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Characterisation of Insulin-like Growth Factor I Receptors of Human Acute Lymphoblastic Leukaemia (ALL) Cell Lines and Primary ALL Cells

Thomas G. Baier, Wolf-Dieter Ludwig, Dieter Schönberg and Klaus K.P. Hartmann

The expression of insulin-like growth factor I (IGF-I) receptors (IGF-IR) on human B-lineage and T-lineage acute lymphoblastic leukaemias (ALL) representing different maturational stages has been studied. Immature (stage I) and mature (stage II) T ALL as well as pre-B ALL cell lines expressed high numbers of IGF-IR with high affinity for IGF-I. In contrast, on T ALL, stage II and B ALL only low specific binding of ^{125}I-IGF-I was detected. No binding of ^{125}I-IGF-I to Burkitt lymphoma cells was found. Primary human T, pre-B and cALL cells also expressed IGF-IR with K_d for IGF-I and IGF-IR number per cell in the same range as the investigated cell lines. Crosslinking of ^{125}I-IGF-I to T and pre-B ALL cells revealed IGF-IR alpha-subunits of 135 and 116 kD for HSB2. Gene expression of IGF-IR could be detected in all T ALL cell lines but was indetectable in SKW6, a B ALL cell line.
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INTRODUCTION

INSULIN-LIKE growth factor (IGF)-I is related to insulin and produced by liver cells after stimulation by growth hormone (GH). It promotes skeletal growth by inducing proliferation of chondrocytes. Further, this peptide acts as a paracrine or autocrine growth factor of many non-transformed and transformed cells and cell lines (for review, see [1]). Physiological actions of IGF-I and IGF-II are mediated by specific interactions with cell surface receptors. IGF-I and IGF-II are ligands for the IGF-I and IGF-II receptors. From both related peptides IGF-I has a crossreactivity with the insulin receptor and IGF-II with the IGF-I receptor. Insulin, with lower affinity, also binds to the IGF-I receptor. Extensive homology exists between the insulin and IGF-I receptor [2], whereas the IGF-II receptor, which

is identical with the cation-independent mannose-6-phosphate receptor, has a completely different structure [3].

The IGF-I receptor is composed of two extracellular alpha subunits (135 kD) which are disulphide linked to two intracellular beta-subunits (95 kD) [2]. Affinity crosslinking studies with radioactive labelled ^{125}I-IGF-II suggest that the IGF-II receptor is a monomer of 260 kD [3]. In addition to many non-transformed and transformed cells, the existence of IGF-I and IGF-II receptors has been proven on human T lymphocytes [4, 5] as well as on human acute lymphoblastic leukaemia (ALL) cell lines of T and B lineage [6]. On T ALL cells the IGF-I receptor has been found, whereas on Burkitt lymphoma and other B lymphoma cells the insulin receptor predominates [6]. It has been previously shown that GH stimulates the growth of human leukaemic T lymphocytes [7] and human T cell leukaemia virus (HTLV)-transformed T lymphoblast cell lines [8]. The biological effect of GH on HTLV-transformed leukaemic cells is mediated via locally generated IGF-I [9].

To clarify which ALL cells express IGF-I receptors and therefore may be influenced by autocrine IGF-I production, we further characterised the IGF-I receptor on human T and B ALL cell lines at various stages of differentiation. We also studied the existence of IGF-I receptors on human primary ALL cells freshly isolated from children with ALL.

MATERIALS AND METHODS

Antibodies and reagents

IgG class of monoclonal antibody alpha-IR3 was provided by Dr Steven Jacobs, Wellcome Research Laboratories, USA, the monoclonal antibody, 2C2, was the gift of Prof. Dr A. Hasilik, Münster, FRG. The monoclonal antibodies of the OKT series were supplied by Ortho Diagnostic, FRG. Monoclonal antibodies directed against CD7, CD10 and CD19 were supplied by Becton-Dickinson, FRG; monoclonal antibody, IOB1, was supplied by Dianova, FRG; and the fluoresceinisothiocyanate (FITC)-labelled antibody directed against human surface IgG was supplied by Behring Werke, FRG.

