Interleukin-4 enhances proliferation of human pancreatic cancer cells: evidence for autocrine and paracrine actions

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Interleukin-4 (IL-4) is an immunomodulatory cytokine, which can inhibit the growth of tumour cells. Pancreatic cancer cells and tissues express high levels of IL-4 receptors. The aim of this study was to characterise the effects of IL-4 on the growth and signalling pathways of pancreatic cancer cells. Cell growth was determined by cell counting and MTT assays in association with fluorescence-activated cell sorter analysis. IL-4 expression using ELISA and real-time PCR techniques, and signal transduction using immunoprecipitation or immunoblot analysis. We now report for the first time that IL-4 significantly enhanced the growth of five out of six cultured pancreatic cancer cell lines in a dose-dependent manner in association with an increased fraction of cells in S-phase. Surprisingly, all six cell lines expressed endogenous IL-4, and IL-4 was detectable in the supernatant. Incubating cells with neutralising IL-4 antibodies resulted in a significant inhibition of basal growth in three cell lines, including IL-4-unresponsive MIA PaCa-2 cells, which however expressed the highest endogenous IL-4 levels. Interleukin-4 enhanced activity of MAPK, Akt-1, and Stat3 in IL-4-responsive, but not in IL-4-unresponsive MIA PaCa-2 cells; however, IL-4 enhanced tyrosine phosphorylation of insulin receptor substrate-1 and -2 in all cell lines. Our results demonstrate for the first time that pancreatic cancer cells produce IL-4 and that IL-4 can act as a growth factor in pancreatic cancer cells. Together with the observation that neutralising IL-4 antibodies can inhibit the growth of these cells, our results suggest that IL-4 may act as an autocrine growth factor in pancreatic cancer cells and also give rise to the possibility that cancer-derived IL-4 may suppress cancer-directed immunosurveillance in vivo in addition to its growth-promoting effects, thereby facilitating pancreatic tumour growth and metastasis.

Keywords: cytokine; growth factor; mitogenic signalling; interleukin; pancreatic cancer

Interleukin-4 (IL-4) is a secreted, potentially glycosylated anti-inflammatory and immunomodulatory 18 kDa cytokine with pleiotropic functions sharing several biologic activities with IL-13. It is produced mainly by a subpopulation of activated T-cells, TH2 CD4(+) helper cells, and promotes the proliferation and differentiation of activated B-cells, the expression of class II MHC antigens, and of low-affinity IgE receptors in resting B-cells (Paul, 1991).

The biological activities of IL-4 are mediated by a specific IL-4 receptor (IL-4R) that is expressed at densities of 100–5000 copies cell⁻¹ (Nelms et al, 1999). IL-4 receptor α (IL-4Rα) was found to be expressed on solid human tumours, including malignant melanoma, breast carcinoma, ovarian carcinoma, mesothelioma, glioblastoma, renal cell carcinoma, head and neck carcinoma, and AIDS-associated Kaposi’s sarcoma (Kawakami et al, 2001; Gooch et al, 2002). It was also reported that several cultured pancreatic cancer cell lines express IL-4Rα (Kornmann et al, 1999a). Using binding assays, it could be demonstrated that PANC-1 pancreatic cancer cells express high numbers of specific IL-4 binding sites (Kawakami et al, 2002). The significance of IL-4Rs expression in pancreatic cancer and other solid tumours is still unknown. In contrast to its growth stimulatory effect on lymphocytes, IL-4 has been shown to have a modest but direct inhibitory effect on the growth of tumour cells of haematopoietic and nonhaematopoietic origin in vitro and in vivo, including those derived from human melanoma, non-Hodgkin’s malignant B-lymphoma, and colon, renal, gastric, and breast carcinoma (Defrance et al, 1992; Gallagher and Zaloom, 1992; Morisaki et al, 1992; Toi et al, 1992, Obiri et al, 1993).

