RECOMBINANT INTERLEUKIN 2 OR 5, BUT NOT 3 OR 4, INDUCES MATURATION OF RESTING MOUSE B LYMPHOCYTES AND PROPAGATES PROLIFERATION OF ACTIVATED B CELL BLASTS

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Several interleukins have previously been implied in some action on B cell responses (1–17). Two of us (Karasuyama, H., and F. Melchers, submitted for publication) have established mouse cell lines by transfection with a bovine papilloma virus–based expression vector carrying full-length cDNAs encoding either IL-2, IL-3, IL-4, or IL-5 that secrete high quantities of these interleukins. In this paper, we test single interleukins and chosen combinations of them for their activity to mature resting B lymphocytes without proliferation (18) and to propagate proliferation of activated B cell blasts at one of three possible restriction points in the B cell cycle (19).

Materials and Methods

Conditioning of Tissue Culture Media with and Partial Purification by DEAE-Cellulose Chromatography of Mouse Interleukin (mIL)[1]-2, -3, -4, and -5. X63-Ag8-653 plasmacytoma cells transfected with the BMGNeo vector containing either mIL-2, -3, -4, or -5 cDNA were grown in serum-containing medium for 48 h and the culture supernatants were collected as conditioned media. The cells were then washed extensively and, thereafter, incubated for 48 hr at $1-2 \times 10^6$ cells/ml in either medium containing transferrin, BSA, and lipids or in medium with no serum, supplemented with only transferrin in concentrations used in the serum-substituted media (20). The conditioned media resulting from these incubations were collected and used as described in Results. Media with only transferrin conditioned by the transformants containing between 10^2 and 10^3 U/ml of the corresponding interleukins, were dialyzed against distilled water. 1 liter of dialyzed medium was run over a 1×5-cm DEAE-cellulose column (DE52, equilibrated with 10^{-3} M K-phosphate, pH 8) and the adsorbed protein and IL activity was eluted with a linear gradient from 10^{-3} to 0.25 M K-phosphate, pH 8. Fractions were assayed for IL activities as described (Karasuyama, H. and F. Melchers, submitted for publication), and active fractions were pooled and used as partially purified IL preparations. An example of an elution profile for IL-5 from DEAE-cellulose similar to that given by Takatsu et al. (21) is shown in Fig. 1. Upon examination of these partially purified IL preparations in SDS-PAGE, it became evident that they were still contaminated by transferrin and by other proteins. They are, therefore, considered to be only partially purified.
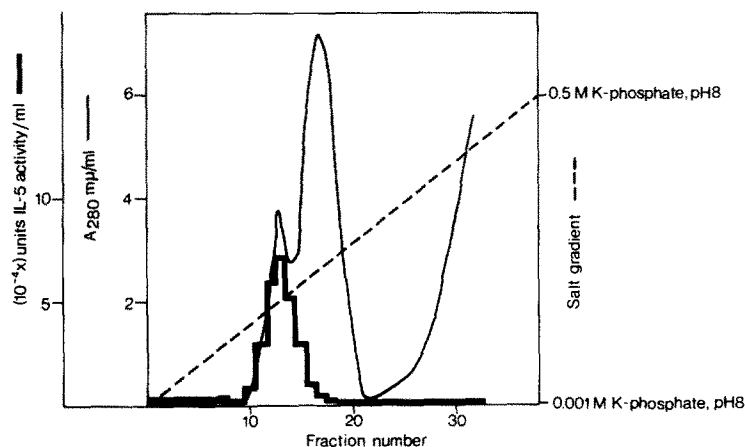
FIGURE 1.   Concentration and partial purification of IL-5 from IL-5-containing conditioned medium by ion-exchange chromatography on DEAE-cellulose. 1 liter of IL-5-containing medium, dialyzed against water, was run over 1 × 5-cm DEAE-cellulose column equilibrated to 10^{-3} M K-phosphate, pH 8. The adsorbed protein and IL-5 activity was eluted with a linear gradient from 10^{-3} M to 0.25 M K-phosphate, pH 8.0. IL-5 activity was assayed as described in this paper by maturation without proliferation of small, resting B cells (see Materials and Methods).

In Vitro Culture of Resting and Activated B Cells..   Spleen cells of C57BL/6 *nu/nu* mice (6–8 wk old, either from the Institut für Biologisch-Medizinische Forschung AG, Füllinsdorf, Switzerland; or from G. Bomholtgaard, Ry, Denmark) were first purified for small resting cells by velocity sedimentation (22). The resting cells were cultured at 5 × 10^5 cells/ml with IL preparations as specified in Results. Supernatants containing the anti-IL-2-R mAbs PC 61 (23) and 7D4 (24) were added to some of these cultures. The development of Ig-secreting cells was monitored by the protein A plaque-forming cell (PFC) assay (25), and their potential proliferation was monitored by uptake of radioactive thymidine. [^{3}H]Thymidine uptake was determined at day 2 of culture by a 4-h pulse with 1 µCi per culture of a [^{3}H]thymidine solution with 2 Ci/mmol (The Radiochemical Centre, Amersham, United Kingdom). For limiting dilution analysis, small resting C57BL/6 *nu/nu* spleen cells were cultured in the presence of 3 × 10^6/ml rat thymocytes as fillers (31) and 10% IL-5-conditioned medium. The development of IgM-secreting cells was monitored on day 5 of culture.

