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Distinct mechanisms may account for the growth-promoting activity of interleukin 3 on cells of lymphoid and myeloid origin

(growth factors/lymphokines/cell metabolism/receptors/cell proliferation)

RONALD PALACIOS* AND JOHN GARLAND†

*The Basel Institute for Immunology, Grenzacherstrasse 487, CH-4005, Basel, Switzerland; and †Department of Immunology, University of Manchester School of Medicine, Manchester M13 9PT, England

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ABSTRACT **We have investigated whether interleukin 3 (IL-3) supports the growth of cells of different lineages by the same mechanism(s). The experiments were carried out with Ea3 cells, a mouse pre-B cell line, and S-480-3 cells, a mouse basophil cell line, both of which are totally IL-3 dependent. We found that Ea3 lymphocytes but not S-480-3 basophils absorb partially purified IL-3. Both Ea3 and S-480-3 cells respond to IL-3 by increasing anaerobic glycolysis as determined by lactic acid production. S-480-3 cells responded to exogenous ATP by maintaining proliferation and reducing lactic acid production, but Ea3 lymphocytes are refractory to exogenous ATP. We conclude that there may be two distinct mechanisms by which cells respond to IL-3, indicated by early events concerning the binding of IL-3 and the effect of exogenous ATP on respiratory metabolism. One appears to be a ligand–receptor-mediated mechanism in lymphoid cells and the other to be a mechanism that is partially replaceable by exogenous ATP in nonlymphoid cells not associated with lymphoid-like receptors. Our findings may explain (*i*) the apparent variety of cell lineages promoted by IL-3 by a widely available mechanism that supports glycolysis and, therefore, enables both proliferation and possibly expression of binding sites for lineage specific differentiation factors and (*ii*) the existence of lymphocytes that express receptors specific for IL-3 and are inducible for other characteristics and functions in a regulated manner.**

Interleukin 3 (IL-3) is a 26- to 28-kilodalton glycoprotein secreted by antigen or lectin-stimulated T lymphocytes (reviewed in refs. 1 and 2) and constitutively by the mouse tumor cell line WEHI-3 (3). Because IL-3 shares most, if not all, of the biochemical and biological properties described for burst-promoting activity and hematopoietic cell growth factor (4–6), all three of these activities may be carried by the same molecular entity.

IL-3 allows the development *in vitro* of cells of myeloid (4–8), lymphoid (9–11), and erythroid (4, 5, 12) lineages. IL-3-dependent cell lines have been classified on the basis of expression of characteristic surface antigens and functional properties such as secretion of interleukin 2 (9–11), antibody production (13), synthesis of hemoglobin (12, 14), and release of histamine (6).

An important question concerning the biological activities of IL-3 is whether the growth of IL-3-dependent cells of different lineages is promoted by the same mechanism. We report here studies indicating that (*i*) IL-3-dependent cells of lymphoid and myeloid lineages share a common respiratory metabolism (anaerobic glycolysis) dependent on IL-3; (*ii*) cells of lymphoid origin reversibly absorb IL-3, but cells of myeloid origin (e.g., basophils) do not; and (*iii*) only for cells of myeloid origin is IL-3 partially replaceable by exogenous ATP.

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MATERIALS AND METHODS

Cell Lines. The origin, isolation, surface, and functional properties of the IL-3-dependent mouse basophil clone S-480-3 and of the mouse pre-B cell line Ea3 have been described (13, 15). Both cell lines are maintained in culture in plastic flasks at a cell concentration of 2.5–4.0 × 10^5 per ml of Iscove's modified Dulbecco medium supplemented with 5% heat-inactivated fetal calf serum (GIBCO), gentamycin (50 μg/ml, Essex Chemie, Lucerne, Switzerland), 50 μM 2-mercaptoethanol, and 0.1 vol of an IL-3-containing supernatant obtained from the mouse tumor cell line WEHI-3. This medium will be referred to as WEHI-3-conditioned medium (WEHI-CM). Before using S-480-3 and Ea3 cells in the assays for proliferative response and absorption of IL-3, the cells were washed three times with balanced salt solution and then suspended in RPMI 1640 serum-free medium supplemented with L-glutamine (2 mM) and gentamycin (50 μg/ml) (culture medium) and incubated at 37°C for 5 hr. The cells were washed again in balanced salt solution and resuspended in culture medium at the desired concentrations.

