Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 0000–0000, December 1984
Immunology

Human interleukin 2 can promote the growth and differentiation of single hapten-specific B cells in the presence of specific antigen

(lymphokines/hapten-gelatin fractionation/T cell-independent antigens/B-cell clones)

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ABSTRACT **B lymphocytes specifically reactive to the hapten fluorescein (FLU) were prepared from normal adult murine spleen by the hapten-gelatin affinity procedure. They were placed in 10 μl of microcultures singly or in small numbers in the absence of any feeder, filler, or accessory cell. The "T cell-independent" antigen FLU-conjugated polymerized flagellin (FLU-POL) or a selected batch of FLU-conjugated Ficoll were used, and these stimulated division and differentiation only in the concomitant presence of lymphokines acting as B-cell growth and differentiation factors (BGDF). It was found that human interleukin 2 (IL-2), prepared by recombinant DNA technology (r-IL-2), was an effective, albeit rather weak, BGDF in this system. When an IL-2-free source of BGDF was used with the antigenic stimulus, addition of r-IL-2 did not augment the response, nor did removal of IL-2 from the crude lymphokine mixture diminish the BGDF activity.**

Lymphokine growth and differentiation factors produced by cloned activated T cells or T-cell hybridomas clearly play a major role in the regulation of B lymphocyte responses to antigen (1–11). The number, molecular characteristics, and modes of action of B-cell growth and differentiation factors (BGDF) are presently controversial. Many lymphokine-rich conditioned media (CM) with activity for B cells contain the T-cell growth factor interleukin 2 (IL-2) (12), but this molecule does not account for all of the observed bioactivity in each CM (1, 3, 10, 11). Some authors have claimed a role for IL-2 itself in B-lymphocyte responses (7, 12–16), but others have disputed this (8, 17). Two possible reasons for this confusion are (*i*) the use of impure preparations of IL-2, which may contain trace amounts of other lymphokines, and (*ii*) culture of lymphocyte populations at high cell density, where a few T cells contaminating a source of B cells could themselves be induced to form lymphokines after appropriate activation.

A definitive resolution of this controversy requires two conditions—namely, a source of pure IL-2 and an assay system where the B cell itself is the unquivocal target of the bioactivity under study. Recently, human IL-2 has been prepared by recombinant DNA technology (r-IL-2) (18–20) and shown to be active in promoting the growth of murine T cells. Furthermore, we have devised a B-lymphocyte cloning system in which a single, hapten-specific murine B cell can be placed into culture (in the absence of any accessory or feeder cell) and stimulated with a combination of specific antigen and B cell-active lymphokines to divide and secrete antibody (1, 21–23). Thus, despite the crossing of a species barrier, it seemed worth testing human r-IL-2 in this system. We now report that r-IL-2 does possess B-cell growth and differentiation-promoting activity.

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MATERIALS AND METHODS

Mice and Preparation of Fluorescein (FLU)-Specific Splenic B Cells. Inbred-specific pathogen-free CBA/CaH/Wehi mice were used at age 8–10 weeks as spleen donors. To prepare hapten-specific B cells, spleen cell suspensions were fractionated on thin layers of FLU-conjugated gelatin as described (24, 25). Adherent FLU-gelatin was removed from the recovered binding cells by collagenase treatment. The binding cell population is 97% B cells, ≈70% FLU-binding, and ≈200-fold enriched for *in vitro* reactivity to FLU conjugates (1, 22, 23).

