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THE SUPPORTIVE EFFECTS OF IL-7 ON EOSINOPHIL PROGENITORS FROM HUMAN BONE MARROW CELLS CAN BE BLOCKED BY ANTI-IL-5

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Human rIL-7 was studied for its effects on myeloid and erythroid progenitors from human bone marrow cells. IL-7 did not support the granulocytic/monocytic or erythroid lineage but exclusively stimulated eosinophil colony formation (CFU-Eo) (4 ± 3 vs 48 ± 17 CFU-Eo/10^5 nonadherent fraction-non-T cell (NAF-NT) cells). This supportive effect was not mediated by T cells or monocytes because similar results were obtained with or without T cell or adherent depleted cell fractions. In addition, it was shown that $CD34^+$ sorted cells could be stimulated by IL-7 (0 vs 15 ± 9 CFU-Eo/3 × 10^3 $CD34^+$ cells) Furthermore studies with IL-3 or granulocyte-macrophage CSF (GM-CSF) demonstrated an additive effect on the IL-7 supported colony formation. Finally, experiments were performed with anti-IL-3, anti-GM-CSF, anti-IL-1, and anti-IL-5 to exclude the possibility that IL-7 indirectly stimulated the eosinophil progenitor cell. Anti-GM-CSF, anti-IL-1, or anti-IL-3 did not influence the supportive effects of IL-7. However, anti-IL-5 did abolish the effects of IL-7 on the eosinophil colony formation (69 ± 15 vs 3 ± 2 CFU-Eo/10^5 NAF-NT, $n = 3$). Similar results were obtained with $CD34^+$ sorted cells. Moreover, IL-5 mRNA expression could be demonstrated in IL-7-stimulated NAF-NT cells. These data suggest that the supportive effects of IL-7 on eosinophil precursors are mediated by the endogenous release of IL-5.

Eosinophil progenitors from human bone marrow cells can be stimulated in vitro to proliferate and differentiate to mature eosinophils in the presence of CSF such as GM-CSF[3], IL-3, or IL-5 (1–4). In addition, these factors modify functional activities of mature eosinophils including prolongation of survival, increased leukotriene production in the presence of a calcium ionophore and enhanced cytotoxic functions (5, 6). In view of these observations eosinophils in bone marrow and peripheral blood seem to be strictly regulated by T cell-derived growth factors. This is further supported by the demonstration of IL-2R on mature eosinophils (7, 8).

IL-7, a stromal-derived growth factor, regulates the proliferation of immature and mature B and T cells (9, 10). In addition there is evidence that IL-7 affects the proliferation of B and T cells from the leukemic counterpart (11) and induces the secretion of IL-6, IL-1, and TNF-α from human monocytes (12). In our study we have investigated whether IL-7 also affects the myeloid or erythroid lineage because most of the cytokines do not act lineage restricted, e.g., IL-2, IL-4, and IL-6 affects the myeloid/monocytic, erythroid, or lymphoid lineage (13–16).

The results demonstrate that IL-7 supports eosinophil colony formation from human bone marrow cells that can be abrogated by the addition of anti-IL-5.

MATERIAL AND METHODS

After informed consent, bone marrow cells were obtained from patients undergoing diagnostic marrow biopsy.

Culture conditions. MNC were isolated by density-gradient centrifugation in Lymphoprep (Nycomed AS, Oslo, Norway). Adherent cells were removed by incubating MNC on plastic dishes for 1 h at 37°C. The contamination of adherent cells in the nonadherent cell fraction was less than 4%, as detected by staining and surface marker analysis. T cell depletion was performed by SRBC. The non-T cell fraction contained less than 4% T cells.

FACS was applied to isolate $CD34^+$ cells. Nonadherent, non-T cells were treated with My-10 (CD34, Becton Dickinson, Sunnyvale, CA) at 4°C for 20 min. The cells were then treated with rat-anti-mouse IgG1/FITC (Zymed Lab, Inc, San Francisco, CA) for 20 min. Cells were maintained at 4°C before and during cell sorting. Analysis and sorting of the CD34-labeled cells was performed using a FACS 440 (B-D FACS systems; Becton Dickinson) with an argon laser set at 488 nm (0.3 W). FITC-fluorescence was measured through a band-pass filter (BP 530/30). Gating windows were established for the strong $CD34^+$ cells. The $CD34^+$ cells in the progenitor fraction was >95% pure as demonstrated by reanalyzing aliquots of sorted cells. Their plating efficacy was 10 to 15%. No $CD3^+$ or $CD14^+$ cells were found in the $CD34^+$ cell fraction.