B cell blasts were generated from resting B cells by a 2-d stimulation with LPS (25 µg/ml, S-form, kindly given to us by Dr. O. Lüderitz and Ch. Galanos, Max-Planck-Institut für Immunbiologie, Freiburg, Federal Republic of Germany) and 10% B cell growth factor α (BCGF-α)-conditioned medium. BCGF-α-conditioned medium was prepared by stimulation of confluent P388DI macrophage line cells for 48 h with 2 µg/ml LPS (26). The resulting B cell blasts were removed from the tissue culture bottles without shaking, allowing for additional accessory (A) cell depletion due to the sticking of A cells to plastic surfaces. The cells were then washed twice at room temperature with warm tissue culture medium, sedimented under earth gravity, and blast cells were collected for reculture (19). Blasts were recultured at ~5 × 10^4 cells/ml. Throughout the in vitro experiments, serum-substituted tissue culture medium (20) was used. Cell concentrations in culture were determined by counting with the aid of a hemocytometer (Bürker, Rieger, Basil, Switzerland). A rat mAb with specificity for µ heavy chains, Ak8, (27) was coupled to Sepharose 4B at 2.5 mg/ml under conditions described previously (28). The beads were used at 0.3–0.5% in culture. βBCGF-containing material was a G100-Sephadex gel chromatography fraction of an (NH₄)₂SO₄-precipitated medium conditioned by Con A–activated rat spleen cells depleted of BCGF-α activity, described previously (19). It was used at 2% in culture.

Results

B Cell Maturation Activity of IL-2, -3, -4, or -5. Resting B lymphocytes appear to have the capacity to mature to Ig-secreting cells even in the absence of proliferation when they are exposed to factors that are secreted by helper T cells and A cells in their Ag-specific, MHC-restricted interactions (18, 29). These factors were termed B cell maturation factors (BMF). The nonreplicating, blast-like, Ig-secreting cells lost their capacity to be induced to proliferate by polyclonal activators, such as LPS, as they matured. Full maturation to IgM-, IgG-, and IgA-secreting cells was observed within 5 d of culture.

To test whether a single IL could induce this maturation without proliferation, we exposed resting B cells to the IL-conditioned media as well as to IL preparations that were partially purified by DEAE-cellulose chromatography as described in Materials and Methods. The results shown in Fig. 2 indicate that IL-3 and IL-4 are inactive as BMF, while IL-2 and IL-5 are active. No synergism was observed for these activities when all possible combinations of two, three, and four different ILs at 10^4-fold different ranges of concentrations were tested (data not shown). The highest concentrations tested were 30% of the conditioned media and 10 μg/ml of partially purified preparations of the four ILs. IL-5, however, was at least 10^2 times more active as BMF than IL-2, assuming that the two IL preparations tested, either as conditioned media or as partially purified protein preparations, contained approximately the same quantities of IL molecules. These results suggest that IL-5 is the major BMF, inducing maturation of resting B cells without proliferation. A more stringent test for the induction of small resting B cells to maturation without proliferation is shown below.
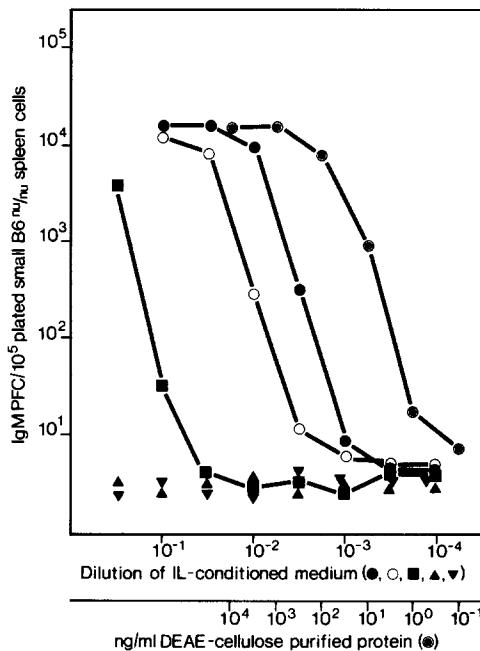

FIGURE 2. Maturation without proliferation to IgM-PFC of small resting C57BL/6J *nu/nu* splenic B cells in the presence of different concentrations of mIL-2 (■), mIL-3 (▲), mIL-4 (▼), and mIL-5 (○,●,⦿). IgM PFC were assayed at day 5 of culture. Proliferation assayed by uptake of radioactive thymidine was at most 5,000 ^{3}H cpm incorporated by 10^5 plated cells in any of the cultures, compared with 75,000 ^{3}H cpm incorporated by the same number of LPS-stimulated cells, all at day 2 of culture. Media were conditioned in serum-substituted medium for 24–48 h by X63-Ag8-653 transformants (1–2 × 10^6/ml) carrying single mIL-cDNA genes (■,▲,▼,●). Conditioning was also done in serum-free medium containing only transferrin (○), from which mIL-5 was partially purified by DEAE-cellulose chromatography (⦿) as described in Materials and Methods.

TABLE I

*Anti-IL-2-R Antibodies Block IL-2-induced, but not IL-5-induced,
Maturation of Small Resting C57BL/6J nu/nu Splenic B Cells*

Antibodies added to cultures	Maturation of small resting B cells to IgM-PFC induced by:	
	IL-2*	IL-5[‡]
None	355	280
Anti-IL-2-R[§]	32	485

* 10^2 U/ml. The number of IgM-PFC was determined at day 5 of culture.
[‡] Conditions as in Fig. 4.
[§] A 10% mix of mAbs PC61 and 7D4.

mAbs PC61 and 7D4 with specificity for the murine IL-2-R inhibited the development of IgM PFC induced by IL-2, but failed to do so in the PFC development induced by IL-5 (Table I). This suggests that induction of maturation without proliferation by the two ILs is mediated by different receptors, and that IL-2 uses the IL-2-R on B cells to do so.