Antigens. The hapten FLU was coupled onto polymerized flagellin (POL), aminoethylcarbamoyl methylated Ficoll (AECM-Ficoll) (Institute of Drug Technology, Melbourne, Australia), and *Brucella abortus* (BA) (Commonwealth Serum Laboratories, Melbourne) as described (23, 25). FLU-POL was used at 50 ng/ml, FLU-AECM-Ficoll with 53 FLU molecules per 400,000 daltons of Ficoll (FLU_{53}-Ficoll) at 0.1 ng/ml, and FLU-BA at $\approx 2 \times 10^8$ organisms per ml.

r-IL-2 and Assay for IL-2 Activity. The gene coding for the human IL-2 protein was obtained from a cDNA library of Jurkat RNA. This gene was expressed in *Escherichia coli*, and the protein produced was purified to apparent homogeneity (19, 20). The lot used in this study (LP210) was lyophilized and reconstituted with sterile water prior to use. The endotoxin level was ≈0.03 ng/10^6 units. The IL-2 activity of the recombinant material was measured at Cetus by using the IL-2-dependent murine cell line HT-2. In Melbourne, IL-2 activity was assessed by using the murine CTL-L line as target cells. Both assay systems were performed in a similar manner as described (26), and a unit of activity was defined as the reciprocal of the dilution that yielded half-maximal incorporation of [^{3}H]thymidine.

EL4 Thyoma Cell-Derived BGDF. Medium conditioned by concanavalin A-stimulated EL4 thymoma cells (EL4-CM) was prepared as described (1) and used as a source of T cell-derived BGDF (EL-BGDF-pik, according to the nomenclature proposed by the 1983 Kyoto workshop). The final concentrations of EL-BGDF-pik specified in the text are dilutions of a ×10 concentrate.

Filler Cell-Free B-Cell Cloning Systems. FLU-specific B cells were cultured in 60-well Terasaki trays in 10 μl of RPMI 1640 medium supplemented with 5% (vol/vol) fetal calf serum and 100 mM 2-mercaptoethanol as described (22, 23). From 1 to 10 FLU-specific B cells were added to all trays in 5 μl of medium, and then the antigen and/or lymphokine was added at twice the required final concentration in another 5 μl.

Abbreviations: BA, *Brucella abortus*; BGDF, B-cell growth and differentiation factor(s); FLU, fluorescein; IL-2, interleukin 2; r-IL-2, recombinant human IL-2; PFC, plaque-forming cell; POL, polymerized flagellin; CM, conditional medium/media; AECM, aminoethylcarbamoylmethylated.

Assessment of Clonal Proliferation and Antibody Formation. After 3–4 days, culture wells were examined with an inverted phase-contrast microscope at 100-fold magnification for the presence or absence of a proliferating B-cell clone as described (1, 22, 23). After assessment of proliferation, wells were scored for the presence or absence of directly hemolytic anti-FLU plaque-forming cell (PFC) clones using an *in situ* plaque-detection method as described (23). The frequency of clonal precursors was determined as described (23, 27, 28).