The different effects of IL-4 on cell proliferation presently remain unclear. Chang and co-workers proposed that binding of IL-4 to its receptor can trigger at least three signalling pathways including insulin receptor substrate (IRS)/signal transducer and activator of transcription 3 (Stat3), Stat6, and Stat1 activation. Whereas the activation of IRS substrates and Stat3 may result in cell proliferation, activation of Stat6 seems to be involved in regulation of the immune system such as T helper cell differentiation and IgE class switching (Chang et al, 2000; Scholz et al, 2003). In contrast, IL-4-mediated activation of Stat1 results in cell growth inhibition (Chang et al, 2000).

Pancreatic cancer is a deadly disease with a poor prognosis and a propensity to rapidly metastasise to tissues and lymph nodes while exhibiting resistance to cancer-directed immune mechanisms. Pancreatic cancers not only overexpress multiple growth factors including members of the insulin-like growth factor (IGF)
family like IGF-I (Korc, 1998), but also overexpress the intra-
cellular signal transducers of this pathway, IRS-1 and IRS-2 (Kornmann et al., 1998). Pancreatic cancer cells and tissues also
express high levels of IL-4Rz (Kornmann et al., 1999a, b; Kawakami et al., 2002).

In view of the abundant production of IL-4 by activated T-
lymphocytes, the proclivity of pancreatic cancer to metastasise and
to avoid cancer-directed immune mechanisms, this project aimed to
collect the effects of IL-4 and neutralising IL-4 antibodies
on the growth of cultured human pancreatic cancer cells, the
expression and secretion of IL-4 by human pancreatic cancer cells,
and IL-4-dependent signalling pathways.

MATERIALS AND METHODS

Materials

The Cycle Test Plus DNA Reagent Kit for fluorescence-activated
cell sorter (FACS) analysis was purchased from Becton Dickinson
Immunocytochemistry Systems (San Jose, CA, USA). Human recom-
binant IL-4 and IGF-1, 3-(4,5-methylthiazol-2-yl)-2,5-diphenylter-
trazolium bromide (MTT), monoclonal mouse anti-β-actin
antibody, and secondary horseradish-conjugated anti-rabbit were
from Sigma Chemical Co. (St Louis, MO, USA). Rabbit polyclonal
anti-phospho-Akt antibody (Ser 473)-R (1:500), mouse monon-
clonal Akt-1 (B-1) antibody (1:500), anti-active mitogen-activated
protein kinase (MAPK) (p-ERK, E-4) mouse monoclonal antibody
(1:5000), ERK-2 (C-14) rabbit polyclonal antibody (1:2000),
mouse monoclonal IgG1 antibody p-Stat3 (A-2) (1:200), rabbit
polyclonal antibody Stat1 p84/p91 (E-23) (1:250), mouse poly-
clonal antibody p-Stat3 (B-7) (1:1000), rabbit polyclonal antibody
Stat3 (K-15) (1:200), rabbit polyclonal antibody Stat6 (1:1000),
mouse monoclonal IgG1 antibody p-Tyr (PY99) (1:2000), and
mouse whole-cell lysate from normal embryo fibroblast cells (NIH/
3T3 cells) were from Santa Cruz Biotechnology (Santa Cruz, CA,
USA). Monoclonal anti-human IL-4 antibody and mouse mono-
clonal anti-human CD5 antibody were obtained from R&D
Systems (Minneapolis, MN, USA). The human IL-4 immunoassay
kit was from BioSource International Inc. (Camarillo, CA, USA).
Rabbit immunooaffinity-purified anti-rat IRS-1 (1:2000) and rabbit
polyclonal anti-mouse IRS-2 (1:1000) were from Upstate Biotech-
nology Inc. (Lake Placid, NY, USA). The RNeasy Mini Kit for RNA
extraction was from Qiagen GmbH (Hilden, Germany). Super-
Script First-Strand Synthesis System for RT – PCR was from
Invitrogen (Carlsbad, CA, USA). The Real-Time PCR Kit for
Human IL-4 Gene Expression was from Maxim Biotech. Inc. (CA,
USA). ASPC-1, CAPAN-1, MIA PaCa-2, and PANCl human
pancreatic cancer cells were purchased from American Type
Culture Collection (ATCC, Rockville, MD, USA). KOLO-357 and
T3M4 human pancreatic cancer cells were a gift from RS Metzgar
(Duke University, Durham, NC, USA).