B Cell Proliferation-Propagating Activity of IL-2, -3, -4 or -5. When B cells are excited from their resting state, they become susceptible to the synergistic proliferation-propagating activity of the two types of B cell growth factors, called BCGF-α and BCGF-β (19). It was previously shown that Ig-specific antibodies insolubilized on Sepharose beads (28) act early in the G_1 phase of every successive cell cycle to allow B cell blasts to become reactive to BCGF-α (19). BCGF-α acts late in the G_1 phase and thereby controls the entry into S phase and G_2 phase. At this point, BCGF-β becomes active to stimulate B cell blasts into mitosis. The cell cycle of activated B cells is, therefore, controlled by three restriction points. This dissection of the cell cycle has provided assay systems that allow the characterization of a given IL as a given B cell proliferation-propagating activity.

Media conditioned by cells producing one of the four ILs and the partially purified preparations of these ILs obtained by DEAE-cellulose chromatography were, therefore, tested for activities replacing either Ig-specific antibodies, BCGF-α or BCGF-β in B cell cycle control. Data in Table II show that neither IL-2, -3, -4, nor -5 can replace either Ig-specific antibodies or BCGF-α in their synergistic proliferation-propagating activities with activated B cell blasts. IL-3 and IL-4, in fact, do not show any B cell proliferation-propagating activities, as they are also inactive in replacing BCGF-β. IL-2 and IL-5, on the other hand, can both replace BCGF-β. It is evident from the data in Fig. 3 that both IL-2 and IL-5 have BCGF-β activities that are comparable; i.e., act at the same low concentrations. Here, we assume again that the conditioned media (as well as the partially purified preparations) contain the same amounts of IL-2 and IL-5. When data presented in Fig. 2 are compared with those in Fig. 3, it becomes evident that half-maximal BCGF-β activity of both IL-2 and IL-5 was obtained at concentrations at which half-maxmial BMF activity of IL-5 was observed. No significant synergism was observed for B cell proliferation-promoting activities of IL-2 or IL-5 when all possible combinations of two, three, and four different

TABLE II

Proliferation of LPS-activated, Purified C57BL/6J nu/nu Splenic B Cell Blasts

Factors further added to culture[‡]	Factors originally in culture[*]						
	None	α	β	Anti-Ig	Anti-Ig + α	Anti-Ig + β	Anti-Ig + α + β
None	7	8	11	10	13	15	<u>75</u>
IL-2	8	9	12	15	<u>85</u>	18	ND
IL-3	8	9	10	10	<u>15</u>	15	ND
IL-4	8	7	10	9	14	16	ND
IL-5	9	9	12	14	<u>80</u>	18	ND
LPS	14	<u>68</u>	20	ND	ND	ND	ND

5×10^4 velocity sedimentation-purified C57BL/6J *nu/nu* LPS-activated B cell blasts per milliliter cultured and counted on day 3 of restimulation as described in Materials and Methods. Positive responses are underlined.

[*] α, BCGF-α; β, BCGF-β; anti-Ig, Ig-specific mAb Ak8 coupled to Sepharose. Numbers in table must be multiplied by 10^4 to obtain the number of cells per milliliter in culture. For details, see Materials and Methods.

[‡] 1% of media conditioned by the appropriate transformants, as described in Materials and Methods.

ILs were tested at 10^4-fold different ranges of concentrations (data not shown).

Filler Effects of mIL-2, -3, -4, or -5 in In Vitro Cultures of Proliferating and Maturing B Cells. The efficiency of cells initiating proliferation in cultures of polyclonally stimulated murine splenic B lymphocytes is cell density dependent (30). Below 10^5 cells/ml, this efficiency starts to drop, so that below 10^4 cells/ml LPS-

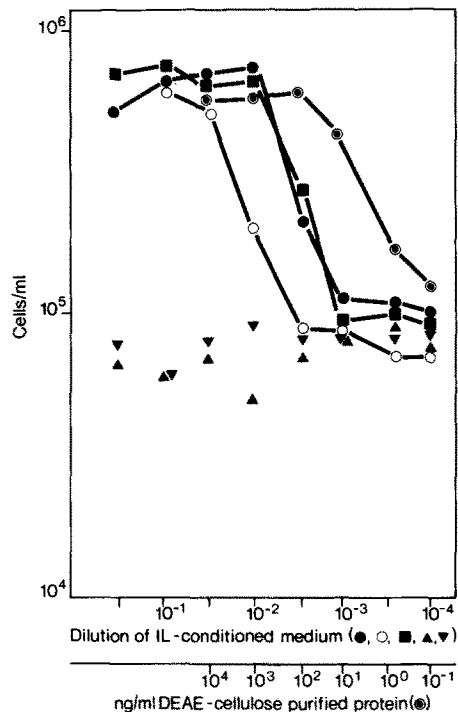FIGURE 3. Proliferation of LPS-activated B cell blasts in the presence of Ig-specific antibodies plus αBCGF and different concentrations of mIL-2 (■), mIL-3 (▲), mIL-4 (▼), and mIL-5 (●,○,⊙). Preparations of the mILs were those described in Fig. 2. Cells were counted after 65 h of culture with the aid of a hemocytometer (Bürker).

or anti-Ig–induced proliferation can no longer be observed. The drop in efficiency is measurable as the ratio of uptake of radioactive thymidine by proliferating B cells over the number of B cells plated (Fig. 4 a). The efficiency of cells initiating polyclonal proliferation upon suitable stimulation is drastically increased to near 100% by the addition of "filler" cells, such as rat thymus cells (31). This has allowed the growth and maturation of single, mitogen-stimulated B cell clones in vitro.