The in vitro colony assay was assessed with 1.1% methylcellulose (Dow Chemical Co, Midland, MI); 20% FCS (HyClone, Logan, UT); 1% deionized BSA (Sigma Chemical Co., Louis, MO); and Iscove's medium (Flow, Rockville, MD). Target cells (1 × 10^5) were plated in methylcellulose in triplicate, and on day-7 or -14 myeloid colony formation (CFU-GM) was counted using an inverted microscope. In cultures with $CD34^+$ cells, 3 × 10^3 cells/ml were plated in methylcellulose. CFU contained more than 40 cells, identified by their distinct morphologic appearance at 100× magnification. Numbers of colonies refer to the means of triplicate cultures. Frequently the nature of the colonies was verified cytologically by staining with May-Grunwald-Giemsa, eosin, or peroxidase after their collection from the plate. The in vitro assay for the BFU-E was performed as previously described (17). Target cells (1 × 10^5) were plated in methylcellulose in the presence of 1 U recombinant erythropoietin/ml (Amgen, Thousands Oaks, CA) and on day 14 BFU-E were scored. In a limited number of experiments, serum-free culture conditions were used (18). In these assays FCS was substituted with deionized BSA and

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[3] Abbreviations used in this paper: GM-CSF, granulocyte-macrophage CSF; BFU-E, burst-forming unit-erytroid; G-CSF, granulocyte-CSF; MNC, mononuclear cells; NAF, nonadherent fraction; NT, non-T cells; PCR, polymerase chain reaction; RT, reverse transcription.

BSA absorbed cholesterol, iron saturated transferrin, insulin, nucleosides, sodium pyruvate, and L-glutamine (Sigma).

RNA studies. Nonadherent, non-T cells (2×10^6/ml) from bone marrow were isolated as described above and cultured in the presence and absence of IL-7 (1/1000) during 18 h. FACS analysis demonstrated that the cell population contained 15% $CD34^+$ cells and <1% CD_2, CD_{14}^+ cells. Peripheral blood cells were obtained from volunteer platelet donors, and mononuclear cell suspensions were prepared by Ficoll-Hypaque density-gradient centrifugation. T lymphocytes were removed by SRBC. The SRBC were lysed in 155 mM NH_4Cl_2, 10 mM $KHCO_3$, and 0.1 mM EDTA and the remaining cell preparation contained more than 98% T cells. T cells were cultured with medium, IL-7 (1/1000), or Con A (25 μg/ml; Calbiochem, La Jolla, CA) plus PMA (50 ng/ml, Sigma). Total cellular RNA was isolated by the guanidium isothiocyanate method, as described (19).

RT and PCR. RT and PCR was performed essentially according to the protocol for RT/PCR by Pharmacia Fine Chemicals, Piscataway, NJ. Total cellular RNA (0.5 μg) in diethylpyrocarbonate treated water was heated for 5 min at 68°C and then cooled on ice. Ten μl of 10 × RT buffer (135 mM Tris-HCl, pH 8.3, 204 mM KCl, 27 mM $MgCl_2$, 5.4 mM each dNTP, 0.24 mg/ml BSA, 14% glycerol), 1 μl of random hexamer mix (0.2 μg/μl), 2 μl dithiothreitol (45 mM), 1 μl of RNA guard (40 U/μl, Pharmacia), 1 μl AMV RT (40 U/μl), and diethylpyrocarbonate-treated water to a final volume of 30 μl, were added. Tubes were kept for 10 min at room temperature and then for 30 min at 42°C.

PCR. To the transcribed RNA was added: 5′ and 3′ primer (50 pmol each), 0.5 μl of Taq DNA Polymerase (5 U/μl), H_2O to a final volume of 100 μl and mineral oil. Forty cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C were performed. The four primers used were 20 mers. The primers used in this study were: 1) 5′-TGT ATGCCATCCCCACAGAA-3′ and 5′-CACCAAGAAACTCTTGCAGG-3′, which spanned a 324-nucleotide fragment of the IL-5 mRNA, 2) 5′-GTGGGGCGCCCCAGGCACCA-3′ and 5′-CTCCTTAATGTCACGC ACGA-3′ that covered a 548-nucleotide fragment of the β-actin mRNA. Aliquots (10 μl each) were taken from each sample after 40 cycles and were run on 2% agarose gel in 2% tris acetate EDTA buffer, stained with ethidium bromide. Photographs were taken at 280 nm illumination on Agfa APX25/15 roll film.

