Conversion of Interleukin-13 into a High Affinity Agonist by a Single Amino Acid Substitution*

Received for publication, November 23, 1999, and in revised form, February 22, 2000

Yasuo Oshima, Bharat H. Joshi, and Raj K. Puri‡

From the Laboratory of Molecular Tumor Biology, Division of Cellular and Gene Therapies, Center for Biologies Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892

We created a novel mutated form of human interleukin-13 (IL-13) in which a positively charged arginine (R) at position 112 was substituted to a negatively charged aspartic acid (D). This mutant, termed IL-13R112D, was expressed in Escherichia coli and purified to near homogeneity. IL-13R112D was found to be a potent IL-13 agonist with 5–10-fold improved binding affinity to IL-13 receptors compared with wild-type IL-13 (wtIL-13). The conclusion of IL-13 agonist activity was drawn on the basis of approximately 10-fold improved activity over wtIL-13 in several assays: (a) inhibition of CD14 expression in primary monocytes; (b) proliferation of TF-1 and B9 cell lines; and (c) activation of STAT6 in Epstein-Barr virus-immortalized B cells, primary monocytes, and THP-1 monocytic cell line. Furthermore, mutant IL-13R112D neutralized the cytotoxic activity of a chimeric fusion protein composed of wtIL-13 and a Pseudomonas exotoxin A (IL-13-PE38) approximately 10 times better than wtIL-13. Based on these results, it was concluded that IL-13R112D interacts with much stronger affinity than wtIL-13 on all cell types tested and that Arg-112 plays an important role in the interaction with its receptors (IL-13R). Thus, these results suggest that IL-13R112D may be a useful ligand for the study of IL-13 interaction with its receptors or, alternatively, in designing specific targeted agents for IL-13R-positive malignancies.

IL-13 is a pleiotropic cytokine that plays a major role in immune response and inflammation (1–3). It can inhibit production of proinflammatory cytokines IL-1, IL-6, and tumor necrosis factor-α and down-regulate the expression of CD14 (a lipopolysaccharide receptor) on monocytes (4). It can also cause the generation of antigen-presenting dendritic cells in combination with granulocyte-macrophage colony-stimulating factor (5). IL-13 also plays a major role in B cells. It can up-regulate CD23, CD72, major histocompatibility complex class II and surface IgM on B cells, drive IgE class switch, and induce production of immunoglobulins by B cells (6).

The function of IL-13 is accomplished through interaction with its plasma membrane receptors (7–9). The IL-13R complex can exist in three different types. In type I IL-13R, IL-13 forms a complex with IL-4Rβ chain (also known as IL-4Rαc, IL-13Rαc (also known as IL-13Rα1), and IL-13Rα (also known as IL-13Rα2). This type of receptor complex is expressed in some non-hematopoietic tumor cell lines such as renal cell carcinoma, AIDS-associated Kaposi’s sarcoma, and glioblastoma multiforme (7, 10–14). In type II IL-13R complex, IL-13Rα chain is not present, and IL-13 binds to IL-4Rβ and IL-13Rα chains. Type II IL-13R complex is expressed on some non-hematopoietic malignant cells such as A431, PA-1, and HT-29 (13–15). In the third type of IL-13R complex, IL-4Rβ and IL-13Rα chains may associate with IL-2Rγ chain (termed γc), shared between the IL-4R, IL7R, IL9R, and IL15R system (16). Type III IL-13R are present on hematopoietic cells such as human erythroblasts cell line TF-1 and healthy human primary monocytes (7, 13–15). Although γc does not interact with IL-13 directly, it modulates IL-13R function through down-regulation IL-13Rα and, to some extent, IL-13Rα chains (17, 18). IL-4R system, which is related to IL-13R system, also exists in three different types. In type I IL-4R, IL-4Rγ chain forms a complex with γc. In type II IL-4R, IL-4Rγ chain forms a complex with IL-13Rα chain, and in type III IL-4R, all three chains are present (7, 19–22). These studies suggested that IL-4Rγ and IL-13Rα chains are shared between IL-4R and IL-13R systems (23).