Proliferative Response to IL-3. Ea3 or S-480-3 cells (2 × 10^4) suspended in (final vol, 200 μl) culture medium were cultured in flat-bottomed microplate wells (Nunclon Delta, Nunc) in the presence or absence of partially purified IL-3 (final dilution, 1:250) (5) (kindly provided by G. Bazill, Paterson Laboratories, Manchester, England). The cultures in triplicate were incubated in humidified 5% CO_2/95% air at 37°C for 24 hr. Cell proliferation was determined by [^{3}H]thymidine uptake (1 μCi per well; 1 Ci = 37 GBq; specific activity 185 MBq; Radiochemical Centre) during the last 6 hr of the culture period. The results are expressed as cpm and they are mean ± SEM of triplicate cultures.

Absorption of IL-2 or IL-3 Activity. Cells (5 × 10^7) of various groups—Ea3 lymphocytes, S-480-3 cells, or the IL-2-dependent cytotoxic T-cell line CTLL (developed by Kendall A. Smith and made available by Max Shreier)—were incubated with either partially purified IL-3 (5) (final dilution, 1:100) or IL-2 (obtained from phorbol 12-myristate 13-acetate-induced EL-4 thymoma cells) (final dilution, 1:10) in a final volume of 0.8 ml for 2 hr on ice in 12 × 75 mm round-bottomed plastic tubes (2054 Falcon). The tubes were centrifuged (2500 rpm, 15 min), and the supernatants were collected, filtered (0.22-μm Acrodisc filters), and tested for their ability to support proliferation of either Ea3 cells (experiment 1 of Table 2) or S-480-3 cells (experiments 2 and 3 of Table 2) (IL-3 activity) or CTLL (IL-2 activity) as detailed before. The results are given for the proliferation induced by 50 μl (experiments 1 and 3 of Table 2) or 25 μl (experiment 2 of Table 2) of the filtered supernatants and are expressed as cpm (mean ± SEM of triplicate cultures) of remaining IL-2 or IL-3 activity after absorption. Similar results were obtained at all concentrations tested (from 5% to 50%).

Abbreviations: IL-3, interleukin 3; WEHI-CM, WEHI-3-conditioned medium.

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Table 1. Proliferative responses of S-480-3 and Ea3 cells to IL-3

Cell line	Characteristics	Stimulus	[^{3}H]Thymidine uptake, cpm		
			Exp. 1	Exp. 2	Exp. 3
Ea3	Lymphocyte	Medium	361 ± 52	436 ± 28	182 ± 30
		IL-3	18,154 ± 597	15,728 ± 1073	21,944 ± 2352
S-480-3	Basophil	Medium	480 ± 35	325 ± 17	516 ± 81
		IL-3	10,683 ± 873	9,501 ± 364	13,172 ± 1025

Cells were incubated in the presence or absence of partially purified IL-3 (final dilution, 1:250) in flat-bottomed microplate wells at 37°C for 24 hr. Cell proliferation was determined by [^{3}H]thymidine uptake during the last 6 hr of the culture period. Results represent mean ± SEM.

Experiments were carried out to recover IL-3 activity absorbed by Ea3 cells. Thus, Ea3 cells or S-480-3 cells (negative control) used in absorption experiments were washed three times in balanced salt solution, resuspended in 0.5 ml of culture medium, and incubated at 4°C for 1 hr. Every 10 min during this period, the cells were vigorously shaken. At the end of the incubation time, the tubes were centrifuged (3000 rpm, 15 min), and the supernatants were collected, filtered, and assayed for their ability to support proliferation of fresh Ea3 cells (IL-3 activity) as described above. The data are expressed as cpm (mean ± SEM of triplicate cultures) of recovered IL-3 activity (experiments 1 and 2 of Table 3).