Cell culture

KOLO-357, MIA PaCa-2, and PANC-1 cells were grown in DME
medium, whereas ASPC-1, CAPAN-1, and T3M4 cells were grown
in RPMI-1640 medium. All media were supplemented with 8%
FBS, penicillin G (100 U ml\(^{-1}\)), and streptomycin (100 \(\mu\)g ml\(^{-1}\)),
term time complete medium. Cells were maintained at 37°C in
humidified air with 5% CO\(_2\). Medium containing antibiotics, 0.1%
BSA, 5 mg l\(^{-1}\) transferrin, and 5 \(\mu\)g l\(^{-1}\) selenious acid was termed
serum-free medium and used when indicated for the specific
assays.

Growth assays

For cell counting, cells (150 000 well\(^{-1}\)) were seeded in 12-well
plates in complete medium (3 ml well\(^{-1}\)). After 24 h, the medium
was replaced by serum-free medium (500 \(\mu\)l well\(^{-1}\)) for the
indicated times in the absence or presence of IL-4 (5 nM) or IGF-
I (5 nM). The medium was changed daily, including the respective
additions. For cell counting, cells were trypsinised, resuspended,
adjusted to 1 ml, and counted under a light microscope using a
haemocytometer.

Cell growth was also determined by the MTT assay as described
(Kornmann et al., 1998). Cells (10 000 well\(^{-1}\)) were seeded in 96-
well plates, incubated for 24 h in complete medium, and then for
48 h in serum-free medium in the absence or presence of the
indicated substrates. The assay was also performed in the presence
of 8% FBS. In these cases, only 5000 cells well\(^{-1}\) were seeded and
IL-4 was added after 24 h for 48 h. To initiate the assay, MTT
reagent was added and cells were incubated for an additional 4 h at
37°C. After removal of the medium and dissolving the crystals with
acidified isopropanol, the samples were analysed using an ELISA
plate reader at 570 nm. The value at 650 nm was subtracted as
background.

Cell cycle analysis: fluorescence-activated cell sorter

To determine the effect of IL-4 and IGF-1 on cell cycle, cells were
seeded in six-well plates, incubated for 24 h in complete medium to
60% confluency, and then serum starved for 24 h. The medium was
replaced by serum-free medium in the absence or presence of IL-4
(5 nM) or IGF-1 (5 nM) for another 24 h. Thereafter, FACS analysis
was performed using a CycleTest Plus kit according to the
instructions of the manufacturer and FACScan (Becton Dickinson)
analysis system equipped with a FACStation, MAC PowerPC
cell, and CellQuest acquisition software as previously described
(Kornmann et al., 1999a, b).

Interleukin-4 ELISA

Total cell lysates were prepared as described (Kornmann et al.,
1998). After adjusting the protein concentration using lysis buffer,
100 \(\mu\)l aliquots were used for the IL-4 sandwich ELISA carried out
according to the protocol of the manufacturer. To detect IL-4 in the
peritoneal cells, were cultured in six-well plates to 70% confluency.
After washing, cells were cultured in 1 ml of serum-free medium
including proteinase inhibitors (Kornmann et al., 1998)
for 48 h. The medium was then harvested and centrifuged before
subjecting 100 \(\mu\)l aliquots to the ELISA. Cells were counted to
determine the amount of IL-4 secreted per cell. In the present
study, the interassay variability was less than 28% and the intra-
assay variability less than 5%.