Recombinant IGF-I and -II were purchased from Boehringer, FRG and insulin was supplied by Eli Lilly, USA. ^{125}I-labelled IGF-1 (specific activity 8.88×10^4 Bq/μg), purified by high-performance liquid chromatography, was supplied by Amersham, UK.

Characterisation of T and B ALL cells

T and B ALL cell lines were characterised by immunofluorescence as described previously [10]. Briefly, 1×10^6 cells were resuspended in culture medium and T ALL incubated with T cell specific antibodies for 30 min at 40°C. Thereafter, T ALL and B ALL were incubated with FITC-labelled anti-human IgG for 30 min at 40°C. Positive cells were analysed in a fluorescence microscope.

Isolation of fresh leukaemic cells

Fresh leukaemic cells were isolated from bone marrow and/or blood samples by "Ficoll-Hypaque" (Pharmacia, FRG) density gradient centrifugation and characterised by immunophenotyping [11].

IGF-I receptor binding studies

10^6 cells were incubated at 10°C for 3 h with 4.5 pM ^{125}I-IGF-I and increasing concentrations of unlabelled IGF-I in 2 ml Hepes buffer, pH 7.4 (in mmol/l: 100 Hepes, 120 NaCl, 5 KCl, 1.2 $MgSO_4$, 10 dextrose, 1 EDTA, 15 sodium acetate; and 1% bovine serum albumin). Binding reactions were stopped by addition of 0.5 ml ice-cold Hepes buffer. The cells were centrifuged and the pellet counted in a gamma-counter. Binding was corrected for non-specific ^{125}I-IGF-I binding as determined in the presence of 130 mmol/l unlabelled IGF-I. Scatchard analysis of the binding data was performed using the Ligand program [12]. No difference in IGF-I receptor number per cell was detected during exponential cell growth phase.

Chemical crosslinking of ^{125}I-IGF-I

Radiolabelled ^{125}I-IGF-I (Amersham) was chemically crosslinked to intact ALL cell lines with di(succinimidyl)suberate (DSS) at a final concentration of 0.4 mM [13]. Briefly, 4–6 × 10^9 cells were incubated at 10°C for 3 h with 2–3 × 10^6cpm ^{125}I-IGF-I. Thereafter, cells were washed twice with binding buffer and at 40°C cells were homogenised and centrifuged at 1500 ***g*** for 30 min and 100,000 ***g*** for 90 min. The resulting membrane pellet was resuspended in Laemmli buffer with 5% v/v 2-mercaptoethanol, separated on a 6% SDS–polyacrylamide gel and subjected to autoradiography.

Northern blotting

Poly (A)+ RNA was isolated from 2×10^8 ALL cells by lysing the cells in 1% SDS in a Tris buffer containing 0.2 mg/ml proteinase K [14]. 5 μg of poly(A)+ RNA were separated on 1% agarose gels containing 2.2 mmol/l formaldehyde and transferred to nitrocellulose. Nitrocellulose blots were hybridised with a human IGF-I receptor cDNA [15] or a human beta-actin cDNA [16] and autoradiography was carried out following a final wash of 0.1 × SSC, 0.1% SDS at 50°C.

RESULTS

Characterisation of human leukaemic T and B cell lines

To determine the stage of differentiation of each cell line we measured binding of T or B lymphocyte-specific antibodies to each cell line by immunofluorescence. According to the criteria of Reinherz *et al.* [10] the T ALL lines could be divided into 3 groups: HSB2 and HUT78, stage I; CEM and MOLT3, stage II and Jurkat, JM886 and JMP, stage III (data not shown). Further Jurkat and JMP cells are OKT3 and OKT4 positive, but OKT8 negative resembling helper T cells. In contrast, JM886 cells are OKT3 and OKT8 positive, but OKT4 negative resembling suppressor T cells. The B ALL line REH could be analysed as pre-B ALL, whereas CESS and SKW6 cells were analysed as B ALL (data not shown).

