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Expression of Interleukin 13 Receptor in Glioma and Renal Cell Carcinoma: IL13Rα2 as a Decoy Receptor for IL13

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SUMMARY: Glioma and renal cell carcinoma (RCC) cells express high affinity interleukin 13 (IL13) binding sites, but only RCC cell proliferation was inhibited by IL13. Both of these two cell types are IL2-receptor γc chain-negative. We thus used these cell models to investigate the patterns of expression of IL13Rα1, IL13Rα2, and IL4Rα chains and the role of IL13Rα2 in the response to IL13. Using new specific antibodies and flow cytometry, we observed a similar surface expression of IL4Rα and IL13Rα1 chains in most RCC and glioma cells, whereas IL13Rα2 was only present on five of six glioma cell lines. In all glioma cell lines, the amount of IL13Rα2 expression was 10 to 30 times higher than that of the two other chains. Although there was no surface or intracellular expression of IL13Rα2, its mRNA was detected in three of seven RCC cell lines. The expression on RCC cells of IL13Rα2 mRNA and/or that of high-affinity IL13 binding sites is not sufficient to predict IL13Rα2 protein expression. Blocking experiments showed that IL4 and IL13 strongly inhibited RCC cell proliferation through a unique receptor composed of IL4Rα and IL13Rα1 chains. Using RCC cells stably transfected with IL13Rα2 cDNA, we showed that the overexpression of IL13Rα2 decreased the response to IL13 but not that to IL4. Our results demonstrate that IL13Rα2 acts as a decoy receptor for IL13 and that it may exert a tight regulation of IL13 activity without impairing the IL4 response of the same cell target. (*Lab Invest 2001, 81:1223–1231*).

Interleukin 13 (IL13) is an immune regulatory cytokine that is produced predominantly by activated Th2 T cells, mast cells, and basophils. It shares many biological properties with IL4 (Zurawski and de Vries, 1994). However, in contrast to IL4, IL13 has no direct effect on T cells. In addition to its major role in inflammatory diseases (Wills-Karp et al, 1998), IL13 may play a prominent role in tumor growth; binding experiments show that high-affinity IL13 receptors (IL13R) are abundant in various human carcinoma and glioma cell lines (Debinski et al, 1999; Maini et al, 1997; Murata et al, 1997b; Obiri et al, 1995; Serve et al, 1996). The cell growth of renal and breast carcinoma cells, but not that of glioma cells, is inhibited by IL13, although all express high-affinity IL13R (Debinski et al, 1995; Obiri et al, 1996; Serve et al, 1996). Receptor cross-competition studies of hematopoietic cells have suggested that receptors for IL4 and IL13 share a common component, the IL4Rα chain (Tony et al, 1994; Zurawski et al, 1995). This was confirmed by biochemical studies showing that IL13R triggering induces the recruitment and tyrosine phosphorylation of IL4Rα (Rolling et al, 1996; Smerz-Bertling and Duschl, 1995; Welham et al, 1995). The genes for two IL13Rα chains have been cloned from mice and humans. The IL13Rα1 chain alone specifically binds IL13 with a low affinity. In association with the IL4Rα chain, it forms a high-affinity IL13R (Aman et al, 1996; Gauchat et al, 1997; Hilton et al, 1996; Miloux et al, 1997), and, thus, IL13Rα1 is essential for IL4/IL13 mediating effects (Murata et al, 1998; Orchansky et al, 1997). The second IL13Rα chain, termed IL13Rα2, binds to IL13 with higher affinity than does IL13Rα1. Coexpression of IL13Rα2 with IL4Rα does not allow the response to IL13, and its coexpression with the IL4Rα/IL13Rα1 complex does not modify the affinity of this complex for IL13 (Caput et al, 1996; Donaldson et al, 1998). Binding experiments and mRNA expression analysis have led to the description of four types of IL13R differently expressed by various cell types (Murata et al, 1997a; Obiri et al, 1997; Vita et al, 1995). However, little information is available regarding the biological relevance of these different receptor types.

To better characterize the IL13R expression and function in tumor cells, we studied seven renal cell carcinoma (RCC) and six glioma cell lines for IL13Rα1, IL13Rα2, and IL4Rα subunit, mRNA and protein. Us-

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ing new specific antibodies and flow cytometry, we found that (a) unlike glioma cells, RCC do not express IL13Rα2 chain, whereas IL13Rα2 mRNA was present in three of the seven RCC cell lines tested; (b) RCC, but not glioma cell proliferation, is inhibited by IL13 and IL4; (c) the effects of IL13 and IL4 on RCC are mediated through a common receptor including the IL4Rα chain; and (d) overexpression of IL13Rα2 in stably transfected RCC cells specifically inhibits the IL13-mediated effect, demonstrating that IL13Rα2 acts as a "decoy target" for IL13.