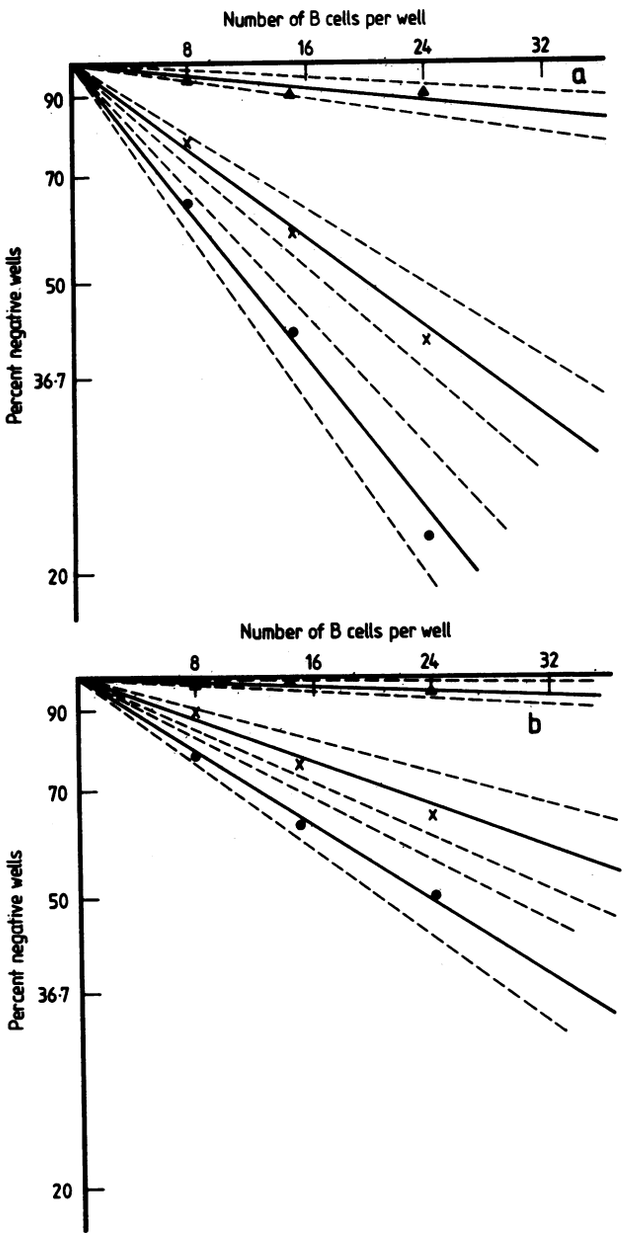

FIG. 1. Limiting dilution analysis of the cloning efficiency of FLU-gelatin-enriched splenic B cells stimulated *in vitro* in the presence of r-IL-2 alone at 10 units/ml (▲), FLU-POL alone at 0.05 μg/ml (×), or both r-IL-2 and FLU-POL (●). Proliferation (*a*) and anti-FLU antibody formation (*b*) are shown. Each point represents a sample of 120 replicate cultures. The frequency values for proliferating and anti-FLU PFC clones, respectively, were: for r-IL-2 alone, 0.51% (95% confidence limits, 0.30–0.72) and 0.06% (0–0.1); for FLU-POL alone, 3.53% (2.95–4.12) and 1.72% (1.35–2.09); for FLU-POL and r-IL-2, 5.87% (5.03–6.71) and 2.97% (2.56–3.49); and for medium alone (not shown) 0.47% (0.25–0.68) and 0. With FLU-POL and EL-BGDF-pik, values of 8.95% for proliferating and 4.07% anti-FLU PFC clones were obtained, and with EL-BGDF-pik alone, the values were 4.86% and 2.28%, respectively. Dashed lines represent the 95% confidence limits.

RESULTS

Synergy of FLU-POL and r-IL-2 in Promoting the Growth and Differentiation of Single Hapten-Specific B Lymphocytes. The ability of r-IL-2 to act in synergy with the T cell-independent antigen FLU-POL to promote proliferation and differentiation of single FLU-specific B cells was investigated. Limiting dilution analysis was performed by using various numbers of FLU-specific B cells cultured in 10 μl of medium alone or in 10 μl of medium containing r-IL-2 alone at 10 units/ml, FLU-POL alone, or both FLU-POL and r-IL-2. Control cultures with EL-BGDF-pik, the standard lymphokine source for this system, were also included. Culture wells were assessed for the presence of proliferating clones and anti-FLU PFC clones after 3 days. Fig. 1 shows a typical experiment demonstrating that human r-IL-2 can act in synergy with specific antigen to promote both proliferation and differentiation of murine splenic B cells. Single-hit kinetics were observed with all combinations used. Proliferation (Fig. 1*a*) and anti-FLU PFC clone formation (Fig. 1*b*), achieved with FLU-POL and r-IL-2 at 10 units/ml acting together, was significantly higher than either that of FLU-POL acting alone or r-IL-2 acting alone in the absence of antigen. Table 1 shows the degree of synergy observed over a wide range of r-IL-2 concentrations; r-IL-2 at 1–100 units/ml significantly elevated the proliferative response above that of antigen alone. With r-IL-2 at 10–100 units/ml, the proportion of cells generating anti-FLU PFC clones also was increased significantly. The dose–response profile was flat, with 1 unit/ml exerting near maximal effects and no further significant increase occurring with up to 100-fold higher concentrations. No significant response above that of medium alone was elicited by r-IL-2 acting alone without antigen (0.51 ± 0.09% proliferation with medium alone compared to 0.65 ± 0.08% for r-IL-2 alone at 10 units/ml). The responses elicited by r-IL-2 and FLU-POL were below those of EL-BGDF-pik and FLU-POL.