We have previously reported that a variety of human solid tumor cells express elevated levels of IL-13Rs (7, 10–12). To target these receptors, we produced a chimERIC protein (IL-13-PE38QQR) composed of wtIL-13 and a mutated form of Pseudomonas exotoxin (PE38QQR). This cytotoxin is highly cytotoxic to IL-13R-expressing cells (10–12). However, the binding affinity of IL-13-PE38QQR was 10 times lower than wtIL-13 (10). To improve the binding affinity of IL-13, we proposed to generate IL-13 muteins by site-directed mutagenesis in which single amino acid substitutions were introduced in the critical region of IL-13 molecule. The selection of single amino acid substitutions was based on structural similarities and reported mutations in a similar cytokine, IL-4. We created and purified human IL-13R112D (in which arginine 112 was changed to aspartic acid) and analyzed its various activities on a variety of cell types. We also tested the capability of IL-13 mutant to inhibit cytotoxicity mediated by IL-13 cytotoxin, IL-13-PE38. We conclude on the basis of these results that IL-13R112D is approximately 5–10 times superior to wtIL-13 in all respects.

EXPERIMENTAL PROCEDURES

Materials—Restriction endonucleases and DNA ligase were obtained from New England Biolabs (Beverly, MA), Life Technologies, Inc., Panvera (Madison, WI), and Roche Molecular Biochemicals. Fast protein

*This work was supported in part by a subsidy from the Ryoichi Naito Foundation for Medical Research to Dr. Yasuo Oshima. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡To whom correspondence should be addressed: Laboratory of Molecular Tumor Biology, Division of Cellular and Gene Therapies, Center for Biologies Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892.Tel.: 301-827-0471; Fax: 1-301-827-0449; E-mail: Purib@ecber.fda.gov.

The abbreviations used are: IL-13, interleukin-13; IL-13R, IL-13 receptor; hIL, human IL; wtIL-13, wild-type IL-13; IL-13-PE38, a recombinant fusion protein composed of wtIL-13 and a truncated form of Pseudomonas exotoxin A; γc, IL-2Rγ chain: IL-13R112D, IL-13 mutant in which arginine 112 was changed to aspartic acid; EMSA, electrophoretic mobility-shift assay; EBV, Epstein-Barr virus.
liquid chromatographic columns and media were purchased from Amersham Pharmacia Biotech. Sequence specific oligonucleotide primers were synthesized at Bioserve Biotechnologies (Laurel, MD). Advantage-HF polymerase chain reaction (PCR) kit was from CLONTECH (Palo Alto, CA).

The pET based expression vector with ampR gene was used for construction of mutein clone. Plasmids were amplified in Escherichia coli (DH5α high efficiency transformation) (Life Technologies, Inc.), and DNA was extracted using Qiagen kits (Chatsworth, CA). TF-1 human erythroleukemia cell line was obtained from ATCC (Manassas, VA), and were cultured in human granulocye-macrophage colony-stimulating factor. B9 mouse plasmacytoma cell line was a kind gift of Giovana Fosato (Center for Biologies Evaluation and Research, Food and Drug Administration, Bethesda, MD), and were grown in human IL-6. PM-RCC renal cell carcinoma cell line was established in our laboratory (19). THP-1 cells were kindly provided by Dr. Ray Donnelly (Center for Biologies Evaluation and Research, Food and Drug Administration, Bethesda, MD). Monocytes were isolated from the peripheral blood mononuclear cells of donors seronegative for human immunodeficiency virus and hepatitis viruses after leukapheresis and purified by countercurrent centrifugal elutriation.