Media conditioned by cells producing one of the four ILs, as well as the partially purified preparations of these ILs, were tested for their capacity not only to synergize in cell cycle control of activated B cells, but also to act as filler activities in polyclonally stimulated B cell cultures. Data in Fig. 4 a show that neither IL-3 nor IL-4 had such activities with either LPS- or anti-Ig–stimulated splenic B cells. In fact, none of the ILs were able to act in this filler capacity in LPS-stimulated B cell cultures. IL-2, as well as IL-5, however, did so, measurable down to 50–100 cells per cultures in anti-Ig–stimulated B cell cultures. We
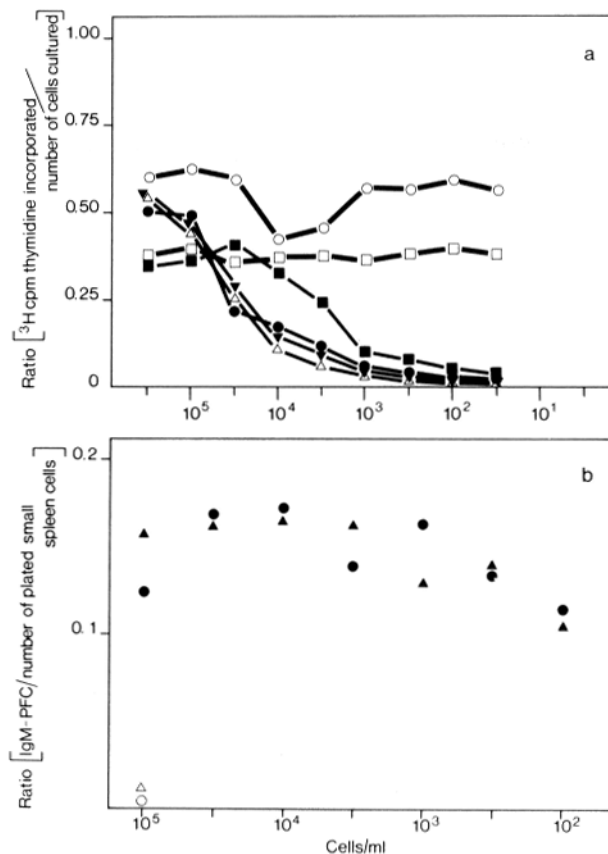

FIGURE 4. (a) Filler effects of mIL-2 and mIL-5 in cultures of B cells stimulated to proliferation in the presence of Ig-specific antibodies and BCGF-α. mIL-2 (□,■), mIL-3, mIL-4 (△,▼; data for IL-3 was indistinguishable from those with IL-4), and mIL-5 (○,●) were contained in serum-substituted media (20) conditioned by X63-Ag8-653 plasmacytoma cells transfected with BMGNeo vectors containing the appropriate mIL-cDNA. They were used at 1% in culture. 200-μl cultures containing serial dilutions of C57BL/6J nu/nu small resting B cells were stimulated with either LPS + BCGF-α (●,■,▼) or Ig-specific antibodies plus αBCGF (○,□,△), and uptake of radioactive thymidine into stimulated cells were determined as described in Materials and Methods. (b) Induction of IgM PFC assayed at day 5 of culture in 200 μl of serum-substituted medium by IL-5 (1% of a conditioned medium [see a]) alone (●) or by IL-5 in the presence of rat thymus cells (5 × 10⁶/ml, ▲) of small spleen cells of C57BL/6J nu/nu mice. Controls are in medium alone (○) or medium containing rat thymus cells (△), both with the highest concentration of small spleen cells cultured. Lewis-strain rats (4–6 wk old) were obtained from the Institut für Biologisch-Medizinische Forschung AG.

conclude from these results that IL-2 and IL-5 will not only act as BCGF-β activity in anti-Ig–stimulated B cells, but also serve as filler activity, which enhances the efficiency of plating of anti-Ig–stimulated B cells by a factor of $>10^2$.

We also tested the capacity of IL-5 to induce small splenic B cells to maturation without proliferation at concentrations of 5×10^5 cells/ml or below in the presence or absence of thymus filler cells. Data in Fig. 4 *b* show that IL-5 induced a constant ratio of IgM PFC over plated small cells in the presence, as well as in the absence, of rat filler cells, measurable down to concentrations of 5×10^2 cells/ml, while no maturation was observed without IL-5 at any cell concentration tested. In these measurements it should, however, be realized that the lower the number of cells assayed, the higher the potential error in the determination of thymidine uptake and in the number of PFC. Thus, 100 cells yield an average of only 50 cpm, respectively, 15 PFC over background. In conclusion, these results indicate that the "filler" effect of rat thymus cells can also be observed when small B cells mature to secreting cells without proliferation, and that this high efficiency of B cell maturation is also observed with IL-5 alone; i.e., in the absence of filler cells.

The Frequency of Resting B Cells that Mature without Proliferation. From the data presented so far, is is not proven that single resting B cells induced by IL-2 or IL-5 mature without division. The residual uptake of radioactive thymidine observed in these assays ($\sim$10% of that induced by the polyclonal activator LPS at day 2 of stimulation) could indicate that a minor population within our preparation of resting B cells could proliferate and mature to IgM-PFC, giving rise within the 5 d of culture to single clones with $\sim$30 cells, all of which might divide and secrete IgM as larger numbers of resting B cells do when stimulated with LPS (30). Maturation without proliferation of larger numbers of resting B cells, on the other hand, would lead to a clone size of one IgM PFC from one
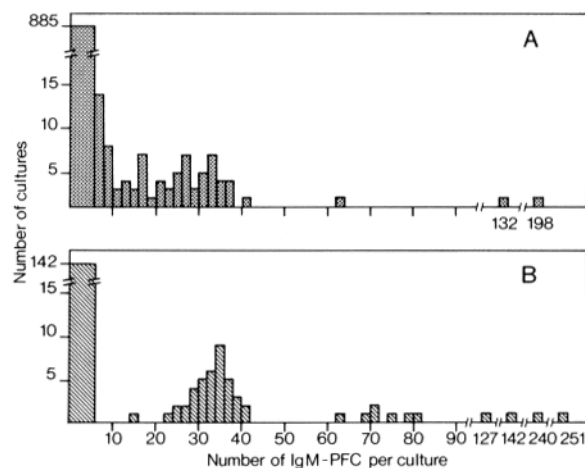