Recombinant human IL-3, IL-5, IL-7, granulocyte-CSF, GM-CSF, anti-GM-CSF, and anti-IL-3 were gifts from Dr. S. C. Clark, Genetics Institute, Cambridge, MA. Human rIL-7 was obtained as conditioned medium from COS cells transfected with a human IL-7 cDNA. The biologic activity was determined in a thymocyte proliferation assay. Half-maximal stimulation was obtained with a dilution of 1/3000. In addition, medium conditioned by COS cells transfected with the same vector but not containing any CSF gene was used as control (Mock-CSF) at dilutions of 1/1000 and 1/200.

Optimal proliferation of myeloid progenitors with additional CSF was examined with the following concentrations or dilutions: IL-3: 10 ng/ml, GM-CSF: 20 ng/ml, G-CSF: 1/1000, IL-5: 1/1000. Polyclonal anti-serum against GM-CSF or IL-3 was used at a dilution of 1/1000. These concentrations were based on blocking experiments with GM-CSF and IL-3 using the AML-193 cell line (20). These dilutions completely neutralize the colony-stimulating activity of GM-CSF (20 ng/ml) and IL-3 (15 ng/ml). The rat anti-murine IL-5 mAb 17 (NC17) neutralized human IL-5 and was used at a dilution of 1/300 (21). This monoclonal demonstrated no cross-competition with IL-7 because the stimulatory effects of IL-7 (1/500) on a pre-B-cell line could not be abolished by addition of anti-IL-5 at a dilution of 1/300. Finally, an anti-human IL-1β antibody was obtained from Dr. S. Gillis, Immunex, Seattle, WA. Anti-IL-1β was used at a dilution of 1/1000 which could completely neutralize the biologic activity of 100 U IL-1/ml.

Statistics. Statistical comparisons were performed using the paired Student's *t*-test.

RESULTS

MNC isolated from normal bone marrow were incubated in vitro with IL-7 (1/1000) during 14 days. A significant increase in colony numbers was observed in comparison to the unstimulated cell fraction (7 ± 2 vs 33 ± 15 CFU/1 × 10^5 MNC, mean ± SD, $p < 0.01$, Fig. 1), whereas no effect was observed of Mock-CSF at a dilution of 1/1000 and 1/200 (4 ± 2, 5 ± 3 CFU/1 × 10^5 MNC, $n = 4$). There was no colony formation when MNC fraction was cultured for 7 days with IL-7. The observed colonies consisted of closely bound cells with a light brown color. The aggregates were picked off and stained with MGG and eosin. Both stains demonstrated the presence of eosinophil promyelocytes and more mature eosinophils. The dose used in these experiments was based on a dose-response curve with variable dilutions of IL-7. Optimal response was observed at a dilution of ≤1/1000 (Table I).

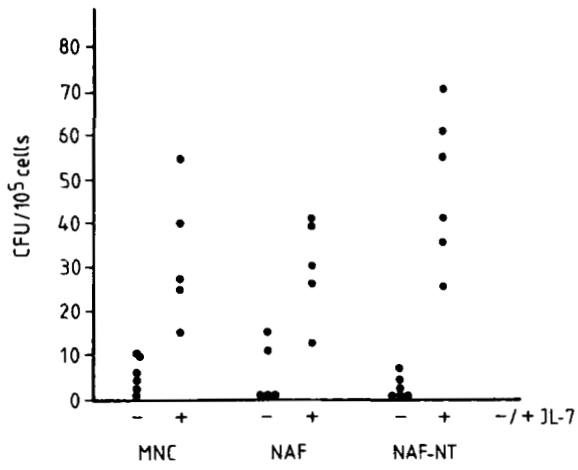

Figure 1. Experiments demonstrate day-14 colony formation/10^5 cells derived from MNC cells, NAF or NAF-NT. The cells were cultured in the absence (−) and presence (+) of IL-7 (1/1000). Values represent the mean of triplicate cultures.

TABLE I

Effects of IL-7 on normal bone marrow cells

Titer IL-7	Expt. I	Expt. II	Expt. III
0	0	0	0
1/5000	13 ± 2	32 ± 2	4 ± 2
1/4000	30 ± 1	ND	17 ± 2
1/2000	52 ± 1	38 ± 1	32 ± 3
1/1000	63 ± 2	68 ± 4	53 ± 4
1/500	ND	59 ± 4	57 ± 6

[a] Dose response curve of IL-7 by using nonadherent, non-T cells. Results are expressed as CFU-Eo/10^5 cells (mean ± SD).