Specific binding of ^{125}I-IGF-I to human leukaemic cell lines

To investigate the specifity of ^{125}I-IGF-I binding to T ALL, competition–inhibition studies were carried out. Specific ^{125}I-IGF-I binding was inhibited by unlabelled IGF-I one-half-maximally at 1.8 ng/ml for HUT78 (Fig. 1a) and 2.3 ng/ml for HSB2 (Fig. 2a). On both cell lines ED_{50} of ^{125}I-IGF-I by unlabelled insulin was 0.6 and 1.5 μg/ml, respectively. On HUT78 ED_{50} of ^{125}I-IGF-I by unlabelled IGF-II was 10 ng/ml (Fig. 1a). In contrast, on HSB2 cells displacement of ^{125}IGF-I

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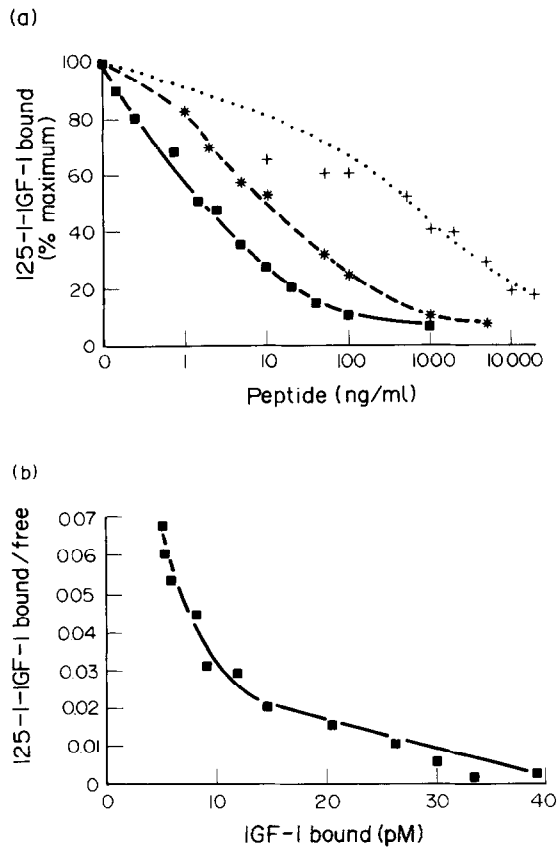

Fig. 1. Specificity of IGF-I ^{125}I-IGF-I binding to HUT78 cells. HUT78 cells were incubated with 4.5 pmol/l ^{125}I-IGF-I and varying concentrations of unlabelled IGF-I (■), IGF-II (*) and insulin (+).