Results

Membrane IL13Rα1, IL13Rα2, and IL4Rα Expression in Glioma and RCC Cell Lines

We studied the surface expression of IL13Rα1, IL13Rα2, IL4Rα, and γc chains by flow cytometry (Table 1). None of the glioma and RCC cell lines expressed the γc chain. IL13Rα2 was expressed in all glioma cell lines except the cell line T98G, but in none of the RCC cell lines. IL13Rα1 was present in all glioma and RCC cell lines, and IL4Rα, which was present on all RCC cell lines, was also detected in four of six glioma cell lines (SF126, SKMG12, U399MG, and T98G). The glioma cell lines (Fig. 1) carried more IL13Rα2 (mean fluorescence intensity [MFI] above 700) than IL13Rα1 (MFI ranging from 20 to 40) or IL4Rα (MFI ranging from 80 to 260). In all cases, the intensity of IL4Rα staining was stronger than that of IL13Rα1.

Expression of IL13Rα1, IL13Rα2, and IL4Rα mRNA in Glioma and RCC Cell Lines

Because most RCC have high-affinity IL13 binding sites and contain IL13Rα2 mRNA, Murata et al (1997a) concluded that RCC carry IL13Rα2 protein at their surface. However, our results showed that RCC did not carry membrane IL13Rα2 (mIL13Rα2). We, therefore, tested for the presence in RCC and glioma cell lines of IL4Rα and IL13Rα chain transcripts by reverse transcription-polymerase chain reaction (RT-PCR) (Fig. 2). As expected from the presence of the proteins on the cell surface, IL13Rα1 and IL4Rα mRNAs were detected in all RCC cell lines. All RCC were devoid of mIL13Rα2, but, surprisingly, IL13Rα2 mRNA was detected in three of seven RCC cell lines. In glioma, the presence of the IL13Rα2, IL13Rα1, and IL4Rα mRNAs correlated with the presence of the corresponding membrane proteins in most cases. However, the SNB19 cell line was devoid of IL4Rα protein but contained the mRNA.

Table 1. IL13R Subunits at the Cell Surface of Various Tumor Cell Lines

	IL13R subunits[a]			
Cell lines	IL13Rα2	IL13Rα1	IL4Rα	γc
Glioma				
SF126	+++	+	++	−
SKMG12	+++	+	++	−
U399MG	+++	+	++	−
U373MG	+++	+	−	−
SNB19	+++	+	−	−
T98G	−	+	++	−
RCC				
ACHN	−	+	++	−
A704	−	+	++	−
RCC1	−	+	++	−
RCC17	−	+	++	−
RCC40	−	+	++	−
RCC42	−	+	++	−
RCC47	−	+	++	−

RCC, renal cell carcinoma.

[a] Staining was evaluated as the mean fluorescence intensity as described in "Materials and Methods": (−) negative, (+) positive, (++) strongly positive and (+++) very strongly positive.

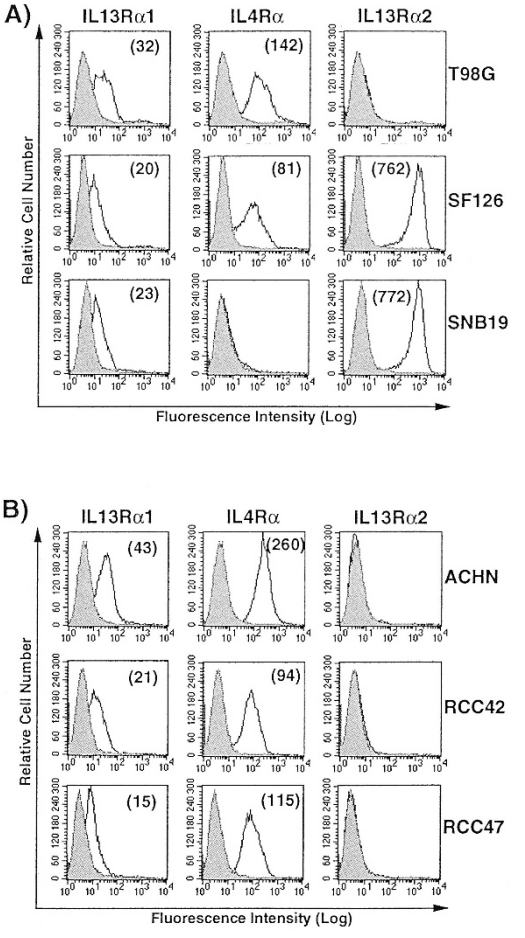

Figure 1.
Representative IL13Rα1, IL13Rα2, and IL4Rα surface immunoreactivity in glioma and renal cell carcinoma (RCC) cells. Glioma (A) and RCC (B) cells were stained with mouse monoclonal antibodies (MoAbs) raised against IL13Rα1, IL13Rα2, and IL4Rα chains and relevant controls (*solid histogram*), as described in "Materials and Methods." Results are given as mean fluorescence intensity (MFI) in brackets. Data are representative of five independent experiments.

