of B cells or to the different degree of contamination in T cells and/or monocytes of B cell-enriched suspension from different sources (tonsil versus spleen and PB B cells).

Although human IL-4 appeared to be as active as mouse IL-4 in the co-stimulation assay with anti-Ig antibodies (provided

that tonsillar B cells were used), it apparently acted, however, in such an assay as a true growth factor for activated B cells. In fact, preincubation with IL-4 did not enhance the response of B cells to the subsequent addition of anti-IgM antibody and IL-4, whereas perincubation of the same cells with anti-IgM and IL-4 rendered them much more reactive to the subsequent addition of IL-4 alone, suggesting that the presence of anti-IgM antibody from the beginning of culture was essential for preparing B cells to respond to IL-4. On the other hand, the finding that preincubation with submitogenic concentrations of soluble anti-IgM antibody alone was a less efficient signal for rendering B cells responsive to IL-4 suggests a possible role of Il-4 in up-regulating the IL-4 receptor itself on activated B cells. In this respect, it is likely that soluble anti-IgM antibody and IL-4 together mimic the activity of optimal concentrations of insolubilized anti-IgM antibody or SAC bacteria, which have been found to be able to render B cells responsive to Il-4 (Yokota *et al.*, 1986; Defrance *et al.*, 1987b). These data suggest that murine and human IL-4 differ with regard to their proliferative activities. IL-4 induces CD23 antigen expression in both mouse and human resting B cells (Defrance *et al.*, 1987a). However, while preincubation of mouse B cells with IL-4 was found to potentiate the proliferative response of these cells to the subsequent addition of anti-IgM antibody, preincubation of human B cells with IL-4 did not display a potentiating effect. Thus, IL-4 seems to act as a growth factor only on activated human B cells. As discussed below, a different behaviour between human and mouse B cells has also been observed in response to IFN-γ when the same co-stimulatory assay with anti-IgM antibody was used (Mond *et al.*, 1985; Defrance *et al.*, 1986; Romagnani *et al.*, 1986b).

The B-cell growth promoting activity of IL-4 on anti-IgM-stimulated B cells was not affected by addition to B-cell cultures of anti-TAC or anti-DR mAbs, which virtually abolished or strongly inhibited, respectively, the B-cell growth promoting activity of IL-2 (Giudizi *et al.*, 1987). These data suggest that IL-4 and IL-2 act on distinct receptors on the same activated B cells or on distinct B-cells subsets. The latter possibility, however, is unlikely for several reasons. First, although IL-4, unlike IL-2, was consistently a more potent BCGF for high- than low-density B cells, both B-cell fractions were usually induced to proliferate by the two lymphokines. In contrast, IFN-γ acted as BCGF on high density B cells only. Second, when the effect of the simultaneous addition of IL-4 and IL-2 to anti-IgM-co-stimulated B cells was assessed, no additional but sometimes even inhibitory effects were observed. Finally, the addition of IFN-γ from the beginning of culture to anti-IgM-co-stimulated B cells or preincubation of B cells with anti-IgM antibody in the presence of IFN-γ promoted an additive proliferative response to the subsequent addition of either IL-2 or IL-4, whereas the reverse did not. These findings confirm previous reports showing that human B cells are more responsive to IFN-γ than IL-2 during the early stages of activation with anti-Ig and, in contrast with the data reported in mice (Rabin *et al.*, 1986b), suggest that the presence of IFN-γ during initial activation with anti-Ig antibodies does not inhibit, but even facilitates, the capacity of activated B cells to proliferate in response to both IL-2 and IL-4. The additive effect of IFN-γ on the IL-4-induced B-cell proliferation reported in these and other (Defrance *et al.*, 1987b) experiments is in contrast with the inhibitory effect of IFN-γ on different activities of IL-4 on human B cells, such as induction of CD23 expression (Defrance *et al.*, 1987a) and induction of IgE production (Del Prete *et al.*, 1988). Furthermore, in the murine system IFN-γ showed an antagonistic effect on IL-4-induced proliferation of anti-IgM-activated B cells (Mond *et al.*, 1985). Mechanisms underlaying these differences deserve further investigation. Taken together, the results of this study suggest that IL-2 and IL-4 may act as growth factors on the same or largely overlapping human B-cell subpopulations. However, the reason why the same subsets of B cells can utilize two different, but at least apparently functionally similar, growth factors is unclear at the present time. Likewise, whether or not the activity of the two factors is related to different B-cell functional pathways still remains speculative.

