Growth Regulation of Multi-Factor-Dependent Myeloid Cell Lines: IL-4, TGF- β and Pertussis Toxin Modulate IL-3- or GM-CSF-Induced Growth by Controlling Cell Cycle Length

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ABSTRACT. The stimulatory effects of lymphokines, interleukin 3 (IL-3), granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin 4 (IL-4), and the inhibitory effects of transforming growth factor β (TGF- β) and the pertussis toxin, islet activating protein (IAP), on multi-factor-dependent myeloid cell lines were examined. The effects of IL-3 on a mast cell progenitor clone, IC2 were indistinguishable from those of GM-CSF with respect to their concentration-response curves for induction of DNA synthesis and capability to maintain cell growth for many months. IL-4 acts differently on IC2 cells: the maximum level of DNA synthesis induced by IL-4 is always lower than that induced by IL-3 or GM-CSF and IL-4-induced proliferation is transient. IL-4, however, synergistically induced DNA synthesis of IC2 cells with limiting concentrations of IL-3 or GM-CSF. When IC2 cells were cultured with saturating concentrations of IL-3, GM-CSF or a combination of both, the doubling time was 25 ± 1 h, whereas it decreased to 17 ± 1 h when IL-4 was further added to the cultures. IAP reduced the DNA synthesis of IC2 cells induced by the above three growth factors. The doubling time of IC2 cells was 30 ± 2 h when IC2 cells were cultured with sufficient concentrations of IL-3 in the presence of IAP. Cell cycle analysis revealed that the fraction of cells in G1 was decreased by IL-4 but was increased by IAP. TGF- β also reduced IL-3-dependent DNA synthesis and increased the fraction of cells in G1. The inhibitory effect on IL-3-dependent growth of IC2 cells was not increased when these cells were exposed simultaneously to TGF- β and IAP. These results suggest that IL-3 and GM-CSF stimulate the growth of IC2 cells through similar pathways and that IL-4 augments the action of IL-3 or GM-CSF by decreasing the G1 period. It is also suggested that IAP and TGF- β retard the growth of IC2 cells by increasing the

Abbreviations used: IL-3, interleukin 3; IL-4, interleukin 4; GM-CSF, granulocyte-macrophage colony-stimulating factor; TGF- β , transforming growth factor β ; IAP, islet activating protein; G-protein, GTP-binding protein; NAD, nicotinamide adenine dinucleotide; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

fraction of cells in G1.

IL-3 is a T-cell-derived growth factor which supports the growth and development of hematopoietic cells *in vitro* and generates multi-lineage colonies from bone marrow cells including granulocyte, macrophage, erythroid, megakaryocyte and mast cell colonies (2, 4, 23, 24, 27). A number of cell lines which grow in response to IL-3 have been established and many of them also respond to another hematopoietic growth factor, GM-CSF (3, 8, 28). In addition, recent studies have revealed that IL-4 also affects the growth of IL-3-dependent cell lines (8, 10, 14, 25). In these cell lines, IL-3, GM-CSF and IL-4 each bind to separate cell surface receptors on the same cells (16, 17, 21, 22, 28). An IL-3-dependent cell line, IC2, was established from a spleen of a DBA/2 mouse and has many features in common with mast cells (6–8).

TGF- β is a factor derived from various cell types which stimulates the growth of some cell types, including osteoblasts and Schwann cells, and inhibits that of others, including fibroblasts, epithelial cells and lymphocytes (26). The pertussis toxin, islet-activating protein (IAP), binds to a class of GTP-binding proteins (G-proteins), inhibiting cell growth of some cell lines (11, 15). Even using the same cell lines, the degree of inhibition varies, however, depending upon the growth factors or agents used for stimulation (11, 15).

In this report, we have shown that IL-4 affected IC2 cells differently from IL-3 or GM-CSF. IL-4 shortens the G1 phase of the cell cycle. In contrast, TGF- β and IAP showed inhibitory effects on the growth of IC2 cells. These two agents elongated the G1 phase but did not cause cell death.

MATERIALS AND METHODS

Materials. RPMI 1640 and FCS were obtained from GIBCO, Grand Island, NY, USA; penicillin G and kanamycin were from Meiji Seika Co., Tokyo; HEPES was from Sigma Chemical Co., St. Louis, MO, USA; propidium iodide was from Boehringer Diagnostics, La Jolla, CA, USA; TGF- β was from Collaborative Research, Inc, Lexington, MA, USA; [methyl- ^3H] thymidine and [α - ^{32}P] NAD were from Amersham International, Amersham, United Kingdom. RPMI 1640 was generally supplemented with 10 mM HEPES, 0.2% sodium bicarbonate, 5×10^{-5} M 2ME, 100 units/ml penicillin G and 0.2 mg/ml kanamycin.