Production of Lactic Acid by Ea3 and S-480-3 Cells. Ea3 and S-480-3 cells were washed and resuspended in RPMI 1640 medium supplemented with 10% dialyzed fetal calf serum, with and without dialyzed WEHI-CM. At various times of culture at 37°C, 0.5 ml of resuspended culture was collected and the cells were removed by centrifugation. The presence of lactic acid in the cell-free supernatants was estimated by the NAD/lactic dehydrogenase method, supplied in kit form by Sigma (kit 826). Optical densities were read in a Beckman spectrometer at 340 nm. Each assay point contained blank and reference samples; and the blank (control medium) values have been subtracted from the values presented in Fig. 1. Reference samples agreed with each other within 5%.

Proliferative Response of S-480-3 and Ea3 Cells to Exogenous ATP. S-480-3 or Ea3 cells (10^5) were cultured in 200 μl of RPMI 1640 medium supplemented with 10% fetal calf serum and glutamine in the presence or absence of different concentrations of ATP. As a positive control, similar cultures were supplemented with 10% IL-3-containing WEHI-CM. After overnight incubation at 37°C, the cultures were pulsed with [^{3}H]thymidine (1 μCi per well), the cells were harvested onto glass fiber filters and extracted with 5% trichloroacetic acid, the acid-precipitated material was washed with 0.9% saline and with absolute alcohol, and its radioactivity was determined. Values are given for mean cpm ± 1 SD.

Effect of Exogenous ATP on the Production of Lactic Acid by S-480-3 and Ea3.13 Clones. S-480-3 and Ea3.13 cells were washed and suspended in 2 ml of RPMI 1640 medium supplemented with 10% dialyzed fetal calf serum containing Na_2ATP (Boehringer Mannheim) (final concentrations, 0.75 and 0.375 mg/ml). After 3 and 5 hr of incubation at 37°C in 5% CO_2/95% air, 0.5-ml samples of these cultures were collected and centrifuged to remove cells, and 0.1-ml portions of the cell-free supernatants were assayed for lactic acid. Lactic acid was measured by conversion of NAD to NADH in the presence of lactic dehydrogenase (Sigma kit 826). Total volumes of test were 1.5 ml, and optical absorbancies were measured at 340 nm. A standard solution of 0.88 mM lactic acid gave an absorbance of 0.580 ± 0.02 (mean ± 1 SD) over the three experiments, and all values are corrected for absorbance of supernatants from initial cell suspensions at time zero (t = 0). Data are given for 3 and 5 hr after reincubation at t = 0, determined by the curves for lactic acid production shown in Fig. 1.

RESULTS AND DISCUSSION

The origin, isolation, and phenotype of the IL-3-dependent clone S-480-3 have been reported (15). S-480-3 cells express the F480 but not the Thy antigen on their surfaces. These cells have Fc fragment receptors for IgG but not for IgE, and they have basophilic granules and histamine in their cytoplasm. Therefore, S-480-3 cells were classified as basophil-like cells. The origin, development, and functional properties of the mouse lymphocyte line Ea3 have been described (13). Ea3 cells were classified as pre-B cells on the following criteria: (*i*) presence of surface antigens characteristic of B cells and absence of surface Ig, (*ii*) rearrangement in Ig genes coding for μ heavy and κ light chains, and (*iii*) Ea3 is inducible *in vitro* to differentiate into IgM-secreting cells. Ea3 cells have been in culture for 17 months and they are absolutely dependent on IL-3. The characteristics of the Ea3.13 clone are identical to those of the parent line (13).