Analysis of IL-4 mRNA

Exponentially growing cells were harvested and pelleted by
centrifugation at 300 g for 5 min after washing twice with PBS.
Total RNA extraction and reverse transcription was then
performed according to the instructions of the manufacturer.
The design of the IL-4 5¢ and 3¢ primers was based on the
sequence with GenBank accession number NM_000589. The design
of β-actin 5¢ and 3¢ primers was based on the sequence with
GenBank accession number NM_001101. Primer sets for IL-4 and
FRET probes for IL-4 with known cDNA copy number were
designed to generate an 81 bp PCR product as described by the
manufacturer (Maxim Biotech Inc., CA, USA). Primers for β-actin
were sense GCCATCCTACCCCTGAAGTA and antisense
GTCAGGCAGCTCGTAGCTCT resulting in a 525 bp PCR product.

Real-time RT – PCR amplification was performed using the
QuantiTect™ SYBR Green PCR Kit (QIAGEN, Germany). Prior to
quantitative analysis, several titration experiments, for MgCl\(_2\), and
efficiency tests were performed to determine optimum amplifica-
tion conditions. Standard curves containing a specific number of
cDNA copies were generated for the IL-4 gene transcript. After the
to the serum-free conditions, the growth of MIA PaCa-2 cells was not altered by IL-4 (5 nM) in the presence of 10% serum (−8.7 ± 4.9% s.e.m.).

To confirm the results obtained by MTT assay suggesting that IL-4 can act like a growth factor in pancreatic cancer cells, cell counting and comparison with the growth-promoting effects of IGF-I was performed next in PANC-1 cells. Interleukin-4 (5 nM) resulted in an increase in cell number in PANC-1 during the whole 4-day incubation period. The maximal increase in proliferation for IL-4 was observed on day 3, resulting in a 39% (±3.5% s.e.m.) increase in cell number (Figure 1B). For IGF-I, a well-known potent mitogenic growth factor, a maximal increase in cell number was observed also on day 3, resulting in a 76% (±31% s.e.m.) increase in cell number (Figure 1B). Flow cytometric analysis revealed that the enhanced proliferation of PANC-1 cells in the presence of IL-4 was associated with a marked increase in the number of cells in S phase comparable to IGF-I (Figure 1C).

Expression of IL-4 in human pancreatic cell lines

In view of the mitogenic effects of IL-4, we next sought to determine whether these cells express this cytokine. Determined by ELISA, the IL-4 protein was detectable in total cell lysates from all six tested cell lines (Figure 2A). Relatively high IL-4 protein levels were found in lysates prepared from COLO-357 and MIA PaCa-2 cells and relatively low IL-4 protein levels were found in lysates prepared from PANC-1 and T3M4 cells (Figure 2A). A subsequent analysis of serum-free medium revealed that IL-4 could also be detected in the supernatant of all six cell lines. Mean ± s.d. (pg ml⁻¹ 10⁻⁵ cells) IL-4 protein concentrations in the supernatant were 1.0 ± 0.7, 0.9 ± 0.3, 1.1 ± 0.9, 1.5 ± 0.6, and 2.5 ± 0.2 for ASPC-1, CAPAN-1, COLO-357, MIA PaCa-2, and T3M4 cells, respectively. Real-time IL-4 mRNA expression in cell number were detectable in PANC-1 cells (5.3 ± 1.7 s.d. (pg ml⁻¹ 10⁻⁵ cells)).

Real-time PCR analysis using specific primers for the IL-4 encoding sequence revealed the presence of IL-4 mRNA transcripts in all six cell lines. Interleukin-4 mRNA levels correlated with IL-4 protein levels after normalisation to β-actin used as internal reference gene in four cell lines; however, relatively high IL-4 mRNA levels and relatively low protein levels were found in CAPAN-1 and especially in PANC-1 cells (Figure 2B).