Mouse recombinant IL-3 (30) and IL-4 (10) were purified from serum-free culture supernatant fluids of COS-7 cells transfected with expression vectors carrying cDNAs for mouse IL-3 and mouse IL-4, respectively. Mouse recombinant GM-CSF (13) was purified from the culture supernatant fluid of yeast cells transfected with an expression vector carrying the cDNA for mouse GM-CSF as described previously (12). The purity of IL-3 and GM-CSF was more than 95% and that of IL-4 was about 50%. Purification of IAP and activation of its A-protomer was performed as described previously (5, 29).

Cells and growth conditions. Conditioned medium from Con A-stimulated mouse splenocytes was prepared as described previously (6). Multi-factor-dependent mast cell progenitor cell lines, IC2 and LT4, were cultured in RPMI 1640 containing 5% FCS, and 50% conditioned medium (6, 7). DNA synthesis was determined in triplicate samples as follows. Exponentially growing IC2 cells after 2 days of culture were washed extensively with RPMI 1640 containing 10% FCS and suspended in the same medium at 10^5 cells/ml. A hundred μl of the suspension was mixed with 100 μl of the above medium containing various concentra-

tions of growth factors and cultured in 96-well microtiterplates for 21 h in a CO₂ incubator. Then the cells were labeled with 1 μ Ci/well of [³H] thymidine for 3 h.

Miscellaneous. Cell cycle analysis was performed as follows. The cells were washed twice with RPMI 1640 containing 10% FCS and mixed with a solution consisting of 50 μ g/ml propidium iodide, 0.05% NP40, 10 μ g/ml RNase A and 0.1% sodium citrate. Flow cytometry was performed using a FACS IV (Becton Dickinson, Mountain View, CA, USA). Substrate(s) of IAP was analyzed by radiolabeling of membrane proteins with [α -³²P]NAD as described previously (18). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described (9).

RESULTS

Difference between IL-4 and other growth factors in the ability to induce DNA synthesis. IC2 cells lose viability within 24 h when cultured in the absence of growth factors. IL-3, GM-CSF and IL-4 have the ability to induce DNA synthesis of IC2 cells (Fig. 1, ref. 8) and maintain viability of IC2 cells after 24 h cultivation. IL-3 and GM-CSF showed similar concentration dependence curves for induction of DNA synthesis in IC2 cells (Fig. 1A). The ED₅₀ (the concentration of IL-3 or GM-CSF required for 50% stimulation of IC2 cells at 5×10^4 cells/ml) was ca. 5 pM. IL-4 stimulated IC2 cells in a different manner. The maximum level of DNA

Fig. 1. DNA synthesis of IC2 cells induced by three growth factors. (A) IC2 cells (10^4 cells in 0.2 ml) were cultured in RPMI 2640-10% FCS with various concentrations of IL-3 (○) or GM-CSF (●) for 21 h in 96-well microtiter plates, after which DNA synthesis was determined by pulse-labeling with 1 μ Ci/well of [³H] thymidine for 3 h. (B) IC2 cells were cultured for 21 h with various concentrations of IL-4 in RPMI 1640-10% FCS (●) or in the same medium containing 1 nM IL-3 (○) or 1 nM GM-CSF (●), after which DNA synthesis was determined by pulse-labeling with [³H] thymidine for 3 h.

Fig. 2. IL-3 and GM-CSF induce DNA synthesis of IC2 cells in a similar manner. (A) IC2 cells were cultured with various concentrations of IL-3 in RPMI 1640-10% FCS (○) or in the same medium containing 3 pM GM-CSF (●) for 21 h followed by 3 h pulse-labeling with $1 \mu\text{Ci/well}$ of $[^3\text{H}]$ thymidine. (B) Various concentrations of GM-CSF were used for stimulation of IC2 cells in the absence (○) or presence of 3 pM IL-3 (●).

synthesis induced by IL-4 alone was always lower than that induced by IL-3 or GM-CSF (Fig. 1B). The ED_{50} of IL-4 was ca. 100 pM, higher than that of IL-3 or GM-CSF. IC2 cells were subcloned by limiting dilution. Each subclone responded

Fig. 3. Synergistic action of IL-4 and IL-3 or GM-CSF. IC2 cells were cultured for 21 h with various concentrations of IL-3 (A) or GM-CSF (B) in RPMI 1640-10% FCS containing 0 (○), 0.03 (◐), 0.3 (◑) or 3 nM (●) IL-4, and DNA synthesis was determined by pulse-labeling with $[^3\text{H}]$ thymidine for 3 h. The symbols in brackets between (A) and (B) indicate DNA synthesis induced by IL-4 in the absence of IL-3 or GM-CSF.