Two further aspects of Table 1 warrant comment. First, the results from the positive control, namely FLU-POL with EL-BGDF-pik, are somewhat higher than previously reported (1, 22, 23). Second, both the antigen acting alone without lymphokine and the lymphokine EL-BGDF-pik acting alone without antigen caused a significant degree of proliferation not noted in previous work. While this could reflect in part batch variation in antigen or EL4-CM, we believe it is also due to some intrinsic change in the activation state of the B

Table 1. Proliferation and differentiation of FLU-specific B cells with r-IL-2 and FLU-POL

Lymphokine added, units/ml	Proliferating clones		Anti-FLU PFC clones	
	%	*P* value*	%	*P* value*
r-IL-2				
0	3.50 ± 0.64	—	1.27 ± 0.28	—
0.1	3.45 ± 0.99	NS	2.46 ± 1.09	NS
1	6.80 ± 1.82	<0.05	2.01 ± 0.74	NS
10	7.39 ± 2.01	<0.05	2.85 ± 0.54	<0.005
100	7.41 ± 1.40	<0.01	2.18 ± 0.36	<0.005
EL-BGDF-pik	10.2 ± 1.35	<0.0025	4.63 ± 0.58	<0.0025

From 1 to 10 FLU-specific B cells were stimulated with FLU-POL and lymphokines as indicated. In the absence of FLU-POL, values for proliferation and antibody formation with medium alone were 0.47 ± 0.14% and 0.06 ± 0.04%, respectively, and with EL-BGDF-pik alone were 4.91 ± 0.78% and 1.79 ± 0.34%. Values shown represent the mean ± SEM of three to five experiments.
*P values obtained by the Student *t* test represent significance from antigen-alone responses. NS, not significant.

Table 2. Dependence upon BGDF of a FLU-Ficoll conjugate for stimulation of single FLU-specific B cells *in vitro*

Additives	Proliferation, %	Anti-FLU PFC clones, %
None	0.62 ± 0.14	0.07 ± 0.07
FLU_{53}-Ficoll	1.05 ± 0.29	0.30 ± 0.13
FLU_{53}-Ficoll/ EL-BGDF-pik	15.9 ± 1.65	6.02 ± 1.21
EL-BGDF-pik	5.40 ± 0.68	1.88 ± 0.31

From 1 to 10 FLU-specific B cells were stimulated with FLU_{53}-Ficoll at 0.1 ng/ml in the presence and absence of 5% (vol/vol) EL-BGDF-pik or with EL-BGDF-pik alone. Values represent the mean ± SEM of five experiments.

lymphocytes of our mouse population, rendering a small proportion of the cells more responsive. In any event, the FLU-POL conjugate of Table 1 behaved more like the T cell-independent antigen FLU-BA, which has been shown (23) to be able to elicit a low response when acting alone, which was significantly increased by the addition of BGDF. We also have used a FLU-POL conjugate that, even with the present cells, stimulates negligible background proliferation (see Table 4).

Lymphokine Dependence of a FLU-Ficoll Conjugate in the Single-Cell System. We recently have classified various T cell-independent antigens on the basis of their requirement for the concomitant presence of lymphokines for effective stimulation of single B cells in this filler cell-free system (23). Among the antigens studied was FLU-AECM-Ficoll, and we reported on two types of conjugates—namely, one type that stimulated FLU-specific B cells in the absence of added EL-BGDF-pik, and another type that failed to stimulate at all, even in the presence of an optimal concentration of BGDF. We now have tested other batches and report here on a FLU_{53}-Ficoll conjugate, the stimulatory capacity of which was found to be almost totally dependent on added lymphokines (Table 2). This particular conjugate, when used as the antigenic stimulus in the presence of EL-BGDF-pik, yields even better proliferation and differentiation than does FLU-POL (Table 1), yet in the absence of BGDF, the background stimulation is very low. Thus, the FLU_{53}-Ficoll conjugate is a particularly favorable antigen to assess putative B cell-active factors. The ability of a wide range of concentrations of r-IL-2 to synergize with FLU_{53}-Ficoll is shown in Table 3. Although significant promotion of division was noted with all concentrations tested, the responses were much lower than seen with EL-BGDF-pik.