We first compared the proliferative rates of S-480-3 and Ea3 cell lines. As shown in Table 1, both are dependent on IL-3. The doubling times were 23 hr for S-480-3 and 16 hr for Ea3 cells during the logarithmic phase. We next investigated the capacity of the two cell lines to absorb IL-3. The results (Tables 2 and 3) show that Ea3 but not S-480-3 cells demonstrably absorbed IL-3 activity from a preparation partially purified from WEHI-CM (5).

Control experiments showed that Ea3 cells did not absorb nonrelated growth factors such as IL-2 and that, under the same conditions, the IL-2-dependent CTLL line removed IL-2 but not IL-3 activity (Table 2). Lack of absorption of IL-3 by S-480-3 cells was confirmed using up to 10^8 cells (twice the number used in the experiments shown in Table 2)

Table 2. Absorption of IL-3 activity by Ea3 and S-480-3 cell lines

Cells	IL-2 activity left after absorption, cpm			IL-3 activity left after absorption, cpm		
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
None	17,183 ± 1041	NT	21,743 ± 1528	33,742 ± 825	26,280 ± 1710	43,810 ± 1309
Ea3	18,011 ± 2137	NT	20,915 ± 2043	15,835 ± 647	12,728 ± 1584	22,340 ± 2018
S-480-3	16,842 ± 2038	NT	NT	34,013 ± 2132	25,778 ± 2316	41,985 ± 3513
CTLL	2,943 ± 341	NT	3,685 ± 743	33,481 ± 2908	27,550 ± 1842	43,312 ± 3693

Cells were incubated with either partially purified IL-3 or IL-2 on ice for 2 hr. Supernatants were collected and assayed for IL-3 activity in either Ea3 cells (experiment 1) or S-480-3 cells (experiments 2 and 3) and for IL-2 activity on CTLL cells. Data are mean ± SEM of remaining IL-2 or IL-3 activity after absorption. NT, not tested.

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Table 3. Absorption of IL-3 activity by Ea3 and S-480-3 cells

Cells	IL-3 activity left after absorption, cpm		IL-3 activity recovered from Ea3 and S-480-3 cells, cpm	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
None	24,373 ± 3138	18,137 ± 1439		
Ea3	16,087 ± 1027	9,025 ± 2107	5527 ± 936	4067 ± 1018
S-480-3	23,181 ± 1974	19,018 ± 1845	839 ± 104	1104 ± 72

Ea3 or S-480-3 cells used in absorption experiments (1 and 2) were washed, resuspended in culture medium, and then incubated at 4°C for 1 hr. Every 10 min the cells were vigorously shaken. The supernatants were collected and tested (final dilution, 1:2) for their ability to support proliferation of fresh Ea3 cells (IL-3 activity). Background in experiment 1 was 718 ± 51 and in experiment 2 was 873 ± 48 cpm. Results are expressed as mean ± SEM of recovered IL-3 activity.

and by performing the experiments at 0°C (Table 2) and 37°C (results not shown). The possibility that Ea3 cells removed IL-3 by degradation or inactivation rather than by absorption is unlikely for two reasons. First, IL-3 activity could be recovered from Ea3 cells used in the absorption experiments. Under the same culture conditions, very little, if any, IL-3 activity was recovered from S-480-3 cells (control). This rules out the possibility that the IL-3 activity recovered from Ea3 cells used in absorption experiments was merely a carry-over of the growth factor (experiments 1 and 2 of Table 3). Second, the absorption experiments were conducted at 0°C. Thus, the data are consistent with the hypothesis that Ea3 lymphocytes but not S-480-3 basophils have receptors for IL-3.