Homology Search and Secondary Structure Analysis of IL-13—A computer program, GCG (Genetics Computer Group, Inc., Madison, WI) was used for a homology search, data base search, and prediction of secondary structure of IL-13 on Silicon Graphics Workstation in Human Genome Center, the Institutes of Medical Science, University of Tokyo (Tokyo, Japan) and the Center for Information Technology, National Institutes of Health (Bethesda, MD). The protein sequence of mouse (IL-13_mouse.swissprot), rat (l26913.gb), Homo sapiens (Caf043334.1.gp main), and Bos taurus (bta132441.1.gp.main) were
obtained from Swiss Prot and GenBank. Since l26913.gb is not a protein sequence but a cDNA sequence, it was used for a homology search after translation into a protein sequence. The IL-13 sequences are shown after deletion of predicted signal sequence of Homo sapiens or the equivalent (see Fig. 1). Hydrophobicity and secondary structure of IL-13 was predicted by the Kyte-Doolittle method and Chou-Fasman method, respectively (24).

**Construction of Plasmids Encoding IL-13R112D**—The mutagenesis of IL-13 gene was performed with a cDNA as a template using sense primer 5'-taa ttt gcc cat atg tcc cca ggc cct gtg cct-3' and antisense primer 5'-taa ttt gcc cga att cag ttg aag tct ccc tcg cg-3' to mutate Arg-112 to Asp (R112D) and incorporate NdeI and EcoRI restriction enzyme sites at 5'- and 3'-termini, respectively. After subcloning the polymerase chain reaction products into pCR2.1 (Invitrogen®, Carlsbad, CA), the plasmid was digested with NdeI and EcoRI. The fragment was inserted into a prokaryotic pET-based expression vector digested with same restriction enzymes. We confirmed the existence of mutation and restriction sites by sequencing of the plasmid.

**Expression and Purification of Recombinant Proteins**—Expression and purification of IL-13R112D and wtIL-13 was carried out by essentially similar techniques as previously reported for IL-4 (25). In the present set of experiments, we used BL21(lDE3)pLys E. coli that contains T7 RNA polymerase under the lac promoter operator in its genome. The protein expression was induced by adding 1 mM isopropyl-b-D-thiogalactopyranoside. wtIL-13 and IL-13R112D were produced in inclusion bodies. After washing, inclusion bodies were solubilized, refolded, and purified by fast protein ion-exchange liquid chromatography. The purified protein showed a single band at 13 kDa in Coomassie Blue-stained SDS-polyacrylamide gel (Fig. 2). In this study, we used IL-13-PE38 fusion protein, which was expressed in E. coli and purified as described previously (26).

**Cell Proliferation Assays**—Proliferation assays were performed as described previously (27). Briefly, TF-1 and B9 cells were washed 2–3 times to remove granulocyte-macrophage colony-stimulating factor and IL-6, and then 1 × 10^3 to 5 × 10^4 cells were cultured in 96-well plates in RPMI complete medium containing 10% fetal bovine serum. Varying concentrations of wtIL-13 and IL-13R112D were added to the wells, and the cells were cultured for 1–2 days. Tritiated thymidine (0.5 mCi) was added to each well 6–9 h before the plates were harvested in a Skatron cell harvester (Skatron, Inc., Sterling, VA). Filter mats were counted in a β plate counter (Wallac, Gaithersburg, MD).

**Protein Synthesis Inhibition Assay**—Protein synthesis inhibition assay was performed as described previously (28). In brief, 1 × 10^3 PM-RCC cells were cultured in 96-well plates in leucine-free medium (Biofluids, Rockville, MD) for 4 h to allow adherence to flat-bottomed microtiter plates.
The DNA-protein complexes were resolved on a 5.0% non-denaturing PAGE gel and visualized by autoradiography of the dried gel.