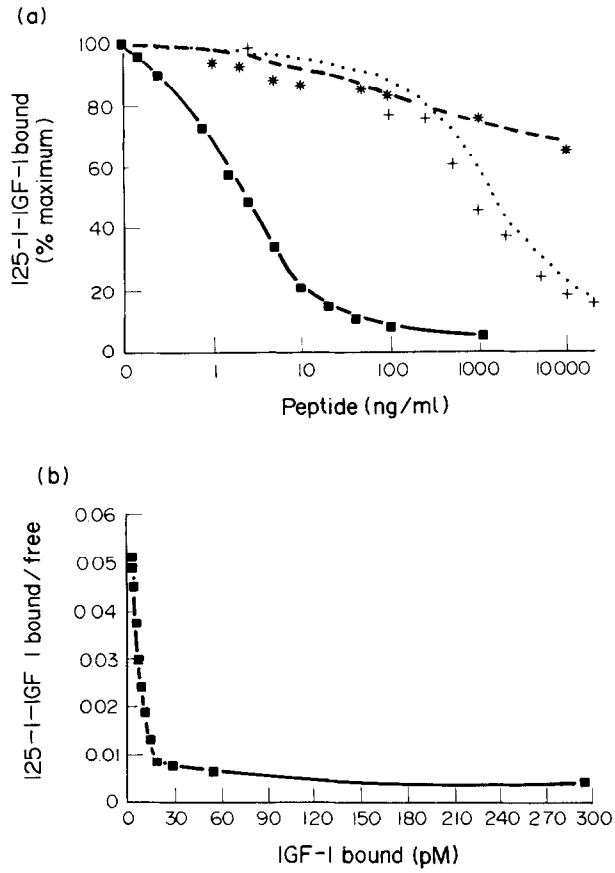

Fig. 2. Specificity of ^{125}I-IGF-I binding to HSB2 cells. For details see Fig. 1. Values are the mean of four separate experiments.

by IGF-II was lower than insulin (Fig. 2a). Scatchard analysis of the binding data revealed a 2-sided model of the binding sites on each cell line (Figs 1b, 2b). Dose displacement of ^{125}I-IGF-I by unlabelled IGF-II and insulin were similar for HUT78, CEM, MOLT3, JM886 and JMP cells, whereas HSB2 and JURKAT cells had similar displacement of ^{125}I-IGF-I by unlabelled IGF-II (data not shown). Binding of ^{125}I-IGF-I was almost completely inhibited in the presence of alpha-IR3 at a concentration of 400 ng/ml alpha IR3 (data not shown). In contrast, 2C2 and RPN538 did not inhibit binding of ^{125}I-IGF-I (data not shown).

Of all tested T ALL cell lines, highest specific binding for ^{125}I-IGF-I was measured for T ALL, stage I, whereas lowest specific affinity was found for T ALL, stage II (Table 1). Scatchard analysis of the binding data revealed high affinity IGF-I receptors for T ALL stages I and III (K_d = 0.15–0.32 nmol/l) but IGF-I receptors with lower affinity for IGF-I on T ALL stage II (K_d = 0.49–0.89 nmol/l) (Table 1). IGF-I receptor number per cell was highest for HSB2 and HUT78, ALL stage I (1400;1800 per cell) and lowest on CEM and MOLT3 (ALL stage II) with 182 and 564 IGF-I receptors per cell, respectively (Table 1).

Also, B ALL specifically bind ^{125}I-IGF-I. On REH-cells dose displacement of ^{125}I-IGF-I by unlabelled IGF-I, IGF-II and insulin revealed an ED_{50} of 3.9, 40 ng/ml and 1 μg/ml, respectively (data not shown). Alpha IR3 completely blocked the binding of ^{125}I-IGF-I at 5 μg/ml, whereas 2C2 and RPN538 had no effect on ^{125}I-IGF-I binding (data not shown). Specific binding of ^{125}I-IGF-I to both REH and NALM-6 cells was in the same range as for T ALL, stage III (Table 1). Both pre-B cell lines express 612 and 720 IGF-I receptors per cell with a K_d of 0.4 and 0.35 nmol, respectively (Table 1). In contrast on B ALL SKW6 and CESS only low specific binding of IGF-I was measured (Table 1). Since K_d for IGF-I was high we cannot exclude the possibility that IGF-I in these cells binds to the insulin receptor. On BJAB, a Burkitt lymphoma cell line, no specific binding of ^{125}I-IGF-I was detected (Table 1).