IL13Rα2 Protein Is Detected in Lysates from Glioma but Not from RCC Cell Lines

To test whether IL13Rα2 mRNA leads to the production of intracellular IL13Rα2 in RCC, IL13Rα2 protein

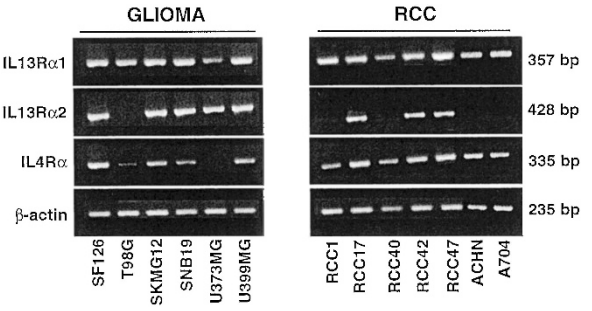

Figure 2.

IL13Rα1, IL13Rα2, and IL4Rα transcripts in glioma and RCC cells. cDNA from each cell line was amplified as described in "Materials and Methods." Data are representative of three independent experiments.

was assayed by ELISA (Fig. 3). No IL13Rα2 was detected in lysates from IGR-RCC17, 42, or 47, although they contained the transcript. As controls, we showed that IL13Rα2 protein was detected in SNB19 and SF126 cell lysates (mIL13Rα2^{+}) but was absent from T98G and IGR-RCC1 lysates (mIL13Rα2^{-}). Similarly, intracellular IL13Rα2 protein was not detected in flow cytometry after permeabilization of RCC cells (data not shown). Thus, glioma, but not RCC, cells expressed IL13Rα2 protein, whereas both cell types similarly expressed membrane IL4Rα and IL13Rα1.

Glioma, but Not RCC, Cells Are Insensitive to IL4 and IL13

To investigate the functional significance of IL13R expression, we compared (^{3}H)-thymidine uptake in glioma and RCC cells cultured with or without IL4 or IL13. Because none of these cell lines produced the IL2R γ chain (Table 1), IL2 was used as control. IL4 and IL13 inhibited the cellular proliferation of the T98G cell line by 20% (Fig. 4A). However, IL4 or IL13 had no effect on the proliferation of SNB19, SKMG12, SF126, U373MG, or U399MG cell lines (Fig. 4A and data not shown). In contrast to glioma cells, IL13 and IL4

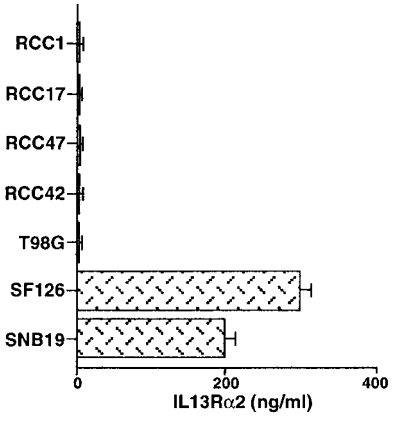

Figure 3.

IL13Rα2 expression in lysates from glioma and RCC cells. Four hundred-microgram aliquots of total cell lysates were assayed for IL13Rα2 protein by ELISA as described in "Materials and Methods." Results are expressed as the mean concentration (ng/ml ± SD) of triplicate determinations. Data are representative of two independent experiments.

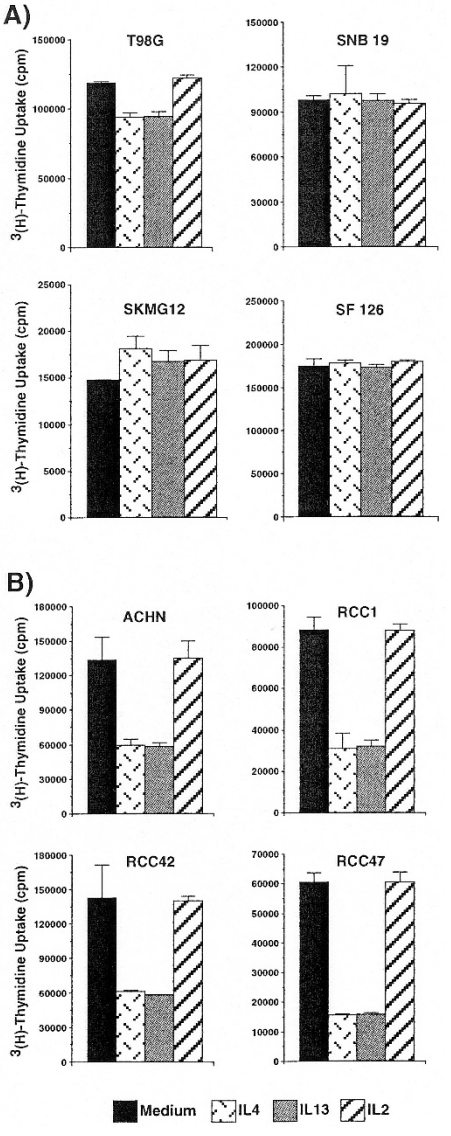

Figure 4.