Effects of neutralising IL-4 antibodies on cell growth

To determine whether there is a potential for IL-4-mediated autocrine growth stimulation, cells were incubated with increasing concentrations of an IL-4 neutralising antibody. The basal growth of COLO-357 and PANC-1 cells was slightly inhibited in a dose-dependent manner, with maximal inhibition of 13% (±2.0% s.e.m.) and 10% (±2.4% s.e.m.), respectively, occurring at 10 μg ml⁻¹ of the anti-IL-4 antibody (Figure 3). The effects were minor, but reproducible (n = 7 for PANC-1 and n = 10 for COLO-357). Interestingly, the basal growth of IL-4-unresponsive Mia PaCa-2 cells, which express high endogenous IL-4 levels, was also inhibited in the presence of IL-4 neutralising antibodies by 17% (±4.19% s.e.m.). A CD5 control antibody (10 μg ml⁻¹) only slightly altered the growth of the six cell lines.

In order to test the specificity of this antibody, the effects of the IL-4 neutralising antibody on cell growth in the presence of exogenous IL-4 were examined. Interleukin-4-stimulated growth (5 nM) of COLO-357 cells was significantly inhibited by incubation with 10 μg ml⁻¹ of neutralising IL-4 antibody. This antibody also attenuated the effects of exogenous IL-4 (5 nM) in the other tested cell lines; however, the effect did not reach significance (Figure 3), demonstrating that this antibody can at least partially block IL-4-mediated proliferative effects.

**IL-4 mediates pancreatic cancer cell growth**

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The effect of IL-4 on signal transduction

Intracellular signalling of IL-4 is mediated via Janus kinases (JAKs), cytoplasmic protein tyrosine kinases that associate with the receptor complex and phosphorylate the receptor as well as other substrates recruited to the receptor complex. First, the IL-4-dependent tyrosine phosphorylation of IRS-1 and -2, over-expressed in human pancreatic cancer, was analysed. Similar to IGF-I, IL-4 (5 nM for 5 min) induced tyrosine phosphorylation of IRS-1 and IRS-2 in PANC-1 (Figure 4A) and the other four IL-4-responsive cell lines (not shown). It also induced tyrosine phosphorylation of IRS-1 in MIA PaCa-2 cells unresponsive to exogenous IL-4 (Figure 4A). In MIA PaCa-2 cells, which express only low levels of IRS-2 (Kornmann et al, 1998), no clear enhancement of IRS-2 tyrosine phosphorylation could be demonstrated.

Mitogen-activated protein kinases mediate mitogenic signalling of several growth factors including IGF-I and cytokines. Both IL-4 and IGF-I enhanced MAPK activity in IL-4-responsive COLO-357 cells (Figure 4B). This effect was less pronounced for IL-4 in comparison to IGF-I, but reproducible (n = 3), and was also observed in the other IL-4-responsive cell lines (not shown). In contrast, in IL-4-nonresponsive MIA PaCa-2 cells, IGF-I, but not IL-4, enhanced MAPK activity (Figure 4B).

Akt is activated by insulin, various growth factors, and survival factors, and functions in a wortmannin-sensitive pathway involving PI-3 kinase (Franke et al, 1997). Both IL-4 and IGF-I enhanced Akt activity in IL-4-responsive COLO-357 cells (Figure 4C) and in the other IL-4-responsive cell lines (not shown). Also the activation of Akt was less pronounced for IL-4 in comparison to IGF-I, but reproducible (n = 3). In IL-4-nonresponsive MIA PaCa-2 cells, IGF-I, but not IL-4, enhanced Akt activity (Figure 4C).