FIGURE 5. Limiting dilution analysis of resting small C57BL/6J nu/nu splenic B cells to respond to IL-5 (A) and to LPS (B) by proliferation and maturation to IgM PFC. (A) Cultures of 0.2 ml contained 50 small resting C57BL/6J nu/nu spleen cells, 6×10^5 rat thymocytes and 10% IL-5-conditioned medium with $>5 \times 10^3$ U. IgM PFC were determined on day 5 of culture. Cultures with 5 IgM PFC are plotted. Frequency of precursors induced by IL-5 giving clone sizes of IgM PFC similar to those induced by LPS (B) as predicted by Poisson's distribution is 1 in 1,500–3,000. (B) Cultures of 0.2 ml contained 3 small resting C57BL/6J nu/nu spleen cells, 6×10^5 rat thymocytes, and 50 µg/ml LPS. IgM PFC were determined on day 5 of culture. Frequency of precursors induced by LPS forming IgM PFC predicted by Poisson's distribution is 1 in 10.

TABLE III

Limiting Dilution Analysis of the Number of Resting B Cells Responding to IL-5 by Maturation without Proliferation to IgM PFC

Numbers of IgM PFC per culture*	Number of cultures	Percent of total cultures	Frequency of precursors forming IgM PFC predicted by Poisson's distribution	Frequency of B cells[‡]
0	492	51.3	(1/70)	1/50
1	187	19.5	(1/200)	1/130
2	102	10.6	(1/70)	1/50
3	56	5.8	(1/50)	1/35
4	32	3.3	(1/40)	1/27
5	16	1.7	(1/25)	1/18
5[§]	74	7.6	See text	$1.5-3 \times 10^3$

* Cultures of 0.2 ml contained 50 small resting C57BL/6J nu/nu spleen cells, 6×10^6 rat thymocytes, and 10% IL-5-conditioned medium with 5×10^3 U/ml, all in serum-substituted media (20). Assays for IgM PFC were done with the protein A plaque assay on day 5 of culture (25).

§ The clone sizes of cultures with >5 IgM PFC are shown in Fig. 5.

‡ Calculated on the basis that ∼ 70% of all resting small cells of C57BL/6J nu/nu were found to be surface Ig^+.

precursor. These two alternate possibilities can be tested experimentally in limiting dilution analyses. Since limiting dilution analyses must be done under tissue culture conditions where the efficiency of plating of precursors is near 100% (31), rat filler cells must be used in the cultures. This then limited the analysis to IL-5 induction of resting B cells since IL-2 induced strong proliferation of rat thymus cells while IL-5 appeared to have no effect on them. The results of a limiting dilution analysis of resting B cells exposed to IL-5 are shown in Fig. 5 and Table III. They distinguish two populations of resting B cells responsive to IL-5. One shows a clone size of one IgM-PFC; these B cells, therefore, mature without division. Their frequency in the resting B cell population of C57BL/6 nu/nu mice is between 1 in 50 and 1 in 100. The same population of resting B cells, in control limiting dilutions with LPS, was shown to contain ∼1 in 10 LPS-reactive B cells (Table III, Fig. 5).

A second population of B cells proliferate and mature to clones of IgM-secreting cells, with clone sizes of IgM-PFC at day 5 of assay that are near those observed in LPS-stimulated single B cell clones (Fig. 5). The frequency of this second population of cells is 15–60 times lower than the first, which matures without proliferation; i.e., 1 in ∼1,500–3,000 resting B cells. These results also indicate that approximately half of the IgM PFC observed in mass cultures of resting B cells are likely to derive from proliferating B cells (compare total numbers of IgM PFC developed in 5 d by the two populations, as shown in Table III and Fig. 5). We conclude that ∼30% of all resting B cells are LPS reactive, ∼1–2% of them mature without division, and ∼0.03–0.06% proliferate and mature in the presence of IL-5.

Discussion

High expression of single ILs in established murine cell lines (Karasuyama, H. and F. Melchers, submitted for publication) has now allowed a reinvestigation

of the role of these molecules in responses of normal resting and activated B cells that, so far, had only been studied with molecules within mixtures with other activities and, in the best cases, with only partially purified preparations. While our preparations of single transformant-made ILs are also only partially pure, they are, at least, free of other known ILs.

As expected, single rmILs had the biological activities in tests that had been used to clone the IL genes and express them as active proteins. Thus, mIL-2 stimulated the CTL-L line, mIL-3 stimulated the IC-2 line, while mIL-4 showed several different biological activities. It induced uptake of radioactive thymidine in a T cell line, a mast cell progenitor line, and a B cell leukemia line, and stimulated the development of IgG1-secreting cells, while inhibiting that of IgG3-secreting cells. It is, therefore, all the more remarkable that only two of the four rILs showed activities in our assays of normal B cell growth and maturation, particularly since these ILs have all been implicated in some activities on cells of the B lineage (1–17, 32, 33). IL-2 has been found to be a growth as well as maturation factor for murine as well as human B cells (4–6, 32, 33). IL-4 (formerly BSF-1) has been found to induce increased expression of MHC class II molecules on B cells, to synergize with Ig-specific antibodies in the stimulation of resting B cells, to induce specific switching to IgG1 and IgE, and to stimulate pre B cell growth (8–13, 16) (and thymocyte growth [34]). IL-5 has been found to serve as a late-acting maturation factor not unlike TRF (14, 15, 17), and to stimulate proliferation as well as maturation of B cells.