Tumor cell proliferative response to IL4 and IL13 stimulation. Glioma cells (A) and RCC (B) were cultured with or without IL4 (20 ng/ml), IL13 (20 ng/ml), or IL2 (20 ng/ml) for 3 days, as described in "Materials and Methods." The proliferative response was measured by following (^{3}H)-thymidine uptake during the last 12 hours of culture. Results are expressed in counts per minute (cpm) (mean of triplicates ± SD). Data are representative of five independent experiments.

strongly inhibited cellular proliferation of all RCC cell lines (Fig. 4B and data not shown). Neither the cell proliferation of T98G nor that of RCC cells was affected by IL2 treatment. These data suggested that IL13Rα2 expression is associated with the absence of the IL13-mediated proliferative response.

IL13 Effects in RCC Are Mediated through the IL4Rα Chain

Recent studies indicate that IL4Rα is a functional subunit of IL13R in transfected cells (Murata et al, 1998; Orchansky et al, 1997). To establish whether the growth inhibitory effects of IL4 and IL13 on RCC cells are mediated through a IL4Rα/IL13Rα1 complex, we

tested the effect of neutralizing antibody directed against human IL4Rα chain. This anti-IL4Rα antibody reversed the growth inhibition induced by IL4 and IL13, whereas a soluble IL13Rα2-Fc fusion protein only antagonized the IL13-mediated effect (Fig. 5). These experiments demonstrate that, in contrast to a previous report (Obiri et al, 1996), IL4Rα participates in the signaling complex used by IL13 in RCC cells.

Modulation of IL13- but Not IL4-Mediated Effects by IL13Rα2

The absence of IL13-mediated response in all glioma cells, other than T98G which did not have a membrane IL13Rα2 chain, suggested that IL13Rα2 is responsible for this lack of response. To test this, we established stable transfectants of IL13Rα2 in the human renal adenocarcinoma cell line, ACHN. Two clones (27 and 28) produced large amounts of the IL13Rα2 chain as detected by flow cytometry (MFI 220 and 190 respectively), whereas the mock cells were negative (Fig. 6). Of note, the presence of IL13Rα2 did not affect the expression of membranes IL13Rα1 and IL4Rα compared with mock cells.

We next examined the impact of the presence of IL13Rα2 on the IL13- and IL4-induced inhibition of cell proliferation. The IL13- and IL4-mediated inhibition in mock-transfected cells was dose-dependent (Fig. 7). Maximum inhibition of these control cells was reached at 5 ng/ml for both cytokines and plateaued thereafter. In contrast, the overexpression of IL13Rα2 in 27 and 28 cells reduced their ability to respond to suboptimal IL13 concentrations (up to 5 ng/ml) but did not affect their response to IL4. In the presence of higher concentrations of IL13 (10–20 ng/ml), the proliferative response of 27 and 28 cells was inhibited as strongly as that of mock cells. These findings are consistent with IL13Rα2 acting as a "decoy target" for IL13.

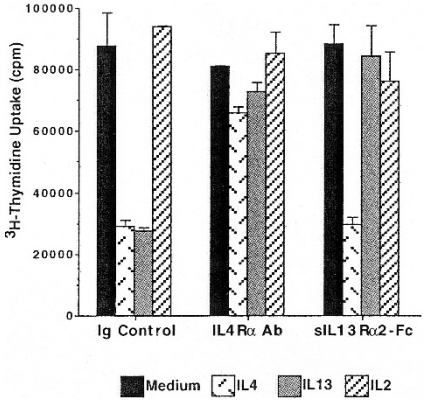

Figure 5.

Effects of anti-IL4Rα antibody and sIL13Rα2-Fc protein on IL4- and IL13-mediated responses in RCC. The RCC cell line ACHN was cultured with or without IL4 (20 ng/ml), IL13 (20 ng/ml), or IL2 (20 ng/ml), and in the absence or the presence of anti-IL4Rα antibody (10 μg/ml), mouse IgG isotype control (10 μg/ml), or sIL13Rα2-Fc protein (1 μg/ml) for 3 days, as described in "Materials and Methods." Proliferative response was measured by following (^{3}H)-thymidine uptake during the last 12 hours of culture. Results are expressed in counts per minute (cpm) (mean of triplicates ± SD). Data are representative of three independent experiments.