Table 1. Specificity of ^{125}I-IGF-I binding to human ALL cell lines

T ALL	^{125}I-IGF-I bound (% of maximum)	K_d (nmol/l)	IGF-I receptor no. (per cell)
HSB2	47	0.17	1400
HUT78	63	0.15	1800
CEM	2	0.89	182
MOLT3	9	0.49	564
Jurkat	19	0.32	1024
JMP	10	0.21	942
JM886	22	0.21	352
Pre-B ALL			
REH	13	0.40	612
NALM6	15	0.35	720
B ALL			
CESS	3	0.50	102
SKW6	5	1.00	41
BJAB	Not detectable		

Table 2. Specificity of ^{125}I-IGF-I binding to fresh leukaemic cells

Cell type	K_d (nmol/l)	IGF-I receptor number (per cell)
T ALL (n = 7)	0.27 (0.18)	150 (72)
Pre-B ALL (n = 2)	0.35 (0.07)	256 (132)
cALL (n = 6)	0.87 (0.41)	752 (492)

Mean (S.D.).

Binding of ^{125}I-IGF-I to fresh leukaemic cells

In comparison to ALL cell lines fresh leukaemic cells with a T or B cell precursor (common, pre-B) phenotype also express IGF-I receptors. All cells were isolated from children with acute lymphoblastic leukaemia. T ALL cells express 150 (S.D. 72) IGF-I receptors per cell with high affinity for IGF-I [0.27 (S.D. 0.18) nmol/l] (Table 2). Similar results were obtained with pre-B ALL cells with 256 (132) IGF-I receptors per cell and a K_d of 0.35 (0.07) nmol for IGF-I. In contrast, on cALL more binding sites for IGF-I were measured [752 (492) IGF-I receptors per cell] but with lower specific affinity for IGF-I [K_d = 0.87 (0.41) nmol] (Table 2).

Structure of IGF-I receptor in ALL cell lines

To determine the size of the alpha-subunit of the IGF-I receptor in leukaemic cell lines we covalently crosslinked ^{125}I-IGF-I to the cell membrane by DSS and separated the membrane fraction of these cells by SDS–electrophoresis under reducing conditions. These experiments revealed alpha-subunits of the IGF-I receptor with a molecular size of 135 kD for the cell lines HUT78, JMP, Jurkat and JM886 (Fig. 3). In contrast, a smaller alpha-subunit of the IGF-I receptor with a molecular size of 116 kD was detected for the cell line HSB2 (Fig. 3a). No detectable crosslinking of ^{125}I-IGF-I to the membrane of CEM cells, which had low specific binding of ^{125}I-IGF-I, was found (Fig. 3b).

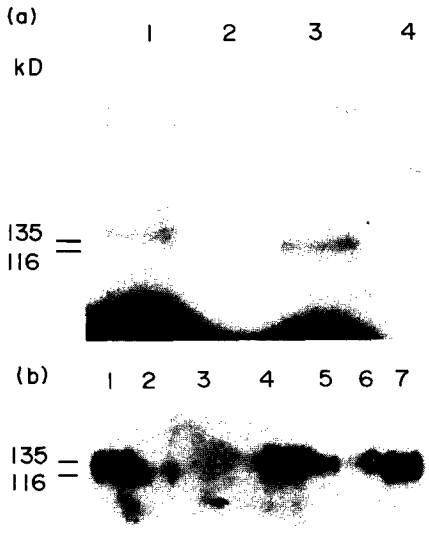

Fig. 3. Chemical crosslinking of ^{125}I-IGF-I to the IGF-I receptor alpha-subunit on ALL membrane. The calculated molecular size of the IGF-I receptor alpha-subunit bands are indicated. (a) 1: Jurkat, 2: Jurkat plus 1 μg/ml non-radioactive IGF-I; 3: HSB2; 4: HSB2 plus alpha-IR3 (50 ng/ml). (b) 1: HUT78, 2: HSB2, 3: CEM, 4: JMP, 5: Jurkat, 6: JM886, 7: REH.