To study the role of accessory cells, experiments were performed with adherent, or adherent- and T cell-depleted cell fractions. Similar results were obtained in all experiments (Fig. 1). In the presence of nonadherent cells the colony numbers increased from 4 ± 2 to 30 ± 11 CFU/1 × 10^5 NAF ($p < 0.005$) whereas in the presence of nonadherent, non-T cells an increase was noticed from 3 ± 2 to 48 ± 17 CFU/1 × 10^5 cells ($p < 0.001$). The small number of colonies in the unstimulated fraction consisted predominantly of macrophages. By stimulating the nonadherent, non-T cells with IL-5 39 ± 11 CFU-Eo/1 × 10^5 cells were obtained ($p < 0.001$). Finally three experiments were performed with $CD34^+$ sorted cells. IL-7 stimulated in all cases the eosinophil colony formation, (0 vs 15 ± 9 CFU-Eo/3 × 10^3 $CD34^+$ cells, mean ± SD, $n = 3$). In these cultures, no CFU-granulocyte, -GM, -macrophage were observed. In response to IL-5 stimulation 9 ± 6 CFU-Eo/3 × 10^3 $CD34^+$ cells were noticed, whereas the combination of IL-3, GM-CSF, and G-CSF supported 162 ± 26 CFU-GM/3 × 10^3 $CD34^+$ cells.

In addition, the effects of serum were studied on the supportive effects of IL-7 by using serum-free culture conditions. There was similar increase in colony numbers with or without the presence of serum 39 ± 2 vs 40 ± 2 CFU-Eo/1 × 10^5 NAF-NT cells (mean ± SD, $n = 2$). The effects of additional CSF such as IL-3, GM-CSF, or G-CSF were studied on the IL-7-induced colony forma-

tion. As shown in Table II, there was no synergistic effect using IL-3, GM-CSF, or G-CSF during the IL-7 supported colony formation but their actions were only additive. Additional experiments were performed with anti-GM-CSF, anti-IL-3, anti-IL-1, and anti-IL-5 to exclude the possibility that IL-7 indirectly stimulated the eosinophil progenitor by releasing IL-3, GM-CSF, IL-1, or IL-5. As depicted in Figure 2 the stimulatory effect of IL-7 could not be inhibited by anti-GM-CSF, anti-IL-1, or anti-IL-3. However, anti-IL-5 abrogated the stimulatory effect of IL-5 (54 ± 4 vs 1 ± 1 CFU/10^5 NAF-NT, $n = 3$) and IL-7 on the eosinophil colony formation. This could not be ascribed to toxic effects because anti-IL-5 did not influence the G-CSF-supported colony formation (data not shown). Similar results were obtained with $CD34^+$ sorted cells. Anti-IL-5 blocked the IL-7 supported eosinophil colony formation completely (28 ± 2 vs 0 CFU-Eo/3×10^3 $CD34^+$ cells).

To demonstrate the induction of IL-5 mRNA in response to IL-7 stimulation, nonadherent, non-T cells were cultured in the absence and presence of IL-7 during 18 h. As depicted in Figure 3 IL-5 mRNA could be demonstrated in IL-7-stimulated nonadherent, non-T cells, whereas in unstimulated nonadherent, non-T cells or IL-7-stimulated T cells no IL-5 mRNA expression was shown. Finally, the effects of IL-7 were studied on erythroid progenitor cells in the presence of erythropoietin. No increase in the BFU-E colony numbers was observed in the presence of IL-7 (200 ± 40 vs 218 ± 34 BFU-E/10^5 nonadherent cells, $n = 4$).

TABLE II

Effects of IL-7 on IL-3, GM-CSF, or G-CSF supported colony formation[a]

± IL-7	IL-7	IL-3		GM-CSF		G-CSF	
	+	−	+	−	+	−	+
Expt.							
1	38	98	125	173	205	95	135
2	37	67	89	107	123	46	75
3	26	72	100	ND	ND	53	72
4	25	70	93	50	74	42	62

[a] Experiments demonstrate the day 14 myeloid/monocytic colony formation of 10^5 cells in the presence of nonadherent cells. The used concentrations or dilutions were: IL-3, 10 ng/ml; GM-CSF, 20 ng/ml; granulocyte-CSF, 1/1000; IL-7, 1/1000. Values represent the mean of triplicate cultures. One SD was 5 to 15% of the mean.