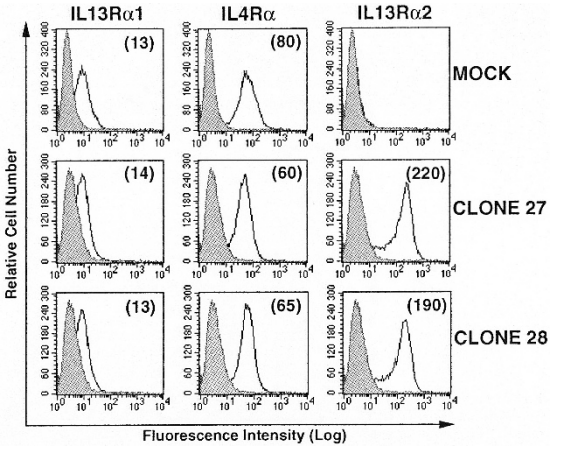

Figure 6.

IL13Rα2 expression in stable transfectant. Stably transfected ACHN clones were obtained and stained with MoAbs raised against IL13Rα1, IL13Rα2, and IL4Rα chains and relevant controls (*solid histogram*), as described in "Materials and Methods." Results are given as mean fluorescence intensity (MFI) in brackets. Data are representative of three independent experiments.

Discussion

Three potential components of human IL13R were identified, namely IL13Rα1, IL13Rα2, and IL4Rα. Although IL4Rα is an essential component of functional IL13R, only IL13Rα1 and IL13Rα2 are able to bind IL13 specifically (Aman et al, 1996; Caput et al, 1996; Orchansky et al, 1997; Zurawski et al, 1995). The IL13Rα1/IL4Rα complex is required for response to IL13, but the contribution of IL13Rα2 chain to this response has not been elucidated and may vary according to the cell target. Primary monocytes and B lymphocytes express small amounts of high-affinity IL13R and large amounts of IL13R of low to intermediate affinity, (Zurawski and de Vries, 1994), whereas binding experiments show that most solid tumor cells mainly express high-affinity IL13R (Debinski et al, 1999; Maini et al, 1997; Murata et al, 1997b; Obiri et al, 1995; Serve et al, 1996). Despite the fact that both express high-affinity IL13 binding sites, glioma and RCC cells are differently sensitive to IL13 (Debinski et al, 1995; Obiri et al, 1996). This and the analysis of the mRNAs IL13Rα1 and IL13Rα2 suggested that these cell types have different functional IL13R configurations (Murata et al, 1997a). We studied membrane IL13Rα1 and IL13Rα2 proteins to characterize IL13R configurations better.

Using new specific antibodies and flow cytometry, we observed a similar surface expression of IL4Rα and IL13Rα1 chains in most RCC and glioma cell lines. In contrast, IL13Rα2 was only present on glioma cells where it was 10 to 30 times more abundant than the two other chains. Our results agree with those of Joshi et al (2000) showing greater expression of IL13Rα2 than IL4Rα and IL13Rα1 chains in primary explants from gliomas. In agreement with the lack of IL13Rα2 chain expression in T98G, Debinski et al (1995) have previously shown that this cell line only expresses 500 IL13 binding sites, whereas U373MG and SNB19, which highly express the IL13Rα2 chain, exhibit more

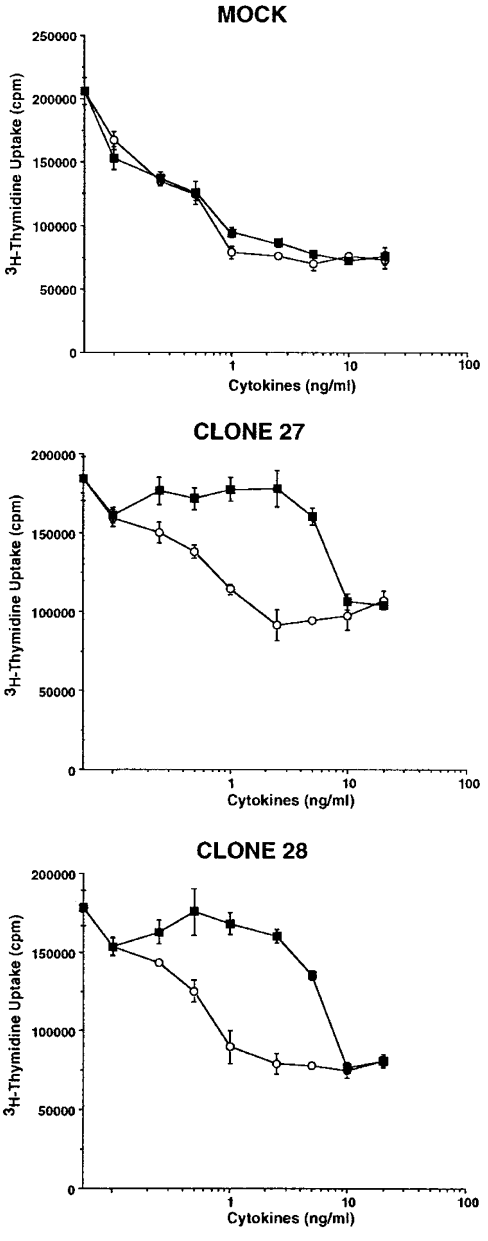

Figure 7.