Fig. 4. Inhibitory effect of IAP on DNA synthesis of IC2 cells induced by IL-3, GM-CSF or IL-4. IC2 cells were cultured with various concentrations of IAP in RPMI-10% FCS containing 100 pM IL-3 (○), 100 pM GM-CSF (●) or 1 nM IL-4 (○) for 21 h and pulse-labeled with [³H] thymidine for 3 h.

Fig. 5. Inhibitory effect of IAP on DNA synthesis induced by various doses of IL-3 in the presence or absence of IL-4. IC2 cells were cultured with various concentrations of IL-3 (○, ●) or a combination of 3 nM IL-4 and various concentrations of IL-3 (□, ■) in RPMI 1640-10% FCS in the absence (○, □) or presence (●, ■) of 100 ng/ml IAP for 21 h and pulse-labeled with [³H] thymidine for 3 h.

similarly to the three growth factors, indicating that individual cells can respond to multiple growth factors.

When IC2 cells were stimulated with a combination of IL-4 and IL-3 or GM-CSF, the level of DNA synthesis was higher than that induced by only IL-3 or GM-CSF (Fig. 1B). In contrast, a combination of IL-3 and GM-CSF did not increase the maximum level of DNA synthesis, although suboptimal doses of IL-3 and GM-CSF additively stimulated the cells (Fig. 2).

IC2 cells were stimulated with IL-3 or GM-CSF in the presence of various concentrations of IL-4 (Fig. 3). The maximum level of DNA synthesis increased in an IL-4-concentration-dependent manner as in Fig. 1B. In addition, the concentration dependence curve of stimulation obtained with IL-3 or GM-CSF shifted to the left in the presence of IL-4. About 1 pM IL-3 or GM-CSF induced the maximum level of DNA synthesis in the presence of 3 nM IL-4.

IAP inhibits growth factor-induced DNA synthesis of IC2 cells. IC2 cells were cultured for 24 h with various concentrations of IAP in the presence of IL-3, GM-CSF or IL-4. IAP inhibited DNA synthesis induced by the growth factors in a concentration-dependent manner (Fig. 4). The inhibitory effect was detected with 10 pg/ml IAP and the maximum inhibition was obtained at 1 ng/ml IAP or more.

Fig. 6. ADP-ribosylation of 41-kDa protein induced by IAP in IC2 cells. IC2 cells were cultured at 5×10^5 cells/ml in RPMI 1640-10% FCS containing 50 pM IL-3 in the absence (a, b) or presence (c, d) of 100 ng/ml IAP for 24 h. The cells were collected and membrane fractions were prepared as described previously (18). Membrane proteins were incubated at 37°C for 60 min in the absence (a, c) or presence (b, d) of 166 μ g/ml preactivated IAP in 2.5 mM MgCl_2 , 1 mM ATP, 10 mM thymidine, 1 mM DTT, 3 mM phosphoenolpyruvate, 10 μ g/ml pyruvate kinase, 10 μ M [α - 32 P] NAD (8.3 Ci/mmol), and 100 mM potassium phosphate (pH 7.6). Samples were resolved by SDS-PAGE and *in vitro* substrates for IAP were detected by autoradiography. The numbers on the left side indicate the positions of molecular weight markers.

TABLE 1. EFFECT OF TGF- β ON DNA SYNTHESIS OF IC2 CELLS INDUCED BY VARIOUS GROWTH FACTORS^a.