The definition of what is a lymphokine for B lymphocytes depends on the assay used. When we measure the effects on activated B cells, we monitor only proliferation and, therefore, restrict ourselves to the biological activity of ILs to promote the progression of B cells through the cell cycle. We monitor this activity by counting cells. We also monitor maturation, but only as the development of large numbers of PFC, and only induced in small resting cells. We rely in both assays on a polyclonal response of a large portion of all B cells. We think that the LPS-stimulated B blasts belong to the newly formed, short-lived, extra-follicular, primary B cell subset, while we do not know whether the B cells induced to maturation without proliferation belong to the same subset. Although we have also done the experiments that are reported in this paper with nu/nu spleen cells and with T cell–depleted and A cell-depleted splenic B cells of normal mice, and have obtained essentially the same results, we cannot exclude that the mILs act on a contaminating "non-B" cell population which, in turn, then acts on resting and/or activated B cells in our assays. Such an indirect action of mILs is, however, also not ruled out in the other assays quoted above.

Responses of other LPS-nonreactive B cell populations may go undetected in our assays of restimulation of LPS-activated B cell blasts in which we monitor the responses of ∼10% of all B cells. We assay a 10-fold lower number of B cells; i.e., 1–2% of all resting B cells, as they mature without proliferation to PFC, although we do not know whether these 1–2% of all B cells are the same as the 10% LPS-reactive B cells. Some of the apparent discrepancies may not only be based on a different readout of the assays, but also be a consequence of the conditions under which the cells are purified and cultured. We use serum-substituted cultures and try to enrich B cells as much as possible in an effort to

reduce influences of contaminating cells and factors. Along these lines, high-rate secretion of single mILs into serum-substituted media, which then can be diluted much more than the traditional conditioned media, further reduces the possible influences of unknown contaminations by other activities produced by the X63-Ag8-653 plasmacytoma cells that secrete the rmILs.

The ideal assay system with which to understand the mechanisms and kinetics of synergistic B cell control at different restriction points in the cycle (19) would be to use a single B cell in isolation in defined, serum-substituted media, and stimulate this single B cell with pure mAbs, pure rαBCGF, and pure rmILs. With the results presented in this paper, we have advanced considerably towards this goal. Not only does it appear possible to purify large quantities of mILs to homogeneity, the surprising "filler" activity of mIL-2 and mIL-5 may make it possible to culture a single B cell in a larger volume of serum-substituted medium. Experiments along these lines are now in progress.

It is already clear that none of the mILs, either alone or in combinations, can replace the actions of either anti-Ig antibodies or αBCGF at their respective restriction points in the cell cycle of an activated B cell. IL-2 and IL-5 both act as βBCGF at similarly low concentrations that are, in fact, as low as the action of mIL-2 on the growth of CTL-L cells. This indicates that the interaction of mIL-2 and mIL-5 with corresponding receptors on activated B cells may be of high affinity, estimated for T cells to be in the range of 0.01 nM (35). The structures of mIL-2 and mIL-5 are sufficiently different to suggest that these two ligands may have, at least in part, different receptors. Purified preparations, which we soon hope to have in hand, should then enable us to do receptor-binding studies to clarify these problems.

While both mIL-2 and mIL-5 are active as BCGF-β, they also are BMFs. IL-2, however, is much weaker than IL-5, which is as active in its BMF activity as in its BCGF-β activity (i.e., maybe at 0.01 nM). Action of the IL-2-R–specific mAbs PC61 and 7D4 indicates that mIL-2 and mIL-5 act through different receptors. The difference in effective concentrations of IL-2 on resting and on activated B cells could suggest that the receptor for mIL-2 on resting cells is in a different state, or maybe a different structure, than that on activated B cells.

The limiting dilution analyses performed in this paper indicate that one resting B cell can mature without proliferation to an IgM PFC. It has been previously shown (18) that this maturation takes as long; i.e., 5 d of culture, as the maturation to IgM PFC, which occurs in cultures of polyclonally stimulated proliferating cells and is optimal at day 5. We, therefore, think that the frequencies determined in Table III will not decrease when the assay is done at earlier or later times of culture. Frequencies of IL-5–responsive B cells calculated with Poisson's distribution from the number of cultures with 0, 1, 2, 3, 4, and 5 IgM PFC in Table III are not exactly the same for all values of IgM PFC and, in fact, appear to increase with their value. The frequencies calculated from the percentage value for one IgM PFC per culture may be lower than expected since the observation of one PFC is particularly difficult. The frequency increase with increasing values for IgM PFC could be explained if a minor population of resting B cells divided once or twice when responding to IL-5. Altogether a value of 1 in 50 resting B cells responding to IL-5 by maturation without proliferation

appears to be a reasonable quantitative estimate from our experimental data. We have no information whether the IL-5–responsive population of resting B cells (1–2% of total) is a separate subpopulation and different from that reactive to LPS or to Ag. It is certainly different from the population of B cells proliferating and maturing in response to IL-5. If occupance of Ig is mandatory for proliferation of B cells (19), it must be asked what occupies surface Igs of the IL-5–responsive proliferating B cells. One candidate is Ag in culture, notably the surface molecules of the rat filler cells, which also are likely to provide α-type BCGF. It is also conceivable that this minor population of B cells represents a yet unknown subpopulation that does not follow the rules of B cell growth control operating with LPS-reactive virgin IgM^+ B cells.

A single mIL, IL-5, can induce both maturation and cell cycle progression through mitosis, i.e., proliferation. This is in agreement with results obtained with purified IL-5 by Takatsu et al. (21) and appears to settle the question as to whether one or several factors are involved in these different reactions of a B cell (19, 36). Final proof will come from a single-cell experiment that now appears experimentally feasible even in the absence of filler cells, since IL-5 also acts as a filler activity in maturation assays of B cells (Fig. 4 *b*).

