Rapid Communication

Interleukin-7 Is a Growth Factor of Precursor B and T Acute Lymphoblastic Leukemia

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We investigated the proliferation-inducing effects of human recombinant interleukin-7 (IL-7) on acute lymphoblastic leukemia (ALL) cells. It is shown that IL-7 stimulates DNA synthesis in ALL cells of B-cell precursor (n = 5) as well as immature T-cell origin (n = 2). Cytogenetic analysis of the cells of four patients proliferating in IL-7-supplemented cultures established the leukemic descent of the IL-7-responsive cells. IL-7 binding experiments with the cells of one patient and with two ALL cell lines showed the presence of two types of IL-7 receptors: one with a high affinity (kd 29 to 51 pmol/L) and one with a low affinity (kd 2.3 to 76 nmol/L) for the ligand. We conclude that IL-7 is one of the cytokines involved in the complex regulation of ALL cell proliferation.

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Materials and Methods

Leukemic cells. Bone marrow or peripheral blood samples of seven untreated ALL patients (five BCP-ALL, two T-ALL) were obtained after informed consent. Immunophenotypic characteristics of these cases are listed in Table 1. Leukemic cells were recovered by Ficoll-Isopaque (Nygaaard, Oslo, Norway) separation. E-rosette forming T lymphocytes were then removed from the ALL samples by rosetting with 2-aminoethylisothiouronium bromide-treated sheep red blood cells (AET-SRBC) and Ficoll separation. This also was feasible in the two cases of T-ALL, as the leukemic cells did not form rosettes with the AET-SRBC. These cell samples contained less than 0.5% E-rosette–forming T cells. Subsequently, cells (10^6/mL culture medium) were incubated in 6-cm Petri dishes (Greiner, Alphen aan den Rijn, The Netherlands) for 1 hour at 37°C to remove plastic adherent monocytes from the cells samples. ALL cell samples were either used fresh or after cryopreservation using a controlled freezing apparatus (Planer Biomed, Sunbury-on-Thames, UK). BCP-ALL cell lines NALM-6 and ALL 202 were maintained in suspension culture as described.

Immunofluorescence and purification of ALL cells by fluorescence-activated cell sorting (FACS). Immunofluorescence procedures, flow cytometric analysis, and monoclonal antibodies (MoAbs) have been described in detail elsewhere. In a majority of the experiments ALL cells were further purified by cell sorting (FACS 440, Becton Dickinson, Mountain View, CA) before incubations in culture. To this end, BCP-ALL were stained with a mixture of CD10/CD19 MoAbs and goat-anti-mouse immunoglobulin (lg) coupled to fluorescein isothiocyanate. Residual CD3+ normal T lymphocytes that could potentially contaminate the cultures were removed from the T-ALL samples by cell sorting as described.

In vitro culture. DNA synthesis in culture was assessed by uptake of tritiated thymidine (‘H-ThdR, 2 Ci/mmol/L, Amersham, UK) essentially as described. However, a major modification is that in the present experiments a serum-free culture medium was used that consists of Iscove's modified Dulbecco's medium supplemented with human iron-saturated transferrin (7.7 × 10^-6 mol/L), insulin (1
Table 1. Induction of DNA Synthesis in ALL Cells

| Patient* | No Factor | IL-7 | IL-3 | IL-3 + IL-7 | Irradiated Cells (30 Gy) |
|----------|-----------|------|------|-------------|-------------------------|
| 1. Pro-B ALL† | 3,045 ± 374 | 6,713 ± 67† | 3,387 ± 84 | 6,592 ± 711 | 108 ± 30 |
| 2. Pre-pre-B ALL† | 3,186 ± 251 | 6,964 ± 396 | 9,023 ± 487 | 8,327 ± 774 | 108 ± 28 |
| 3. Pre-pre-B ALL | 17,738 ± 1,687 | 21,697 ± 889 | 8,440 ± 2,388 | 24,252 ± 1,236 | 124 ± 12 |
| 4. Pre-B ALL† | 2,455 ± 149 | 9,756 ± 360 | 3,075 ± 280 | 8,510 ± 442 | 45 ± 17 |
| 5. Pre-B ALL | 685 ± 83 | 25,916 ± 1,554 | 1,613 ± 132 | 28,201 ± 987 | 189 ± 35 |

*Immunologic subtypes of ALL:† Pre-B ALL: CD34+, CD19+, CD10+, Cyμ+, TdT†; pre-pre-B ALL: CD19+, CD10+, Cyμ+, SmIg-, TdT†; Pre-B ALL: CD19+, CD10+, Cyμ+, SmIg-, TdT†; immature thymocytic ALL: CD1+, CD2+, SmCD3+, CD4+, CD7-, TdT+ (case 6); CD1+, CD2+, SmCD3+, CD4+, CD7+, TdT+ (case 7).

†FACS purified cell samples (see Materials and Methods).

‡Mean dpm ± SD of 3H-TdR uptake in triplicate cultures.