Relative Weakness of r-IL-2 as a BGDF Confirmed with Various Antigens. Table 4 draws together data from a series of four experiments in which the B-cell growth-promoting activity of EL-BGDF-pik and r-IL-2 were directly compared

Table 3. Proliferation and differentiation of FLU-specific B cells with r-IL-2 and FLU_{53}-Ficoll

Lymphokine added, units/ml	Proliferating clones		Anti-FLU PFC clones	
	%	*P* value	%	*P* value
r-IL-2				
0	0.92 ± 0.52	—	0.50 ± 0.07	—
1	2.79 ± 0.44	<0.025	1.22 ± 0.22	<0.025
10	3.75 ± 0.67	<0.025	1.82 ± 0.21	<0.0125
100	4.25 ± 0.73	0.05	0.77 ± 0.17	NS
EL-BGDF-pik	17.2 ± 0.89	<0.0005	7.20 ± 1.00	<0.01

Responses were generated with FLU_{53}-Ficoll at 0.1 ng/ml and lymphokines as indicated. Respective values for proliferation and antibody formation for medium alone were 0.60 ± 0.20% and 0.07 ± 0.07%, respectively, and for EL-BGDF-pik alone were 5.85 ± 0.80% and 2.14 ± 0.23%. Values represent the mean ± SEM of three experiments. NS, not significant.

Table 4. Synergy of r-IL-2 with various T cell-independent antigens

Antigen	No lymphokine	r-IL-2	r-IL-2 with EL-BGDF-Pik
None	0.32 ± 0.22	0.43 ± 0.20 (NS)	3.96 ± 1.43 (<0.05)
FLU-POL			
No. 14	2.33 ± 0.47	3.22 ± 0.33 (0.05)	7.80 ± 1.71 (<0.025)
No. 13	0.37 ± 0.05	1.14 ± 0.32 (<0.05)	7.42 ± 2.17 (<0.025)
FLU-BA	5.68 ± 0.96	7.78 ± 0.32 (<0.025)	21.1 ± 5.07 (<0.025)
FLU_{53}-Ficoll	1.00 ± 0.25	2.60 ± 0.79 (<0.05)	14.1*

Proliferating clone frequency values from four experiments where FLU-specific B cells were cultured at limit dilution with various T cell-independent antigens in the absence of added lymphokines, with r-IL-2 at 10 units/ml, or with 5% (vol/vol) EL-BGDF-pik. Numbers in parenthesis are *P* values, which represent significance from the no-lymphokine antigen-alone response.
*Two experiments only.

by using a variety of antigenic stimuli. The results with FLU-POL batch 14 and FLU_{53}-Ficoll confirm the data of Tables 1–3. FLU-POL batch 13 was chosen as a conjugate entirely dependent on lymphokine copresence for activation, even with the present, more readily activatable B cells. FLU-BA was chosen as an antigen both partially dependent and partially independent of BGDF (23). In both cases, the effect of r-IL-2, although significant, was quite modest.