These cells then received various concentrations of IL-13-PE38 incubated for 20–24 h at 37 °C and then 1 μCi of [3H]leucine (NEN Life Science Products) was added to each well and cultured for an additional 4 h. For blocking experiments, 2 μg/ml of wtIL-13 or IL-13R112D was added before the addition of IL-13-PE38. Finally, cells were washed and harvested on a fiberglass filter mat, and cell-associated radioactivity was measured in a β plate counter. The concentration of IL-13-PE38 at which 50% inhibition of protein synthesis (IC50) occurred was calculated.

IL-13 Receptor Binding Studies—Recombinant human IL-4 and rhIL-13 were labeled with 125I (Amersham Pharmacia Biotech) by using IODO-GEN reagent (Pierce) according to the manufacturer’s instructions. The specific activity of radiolabeled IL-4 and IL-13 ranged from 41,500 to 220,000 cpm/μg and 17.6 to 18.0 cpm/μg, respectively. The equilibrium binding studies were carried out as described elsewhere (7, 19). Briefly, 1 × 106 cells in 100 μl of binding buffer were incubated at 4 °C for 2 h with [125I]IL-4 (500 pM) or [125I]IL-13 (500 pM) in the absence or presence of increasing concentrations (10 pM-200 nM) of unlabeled wtIL-13 or IL-13R112D. Duplicate samples of cells associated with [125I]IL-4 or [125I]IL-13 were separated from free [125I]IL-4 or [125I]IL-13 by centrifugation through a cushion of phthalate oils. The cell pellets were counted in a γ counter (Wallac, Gaithersburg, MD).

Electrophoretic Mobility-shift Assay (EMSA)—EMSA was performed as described before (21, 23). After incubation with various concentrations of wtIL-13 or IL-13R112D for 10 min, THP-1 cells, primary monocytes, and Tory EPV-immortalized B cells (a gift from Dr. Giovanna Tosato, Center for Biologies Evaluation and Research, Food and Drug Administration) were washed with cold phosphate-buffered saline and solubilized with cold whole-cell extraction buffer (1 mM MgCl2, 20 mM HEPES, pH 7.0, 10 mM KCl, 300 mM NaCl, 0.5 mM dithiothreitol, 0.1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na3VO4, and 20% glycerol). DNA-protein interactions were assessed by EMSA using the bandshift kit from Amersham Pharmacia Biotech. Briefly, 40 μg of sample proteins were incubated for 20 min at room temperature with 1 ng of 32P-labeled double-stranded oligonucleotide probe STAT-binding element-1 (sense oligo 5′-gat cgc tct tct tcc tcc gaa ctc aat g-3′, antisense oligo 5′-tgc aca ttg act ttc tcc tgg gaa gaa gag c-3′) in binding buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.5 mM dithiothreitol, 10% glycerol, 0.05% Nonidet P-40, 0.05 mg/ml poly(dI-dC)2). In some experiments, a 200-fold excess of unlabeled ILs. Total [125I]IL-13 (A) and [125I]IL-4 (B) bound to PM-RCC at 2962 ± 123 and 1706 ± 141 cpm ± SD, respectively. Data are represented as mean ± S.D. of duplicate determination; bars, S.D., shown when larger than symbols.

**Fig. 5.** Wild-type IL-13 and IL-13R112D activate STAT-6. EMSAs were performed to determine whether signaling of IL-13R112D is stronger than wtIL-13. Primary monocytes (A), monocytic cell line THP-1 (B), or EBV-immortalized B cells (C) were treated with or without 1, 10, or 50 ng/ml wtIL-13 or IL-13R112D, and cellular extracts were prepared after 15 min (A) or 10 min (B and C) post-treatment. The activation of STAT-6 by IL-13 or its mutant was determined by EMSA using a radioactively labeled STAT-6-specific probe, STAT-binding element-1. The DNA-protein complexes were resolved on a 5.0% non-denatured PAGE gel and visualized by autoradiography of the dried gel.