Expression and activation of Stat by IL-4

In addition to IRS-1 and IRS-2, IL-4 has been reported to be able to activate the transcription factors Stat1, Stat3, and Stat6. At first, we examined whether pancreatic cancer cell lines express Stat1, Stat3, and Stat6. The Stat1 protein was expressed in all six cell lines at various levels (Figure 5A). Highest levels of Stat1 (85 kDa) were observed in MIA PaCa-2 cells. The Stat3 immunoblot analysis revealed a band of 92 kDa readily visible in CAPAN-1, MIA PaCa-2, and PANC-1 cells corresponding to the Stat3 isoform. A very faint Stat3 band was also detectable in the other cell lines after a long exposure; Stat6 (120 kDa) was also expressed in pancreatic cancer cells at various levels, with the highest levels found in COLO-357 cells (Figure 5A).

Phosphorylation of Stat occurs in response to a broad spectrum of physiologic stimuli, including activation of cytokine receptors and receptor-tyrosine kinases. Next, we examined activation of Stat1, Stat3, and Stat6 after incubation with IL-4 by immuno-
Interleukin-4 is an anti-inflammatory cytokine involved in various immune responses (Nelms et al., 1999). Nevertheless, in addition to its expression and functions in immune cells, IL-4Rα receptors were found to be widely expressed on solid human tumours including pancreatic cancer (Kawakami et al., 2001).

Initially, it was hypothesised on the basis of upregulation of IL-4R molecules in malignant tumours of epithelial origin that IL-4R may be a product of an oncogene that could be involved in the process of carcinogenesis (Al Jabaari et al., 1989). However, in most of the examined tumour cell lines, IL-4 induced growth inhibition (Hoon et al., 1991; Gallagher and Zaloom, 1992; Morisaki et al., 1992; Toi et al., 1992, Obiri et al., 1993). Only in Burkitt’s lymphoma (Chang et al., 2000), in three of 35 malignant B-samples (Taylor et al., 1990), and in human head and neck squamous cell carcinoma cells (Myers et al., 1996), IL-4 was reported to stimulate cell proliferation. The present study revealed for the first time that IL-4 also exerts proliferative effects in the majority of pancreatic cancer cell lines. Flow cytometry and cell counting in comparison to the potent mitogenic growth factor IGF-1 revealed that IL-4 is less potent, but exerts effects similar to IGF-1 in increasing cell number and S-phase fraction.

Myers et al. (1996) proposed a possible explanation for the disparity in the observed effects of IL-4 on the growth of tumour cell lines. Various IL-4R-expressing tumour types may have different downstream mediators of IL-4 action. To date, a number of cytoplasmic signalling proteins have been shown to be phosphorylated in response to IL-4 stimulation, including Jak1, Stat1,Stat3,Stat6,IRS-2, and others (Zamorano and Keegan, 1998; Nelms et al., 1999; Chang et al., 2000).

Insulin receptor substrate proteins are large adaptor PTB domain proteins involved in insulin and IGF-1 signalling (White and Yenush, 1998); IRS-2, in particular, was also identified as one of the predominant proteins phosphorylated in response to IL-4 (Sun et al., 1995). Recruitment of IRS-2 to the activated IL-4Rα chain results in its phosphorylation and subsequent activation of downstream signalling proteins, including phosphatidylinositol-3 (PI-3) kinase (Sun et al., 1995). We have shown that both IGF-1 and IL-4 induced the tyrosine phosphorylation of IRS-1 and IRS-2 in five IL-4-responsive cell lines. Interestingly, IL-4 also induced the tyrosine phosphorylation of IRS-1 in IL-4-unresponsive MIA PaCa-2 cells, demonstrating that the initial IL-4 signal transduction is functional in MIA PaCa-2 cells.