Proliferative response to IL13 and IL4 stimulation of transfected cells. The ACHN-transfected cells expressing (clones 27 and 28) the IL13Rα2 chain and mock-transfected cells were cultured with or without one of a series of concentrations of IL4 (*black squares*) or IL13 (*open circles*) for 3 days, as described in "Materials and Methods." The proliferative response was measured by following (^{3}H)-thymidine uptake during last 12 hours of culture. Results are expressed in counts per minute (cpm) (mean of triplicates $\pm$ SD). Data are representative of three independent experiments.

than 16,000 IL13 binding sites. Although there was no surface IL13Rα2 protein on RCC, its mRNA was detected in three of seven RCC, in agreement with previous reports (Murata et al, 1997a; Obiri et al, 1997). Like Kawakami et al (2000), we did not detect IL13Rα2 at the cell surface of Caki-1 cells (not shown), a RCC cell line that contains the corresponding mRNA (Caput et al, 1996). Despite the presence of IL13Rα2 mRNA, we failed to detect an intracellular form of IL13Rα2 in RCC cells.

To assess whether IL13R was functional, we tested the proliferative responses of RCC and glioma cell lines to IL4 and IL13. Only the proliferation of one glioma cell line of six (T98G) was weakly inhibited by IL4 and IL13, whereas the growth of all RCC cell lines was strongly inhibited by both cytokines. Several studies have already reported that IL13 and IL4 were neither able to modulate cell proliferation nor signal transducer and activator of transcription (STAT)6 activation in glioma cells (Debinski et al, 1995, 1996; Liu et al, 2000a). In addition, we also found that IL13 and IL4 did not modulate the production of IL6 or IL8 in these glioma lines (data not shown). Thus, other than the T98G cell line, glioma cells seem to be unresponsive to IL4 and IL13. The lack of responsiveness to IL4 has been previously reported (Debinski et al, 1996) and seems a hallmark of the progression towards high grade tumors (Debinski et al, 1999). Although all cell lines used in the present study are classified as high grade glioma, express similar levels of epidermal growth factor-receptor (EGF-R) and insulin-like growth factor 1 receptor (IGF1-R), and produce similar levels of IL8 (data not shown), it remains possible that they correspond to different stages of tumor progression. The lack of responsiveness to IL4 is easily explained by the lack of IL4Rα chain expression in the U373MG and SNB19 cell lines. In contrast, IL4Rα and IL13Rα1 chains are expressed by SF126, SKMG12, and U399MG cells, suggesting that defects in the IL4R cell signaling pathway occur in these cells. Accordingly, Liu et al (2000a) have described abnormal patterns of STAT6 phosphorylation in various glioma cells. Liu et al (2000b) have also shown that IL4 responsiveness in astrocytoma requires unmutated p53 and involves regulation of p21 (waf1/cip1) and p27 (Kip1) expression. Besides IL4R signaling impairments, the strong expression of IL13Rα2 ($680 < \text{MFI} < 772$) in SF126, SKMG12, and U399MG cells may induce the formation of nonfunctional, ligand-independent heterotrimeric receptors. In our IL13Rα2-transfected clones, the expression of IL13Rα2 is weaker than that in glioma ($190 < \text{MFI} < 220$), and, thus, IL4R, including or not including a IL13Rα2 chain, may coexist, allowing a response to low doses of IL4. To verify whether IL13Rα2 expression is also responsible for the lack of response to IL4 in glioma cells, we are currently testing an mRNA antisense strategy to "switch off" the IL13Rα2 production in these cells. Alternatively, we would transfect the IL13Rα2 chain in the T98G cell line or in IL4-sensitive low-grade astrocytoma (Liu at al, 2000a).

Blocking experiments showed that IL4 and IL13 exerted a strong inhibition of RCC cell proliferation through a unique receptor composed of the IL4Rα and IL13Rα1 chains. This was confirmed by the lack of additive effect of IL4 and IL13 on the growth of RCC cells (data not shown). Our results constrasted with those of Obiri et al (1996), who reported that IL13-mediated effects on RCC cells are IL4Rα-independent. This discrepancy could be explained by the use of different anti-IL4Rα antibodies that nevertheless exert a similar antagonistic activity on the IL13

response of lymphoid cells. Thus, our results with RCC showed that the expression of IL13Rα2 mRNA and/or that of high-affinity IL13 binding sites is not sufficient to predict IL13Rα2 protein expression. Interestingly, we showed that IL13 is a potent inhibitor of cell proliferation of primary (IGR-RCC17, 40, and 42) and metastatic (IGR-RCC1) renal nonpapillary or papillary (IGR-RCC47) tumors.