μg/mL), bovine serum albumin (BSA, 15 mg/mL), cholesterol (8 μg/mL), linoleic acid (3 μg/mL), sodium selenite (10−6 mol/L) and β-mercaptoethanol (10−4 mol/L). For immunophenotypic and cyto genetic analysis of cultured cells, the cells were incubated at concentrations of 0.5 to 1.0 × 105/mL in the same medium in 12-mL culture tubes (Greiner).

Recombinant growth factors. Purified recombinant IL-7 (lot no. 1899-44-1, approximate activity 106 U/μg) used throughout these experiments was prepared at Immunex (Seattle, WA). In certain experiments, IL-7 containing COS cell supernatant (Genetics Institute, Cambridge, MA) was included in parallel. Pure recombinant IL-2 was a gift from Cetus Corp (Emeryville, CA) and added to the cultures at a concentration of 25 U/mL. Pure IL-3 and IL-4 containing COS cell supernatant were donations from the Genetics Institute. IL-3 was used at a concentration of 100 U/mL and IL-4 at a final dilution of 1:5,000, which optimally induced 3H-TdR uptake in IL-4-responsive T-ALL cells.

Radioiodination of IL-7 and binding experiments. The pure IL-7 was labeled with 125I using the Bolton-Hunter reagent (Amer sham), exactly as has been described for IL-3 and granulocyte macrophage colony-stimulating factor (GM-CSF).13 Sodium dodecyl sulfate polyacrylamide (15%) gel electrophoresis under reducing conditions and subsequent autoradiography did not indicate the presence of carrier proteins in the IL-7 preparation. Self-displacement analysis was performed to determine the specific radioactivity (61 to 185 × 103 cpm/ng) of the labeled IL-7 samples. Binding capacity of 125I-IL-7 was determined according to the method of Calvo et al.14 To estimate the number of IL-7 receptors and their affinity for the ligand, cells (0.5 to 1.0 × 106) were incubated for 1 hour at 37°C in 100 μL α minimal essential medium (α-MEM) + 1% wt/vol BSA with 10 to 3,500 pmol/L radiolabeled IL-7. Equilibrium binding of the 125I-IL-7 occurred under these conditions. To determine nonspecific binding, incubations in the presence of excess (300 pmol/L) nonlabeled IL-7 were performed in parallel. Experimental procedures and calculations were identical to those described previously.12 Two-affinity receptor analysis was performed using the ENZ FITTER data analysis program (Sigma Chemical Co, St Louis, MO).

RESULTS

IL-7-induced proliferative response in ALL cells. First, IL-7 titration experiments were performed with the cells of patients nos. 4 (pre-B ALL) and 6 (T-ALL) to assess the optimal concentration for stimulation of DNA synthesis (Fig 1). In both pre-B ALL and T-ALL cells 30 U/mL of IL-7, which is equivalent to approximately 170 pmol/L, induced a
Table 2. Cytogenetics of ALL Cells Cultured With IL-7

| Patient Type | Leukemic Karyotype | Ratio of Leukemic/Normal Metaphases in Cultured Cells |
|--------------|--------------------|------------------------------------------------------|
| 1. Pro-B ALL | 47, XY, 7q-, 13q-, +M | 15/0                                                 |
| 2. Pre-pre-B ALL | 51, XXY, +8, +17, +21, +21, 14q+ | 15/0                                                 |
| 3. Pre-B ALL | 47, XX, 1q-, 2p+, 3p+, 4p+, 6q-, +M | 15/0                                                 |
| 4. Pre-B ALL | 47, XX, 7p-, 11q- | 14/1                                                 |

After removal of E-rosette forming T lymphocytes and plastic adherent monocytes, cells were cultured in suspension in serum-free culture with 100 U/mL IL-7. Metaphase spreads were prepared after 2 to 3 days of culture as described.

maximal proliferative response. Increase of the IL-7 concentration up to $10^4$ U/mL did not further elevate $^3$H-TdR uptake. The IL-7 containing COS cell supernatant (Genetics Institute) optimally stimulated DNA synthesis at a dilution of 1:1,000. In subsequent experiments, IL-7 was used at a concentration of 50 U/mL. Data of $^3$H-TdR uptake assays in the seven cases of ALL are listed in Table 1. A significant proliferative response to IL-7 was evident in all cases with the exception of patient no. 3. The cells of the latter patient proliferated spontaneously, and addition of IL-7 to the culture only slightly elevated the rate of DNA synthesis. Because IL-3 has been reported to provoke a proliferative response in BCP-ALL, the effects of IL-7 on BCP-ALL were also tested in combination with IL-3. No additional or synergistic effects of IL-3 on the action of IL-7 were noted. Synergistic effects of IL-2 or IL-4 on the IL-7-induced proliferative response in the T-ALL cells also were not apparent.

Cytogenetic analysis of cells proliferating in IL-7-containing cultures. To rule out the possibility that the cells which responded to IL-7 were contaminant normal cells, cytogenetic analysis was performed on cells of four patients that had been cultured for 2 to 3 days in the presence of IL-7.

The data summarized in Table 2 demonstrate that only the leukemic cells entered metaphasis under the applied in vitro conditions.