Since mIL-5 acts with the same apparent high avidity in both reactions, it may well do so through the same receptor. It is reasonable to expect that it is the relative position of a B cell in the cell cycle, rather than the nature of the receptor or the ligand, that determines this difference in reaction. A B cell blast in G_2 phase appears to be stimulated by mIL-5 to enter mitosis and end in a phase of G_1 where maturation occurs. It should be mentioned that we have not seen any effect of IFN-γ on B cell maturation in our assay system, which previously has been reported to act synergistically with other helper T cell–derived factors in the induction to maturation (37). A resting G_0 phase B cell appears to be stimulated by mIL-5 to enter a very similar phase of G_1, where maturation occurs. Both types of B cells can be drawn into the mitotic cycle if anti-Ig antibodies excite them via surface Ig, and if BCGF-α then stimulate them to enter S phase (19, 29).

In conclusion, B cells appear to have an option in the G_1 phase of the cell cycle either to mature or to enter the mitotic cycle. This duality of reactions has been observed in LPS-stimulated B cells (30, 38). The option to either mature or enter S phase should also be influenced by relative amounts of mIL-2 vs. mIL-5 available to an activated B cell, where mIL-5 would be more efficient in driving B cells to maturation.

Summary

Plasmacytoma transformants of the X63-Ag8-653 cell line carrying an expression vector with either IL-2, -3, -4, or -5 cDNA were established that secrete the corresponding ILs at high rates. The four mouse ILs (mILs) were then tested as single ILs and in combinations for their effects on the maturation of resting and proliferation of activated normal mouse splenic B cells. mIL-3 and mIL-4 were inactive in all assays. mIL-2, as well as mIL-5, synergized with Ig-specific antibodies and B cell growth factor α (BCGF-α) to stimulate successive rounds of B cell division with LPS-activated B cells. This activity as BCGF-β was effective

at concentrations similar to those at which mIL-2 induced proliferation of the CTL-L T cell line, indicating a high-affinity interaction of both mIL-2 and mIL-5 with their corresponding receptors on activated B cells.

mIL-5 and maybe IL-2 also induced maturation of resting B cells to Ig-secreting cells without proliferation. This B cell maturation factor (BMF) activity of mIL-5 was as effective as its BCGF-β activity, while the BMF activity of mIL-2 was at least 10^2-fold less effective. BMF activity of mIL-2, but not mIL-5, was blocked by anti-IL-2-R antibodies, indicating that mIL-2 and mIL-5 use separate receptors for B cell signaling. mIL-2, as well as mIL-5, furthermore, acted as filler activities when proliferation in the presence of Ig-specific antibodies and BCGF-α was measured with as little as 500 B cells. In the case of mIL-5, this was also true for maturation of that few cells. Limiting dilution analyses showed that $\sim$1–2% of the resting B cells matured without division, while 30–100-fold fewer cells (0.03–0.06%) proliferated and matured in response to IL-5. A single IL, therefore, is capable of inducing maturation and of stimulating mitotic cell cycle progression of normal B cells.

References

1. Howard, M., and W. E. Paul. 1983. Regulation of B-cell growth and differentiation by soluble factors. *Annu. Rev. Immunol.* 1:307.
2. Hoffmann, M. K., S. B. Mizel, and J. A. Hirst. 1984. IL-1 requirement for B cell activation revealed by use of adult serum. *J. Immunol.* 133:1566.
3. Hirano, T., K. Yasukawa, H. Harada, T. Taga, Y. Watanabe, T. Matsuda, S. Kashiwamura, K. Nakajima, K. Koyama, A. Iwamatsu, S. Tsunasawa, F. Sakiyama, H. Matsui, Y. Takahara, T. Taniguchi, and T. Kishimoto. 1986. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature (Lond.).* 324:73.
4. Zubler, R. H., J. W. Lowenthal, F. Erard, N. Hashimoto, R. Devos, and H. R. MacDonald. 1984. Activated B cells express receptors for, and proliferate in response to, pure interleukin 2. *J. Exp. Med.* 160:1170.
5. Pike, B. L., A. Raubitschek, and G. J. V. Nossal. 1984. Human interleukin 2 can promote the growth and differentiation of single hapten-specific B cells in the presence of specific antigen. *Proc. Natl. Acad. Sci. USA.* 81:7917.
6. Tsudo, M., T. Uchiyama, and H. Uchino. 1984. Expression of TAC antigen on activated normal human B cells. *J. Exp. Med.* 160:612.
7. Palacios, R., G. Henson, M. Steinmetz, and J. P. McKearn. 1984. Interleukin-3 supports growth of mouse pre-B-cell clones in vitro. *Nature (Lond.).* 309:126.
8. Howard, M., J. Farrar, M. Hilfiker, B. Johnson, K. Takatsu, T. Hamaoka, and W. E. Paul. 1982. Identification of a T cell–derived B cell growth factor distinct from interleukin 2. *J. Exp. Med.* 155:915.
9. Noelle, R., P. H. Krammer, J. Ohara, J. W. Uhr, and E. S. Vitetta. 1984. Increased expression of Ia antigens on resting B cells: an additional role for B-cell growth factor. *Proc. Natl. Acad. Sci. USA.* 81:6149.

10. Roehm, N. W., J. Liebson, A. Zlotnick, J. Kappler, P. Marrack, and J. C. Cambier. 1984. Interleukin-induced increase in Ia expression by normal mouse B cells. *J. Exp. Med.* 160:679.

11. Vitetta, E. S., K. Brooks, Y. W. Chen, P. Isakson, S. Jones, J. Layton, G. C. Mishra, E. Pure, E. Weiss, C. Wood, D. Yuan, P. Tucker, J. W. Uhr, and P. H. Krammer. 1984. T cell-derived lymphokines that induce IgM and IgG secretion in activated murine B cells. *Immunol Rev.* 78:137.

12. Vitetta, E. S., J. Ohara, C. Myers, J. Layton, P. H. Krammer, and W. E. Paul. 1985. Serological, biochemical, and functional identity of B cell–stimulatory factor 1 and B cell differentiation factor for IgG1. *J. Exp. Med.* 162:1726.