Unlike that of IL13Rα1, the function of IL13Rα2 is still unknown. Our observation that cell lines responsive to IL13 and IL4 did not express IL13Rα2 (RCC and T98G glioma cell) and the results of Feng et al (1998) in synovial fibroblast cells raise the possibility that IL13Rα2 acts as a dominant negative inhibitor or a decoy receptor for IL13. To assess this possibility, we constructed stably transfected IL13Rα2^{+} clones of the RCC cell line ACHN and compared their proliferative response with that of IL4 and IL13. Overexpression of IL13Rα2 reduced the response to IL13 without affecting the response to IL4. This phenomenon was only observed at suboptimal IL13 concentrations (up to 5 ng/ml), suggesting that the IL13Rα1/IL4Rα complex on these cells remains functional. Our results demonstrate that IL13Rα2 acts as a membrane decoy receptor for IL13, but do not rule out the possibility that a soluble form of IL13Rα2 is released into the supernatants of transfected cells. As described for the IL1 type II receptor (Bossù et al, 1995; Colotta et al, 1993), both membrane and soluble IL13Rα2 could have antagonistic activities. A natural soluble form of IL13Rα2 has been described in mouse serum and urine (Zhang et al, 1997). The protein sequence of this soluble form matches the N terminal part of the mIL13Rα2 (Donaldson et al, 1998), suggesting that it is produced by proteolytic cleavage or alternative splicing. Recent data describe that alternative mRNA splicing of the murine IL13Rα1 mRNA occurs, generating variant transcripts that encode sIL13Rα1 (Osawa et al, 2000).

In conclusion, we demonstrate that IL13Rα2 is overexpressed in glioma cells but not in RCC cells that expressed only one IL13R type composed of IL13Rα1 and IL4Rα chains. IL13Rα2 overexpression correlates with a lack of sensitivity to IL13, suggesting that IL13Rα2 acts as a decoy receptor for IL13. Through its expression, IL13Rα2 may regulate IL13 activity without impairing the IL4 response of the same target cell.

Materials and Methods

Cell Lines

The RCC lines, ACHN and A704, were purchased from the American Type Culture Collection (Manassas, Virginia). IGR-RCC 1, 17, 40, 42, and 47 cell lines were obtained from Dr. E. Angevin (Institut Gustave-Roussy, Villejuif, France) and are described in Angevin et al (1999). Glioma cell lines (SNB19, SF126, T98G, U399MG, U373MG, and SKMG12) were a gift from Dr. P. Horellou (INSERM EMI 00-20, Clamart, France). These cell lines were cultured in RPMI (RCC) or DMEM (glioma) supplemented with 10 mM HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate, and 10% heat-inactivated FCS (Gibco BRL, Paisley, Scotland). The cell lines were subcultured twice a week at 1×10^5 cells/ml.

RNA Isolation and RT-PCR Analysis

RNA extraction (from 5×10^6 cells) and cDNA preparation were previously described in Krzysiek et al (1999). cDNA (200 ng for β-actin, or 400 ng for IL13Rα1, IL13Rα2, and IL4Rα) was amplified by standard PCR using IL13Rα1 primers (sense: 5'-AGGATGACAAACTCGGAG, antisense: 5'-CTCAAGGTCCACAGTGAGGG) that amplified a product of 357 bp; IL4Rα primers (sense: 5'-GACCTGGAGCAACCCGTATC, antisense: 5'-CATAGCACAACAGGCAGACG) that amplified a product of 335 bp (Reinecker and Podolsky, 1995); and IL13Rα2 primers (sense: 5'-ATACCTTTGGGACCTATTCC3', antisense: 5'-TGAACATTTGGCCATGACTG3') that amplified a 428 bp product. PCR conditions were 35 cycles of denaturation at 94° C for 1 minute, annealing at 58° C for 1 minute, and extension at 72° C for 2 minutes and 30 seconds (for IL13Rα1 and IL13Rα2), or annealing at 63° C for 50 seconds and extension at 72° C for 1 minute (for IL4Rα). IL13Rα primers and PCR conditions were provided by Dr. P. Ferrara (Sanofi, Labège, France). Primers and PCR conditions for β-actin were described in Krzysiek et al (1999). Ten-microliter aliquots of amplified products were subjected to electrophoresis in 2% agarose gel stained with ethidium bromide. The gels were photographed under ultraviolet light, and bands were analyzed with FotoLook SA 2.0 software (AGFA-Gevaert, Munich, Germany).

Production and Characterization of Anti-IL13Rα Chain Ab

Monoclonal antibody E-74 raised against the IL13Rα1 chain, a gift from Glaxo Wellcome (Stevenage, United Kingdom), was fully described in Graber et al (1998). Monoclonal antibody B-E34 raised against the IL13Rα2 chain was a gift from Diaclone Research (Besançon, France) and was described in Vermot-Desroches et al (2000).

