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CHARACTERIZATION OF HUMAN INTERLEUKIN-3 RECEPTORS ON A MULTI-FACTOR-DEPENDENT CELL LINE

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SUMMARY. Recombinant human interleukin-3 (hIL-3) was radioiodinated by Bolton-Hunter method with maintenance of biological activity. Using ^{125}I-hIL-3, hIL-3 receptors were characterized on a multi-factor-dependent cell line TF-1. Equilibrium binding studies revealed the existence of a single class of binding sites (667 ± 306 sites/cell) with a Kd of 173 ± 25 pM. Affinity labeling of TF-1 cells with ^{125}I-IL-3 yielded two bands of 150 kDa and 85 kDa, implying molecular weights of 135 kDa and 70 kDa for the hIL-3 receptors.

Interleukin-3 (IL-3) is a lymphokine which stimulates the proliferation and differentiation of a wide variety of hematopoietic progenitor cells (1). Murine IL-3 (mIL-3) was purified to homogeneity (2) and cDNA for mIL-3 was isolated (3, 4). In contrast, it had been difficult to prove the existence of a human homologue of mIL-3. Recently, a cDNA for hIL-3 was isolated on the basis of a significant homology of sequences between human and gibbon IL-3 (5). The cDNA for hIL-3 was shown to have a significant but low homology (29% at the amino acid level, 45% at the nucleotide level) with the sequence of mIL-3 (5).

The biological effects of the hematopoietic growth factors are known to be mediated through interactions with specific

The abbreviations used are: Il-3, interleukin-3; GM-CSF, granuloctyte-macrophage colony-stimulating factor.

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receptors on target cells. In the murine systems, mIL-3 receptors with high affinity have been identified on several murine cells (6,7,8,9), but little is known about hIL-3 receptors except for the reports using ^{35}S-metabolically labeled hIL-3 (10,11). We have made radioiodinated hIL-3 with Bolton-Hunter reagent and have established a novel human multi-factor-dependent cell line, TF-1 (12), and chracterized the hIL-3 receptor on TF-1 cells. This is the first report which has identified and characterized the specific receptors for hIL-3 by cross-linking using radioiodinated hIL-3.

MATERIALS AND METHODS

GROWTH FACTORS. Chemically synthesized hIL-3 and human granulocyte/macrophage colony-stimulating factor (GM-CSF) genes were expressed in E. Coli. These recombinant growth factors were extracted from E. Coli cells and purified to homogeneity.

CELL LINE AND CELL CULTURE. Human multi-factor-dependent cell line TF-1 was established from a patient with erythroleukemia (12). TF-1 cells, showing strict dependency on either human GM-CSF or IL-3, were cultured in RPMI 1640 medium (GIBCO, NY) containing GM-CSF (2 ng/ml) and 10% fetal bovine serum (HAZLETON, KS) at cell densities of 10^5-10^6 cells/ml.

PREPARATION OF RADIOIODINATED HUMAN IL-3. Purified hIL-3 was iodinated using a Bolton-Hunter reagent (ICN, CA) (13). Human IL-3 (5 µg) in 10 µl of sodium phosphate buffer (0.3M, pH 8.0) was incubated with 1 mCi of the Bolton-Hunter reagent for 15 hr at 4°C. After the incubation, the reaction was quenched by adding 50 µl of 5 mg/ml glycine in PBS (pH 6.5). Iodinated hIL-3 was separated from reactants by passing through a PD-10 column (Pharmacia, Uppsala, Sweden) with PBS (pH 6.5) containing 0.1% gelatin. The specific radioactivity of ^{125}I-IL-3 was determined by self-displacement analysis (14).