**Fig. 6.** Inhibition of binding of [125I]IL-13 and [125I]IL-4 on PM-RCC cells. Cells (1 × 106) were incubated with 500 pm [125I]IL-13 (A) or 500 pm [125I]IL-4 (B) with or without increasing concentrations (up to 10 nM) of wtIL-13 or IL-13R112D. Bound radioactivity was determined as described under “Experimental Procedures.” Data are presented as the mean percentage of maximal specific binding without unlabeled ILs. Total [125I]IL-13 (A) and [125I]IL-4 (B) bound to PM-RCC at 2962 ± 123 and 1706 ± 141 cpm ± SD, respectively. Data are represented as mean ± S.D. of duplicate determination; bars, S.D., shown when larger than symbols.
and the assay was repeated several times. Data are means; cultured with various concentrations of wtIL-13 or IL-13R112D (0, 0.1–1000 ng/ml) or without 2000 ng/ml wtIL-13 or IL-13R112D.

B: cultured with various concentrations of IL-13-PE38 (0, 0.1–1000 ng/ml) for 2 h and autoradiographed overnight at room temperature.

The crystal structure of IL-13 is not known. According to the conventional prediction algorithm, which predicts approximately 56% accuracy, there may be four major α-helices (A, B, C, and D) in the IL-13 molecule. The secondary structure of IL-13 was predicted based on the homology with IL-4 (1) and multiple alignments between human IL-4, human IL-2, granulocyte-macrophage colony-stimulating factor, granulocyte-colony-stimulating factor, and growth hormone (29).

Based on these predictions, the locations of α-helices A and D were found to be at similar positions, whereas the location of α-helices B and C could not be accurately predicted in these models. The sequence homology alignment of IL-13 between species revealed that human IL-13 sequence is prominently conserved between four species (Fig. 1A). For example, the consensus sequence shows that a total of 87 amino acid residues are highly conserved between species. It has been shown that a cluster of amino acid residues in α-helix A, B, and C of IL-4 binds to IL-4Rβ chain, whereas a functional cluster that interacts with αc is suggested to be located in helix A and D, particularly amino acids Ile-11, Asn-15, Arg-121, Tyr-124, and Ser-125 (30–32). Since most (approximately 70%) of the conserved and charged residues are located in predicted α-helix regions of IL-13, it is hypothesized that these residues may be essential in IL-13 binding to its receptors. Because IL-4Rβ chain and IL-13Rα chains are shared between IL-4R and IL-13R systems and IL-2Rγ chain does not interact with IL-13 (15, 23), it was predicted that amino acids in helix D interact with IL-13Rα chain. We further hypothesized that 1) hydrophilic residues (with acidic or basic side chains) are usually exposed at the surface of a protein that might be expected to be involved in receptor binding and 2) highly conserved residues between species might be important for IL-13 to be an IL-13. Based on these hypotheses and the fact that the residue R112 of human IL-13 is hydrophilic and positive charge at this position is conserved between three species, we decided to mutate this amino acid and test the biological activities (Fig. 1B).

WtIL-13 and IL-13R112D were expressed and purified in an identical manner. As shown in Fig. 2 (lanes 1 and 2 in A and B), isopropyl-β-D-thiogalactopyranoside induced protein expression very efficiently. Purification of proteins from inclusion bodies revealed a major band of 13 kDa (Fig. 2, lane 3 of A and B). Upon purification on cation exchange chromatography, a highly purified protein (>95% pure) was obtained (Fig. 2, lane 4 of A and B). Thus, one-step purification provided highly purified proteins. One liter of each bacterial culture yielded several milligram of each type of pure proteins. To confirm the identity, the protein was shown to react with anti-human IL-13 antibodies on Western blot analysis (results not shown). In some purifications, we also observed a minor 26-kDa protein in SDS-gel that is regarded as dimerized IL-13R112D or wtIL-13 (results not shown).