Lack of Synergy of r-IL-2 with EL-BGDF-pik. The question was asked as to whether the addition of r-IL-2 could enhance the more vigorous response obtained with antigen and EL-BGDF-pik. As El-BGDF-pik is contained with a crude CM, this necessitated first determining its own content of (murine) IL-2. This was found to be 18 units/ml for the 10-times-concentrated CM and, thus, 0.9 units/ml for the 5% (vol/vol) concentration used as the optimal concentration for B-cell stimulation. Accordingly, EL-BGDF-pik was absorbed with cells from the IL-2-dependent murine CTL-L line (26) at 2×10^7 cells per ml for 2 hr at 37°C, which reduced its IL-2 content by a factor of 30 (data not shown). FLU-specific B cells were stimulated with either FLU-POL or FLU-Ficoll in the presence of either CTL-L cell-absorbed or unabsorbed EL-BGDF-pik both with or without r-IL-2 at 10 units/ml. The first point to note from the results obtained with FLU-POL as the antigenic stimulus (Table 5) is that the absorption procedure did not significantly alter the stimulatory capacity of EL-BGDF-pik. Secondly, no evidence of synergy be-

Table 5. Lack of enhancement of BGDF–antigen synergy by r-IL-2

EL-BGDF-pik, % (vol/vol)	IL-2, units/ml	FLU-specific B cells proliferating, %	
		Without r-IL-2	With r-IL-2
Unabsorbed			
5%	0.9	6.27 ± 1.32	6.42 ± 1.74
1%	0.2	3.77 ± 1.08	2.49 ± 1.02
Absorbed			
5%	0.03	6.47 ± 0.88	4.41 ± 1.82
1%	0.006	2.95 ± 0.87	3.39 ± 1.30

FLU-specific B cells were stimulated with FLU-POL at 50 ng/ml in medium containing EL-BGDF-pik as indicated with or without r-IL-2 at 10 units/ml. Values represent the mean ± SEM from three experiments. In the absence of EL-BGDF-pik, proliferation with FLU-POL alone was 0.74 ± 0.20% and with FLU-POL and r-IL-2 was 1.27 ± 0.27%.

tween EL-BGDF-pik and r-IL-2 was noted. Similar findings were observed when FLU-Ficoll was used as the antigenic stimulus, and polyclonal activation by EL-BGDF-pik acting alone without antigen was similarly unaffected (data not shown). With both antigens, antibody formation was not significantly affected either by the absorption of the EL-BGDF-pik or by the addition of r-IL-2 (data not shown). In other words, although IL-2 acting alone has weak BGDF activity, its addition to a specific BGDF source could not augment, nor its removal diminish, the total bioactivity observed.

DISCUSSION

The results presented provide unequivocal evidence that IL-2, a defined T-cell growth factor, is able to act directly on murine B cells as demonstrated by its ability to synergize with specific antigen to trigger single, isolated antigen-specific B lymphocytes to proliferate to form clones, many of which contain antibody-forming cells. The question of a role for IL-2 in B-cell activation and differentiation has been addressed by others, some workers favoring a role for IL-2 (acting in synergy with other factors) (7, 12–16), while others dispute its involvement (8, 17). These earlier studies were performed with nonclonal assay systems, allowing possible contributions by contaminating non-B-cells within the enriched B-cell population, and with purified IL-2 sources, which possibly could contain some other B cell-active factors. In the present studies, we have circumvented the difficulties inherent in previous attempts to seek a role for IL-2 acting directly on B cells. First, a single antigen-specific B cell was used as the unequivocal target cell of the added lymphokine. Second, the source of lymphokine, namely r-IL-2, was prepared by recombinant DNA technology, thus eliminating the possibility of detected activity being a consequence of traces of contaminating B cell-active factors, as would be the case with IL-2 purified from culture supernatants. Thus, despite the disadvantage of using a human lymphokine to act on murine cells, a genuine though modest stimulatory role for IL-2 acting on B cells was confirmed.

Dose–response studies (Tables 1 and 3) showed that only minor increments in bioactivity could be achieved by increasing the r-IL-2 concentration beyond the optimum for T-cell stimulation. This is interesting because, if the receptors on the B-lymphocyte surface capable of binding IL-2 were really designed to recognize a different ("cross-reactive") lymphokine, one might have anticipated that raising the ligand concentration might have increased the degree of its binding and, thus, the bioeffect. The results suggest that B cells possess authentic receptors for IL-2, though perhaps in smaller numbers than do T cells.