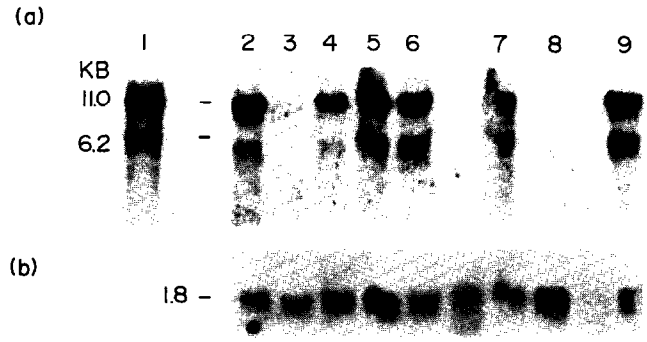

Fig. 4. Northern blot analysis of 5 μg poly(A) + RNA with (a) a human IGF-I receptor cDNA [15] and (b) a human beta-actin cDNA [16]. The calculated molecular sizes of the specific mRNA bands of the IGF-I receptor and beta-actin are indicated. 1: HSB2, 2: HUT78, 3: CEM, 4: MOLT3, 5: Jurkat, 6: JM886, 7: REH, 8: SKW6, 9: IM9.

IGF-I receptor gene expression in ALL cell lines

To determine IGF-I receptor gene expression in these cell lines we performed northern blot analysis of 5 μg poly A+ RNA of each cell line. Two IGF-I receptor mRNA bands with a size of 11 and 6.2 kB could be seen for T ALL HSB2, HUT78, Molt3, CEM, Jurkat, JMP and REH cells, whereas IGF-I receptor mRNA in SKW6 cells was not detectable (Fig. 4).

DISCUSSION

It is well known that human non-transformed and transformed lymphocytes express IGF-I receptors [4, 6, 8, 9, 17, 18]. Mitogenic stimulation of non-transformed peripheral T lymphocytes is accompanied by an increase in IGF-I receptor number [4, 18], suggesting that this receptor is involved in mitogenic proliferation of these cells. It has been previously shown that T ALL preferentially express IGF-I receptors, and B ALL insulin receptors, but lack IGF-I receptors [6]. Our data show that leukaemic T-cells with an immature or mature phenotype as well as freshly isolated primary T ALL express IGF-I receptors. Compared with peripheral non-transformed T lymphocytes [18] the affinity for IGF-I is similar in T ALL stage I and III but different in T ALL stage II. However, compared to non-transformed T lymphocytes [18] in T ALL stages I and III, the IGF-I receptor number per cell is increased 3–5-fold. Dose displacement of ^{125}I-IGF-I by unlabelled IGF-II revealed the existence of different IGF-I receptors on HSB2 and Jurkat cells. Since IGF-II in these cells displaces ^{125}I-IGF-I less than insulin, we assume the existence of IGF-I/insulin receptor hybrids in these cells. On both cell lines the existence of insulin receptors was measured (unpublished data). Further Jurkat and HSB2 cell express in addition to IGF-I receptor mRNA, insulin receptor mRNA (unpublished data). The existence of insulin/IGF-I receptor hybrids has been described on IM9 lymphocytes, a pre-B ALL cell line [19, 20]. Furthermore, insulin/IGF-I hybrid receptors composed of an insulin alpha-beta heterodimer and an IGF-I alpha-beta heterodimer half receptor can be assembled *in vitro* by either treatment with Mn/MgATP or with a combination of insulin and IGF-I [21].

In addition, the alpha subunit of the IGF-I receptor in HSB2

cells was approximately 20 kD smaller than in other T ALL cells or non-transformed T lymphocytes which also express a 135 kD IGF-I receptor alpha-subunit [18]. The human brain expresses structurally distinct IGF-I receptor alpha-subunits with a molecular size of 120 kD [22]. Truncated forms of the extramembrane part of the receptor have been described for EGF receptor [23]. Human Schwann cells shed a truncated form of the NGF receptor into their cell culture medium [24]. Furthermore, the extramembrane part of the HGH receptor circulates as HGH binding protein in human serum [25].