It has been shown that basophil lines increase their rate of anaerobic glycolysis after IL-3 treatment and that oxidative phosphorylation does not significantly contribute to the energy metabolism of the cells (unpublished data). We investigated the effect of IL-3 on the generation of lactic acid by Ea3 cells. Lactic acid production should be a good measure of anaerobic glycolysis. As shown in Fig. 1, the rate of lactic acid production by both Ea3 and S-480-3 cells is about twice as high in the presence of IL-3 as it is in the absence of IL-3. As would be expected from their faster growth rate, the absolute rate of lactic acid production was much greater for Ea3 than for S-480-3 cells. Thus, both Ea3 lymphocytes and S480-3 basophils appear to have the same metabolism promoted by IL-3.

Recently, Whetton and Dexter (16) reported that exogenous ATP could support proliferation and maintain (or increase) intracellular levels of ATP in the myeloid cell line FDC-P1, which responds to hematopoietic cell growth factor. Since FDC-P1 cells also respond to IL-3 (2), we considered it important to determine whether there was a difference in the response of IL-3-dependent Ea3 lymphocytes and S-480-3 basophils to exogenous ATP. The results show that S-480-3 cells respond to exogenous ATP by proliferation as determined by [^{3}H]thymidine uptake and viability whereas Ea3 lymphocytes are totally refractory (Table 4 and data not shown). To investigate this difference further, we compared the effect of exogenous ATP on the generation of lactic acid in the absence of IL-3 by S-480-3 and Ea3 cells.

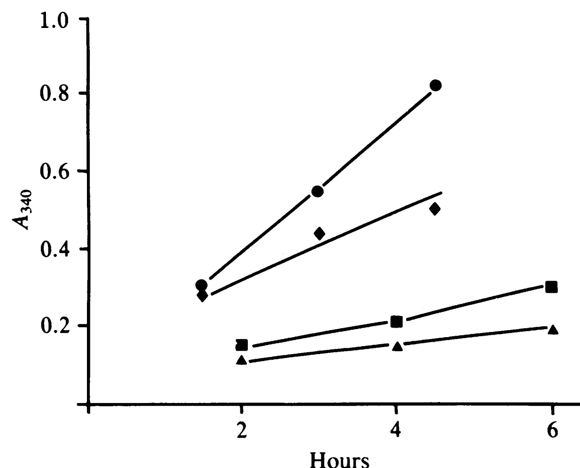

FIG. 1. Lactic acid production by S-480-3 and Ea3 cells. Cells were resuspended at 10^6/ml after washing twice in RPMI 1640 medium with 10% dialyzed fetal calf serum with or without dialyzed WEHI-CM. At various times, 0.5 ml of resuspended culture was removed, the cells were spun out, and lactic acid in the cell-free supernate was estimated by the NAD/lactic dehydrogenase method, supplied in kit form by Sigma. Absorbances were read on a Beckman spectrophotometer at 340 nm. Each assay point contained blank and reference samples, and results are given with blank (control medium) values subtracted. Agreement of reference samples was within 5% error. ●, Ea3 cells with IL-3; ♦, Ea3 cells without IL-3; ■, S-480-3 cells with IL-3; ▲, S-480-3 cells without IL-3.

The results are shown in Table 5. Using equivalent numbers of cells (experiment 1), we found that, although Ea3.13 lymphocytes were completely unaffected by ATP and the rate of production of lactic acid was not different from con-

Table 4. Proliferative responses of S-480-3 and Ea-3 cells to exogenous ATP

Exogenous ATP, mg/ml	[^{3}H]Thymidine uptake, cpm: S-480-3 cells, Exp. 1	S-480-3 cells, Exp. 2	Ea3 cells, Exp. 1	Ea3 cells, Exp. 2
None	222 ± 12	829 ± 160	963 ± 571	1060 ± 183
3	166 ± 90	156 ± 23	200 ± 2	223 ± 20
1.5	123 ± 16	168 ± 37	132 ± 1	416 ± 183
0.75	411 ± 201 (0.15)	875 ± 418	264 ± 19	460 ± 227
0.375	3843 ± 555 (0.005)	7,771 ± 1372 (<0.01)	596 ± 85	488 ± 75
0.18	428 ± 75 (0.01)	2,627 ± 487 (0.015)	867 ± 140	547 ± 57
0.09	261 ± 58	626 ± 49	833 ± 50	554 ± 47
WEHI-CM (positive control)	8796 ± 696	17,086 ± 2954	7287 ± 200	4958 ± 433

Cells were incubated in the presence or absence of different concentrations of ATP or IL-3-containing medium (WEHI-CM). After overnight incubation at 37°C, cultures were pulsed with [^{3}H]thymidine for 3 hr to measure cell proliferation. Results represent mean cpm ± SEM. Numbers in parentheses represent P values (Student's t test).