The failure of IL-2 to augment the response to optimal concentrations of EL-BGDF-pik rendered IL-2-free through absorption requires comment. First, this indicates that responsiveness to IL-2 is not due to some minor B-cell subset not possessing BGDF receptors. Second, it suggests that a B cell may be activated by alternative pathways, the "BGDF receptor(s)" and the IL-2 receptors, with the same end result. In that case, the lower response to IL-2 may simply indicate that some B cells that possess BGDF receptors lack IL-2 receptors. This point must remain speculative until B-cell stimulatory factors are available in pure form. The results do show, however, that B cells possess receptors for more than one stimulatory lymphokine.

The present studies differ in one important respect from our previous work in the filler cell-free system (1, 22, 23, 25). A substantial degree of cell division and a lesser but nevertheless not negligible degree of antibody formation was initiated by EL-BGDF-pik acting alone in the absence of antigen. This was not batch-dependent and, indeed, was noted with the same batch that had been found previously to lack this capacity. We have considered whether the results could be due simply to higher cloning efficiencies due to progressively greater experience with this demanding tissue culture system. This seems unlikely, as the polyclonal-activating effects are noted even in experiments where the overall cloning efficiency is low. Our working hypothesis is that there has been a change in the mouse population itself, with perhaps a greater degree of *in vivo* environmental priming of B cells. If this is so, our mice now may be more akin to those used by other investigators, who note extensive polyclonal activation of B cells in dense culture by various lymphokines (4, 6, 7, 29).

The availability of a batch of FLU-Ficoll that is entirely dependent on lymphokines (Table 2) for its stimulatory capacity is a useful development. The marked variation in behavior of different FLU-Ficoll batches prepared empirically and not size-fractionated is consistent with the work of Dinztis *et al.* (30), suggesting that "T-cell independent" antigens must possess a certain minimum critical number of epitopes per molecule before stimulating the B cell.

IL-2, like other B cell-active factors, acting in concert with antigen encouraged both growth and differentiation in this system. We shall report elsewhere on the results we have obtained on various "growth" and "differentiation" factors sent to us in highly purified form from different laboratories for assessment of their effects on single cells. Suffice it to say that we have not yet identified any factor active on B cells that supports growth without differentiation or vice versa. Therefore, the currently favored paradigm, which sees factors acting in sequence—some promoting growth and others differentiation, will require modification. This question will only be answered when the BGDF are cloned and sequenced. Similarly, the question as to whether two distinct sets of B cell-active lymphokines exist, namely growth-promoting as distinct from differentiation-promoting, will not be unequivocally answered. The data presented clearly show that B-cell activation into terminal differentiation can be influenced by at least two separate sets of lymphokines.

The excellent technical assistance of Maureen Zanoni and Mandy Ludford is gratefully acknowledged. This work was supported by the National Health and Medical Research Council, Canberra, Australia; by Grant AI-03958 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service; and by the generosity of a number of private donors to The Walter and Eliza Hall Institute.