Flow Cytometric Analysis of IL4Rα and IL13Rα Chain Expression

Cells were detached from culture flasks by brief exposure to 1XPBS/4 mM EDTA. Cell surface staining was performed using the Enzymatic Amplification Staining Kit (Flow-Amp Systems, Ltd., Cleveland, Ohio) according to the manufacturer's recommendations. Cells (5×10^5) were incubated with mouse monoclonal antibodies (MoAbs) (10 μg/ml) directed to IL4Rα (CD124, MB02, IgG2a; R&D Systems, Inc., Abingdon, United Kingdom), IL13Rα2 (CD213a2, B-E34, IgG1), γc (CD132, TUGh4, IgG1; Pharmingen, San Diego, California), or IL13Rα1 (CD213a1, E-74, IgG1). Mouse isotype-matched IgG were used as controls. Immunostaining acquisition and analysis were performed on

10,000 events gated on viable cells using a FACScan flow cytometer with CellQuest software (BD Biosciences, Franklin Lakes, New Jersey). Results are expressed as mean fluorescence intensity (MFI).

Cell Lysates Preparation and IL13Rα2 Protein Expression

Confluent cells were washed and lysed as previously described (Rolling et al, 1995). Protein concentration was determined using a protein assay kit (BIO-RAD, Munich, Germany) according to the manufacturer's recommendations. Lysates were stored at −80° C until tested. The presence of IL13Rα2 protein was detected by ELISA using different mAb CD213a2 (Diaclone SA, Besançon, France). Briefly, 96-well plates (Nunc, Roskilde, Denmark) were coated with 1 μg/well of purified MoAb1 overnight at 4° C. The plates were washed and blocked with 5% bovine serum albumin (Sigma Aldrich, Steinheim, Germany) for 2 hours at room temperature (RT). Then, 400 μg of cell lysate samples or serial dilutions of standard recombinant IL13Rα2-Fc (R&D Systems) were added to wells and incubated at RT for 2 hours. After four washes in 0.5% Tween-20-PBS, 100 ng/well of biotinylated MoAb2 was added, and the plates were incubated for 1 hour at RT. Following four washes, 100 μl of avidin-horseradish peroxidase anti-mouse immunoglobulin was added, and the plates were incubated for 20 minutes at RT. Wells were then washed and TMB (Sigma) was added. The reaction was stopped with sulfuric acid (H_2SO_4, 1 N) and read at 492 nm in a microtiter ELISA reader (DYNEX Technology, Chantilly, Virginia). The concentration of IL13Rα2 in lysates was calculated by linear regression analysis from standard titration curves. Results are expressed as the mean concentration (ng/ml, ± SD) of triplicate determinations.

Proliferation Assays

Cells were seeded at 5×10^3 cells/well in 96-well microtiter plates (Costar, Cambridge, Massachusetts) and cultured in medium with 10% FCS for two days and then for 3 days in the absence or in the presence of IL4 (Schering-Plough, Kenilworth, New Jersey), IL13 (R&D Systems), or IL2 (Chiron, Amsterdam, The Netherlands) at various concentrations (see figure legends) in 200 μl of medium with 2% FCS. For inhibition experiments, anti-IL4Rα antibody (MOB2; R&D Systems; 10 μg/ml) was added 30 minutes before the cytokines, whereas sIL13Rα2-Fc protein (R&D Systems; 1 μg/ml) was incubated with cytokines for 30 minutes before its addition to the cells. Proliferation was measured by supplying the culture with 0.5 μCi/well of (methyl-^{3}H) thymidine (Amersham, les Ulis, France) for the last 12 hours of the third day of culture. The plates were then frozen and thawed and the DNA was harvested onto a filtermat. Incorporated radioactivity was measured on a Wallac 1205 Betaplate counter (Wallac, Gaithersburg, Maryland) and results are expressed in counts per minute (cpm) (mean of triplicates ± SD).

Transfection of ACHN Cells

ACHN cells (2×10^5 cells/well) were plated in 6-well culture plates (Costar) and cultured overnight. Ten micrograms of empty pSE1 vector or the vector containing the IL13Rα2 cDNA were cotransfected with 1 μg of the selectable plasmid pSV40 containing the neomycin transferase cDNA by Lipofectamine (Gibco BRL) according to the manufacturer's instructions. Neomycin-resistant transfectants were selected by inclusion of 800 μg/ml of active geneticin (Gibco BRL) in the cultures for 3 weeks. Selected cells were examined by flow cytometry to identify clones producing the IL13Rα2 chain. Five such clones were identified and exhibited similar intensities of IL13Rα2 immunoreactivity and similar results in proliferation experiments. Two of these clones, named 27 and 28, are described in this paper. Clones transfected with the neomycin resistance vector and an empty vector (mock) were used as negative controls.

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