ACKNOWLEDGMENTS

This work has been supported by grants from C.N.R. (Finalized Project, Infectious diseases, n.83.00686.52), the Ministero Pubblica Istruzione (n. 02938), and the National Institutes of Health (AI21384 and CA38512).

REFERENCES

ALMERIGOGNA F., BIAGIOTTI R., GIUDIZI M.G., DEL PRETE G.F., MAGGI E., MAZZETTI M., ALESSI A., RICCI M. & ROMAGNANI S. (1985) Different reactivity of activated human B cells to B-cell growth factor and interleukin 2 in the costimulation assay with anti-IgM antibody and in the preactivation assay with *Staphylococcus* bacteria. *Cell. Immunol.* **95,** 358.

CRAWFORD R.M., FINBLOOM D.S., OHARA J., PAUL W.E. & MELTZER M.S. (1987) B cell stimulator factor-1 (interleukin-4) activates macrophages for increased tumoricidal activity and expression of Ia antigens. *J. Immunol.* **139,** 135.

DEFRANCE T., AUBRY J.P., ROUSSET F., VANBERVLIET B., BONNEFOY J., ARAI N., TAKEBE Y., YOKOTA T., LEE F., ARAI, K., DE VRIES J. & BANCHEREAU J. (1987a) Human recombinant interleukin 4 induces Fcε receptors (CD23) on normal human B lymphocytes. *J. exp. Med.* **165,** 1459.

DEFRANCE T., AUBRY J.P. VANBERVLIET B. & BANCHEREAU J. (1986) Human interferon-γ acts as a B cell growth factor in the anti-IgM antibody costimulatory assay but has no direct B cell differentiation activity. *J. Immunol.* **137,** 3861.

DEFRANCE T., VANBERVLIET B., AUBRY J.P., TAKEBE Y., ARAI N., MIYAJIMA A., YOKOTA T., LEE F., ARAI K., DE VRIES J. & BANCHEREAU J. (1987b) B cell growth promoting activity of recombinant human IL-4. *J. Immunol.* **139,** 1135.

DEL PRETE G.F., MAGGI E., PARRONCHI P., CHRÈTEIN I., TIRI A., MACCHIA D., RICCI M., BANCHEREAU J., DE VRIES J. & ROMAGNANI S. (1988) IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J. Immunol.* **140,** 4193.

GIUDIZI M.G., BIAGIOTTI R., ALMERIGOGNA F., ALESSI A., TIRI A., DEL PRETE G.F., FERRONE S. & ROMAGNANI S. (1987) Role of HLA class I and class II antigens in activation and differentiation of B cells. *Cell. Immunol.* **108,** 97.

GRABSTEIN K., EISEMAN J., MOCHIZUKI D., SHANEBECK K., COULON P., HOPP T., MARCH C. & GILLIS S. (1986) Purification to homogeneity of B cell stimulating factor. *J. exp. Med.* **163,** 1405.

HOWARD M., FARRAR J., HILFIKER M., JOHNSON B., TAKATSU K., HAMAKA T. & PAUL W.E. (1982) Identification of a T cell-derived B cell growth factor distinct from interleukin-2. *J. exp. Med.* **155,** 914.

KISHIMOTO T. (1987) B-cell stimulatory factors (BSFs): molecular structure, biological function, and regulation of expression. *J. clin. Immunol.* **7,** 343.

KURNICK J.T., OSBERT L., STEGAGNO M., KIMURA A.K., ORM A.& SJOBERG O. (1979) A rapid method for the separation of functional

lymphoid cell populations of human and animal origin on PVP-silica (Percoll) density gradient. *Scand J. Immunol.* **10,** 563.

LEE F., YOKOTA T., OTSUKA T., MEYERSON P., VILLARET D., COFFMAN R., MOSMANN T., RENNICK D., ROEHM N., SMITH C., ZLOTNIK A. & ARAI K. (1986) Isolation and characterization of a mouse interleukin cDNA clone that expresses B-cell stimulatory factor 1 activities and T-cell and mast-cell-stimulating activities. *Proc. natl Acad. Sci. U.S.A.* **83,** 2061.

MINGARI M.C., GEROSA F., CARRA G., ACCOLLA R.S. MORETTA A., ZUBLER R.H., WALDMAN T.A. & MORETTA L. (1984) Human interleukin-2 promotes proliferation of activated B cells via surface receptors similar to those of activated T cells. *Nature (Lond.)* **312,** 641.