$^{125}$I-IL-7 binding experiments. Binding of titrated concentrations of radiolabeled IL-7 to the ALL cells of patient no. 3 are shown in Fig 2 (left panel). Scatchard conversion of these data produced a biphasic plot, indicative of the existence of high and low affinity binding sites (Fig 2, right panel). Similar IL-7 binding characteristics were observed with the ALL cell lines NALM-6 and ALL 202. Estimations of IL-7 receptor numbers and affinities are listed in Table 3.

DISCUSSION

Insights into the mechanisms controlling the proliferation and maturation of normal and neoplastic human B- and T-cell precursors have remained limited. This has been due to a lack of purified growth stimuli that specifically act on early human lymphoid cells. Although evidence has been obtained for a stimulatory role of IL-3 in inducing DNA synthesis in certain cases of BCP-ALL, in another group of patients no consistent proliferation inducing effects could be attributed to a panel of recombinant growth factors that included the ILs 1, 2, 3, 4, and 6, as well as GM-, G-, and M-CSF. A similar deficiency in growth factor responses has been noted in T-ALL, although variable proliferative responses to IL-2 have been reported.

Based on the initial reports on the activity of murine IL-7, we speculated that the human homologue could be a candidate regulator of ALL cell proliferation. In the present study we have confirmed this suggestion and showed that IL-7 is capable of inducing a significant, although generally quite moderate, proliferative response in BCP-ALL as well as T-ALL cells. This relatively low responsiveness probably illustrates that IL-7 is only one of the factors among others controlling the growth of normal and neoplastic lymphoid precursors. The observations that IL-3 stimulated DNA synthesis in BCP-ALL patient nos. 2 and 4, and that some stimulatory effects of IL-2 and IL-4 in T-ALL case 6 were noted, support this idea. In view of this concept, the possibil-
ality that the ILs 1, 2, 4, 5, 6, and tumor necrosis factor cooperate with IL-7 in inducing a proliferative response in ALL cells also has been addressed. We have not yet obtained evidence for such synergistic actions between IL-7 and other factors in ALL (Table 1 and data not shown).

In a previous study in which a serum containing culture was applied, we encountered the outgrowth of contaminant normal cells as a serious problem for evaluating weakly induce/enhance the expression of CD1, induced maturation of the cells. Purification and characterization of a growth factor active on thymocytes proliferate in direct response to interleukin-7. Blood 74:1368, 1989

It has also been examined in this study as to whether IL-7 induced maturation of the ALL cells. In three cases of BCP-ALL, ie, patient nos. 1, 2, and 4, IL-7 appeared to weakly induce/enhance the expression of CD20 on the cell membrane (data not shown), which is of interest given the evidence that the CD20 antigen is involved in the activation of B cells. No other immunophenotypic alterations indicative of progressive maturation during culture, such as the acquisition of cytoplasmic or surface Ig, evidence for such synergistic actions between IL-7 and other factors in ALL (Table 1 and data not shown). Thus, we consider the maturation-inducing effects of IL-7 on ALL cells very limited.

In a previous study in which a serum containing culture assay had been applied, we encountered the outgrowth of contaminant normal cells as a serious problem for evaluating the proliferative response of BCP-ALL cells. Typically, the effects of a crude source of B-cell growth factors (BCGF) could only be measured when the ALL cells had been purified by cell sorting before culture. Without this purification step, only nonleukemic cells proliferated in response to BCGF. To circumvent this potential pitfall, we performed a major portion of our present experiments with FACS purified cell samples. However, it should be noted that under the currently applied serum-free conditions, exclusively the leukemic cells entered metaphase in IL-7 supplemented cultures, even when FACS purification had been omitted (Table 2).

The data of radiolabeled IL-7 binding experiments (Fig 2, Table 2) point toward the presence of both high and low affinity type IL-7 receptors on ALL cells. Presently we have no experimental data to explain the dual affinity characteristics of IL-7 receptors. One possibility that emerges is that this phenomenon reflects the involvement of di- or oligomerization of receptor chains in the formation of high affinity binding sites, and that low affinity binding occurs to single receptor chains, as has been proposed for a number of other growth factor receptors, eg, IL-2 receptors.

Over the years, the application of in vitro cultures for human leukemic lymphoid precursor cells has remained particularly troublesome due to the lack of well-defined assays. Our finding that IL-7 is one of the factors stimulating ALL cell proliferation illustrates that the current interest in the molecular cloning and large-scale production of new growth factors is of vital importance for the development of reliable culture assays for ALL.

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Table 3. 125I-IL-7 Binding to BCP-ALL Cells

| Sites/Cel| kd (pmol/L) | Sites/Cel| kd (pmol/L) |
|---------|-----------|---------|-----------|
| High Affinity Receptors | Low Affinity Receptors | | |
| BCP-ALL (patient 3) | 265 | 29.5 | 1,841 | 4.99 |
| NALM-6 | 87 | 51.5 | 1,431 | 2.33 |
| ALL-202 | 205 | 38.1 | 10,627 | 75.7 |
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