RADIORECEPTOR ASSAY. Cells were collected and washed three times in Hanks' balanced salt solution (20 mM Hepes, pH 7.4), and incubated with the ^{125}I-hIL-3 before resuspending in 200 µl of binding buffer (Hanks' balaced salt solution-20 mM Hepes, 0.1% BSA and 0.02% sodium azide, pH 7.4). After incubation, duplicate 80-µl cell suspensions were transferred onto 100 µl of n-butyl phtalate (Wako, Osaka, Japan) in 0.4-ml polyethylene centrifuge tubes. The cells were then sedimented and separated from the unbound ^{125}I-hIL-3. Specific binding was defined as the amount of binding blocked by competition with a 40-fold excess of unlabeled hIL-3.

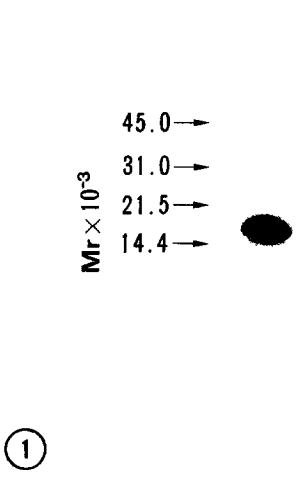
CROSS-LINKING OF RADIOIODINATED HUMAN IL-3 TO ITS RECEPTORS. ^{125}I-IL-3 was incubated with TF-1 cells (1 x 10^7) in the presence or absence of unlabeled hIL-3 for 2 hr at 15°C. After washing twice with ice-cold PBS, the cell surface proteins were cross-linked with 0.2 mM disuccinimidyl suberate (Pierce, IL) for 15 min at 0°C. The cells were solubilized by incubation with 100 µl of lysing buffer (1% Triton X-100, 50 mM Hepes-pH 7.4, 500 U/ml aprotinin, 1mM phenylmethylsulfonyl fluoride) for 15 min at

0°C. The cell extract (80 µl) was mixed with 1/5 volume of 5-fold concentrated Laemmli's sample buffer (15), and was boiled for 3 min in the presence of 50 mM dithiothreitol. Electrophoresis was performed by using 7.5% (W/V) polyacrylamide gel. After electrophoresis, the gel was fixed, stained with Coomassie blue, dried, and autoradiographed for 14 days at -70°C using a Kodac RP-XOMAT film.

RESULTS

PREPARATION AND CHARACTERIZATION OF RODIOIODINATED HUMAN IL-3

The chloramine-T and lactoperoxidase methods resulted in very low incorporations of ^{125}I into the hIL-3 (data not shown). In contrast, using the Bolton-Hunter reagent (13), hIL-3 was iodinated to high specific activity. The ^{125}I-IL-3 migrated as a single species (Fig. 1) with an apparent molecular weight of 15,000 on analysis by SDS-PAGE and autoradiography. The specific radioactivities of ^{125}I-IL-3 were determined by self-displacement analysis (14), and ranged from 0.5 x 10^5 to 1.2 x



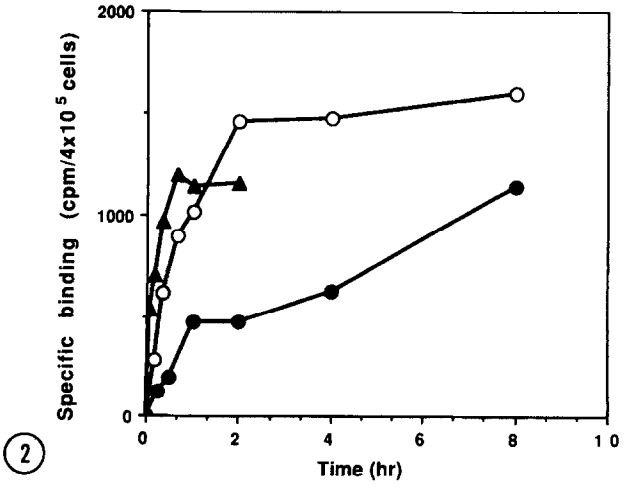
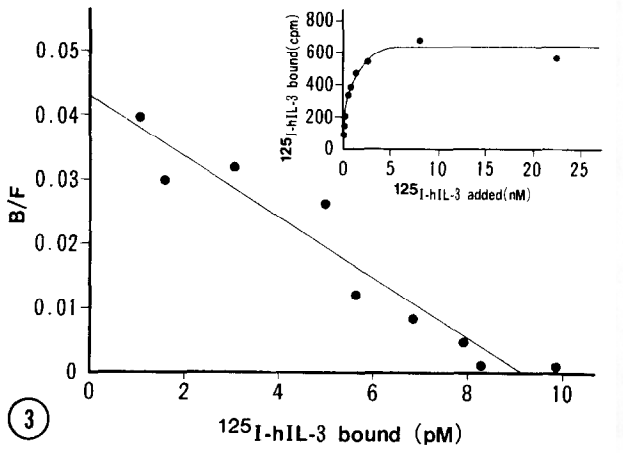