Proliferation Activity of wtIL-13 and IL-13R112D on Hematopoietic Cell Lines—After purification of wtIL-13 and IL-13R112D, the goal was to compare their biological activities on various cell types that express different types of IL-13R. First we tested their mitogenic activity. wtIL-13 has been shown to induce proliferation of TF-1 human erythroleukemia cell line (26, 33). We tested the proliferative activity of IL-13R112D on TF-1 cell line. As shown in Fig. 3A, proliferative activity of IL-13R112D was more than 10 times better than induced by wtIL-13. The concentration of wtIL-13 that produced half-maximal proliferation (ED50) was about 2 ng/ml compared with less than 0.2 ng/ml for IL-13R112D. Similarly, IL-13R112D stimulated mouse plasmacytoma cell line B9 much stronger than wtIL-13. IL-13R112D was 5.7–19-fold better than wtIL-13 in proliferation assays. Thus, proliferation activity of IL-13R112D on hematopoietic cells that express type III IL-13R is about one log greater than wtIL-13.

Down-regulation of CD14 Expression on Monocytes by IL-13R112D—Since IL-13 has been shown to down-regulate CD14 expression on monocytes (4), we investigated whether IL-13R112D mutant has stronger activity compared with wtIL-13. As shown in Fig. 4, IL-13R112D and wtIL-13 suppressed CD14 expression on monocytes in a dose-dependent manner. IL-13R112D was 10 times superior to wtIL-13 in down-regulation of CD14. For example, 1 ng/ml IL-13R112D induced down-regulation of CD14, which was similar to that induced by 10 ng/ml wtIL-13.

STAT 6 Activation in THP-1 Cells, Monocytes, and EBV-immortalized B Cells—IL-13 has been shown to phosphorylate and activate STAT6 protein for signal transduction in various cell types (21, 23, 34, 35). Therefore, we compared the strength of wtIL-13 and IL-13R112D in the stimulation of STAT6 in monocytic cell line THP-1, primary monocytes, and EBV-immortalized B cell line, which express type III IL-13R (13, 14). The cytokine concentration at which IL-13R112D and wtIL-13 stimulated maximal activation of STAT6 in primary monocytes (Fig. 5A) or EBV-immortalized B cells (Fig. 5C) were 10 ng/ml and 50 ng/ml, respectively. However, one-tenth of IL-13R112D could easily stimulate STAT6 in THP-1 cells as compared with
High Affinity Interleukin-13 Agonist

wtIL-13 (Fig. 5B). These studies demonstrate that IL-13R112D has approximately 5–10 times better activities than wtIL-13 on human mononuclear cells and B lymphocytic cells.

Inhibition of [125]IL-13 and [125]IL-4 Binding by IL-13 Mutant—The activity of the IL-13 mutant to compete for [125]IL-13 and [125]IL-4 binding was next tested in PM-RCC cells, which express type I IL-13R. The concentration of IL-13R112D that inhibited [125]IL-13 binding by 50% (ED50) was 150 pm as compared with 650 pm by wtIL-13 (Fig. 6A). Similar results were obtained in another solid tumor cell line (U251) (data not shown). On the other hand, the ED50 of wtIL-13 and IL-13R112D to replace [125]IL-4 binding was 800 pm and 100 pm, respectively (Fig. 6B). Thus, IL-13R112D interacted with higher affinity with IL-4R and IL-13R than wtIL-13.