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Table 5. Effect of exogenous ATP on production of lactic acid

		Lactic acid production, % of control without IL-3						
		At 3 hr			At 5 hr			
Cell line	ATP, mg/ml	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	P*
S-480-3	0.75	71 (0.77)	88 (0.95)	44 (0.86)	66 (1.2)	77 (1.2)	52 (1.1)	<0.025
	0.375	88	114	77	82	100	81	<0.05
Ea3.13	0.75	91 (0.5)	215 (0.78)	100 (0.63)	96 (0.78)	145 (1)	130 (0.71)	<0.1
	0.375	94	114	105	99	136	124	<0.1

Lactic acid was measured by conversion of NAD to NADH in the presence of lactic dehydrogenase. Total volumes of test solutions were 1.5 ml; absorbances were measured at 340 nm. A standard solution of 0.8 mM lactic acid gave an absorbance reading of 0.580 ± 0.02 (mean ± SD) over the three experiments, and all values are subtracted for absorbance of supernates from initial cell suspensions at $t = 0$. Values in parentheses are concentrations of lactic acid (mM) in control cultures. Data are given for 3 and 5 hr after reincubation at $t = 0$, determined by the curves for lactic acid production given in Fig. 1. S-480-3 cells were used at 1×10^6/ml in all experiments, and Ea3 cells were used at 1×10^6/ml in experiment 1 and 2×10^6/ml in experiments 2 and 3.
*Student's *t* test.

trol cells starved for IL-3, in S-480-3 cells the rate of lactic acid production was considerably reduced in the presence of exogenous ATP. To accentuate any changes in Ea3.13 cells, they were investigated at twice the cell density (experiments 2 and 3 of Table 5). The rate of lactic acid production was not significantly increased over control cells, enhancing the difference with S-480-3 cells. It should be noted that the concentration of exogenous ATP promoting S-480-3 cells is very sharply defined, and the level of ATP affecting glycolysis is not the same as that promoting limited proliferation (this study and unpublished data). Thus, the data show that the lymphoid cells behave quite differently from the basophil cells in which exogenous ATP appears to feedback regulate the level of glycolysis in cells deprived of IL-3.

We conclude that there appear to be two distinct mechanisms associated with cellular responses to IL-3, indicated by differences in binding and the effects of exogenous ATP on respiratory metabolism—i.e., a ligand-receptor mechanism in lymphoid cells and one without specific receptor expression in nonlymphoid cells in which IL-3 can be partially replaced by ATP. Ihle *et al.* (17) have reported the binding of iodinated IL-3 to the myeloid-like line FDC-P1. Although this binding was specific to factor-dependent cells, the amounts bound were extremely low, approximately 0.04 unit per 10^6 cells; at the projected molar activity of IL-3, this is equivalent to <1000 molecules per cell, in keeping with our findings for S-480-3 cells. We suggest that, in myeloid cells, interaction of IL-3 with the cell membrane is required, but the membrane domain is not the same as that involved in lymphoid cells and may indicate the creation of alternative routes to enhanced glycolysis. These observations may explain the wide variety of cells capable of responding to IL-3, which enables both continued proliferation and expression of receptors for lineage-specific differentiation factors; not only does IL-3 promote freshly isolated multipotential stem cells (18) but also certain IL-3-dependent cell lines are capable of differentiation into mature cell types (13, 14).

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