Reagents	[³ H] thymidine incorporation	
	—	+ TGF- β (100 pM)
—	238 $\pm$ 34	312 $\pm$ 12
IL-3 (10 ⁻¹⁰ M)	10234 $\pm$ 646	6458 $\pm$ 342
GM-CSF (10 ⁻¹⁰ M)	13231 $\pm$ 574	8468 $\pm$ 171
IL-4 (10 ⁻⁹ M)	8607 $\pm$ 236	2151 $\pm$ 91
IL-3 + IL-4	17091 $\pm$ 1852	10991 $\pm$ 859
GM-CSF + IL-4	19247 $\pm$ 602	13039 $\pm$ 265

^aDNA synthesis was determined in triplicate samples according to the method described in Materials and Methods and is expressed with SEM.

The inhibition by IAP was always incomplete, however (Fig. 4), and inhibition was observed at all doses of IL-3 (Fig. 5). In addition, IAP also partially inhibited DNA synthesis induced by IL-3 and IL-4 (Fig. 5). IAP at ng/ml orders inhibited IL-4-induced DNA synthesis by 70% (Fig. 4). IAP affected to lesser extents DNA synthesis dependent upon IL-3 or GM-CSF. IAP treatment did not decrease the viability of IC2 cells, indicating that the inhibitory effect of IAP was not due to toxicity of the reagent. We have excluded the following possibilities (data not shown): (a) IAP inhibits binding of growth factors to IC2 cells, (b) IAP inhibits uptake of [³H] thymidine into stimulated cells. IAP inhibited growth of IC2 cells as measured by cell number (see below).

To confirm that IAP ADP-ribosylated membrane proteins during the incubation, we performed *in vitro* ADP-ribosylation using a membrane fraction prepared from IC2 cells cultured for 24 h with 1 nM IL-3 in the presence or absence of 100 ng/ml

Fig. 7. Inhibition of DNA synthesis of IC2 cells by TGF- β . (A) IC2 cells were cultured with various concentrations of IL-3 in RPMI 1640-10% FCS (○) or in the same medium containing 100 pM TGF- β (●) for 21 h followed by 3 h pulse-labeling with 1 μ Ci/well [³H] thymidine. (B) IC2 cells were cultured with various concentrations of TGF- β in RPMI 1640-10% FCS containing 100 pM IL-3 for 21 h followed by 3 h pulse-labeling with 1 μ Ci/well of [³H] thymidine.

Fig. 8. Long-term culture of IC2 cells with various growth factors. IC2 cells were cultured at 5×10^4 cells/ml in RPMI 1640-10% FCS containing 1 nM IL-3 ($\circ$), 1 nM IL-4 ($\triangle$), 1 nM IL-3 and 1 nM GM-CSF ($\bullet$), 1 nM IL-3 and 100 ng/ml IAP ($\odot$) or 1 nM IL-3 and 1 nM IL-4 ($\bullet$). Cell numbers were determined 2 days after cultivation began and the cells were cultured further under the same conditions.

IAP. As shown in Fig. 6 (lane b), IAP ADA-ribosylated a 41-kDa protein *in vitro* in a membrane fraction from cells which had been cultured in the absence of IAP. In contrast, the 41-kDa protein was not ADP-ribosylated *in vitro* with membrane fractions from cells cultured in the presence of IAP (Fig. 6, lane d). This indicates that the protein was fully ADP-ribosylated *in vivo*.

TGF- β inhibits DNA synthesis of IC2 cells. IC2 cells were stimulated for 24 h with various growth factors in the presence or absence of 100 pM human TGF- β .

Fig. 9. Cell cycle analysis of IC2 cells stimulated by various growth factors. IC2 cells which had been cultured for 3 days without changing the conditioned medium were collected (a). Cells cultured with IL-3 (b), with IL-3 and IL-4 (c) or with IL-3 and IAP (d) were collected on day 8. The cells were washed and mixed with a solution consisting of 50 μ g/ml propidium iodide, 0.05% NP40, 10 μ g/ml RNase A and 0.1% sodium citrate. Cell cycle analysis was performed by flow cytometry using a FACS IV.

TGF- β inhibited DNA synthesis induced by IL-3, GM-CSF, IL-3 plus IL-4 or GM-CSF plus IL-4 by 30 to 40%, but more severely (ca. 70%) inhibited that induced by IL-4 (Table 1). Inhibition of DNA synthesis by TGF- β was dose-dependent and reached a plateau level at 100 pM (Fig. 7).