MOND. J.J., FINKELMAN E.D., SARMA C., OHARA J. & SERRATE S. (1985) Recombinant interferon γ inhibits the B cell proliferative response stimulated by soluble not by Sepharose-bound anti-immunoglobulin antibody. *J. Immunol.* **135,** 2513.

MOSMANN T.R., BOND M.W., COFFMAN R.L., OHARA J. & PAUL W.E. (1986) T-cell and mast cell lines respond to B-cell stimulatory factor 1. *Proc. natl. Acad. Sci. U.S.A.* **83,** 5654.

MURAGUCHI A., KEHRL J.H., LONGO D.L., VOLKMAN D.J., SMITH K.A. & FAUCI A.S. (1985) Interleukin-2 receptors on human B cells. Implications for the role of interleukin 2 in human B cell function. *J. exp. Med.* **161,** 181.

OHARA J. & PAUL W.E. (1987) Receptors for B-cell stimulatory factor-1 expressed on cells of haematopoietic lineage. *Nature (Lond.)* **325,** 537.

RABIN E.M., MOND J.J., OHARA J. & PAUL W.E. (1986) B cell stimulatory factor (BSF)-1 prepares resting B cells to enter S phase in response to anti-IgM and to lipopolysaccharide. *J. exp. Med.* **164,** 517.

RABIN E.M., MOND J.J., OHARA J. & PAUL W.E. (1986b) Interferon-γ inhibits the action of B cell stimulatory factor (BSF)-1 on resting B cells. *J. Immunol.* **137,** 1573.

RABIN E.M., OHARA J. & PAUL W.E. (1985) B cell stimulatory factor (BSF)-1 activates resting B cells. *Proc. natl. Acad. Sci. U.S.A.* **82,** 2435.

ROMAGNANI S., GIUDIZI M.G., ALMERIGOGNA F. & RICCI M. (1980) Interaction of staphylococcal Protein A with membrane components of IgM- and/or IgD-bearing lymphocytes from human tonsil. *J. Immunol.* **124,** 1620.

ROMAGNANI S., GIUDIZI M.G., MAGGI E., ALMERIGOGNA F., BIAGIOTTI R., DEL PRETE G.F., MAZZETTI M., ALESSI A., VERCELLI D. & RICCI M. (1985) Synergy of B cell growth factor and interleukin-2 in the proliferation of activated human B cells. *Eur. J. Immunol.* **15,** 1158.

ROMAGNANI S., GIUDIZI M.G., BIAGIOTTI R., ALMERIGOGNA F., MINGARI C., MAGGI E., LIANG C.-M. & MORETTA L. (1986ab) B cell growth factor activity of interferon-γ. Recombinant human interferon-γ promotes proliferation of anti-IgM-activated human B lymphocytes. *J. Immunol.* **136,** 3513.

ROMAGNANI S., GIUDIZI M.G., ALMERIGOGNA F., BIAGIOTTI R., ALESSI A., MINGARI C., LIANG C.-M., MORETTA L. & RICCI M. (1986a) Analysis of the role of γ-interferon, interleukin-2 and a third factor distinct from interferon-γ and interleukin-2 on human B-cell proliferation. Evidence that they act at different times after B-cell activation. *Eur. J. Immunol.* **16,** 623.

SHARMA S., METHA S., MORGAN J. & MAIZEL A. (1987) Molecular cloning and expression of a human B-cell growth factor gene in *Escherichia coli. Science,* **235,** 1489.

SMITH C.A. & RENNICK D.M. (1986) Characterization of a murine lymphokine distinct from interleukin-2 and interleukin-3 possessing a T cell growth factor activity and a mast cell growth factor that synergizes with IL-3. *Proc. natl. Acad. Sci. U.S.A.* **83,** 1857.

TSUDO M., UCHIYAMA T. & HUCHINO H. (1984) Expression of TAC antigen on activated normal human B cells. *J. exp. Med.* **160,** 612.

VITETTA E.S., OHARA J., MYERS C., LAYTON J., KRAMMER P.H. & PAUL W.E. (1985) Serological, biochemical, and functional identity of B cell-stimulatory factor 1 and B cell differentiation factor for IgGl. *J. exp. Med.* **162,** 1726.

YOKOTA T., OTSUKA T., MOSMANN T., BANCHEREAU J., DEFRANCE T., BLANCHARD, D., DE VRIES J. & ARAI K. (1986) Isolation and characterization of a human interleukin cDNA clone, homologous to mouse B-cell stimulatory factor 1, that expresses B-cell- and T-cell-stimulating activities. *Proc. natl. Acad. Sci. U.S.A.* **83,** 5894.