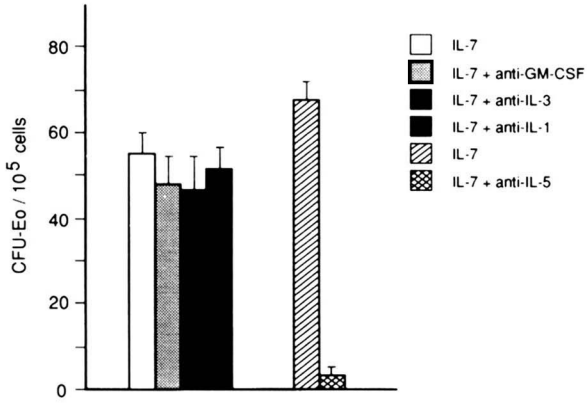

Figure 2. NAF-NT from three different bone marrow samples were cultured with IL-7 in the absence or presence of anti-GM-CSF, anti-IL-3, anti-IL-1, or anti-IL-5. Results are expressed as CFU-Eo/10^5 cells. Used dilutions were: IL-7, 1/1000; anti-GM-CSF, 1/1000; anti-IL-3, 1/1000; anti-IL-1, 1/1000; anti-IL-5, 1/300.

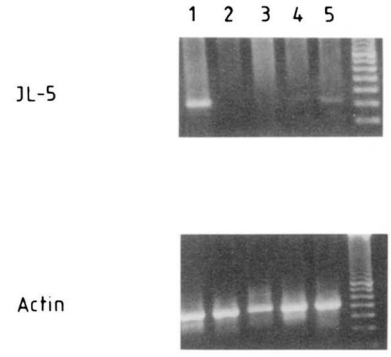

Figure 3. Agarose gels of PCR amplified mRNA from T cells stimulated with Con A plus PMA (*1*); T cells cultured with medium (*2*) or IL-7 during 18 h (*3*); NAF-NT cultured with medium (*4*), or IL-7 during 18 h (*5*).

DISCUSSION

IL-7 was originally described as a growth factor for T and B cells (9, 10). Our study extends these observations and demonstrates that IL-7 also acts on eosinophil progenitors from human bone marrow without affecting the granulocytic/macrophage or erythroid lineage. However, it appeared that the stimulatory effect of IL-7 was mediated by the endogenous release of IL-5 because anti-IL-5 did abrogate the supportive effects of IL-7 on the eosinophil colony formation, whereas with anti-IL-3, anti-IL-1, or anti-GM-CSF no change was observed.

It is unlikely that T cells or monocytes are the cells responsible for the production of IL-5 because their depletion did not influence the effects of IL-7. In addition IL-5 mRNA could exclusively be demonstrated in IL-7-stimulated nonadherent, non-T cells. Moreover $CD34^+$ cells could be stimulated by IL-7, suggesting that IL-7 stimulates early hematopoietic progenitor cells for the release of IL-5. These data indicate that IL-7 has a broader spectrum of activity than previously was supposed. A recent study of Alderson et al. (12) also indicated that IL-7 cannot be considered as a lineage-restricted growth factor for the lymphoid lineage because human monocytes exposed to IL-7 did secrete IL-6 and TNF-α. The observed effects of IL-7 to some extent resemble the effects of IL-1 on the myeloid lineage. IL-1 affects accessory cells, e.g., IL-1 induces the secretion of IL-1, IL-6, and G-CSF from human monocytes (22, 23). Moreover IL-1 supports the proliferation of $CD34^+$ cells which is mediated by the end endogenous release of GM-CSF (24).

Different CSF are known that stimulate the eosinophil progenitor cell at different stages of differentiation. In addition to the eosinophils, GM-CSF and IL-3 stimulate the myeloid/macrophage colony formation, whereas IL-5 stimulates exclusively the eosinophil progenitor cell (1–4). Moreover, the interactions between IL-5 and IL-3 or GM-CSF are additive, suggesting that IL-5 acts on a subpopulation of eosinophil precursor cells (4). Our results with IL-7 indicate that additional growth factors can also modulate the development of the eosinophilic lineage. However, this probably occurs indirectly by the endogenous release of IL-5. These results indicate that at least four hematopoietic growth factors influence the eosinophil progenitor cell. In view of these findings it will be of interest to study how far these factors can be differentially induced in lymphocytes and eosinophils (25). Limited information is available regarding the regulation of IL-3, GM-CSF, and IL-5 at the mRNA level. IL-3 and GM-

CSF can be induced in human T cells in response to anti-CD3 and IL-1 (26, 27). In murine Th cells it has been shown that a combination of GM-CSF and IL-5 are induced in response to anti-CD3 and IL-1 stimulation whereas IL-2 selectively induced IL-5 expression (28). It is conceivable that more activation signals exist, resulting in a selective secretion and subsequent action of these factors on eosinophil progenitors.

In summary, these data demonstrate that the growth supportive effects of IL-7 on the eosinophil progenitor cell from human bone marrow is mediated by IL-5.

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