Figure 1. Characterization of radioiodinated hIL-3 by SDS-PAGE. ^{125}I-hIL-3 (3,000 cpm) was mixed with 20 µl of Laemmli's sample buffer (15), boiled for 3 min in the presence of 50 mM dithiothreitol and was electrophoresed through 7.5% polyacrylamide gel. The gel was fixed, stained with Coomassie blue, dried and autoradiographed for 2 days at -70°C using a Kodac RP-XOMAT film.

Figure 2. Time and temperature dependence of specific binding of radioiodinated hIL-3 to TF-1 cells. ^{125}I-hIL-3 (1 x 10^5 cpm) was incubated with TF-1 cells (1 x 10^6) in 200 µl of binding buffer at 4°C (●), 15°C (○) and 37°C (▲). At the indicated time, the specific binding of ^{125}I-hIL-3 was determined as described in the "Materials and Methods".



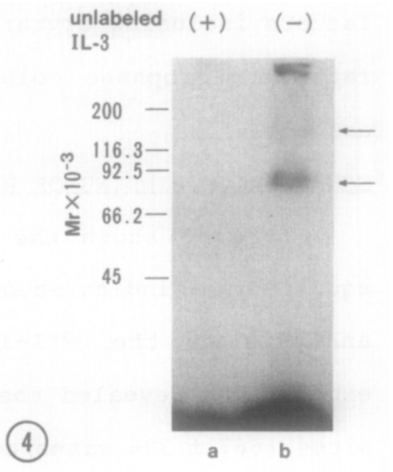


Figure 3. Equilibrium binding of ^{125}I-hIL-3 to TF-1 cells and Scatchard analysis of the binding data. TF-1 cells ($1x10^6$ cells) were incubated with increasing concentrations of ^{125}I-hIL-3 in the presence or absence of 40-fold unlabeled hIL-3 for 2 hr at 15°C. The experiments were performed three times, and this figure is one of the representative.

Figure 4. Affinity cross-linking of ^{125}I-hIL-3 to TF-1 cells. TF-1 cells ($1x10^7$ cells) were incubated with ^{125}I-hIL-3 ($5x10^5$ cpm) in the presence or absence of unlabeled hIL-3 for 2 hr at 15°C. The proceeding of affinity cross-linking, SDS-PAGE and autoradiography are described in the "Materials and Methods". Lane a: In the presence of unlabeled hIL-3. Lane b: In the absence of unlabeled hIL-3.

10^5 cpm/ng for different preparations. ^{125}I-IL-3 could be stored at 4°C for at least two weeks with no loss of specific binding capacity.

TIME- AND TEMPERATURE-DEPENDENCE OF THE BINDING OF HUMAN RADIOIODINATED HUMAN IL-3 TO TF-1 CELLS

Time-course of specific ^{125}I-IL-3 binding to TF-1 cells is shown in Fig. 2. Binding at 15°C reached to the steady-state at 2 hr. At 37°C, binding was rapid and appeared to be maximal by 40 min, and decreased slightly after that period. Binding at 4°C was less than that at 15°C even after 8 hr of incubation. All subsequent binding studies were performed for 2hr at 15°C.