Effects of IL-3, GM-CSF, IL-4, IAP and TGF- β on long-term growth of IC2 cells. The abilities of the three growth factors to support long-term growth of IC2 cells were examined (Fig. 8). IC2 cells grew continuously in the presence of IL-3 or GM-CSF when appropriately diluted every two days. The doubling time was ca. 25 h for cultures with a sufficient concentration of IL-3 or GM-CSF. In contrast, IL-4 did not support the continuous growth of IC2 cells. In the presence of IL-4, the cells survived for 24 h and divided once, but eventually died after 2 days of incubation. IL-4, however, enhanced the IL-3- or GM-CSF-dependent growth of IC2 cells. Sufficient concentrations of IL-3 or GM-CSF in the presence of 1 nM IL-4 allowed IC2 cells to grow rapidly, with a doubling time of ca. 17 h. When IC2 cells were cultured with a sufficient concentration of IL-3 in the presence of 100 ng/ml IAP, the cells grew exponentially with a doubling time of ca. 30 h.

Cell cycle analysis was performed with exponentially growing IC2 cells under various conditions by staining DNA with propidium iodide (Fig. 9). While the fraction of G1 cells was 59% for a culture stimulated by IL-3 (Fig. 9b), it decreased to 49% for that co-stimulated by IL-3 and IL-4 (Fig. 9c). On the other hand, IAP increased the fraction of G1 cells growing with IL-3 to 70% (Fig. 9d) and that of those growing with IL-3 and IL-4 to 56% (see Table 2). From these results, we calculated the length of G1 phase in the cell cycle (Table 2, Exp. 1). The results demonstrated that the length of G1 phase varied with treatment, whereas the length of S + G2 + M

TABLE 2. EFFECTS OF IL-4, TGF- β AND IAP ON THE CELL CYCLE OF IC2 CELLS.

reagent	Td (h) ^a	G1(%) ^b	cell cycle length (h) ^c	
			G1	S + G2 + M
Experiment 1				
IL-3	25 $\pm$ 1	59 $\pm$ 5	13 $\pm$ 1	12 $\pm$ 1
IL-3 + IL-4	17 $\pm$ 1	49 $\pm$ 5	7 $\pm$ 1	10 $\pm$ 1
IL-3 + IAP	30 $\pm$ 2	70 $\pm$ 5	19 $\pm$ 2	11 $\pm$ 1
IL-3 + IL-4 + IAP	23 $\pm$ 1	56 $\pm$ 5	11 $\pm$ 1	12 $\pm$ 1
Experiment 2				
IL-3	20 $\pm$ 2	45 $\pm$ 5	8 $\pm$ 1	12 $\pm$ 2
IL-3 + TGF- β	26 $\pm$ 2	57 $\pm$ 5	13 $\pm$ 2	13 $\pm$ 2
Experiment 3				
IL-3	26 $\pm$ 2	nd ^d	nd	nd
IL-3 + TGF- β	30 $\pm$ 3	nd	nd	nd
IL-3 + IAP	29 $\pm$ 1	nd	nd	nd
IL-3 + TGF- β + IAP	30 $\pm$ 2	nd	nd	nd

^a The mean doubling time (Td) was determined from growth line slope (Fig. 8) and is expressed with SE caused in determination of the slope.

^b The fraction of cells in G1 phase was determined by flow cytometry with duplicate samples (Fig. 9) using Dean's method (1).

^c The length of the G1 period was calculated from populations of cells in G1 phase according to the equation $Td \times [1 - \log\{2 - F(G1)\} / \log 2]$, in which F(G1) is the fraction of cells in G1 phase (31).

^d Not determined.

Fig.10. Effects of IL-3, IL-4 and IAP on the growth of LT4 cells. Long-term cultures of LT4 cells with 1 nM IL-3 (○), 1 nM IL-3 and 1 nM IL-4 (●), and 1 nM IL-3 and 100 ng/ml IAP (◐) were examined as described in Fig. 8.

was constant.

TGF- β also reduced the growth rate of IC2 cells (Table 2). It should be noted that the growth rate of control IC2 cells incubated with IL-3 alone varied from experiment to experiment (Table 2, Exp. 2 and 3). This variation did not affect of the major conclusions of this study, however. Simultaneous addition of IAP and TGF- β did not cause stronger inhibition than that caused by either one alone.

Another multi-factor-dependent myeloid cell line, LT4 (7), grows more rapidly than IC2, with a doubling time of ca. 14 h (Fig. 10). The IL-3-dependent growth of LT4 cells was not affected by IL-4, although DNA synthesis of these cells was stimulated by IL-4 alone. IAP inhibited the growth of LT4 cells. The doubling time of LT4 cells cultured with sufficient concentrations of IL-3 in the presence of 100 ng/ml IAP was ca. 19 h (Fig. 10), indicating that IL-3-induced growth of LT4 cells is also IAP-sensitive.