The MAPK and Akt pathways are also key components in the signal transduction of many mitogenic growth factors such as IGF-1 and are known to regulate proliferation and apoptosis in different cancers. They are classically activated following ligand–receptor interaction by the sequential activation of a linear cascade of protein kinases, including Ras, Raf-1, MEK, and the MAPKs ERK-1 and ERK-2 (Puri and Siegel, 1993; Smerz-Bertling and Duschl, 1995) on the one hand and PI 3-kinase and Akt on the other hand (Franke et al., 1997; Fahy et al., 2003). Activation of the MAPK and Akt cascades has been observed for several other cytokines, including IL-3, IL-12, and IL-13. In the present study, we demonstrated for the first time that IL-4 activates MAPK and Akt in IL-4-responsive pancreatic cancer cells. In IL-4-nonresponsive MIA PaCa-2 cells, IGF-1 but not IL-4 enhanced MAPK and Akt activity, suggesting that MAPK

**DISCUSSION**

not in IL-4-nonresponsive MIA PaCa-2 cells (Figure 5B). We did not observe an increase of Stat1 or Stat6 phosphorylation on incubating any of the cell lines with IL-4 even after long exposure and using positive phosphorylation controls provided by the manufacturer (not shown).
and Akt activation are essential for IL-4-induced cell proliferation in pancreatic cancer cells.

Interleukin-4 may exert various effects dependent on the activation of specific Stat transcription factors as proposed by Chang et al. (2000). All human pancreatic cancer cells tested expressed various levels of Stat1, Stat3, and Stat6 proteins. However, IL-4-induced activation was only observed for Stat3 in IL-4-responsive cells similar to MAPK and Akt, while in IL-4-nonresponsive cells, IL-4 did not induce phosphorylation of Stat3. This finding is especially exciting in regard to the recently published results of Scholz et al. (2003), demonstrating that activated Stat3 can promote the malignant phenotype of human pancreatic cancer by acceleration of G1/S-phase progression and thus supporting our present results.

The unresponsiveness of MIA PaCa-2 cells to exogenous IL-4 remains to be determined. However, there are several possible explanations. First, as shown by Northern blot analysis, MIA PaCa-2 cells express low levels of IL-4R (Kornmann et al, 1999a). In association with the high endogenous IL-4 levels, this may result in unresponsiveness to exogenous IL-4. This is also supported by the observation that neutralising IL-4 antibodies significantly inhibited the basal growth of MIA PaCa-2 cells. Second, MIA PaCa-2 cells have shown only a slight activation of IRS-2 as compared to the other cell lines resulting in subsequent nonactivation of the MAPK, Akt, and Stat3 pathways. Additional other alternative pathways, however, cannot be ruled out at this point. It is also possible that the expression of the IL-13Rα1 chain, which associates with the IL-4Rα chain to form a functional receptor, may influence the effects of exogenous IL-4 although MIA PaCa-2 cells as well as the other pancreatic cancer cells lines express IL-13Rα1 (Kornmann et al, 1999a).

Our present study not only demonstrated that IL-4 can exert growth stimulatory effects in pancreatic cancer cells but that they themselves express different amounts of IL-4. To our knowledge, this is a novel finding, which has thus far only been demonstrated.
in lymphoma cells (Taylor et al, 1990; Ford et al, 1993). Several lines of evidence in our study suggest that IL-4 has the potential to exert autocrine growth stimulatory effects. First, IL-4 stimulated growth of five pancreatic cancer cell lines. Second, pancreatic cancer cells expressed IL-4. Third, anti-IL-4 neutralising antibodies partially inhibited basal and IL-4-stimulated growth. Together with the observation that pancreatic cancer cells express high levels of IL-4Rs (Kornmann et al, 1999a), our results raise the possibility that IL-4 may have a dual effect in human pancreatic cancers. First, IL-4 may act as an autocrine growth factor in pancreatic cancer cells. Second, cancer cell-derived IL-4 may exert paracrine functions on surrounding infiltrating immune cells, inhibiting immune responses.

Overall, our observations may help to better understand the pathobiology of pancreatic cancer in the future giving rise to the possibility that IL-4 and other related cytokines may be important suppressors of cancer-directed immunosurveillance in addition to their growth-promoting effects, thereby facilitating primary pancreatic tumour growth and metastasis. However, to confirm this hypothesis, further studies are necessary.

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