1. Pike, B. L., Vaux, D. L., Clark-Lewis, I., Schrader, J. W. & Nossal, G. J. V. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6350–6354.
2. Swain, S. L., Wetzel, G. D., Saubiran, P. & Dutton, R. W. (1982) *Immunol. Rev.* **63**, 111–128.
3. Howard, M., Farrar, J., Hilfiker, M., Johnson, B., Takatsu, K., Hamaoka, T. & Paul, W. E. (1982) *J. Exp. Med.* **155**, 914–923.
4. Takatsu, K., Tanaka, K., Tominaga, A., Kumahara, Y. & Hamaoka, T. (1980) *J. Immunol.* **125**, 2646–2653.
5. Marrack, P., Graham, S. D., Kushnir, E., Leibson, H. J., Boehm, N. & Kappler, J. W. (1982) *Immunol. Rev.* **63**, 33–49.
6. Pure, E., Isakson, P. C., Takatsu, K., Hamada, T., Swain, S. L., Dutton, R. W., Dennert, G., Uhr, J. H. & Vitetta, E. S. (1981) *J. Immunol.* **127**, 1953–1958.
7. Farrar, J. J., Benjamin, W. R., Hilfiker, M., Howard, M., Farrar, W. L. & Fuller-Farrar, J. (1982) *Immunol. Rev.* **63**, 129–166.
8. Kishimoto, T., Yoshizaki, K., Kimoto, M., Okada, M., Kuritani, T., Kikutani, H., Shimizo, K., Nakagawa, T., Nakagawa, N., Miki, Y., Kishi, H., Fukunaga, K., Yoshibuko, T. & Taga, T. (1984) *Immunol. Rev.* **78**, 97–118.
9. Lernhart, W., Corbel, C., Wall, R. & Melchers, F. (1982) *Nature (London)* **300**, 355–357.

10. Farrar, J. J., Howard, M., Fuller-Farrar, J. & Paul, W. E. (1983) *J. Immunol.* **131,** 1838–1842.
11. Leanderson, T., Lundgren, E., Ruuth, E., Borg, H., Persson, H. & Coutinho, A. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7455–7459.
12. Watson, J., Gillis, S., Marbrook, J., Mochizuki, D. & Smith, K. A. (1979) *J. Exp. Med.* **150,** 849–861.
13. Swain, S., Dennert, G., Warner, J. & Dutton, R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2517–2521.
14. Leibson, H., Marrack, P. & Kappler, J. (1981) *J. Exp. Med.* **154,** 1681–1693.
15. Parker, D. C. (1982) *J. Immunol.* **129,** 469–474.
16. Roehm, N. W., Marrack, P. & Kappler, J. W. (1983) *J. Exp. Med.* **158,** 317–333.
17. Pure, E., Isakson, P. C., Paetkall, V., Caplan, B., Vitetta, E. S. & Krammer, P. H. (1982) *J. Immunol.* **129,** 2420–2425.
18. Taniguchi, T., Matsui, H., Fujita, T., Takaoka, C., Kashima, N., Yohimoto, R. & Hamuro, J. (1983) *Nature (London)* **302,** 305–310.
19. Rosenberg, S. A., Grimm, E. A., McGrogan, M., Doyle, M., Kawasaki, E., Koths, K. & Mark, D. F. (1984) *Science* **223,** 1412–1415.
20. Wang, A., Lu, S.-D. & Mark, D. F. (1984) *Science* **224,** 1431–1432.
21. Vaux, D. L., Pike, B. L. & Nossal, G. J. V. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 7702–7706.
22. Pike, B. L., Vaux, D. L. & Nossal, G. J. V. (1983) *J. Immunol.* **131,** 544–560.
23. Pike, B. L. & Nossal, G. J. V. (1984) *J. Immunol.* **132,** 1687–1695.
24. Nossal, G. J. V. & Pike, B. L. (1976) *Immunology* **30,** 189–202.
25. Nossal, G. J. V., Pike, B. L. & Battye, F. L. (1978) *Eur. J. Immunol.* **8,** 151–157.
26. Gillis, S., Ferm, M. M., Ou, W. & Smith, K. A. (1978) *J. Immunol.* **120,** 2027–2032.
27. Lefkovits, I. & Waldman, H. (1979) in: *Limiting Dilution Analysis of Cells in the Immune System* (Cambridge Univ. Press, Cambridge, England).
28. Good, M. F., Boyd, A. W. & Nossal, G. J. V. (1983) *J. Immunol.* **130,** 2046–2055.
29. Melchers, F., Andersson, J., Lernhardt, W. & Schreier, M. H. (1980) *Eur. J. Immunol.* **10,** 679–685.
30. Dinztis, R. Z., Middleton, M. H. & Dinztis, H. M. (1983) *J. Immunol.* **131,** 2196–2203.