13. Coffman, R. L., J. Ohara, M. W. Bond, J. Carty, E. Zlotnick, and W. E. Paul. 1986. B cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide-activated cells. *J. Immunol.* 36:4538.

14. Nakanishi, K., M. Howard, A. Muraguchi, J. Farrar, K. Takatsu, T. Hamoaka, and W. E. Paul. 1983. Soluble factors involved in B cell differentiation: identification of two distinct T cell-replacing factors (TRF). *J. Immunol.* 130:2219.

15. Harada, N., Y. Kikuchi, A. Tominaga, S. Takaki, and K. Takatsu. 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.

16. Sideras, P., S. Bergstedt-Lindquist, H. R. MacDonald, and E. Severinson. 1985. Secretion of IgG1 induction factor by T cell clones and hybridomas. *Eur. J. Immunol.* 15:586.

17. Swain, S. L., M. Howard, J. Kappler, P. Marrack, J. Watson, R. Booth, G. D. Wetzel, and R. W. Dutton. 1983. Evidence for two distinct classes of murine B cell growth factors with activities in different functional assays. *J. Exp. Med.* 158:822.

18. Melchers, F., J. Andersson, W. Lernhardt, and M. H. Schreier. 1980. H-2-unrestricted, polyclonal maturation without replication of small B cells induced by antigen-activated T cell help factors. *Eur. J. Immunol.* 10:669.

19. Melchers, F., and W. Lernhardt. 1985. Three restriction points in the cell cycle of activated murine B lymphocytes. *Proc. Natl. Acad. Sci. USA.* 82:7681.

20. Iscove, N. N., and F. Melchers. 1978. Complete replacement of serum by albumin, transferrin, iron, and soybean lipid in cultures of lipopolysaccharide-activated B lymphocytes. *J. Exp. Med.* 147:923.

21. Takatsu, K., N. Harada, Y. Hara, Y. Takahama, G. Yamada, K. Dobashi, and T. Hamaoka. 1985. Purification and physicochemical characterization of murine T cell replacing factor (TRF). *J. Immunol.* 134:382.

22. Miller, R. G., and R. A. Phillips. 1969. Separation of cells by velocity sedimentation. *J. Cell. Physiol.* 73:191.

23. Lowenthal, J. W., R. H. Zubler, M. Nabholz, and H. R. MacDonald. 1985. Similarities between interleukin-2 receptor number and affinity on activated B and T lymphocytes. *Nature (Lond.)* 315:669.

24. Malek T. R., R. J. Robb, and E. M. Shevach. 1983. Identification and initial characterization of a rat monoclonal antibody reactive with murine interleukin 2 receptor ligand complex. *Proc. Natl. Acad. Sci. USA.* 80:5694.

25. Gronowicz, E., A. Coutinho, and F. Melchers. 1976. A plaque assay for all cells secreting Ig of a given type or class. *Eur. J. Immunol.* 6:588.

26. Corbel, C., and F. Melchers. 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.

27. Leptin, M., M. J. Potash, M. Grützmann, C. Heusser, M. Schulman, G. Köhler, and F. Melchers. 1984. Monoclonal antibodies specific for murine IgM. I. Character-

ization of antigenic determinants on the four constant domains of the μ heavy chain. *Eur. J. Immunol.* 14:534.

28. Leptin, M. 1985. Monoclonal antibodies specific for murine IgM. II. Activation of B lymphocytes by monoclonal antibodies specific for the four constant domains of IgM. *Eur. J. Immunol.* 15:131.

29. Melchers, F., and J. Andersson. 1984. B cell activation: three steps and their variations. *Cell.* 37:715.

30. Andersson, J., A. Coutinho, W. Lernhardt, and F. Melchers. 1977. Clonal growth and maturation to immunoglobulin secretion in vitro of every growth-inducible B lymphocyte. *Cell.* 10:27.

31. Lernhardt, W., J. Andersson, A. Coutinho, and F. Melchers. 1978. Cloning of murine transformed cell lines in suspension culture with efficiencies near 100%. *Exp. Cell Res.* 111:309.

32. Nakagawa, T., T. Hirano, N. Nakagawa, K. Yoshizaki, and T. Kishimoto. 1985. Effect of recombinant IL-2 and γ-IFN on proliferation and differentiation of human B cells. *J. Immunol.* 134:959.

33. Nakanishi, K., T. R. Malek, K. A. Smith, T. Hamaoka, E. M. Shevach, and W. E. Paul. 1984. Both interleukin 2 and a second T cell–derived factor in EL-4 supernatant have activity as differentiation factors in IgM synthesis. *J. Exp. Med.* 160:1605.

34. Palacios, R., P. Sideras, and H. von Boehmer. 1987. Recombinant interleukin 4/BSF-1 promotes growth and differentiation of intrathymic T cell precursors from fetal mice in vitro. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:91.

35. Riendeau, D., D. G. Harnish, R. C. Bleackley, and V. Paetkau. 1983. Purification of mouse interleukin 2 to apparen homogeneity. *J. Biol. Chem.* 258:12114.

36. Andersson, J., M. H. Schreier, and F. Melchers. 1980. T cell-dependent B cell stimulation is H2-restricted and antigen dependent only at the resting cell level. *Proc. Natl. Acad. Sci. USA.* 77:1612.

37. Sidman, G. L., J. D. Marshall, L. D. Schultz, P. W. Gray, and H. M. Johnson. 1984. Gamma-interferon is one of several direct B cell-maturing lymphokines. *Nature (Lond.).* 309:801.

38. Melchers, F., and J. Andersson. 1973. Synthesis, surface deposition and secretion of immunoglobulin M in bone marrow-derived lymphocytes before and after mitogenic stimulation. *Transplant. Rev.* 14:76.