DISCUSSION

IL-3 and GM-CSF specifically bind to distinct receptors (16, 17, 21, 22, 28). The IC2 cells used in this study express both IL-3 and GM-CSF receptors. We have recently shown that IL-3 but not GM-CSF rapidly induces tyrosine phosphorylation of a membrane glycoprotein, gpp150 (8). Although the receptors for these two growth factors and the early biochemical events induced by them are distinct, the stimulatory effects of IL-3 and GM-CSF on IC2 cells appear quite similar, as described above. These two growth factors induced DNA synthesis of IC2 cells in a similar concentration-dependent manner and could support the long-term growth of IC2 cells. When the cells were co-stimulated with a combination of IL-3 and GM-CSF, the total concentrations of the two growth factors required for the induction of DNA synthesis to 50% of the maximum were always ca. 5 pM regardless of the ratio of the two growth factors. This value is the same as that obtained with either IL-3 or

GM-CSF alone, suggesting that IL-3 and GM-CSF induce DNA synthesis of IC2 cells through common downstream processes although the initial events are different.

IL-4 induced transient DNA synthesis but could not support long-term growth of IC2 cells. Cells incubated with IL-4 alone died within 2 days, suggesting that these cells initiated DNA synthesis and completed the cell cycle once but could not enter the next round of the cell cycle. IL-4 augmented the effect of IL-3 and GM-CSF on IC2 cells: 1) the maximum rate of DNA synthesis increased, 2) sensitivity to IL-3 or GM-CSF increased, and 3) the length of the G1 phase was shortened. Furthermore, IL-4-induced DNA synthesis is more susceptible to inhibition by IAP and TGF- β than other growth factor-induced DNA synthesis. It is thus suggested that IL-4 affects IC2 cells through a unique mechanism.

When IC2 cells were cultured for 3 days or more without changing the conditioned medium, the viability of the cells gradually decreased and the fraction of cells in G1 phase increased (Fig. 9a). This suggested that IC2 cells could not enter S phase but died in G1 phase in the absence of sufficient amounts of growth factors. It has been proposed that every cell passes through a restriction point in the late G1 phase before entering S phase (19, 20). Only cells that receive sufficient stimulating signals can go through the restriction point; others stay in G1 phase. IL-3 and GM-CSF are considered to be progression factors which stimulate IC2 cells to go through the restriction point and to round the cell cycle. The threshold of the restriction point to enter S phase may be lowered by IL-4 (Figs. 1B, 3). Augmentation of the growth rate by IL-4 was not observed in LT4 cells, although these cells express receptors for IL-4 and can respond to IL-4 alone (unpublished observation). Similar results were obtained with a variant of IC2 which also grew more rapidly than IC2 with a doubling time of ca. 14 h in a sufficient concentration of IL-3 alone (unpublished observation). The hypothetical growth enhancing signal(s) in IC2 cells induced by stimulation with IL-4 in IC2 cells might be constitutively generated in LT4 and the variant of IC2.

IAP has been shown to inhibit DNA synthesis induced by certain growth factors in fibroblast cells (11, 15). In contrast, IAP did not inhibit DNA synthesis of an interleukin 2-dependent T cell clone, K23, at any concentration (unpublished observation). As shown above, IAP commonly inhibited DNA synthesis in myeloid cells induced by either one of or a combination of IL-4, IL-3 and GM-CSF. An interesting feature of the IAP effect is that IAP did not completely inhibit DNA synthesis, although a 41-kDa substrate was fully ADP-ribosylated in IAP-treated cells (Figs. 4-6). A similar IAP effect was observed with fibroblasts (11, 15). Other features of the IAP effect are: 1) IAP did not cause cell death, 2) the cells grew slowly in the presence of IAP, and 3) IAP increased the fraction of cells present in the G1 phase of the cell cycle. The effect of TGF- β on IC2 cells is apparently similar to that of IAP. However, TGF- β has been reported to inhibit the proliferative response of T cells (26) whereas IAP did not (unpublished observation). This suggests that the target biochemical reactions of the two growth-inhibitory agents in IC2 cells are different. The growth inhibition by IAP does not necessarily suggest that an IAP-sensitive G protein(s) is directly involved in regulation of the cell cycle.

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