SPECIFICITY OF THE BINDING OF RADIOIODINATED HUMAN IL-3 TO TF-1 CELLS

The specific binding of ^{125}I-hIL-3 to TF-1 cells was inhibited by unlabeled hIL-3 but not by 50nM of other hematopoietic

factors including erythropoietin, granulocyte colony-stimulating factor, macrophage colony-stimulating factor and GM-CSF (data not shown).

EQUILIBRIUM BINDING OF RADIOIODINATED HUMAN IL-3 TO TF-1 CELLS

Fig. 3 shows the characterization of hIL-3 receptors by equilibrium-binding study of ^{125}I-IL-3 to TF-1 cells. Scatchard analysis of the ^{125}I-IL-3 binding data from four binding experiments revealed the existence of a single class of binding sites (667 ± 306 sites/cell) with a Kd of 173 ± 25 pM.

AFFINITY CROSS-LINKING OF RADIOIODINATED HUMAN IL-3 TO TF-1 CELLS

The hIL-3 receptors on TF-1 cells were further characterized by affinity cross-linking. Fig. 4 demonstrates the autoradiogram analyzed by SDS-PAGE under reducing condition. It revealed two radioactive bands with molecular weights of approximately 150 kDa and 85 kDa, which was competed by unlabeled hIL-3.

DISCUSSION

It is known that several mIL-3-dependent cell lines express a relatively low number of receptors varying between 450 and 2500 sites/cell, except B6SUtA, which was found to express over 100,000 of high affinity binding sites for mIL-3 (7,16). In human systems, hIL-3 binding sites have been analyzed by metabolically labeled ^{35}S-hIL-3 (10,11). In the present report, we have shown the radioiodination of hIL-3 by Bolton-Hunter reagent. Since hIL-3 has only one tyrosine residue, iodination using the chloramine-T or lactoperoxidase method proved to be unsatisfactory. In contrast, using Bolton-Hunter method sufficient amounts of ^{125}I-molecule was reproducibly incorporated into hIL-3.

By using this iodinated hIL-3, the number and affinity of hIL-3 receptor on TF-1 cells were charcterized. The binding of

^{125}I-hIL-3 to TF-1 cells revealed the existence of a single class of binding sites (667 ± 306 sites/cell) with a Kd of 173 ± 25 pM, indicating that the binding characteristics of hIL-3 receptor on TF-1 cells were similar to those of murine and human IL-3 receptors as reported. Gesner et al. reported that GM-CSF competed the binding of the labeled hIL-3 to KG-1 cells (10), but we could not observe this interaction with TF-1 cells. Since we examined the binding of ^{125}I-hIL-3 using TF-1 cells maintained with the medium containing GM-CSF, there was a possibility that the effect of GM-CSF to TF-1 cells was remained.

Using the ^{125}I-hIL-3, the basic structural information of hIL-3 receptors could be obtained. Affinity cross-linking studies suggested the similarity between murine and human IL-3 receptors. The electrophoretic patterns of mIL-3-mIL-3 receptors complexes demonstrated a major 90-kDa band, implying a molecular weight of 65 kDa for the mIL-3 receptor (6,7,8,9). In addition, a minor band was observed in murine systems at the position of the higher molecular weight of 138-200 kDa (6,8). Recently it has been suggested that the 140-150 kDa membrane glycoprotein was phosphorylated on tyrosine upon mIL-3 binding to its receptors (17,18). Furthermore, Isfort et al. suggested that the affinity labeled minor band at the higher moleculer weight is related to the protein to be phosphorylated (18). The correlation between 135-kDa molecule on TF-1 and 140-150 kDa membrane protein which was phosphorylated is remained to be elucidated.

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