Inhibition of IL-13 Toxin Mediated Cytotoxicity by IL-13 Mutant—We previously demonstrated that IL-13 toxin (IL-13-PE38QQQR) is specifically highly cytotoxic to the PM-RCC cell line (10). To determine the superiority of IL-13R112D over wtIL-13, we compared the activity of wtIL-13 and IL-13R112D as it displaced cytotoxicity mediated by IL-13-PE38 in PM-RCC cells. As shown in Fig. 7A, IL-13R112D appeared to be better than wtIL-13 in blocking the cytotoxicity of IL-13-PE38. IL-13-PE38 was highly cytotoxic to these cells with a concentration that inhibited protein synthesis by 50% (IC50) was less than 0.1 ng/ml. In the presence of 2 μg/ml wtIL-13, the IC50 increased to 60 ng/ml, whereas in the presence of IL-13R112D, the IC50 reached to 105 ng/ml. To carefully determine the extent of superiority of IL-13R112D in blocking cytotoxicity of IL-13-PE38, we used varying concentrations of cytokines in the presence of a fixed concentration of IL-13-PE38 (Fig. 7B). In this assay, IL-13R112D appeared to be approximately 10 times better than wtIL-13 in blocking the cytotoxicity (Fig. 7B).

IL-13 is a central mediator of asthma and may play a major role in cancer biology, because a variety of solid tumor cells express abundant numbers of receptors for IL-13 (3, 7, 10–12, 17, 18, 21, 36–40). We hypothesize that elimination of IL-13-expressing cells may provide therapeutic benefit for disease processes where IL-13 and IL-13R are involved. To achieve that goal, we produced a IL-13 cytotoxin that eliminated IL-13R-expressing cells, but this molecule had 10-times lower binding affinity to IL-13 than wtIL-13 (10). To improve the binding affinity, in the current study we made IL-13R112D that not only bound better than wtIL-13 to IL-13R but turned out to be a IL-13 agonist with improved binding activity and cytotoxicity to IL-13R-expressing cells.

Acknowledgment—We thank Pamela Leland for technical assistance, Drs. S. Rafat Husain and Koji Kawakami for providing radiolabeled IL-13, and Drs. Ray Donnelly and Subhash Dhawan for critical review of the manuscript.

REFERENCES

1. Minty, A., Chalon, P., Derozo, J. M., Dumont, X., Guillotet, J. C., Kaghad, M., Labit, C., Leplatois, P., Liazuzun, P., Miloux, B., Minty, C., Casellas, P., Loison, G., Lupker, J., Shire, D., Ferrara, P., and Caput, D. (1993) Nature 362, 248–250
2. McKenzie, A. N. J., Culpepper, J. A., Malefyt, R. D., Briere, F., Pumponnen, J., Aversa, G., Sato, A., Dang, W., Cocks, B. G., Menon, S., Devries, J. E., Banchereau, J., and Zarrawski, G. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3725–3739
3. Wille-Karp, M., Luytimari, J., Xu, X. Y., Schofield, B., Neben, T. Y., Karp, C. L., and Donaldson, D. D. (1998) Science 282, 2258–2261
4. Cosentino, G., Soprana, E., Thiennes, A. C., Siccardi, A. G., Viale, G., and Vercelli, D. (1995) J. Biol. Chem. 270, 8797–8804
5. Vila, N., Lefort, S., Laurent, P., Caput, D., and Ferrara, P. (1995) J. Biol. Chem. 270, 3512–3517
6. Murata, T., Obiri, N. I., Gill, P., Zheng, T., Pastan, I., Debinski, W., and Puri, R. K. (1997) Clin. Cancer Res. 3, 151–156
7. Obiri, N. I., Debinski, W., Leonard, W. J., and Puri, R. K. (1995) J. Biol. Chem. 270, 3626–3632
8. Friberg, T., Obiri, N. I., DeBanti, G. N. J., Culpepper, J. A., Briere, F., Punnonen, J., McKenzie, A. N. J., Menon, S., Devries, J. E., Banchereau, J., and Zarrawski, G. (1993) J. Biol. Chem. 268, 13869–13874
9. Zurawski, S. M., Chomarat, P., Djossou, O., Bidaud, C., McKenzie, A. N. J., Menon, S., Debinski, W., and Puri, R. K. (1997) J. Clin. Cancer Res. 3, 151–156
10. Obiri, N. I., Debinski, W., and Puri, R. K. (1998) J. Biol. Chem. 273, 20251–20258