As on IM9 cells [19, 20], the IGF-I receptor was also detected on other pre-B ALL cell lines—REH and Nalm6—and on primary pre-B ALL cells. In addition, primary cALL express IGF-I receptors. On B ALL cells only low specific binding of ^{125}IGF-I was measured, suggesting binding to the insulin receptor. In contrast, on Burkitt type ALL lines the existence of IGF-I receptors has been shown [26].

Fresh isolated leukaemic cells also express IGF-I receptors. Affinity constant for IGF-I in both cell types, primary T ALL and T ALL cell lines, is similar. However, IGF-I receptor number is increased in T ALL cell lines, whereas on primary T ALL cells IGF-I receptor number is in the same range as in non-transformed T lymphocytes [18]. Similar results were obtained for primary pre-B ALL cells and the pre-B ALL cell line REH. In contrast, primary cALL cells as B ALL cell lines bind IGF-I with low specific affinity, suggesting that IGF-I could bind to insulin receptors or different IGF-I receptors on these cells. The fact that GH induces IGF-I production in IM9 lymphoblasts [27] and HTLV-transformed T lymphocytes [9] raises the question whether IGF-I for these cells is an autocrine growth factor acting via the IGF-I receptor. We previously found that IGF-I acts as a paracrine growth factor for HSB2 and as an autocrine growth factor for HUT78 and REH cells [28]. In contrast, the other T ALL cell lines, which also express IGF-I receptors, do not use IGF-I as a cellular growth factor [28]. However, our data show that insulin, acting via the insulin receptor, is involved in proliferation of the T ALL CEM and MOLT3 and the B ALL SKW6 and CESS [28]. The role of the IGF-I receptor for growth of T-ALL JURKAT, JMP and JM886 remains unclear, since neither insulin nor IGF-I is involved in cell proliferation. Possibly the IGF-I receptor itself, without the ligand IGF-I, is involved in cellular proliferation of JURKAT and JMP, since alpha-IR3 but not IGF-I antibody inhibits DNA synthesis in these cells [28]. Binding studies with ^{125}I-IGF-I revealed the existence of distinct receptors on JURKAT cells. In contrast to HSB2 cells, the alpha-subunit of JURKAT cells was not different compared with the other T ALL lines or compared with normal non-transformed T lymphocytes [18]. In all T ALL cells expressing the IGF-I receptor, IGF-I receptor mRNA was also detected. Using a IGF-I receptor cDNA from the alpha-subunit of the IGF-I receptor [1] in all T ALL cell lines, two IGF-I receptor mRNA bands with a molecular size of 11 and 6.2 kB were found. On fibroblasts [1] and human breast cancer cell lines [29] northern blot analysis revealed the existence of two IGF-I receptor mRNA bands with a molecular size of 11 and 7 kB. Both mRNA bands were also seen when poly(A+) RNA of HSB2 cells was hybridised with a 3′ IGF-I receptor cDNA corresponding to the beta-subunit of the IGF-I receptor (manuscript in preparation). However, in contrast to HSB2 cells when poly(A+) RNA of JURKAT cells was hybridised with this 3′ IGF-I receptor cDNA three additional IGF-I receptor mRNA bands were seen (manuscript in preparation). To determine whether this fact is due to a difference in the length of the IGF-I receptor mRNA or due to a difference of mRNA splicing and therefore responsible for a distinct IGF-I receptor in JURKAT cells we are attempting to isolate further IGF-I receptor cDNAs from a JURKAT cDNA library.

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Induction of MHC Antigens by Tumour Cell Lines in Response to Interferons

A.M.E. Nouri, R.F. Hussain, A.V.L. Dos Santos, D.J. Gillott and R.T.D. Oliver

The induction of major histocompatibility complex antigens by interferons (IFN) on 17 established tumour cell lines was investigated by radio binding. One bladder (Fen) and two testis lines (Tera I and Ha) lacked class I antigens and IFN-γ failed to induce their expression. However, IFN-γ upregulated these antigens on lines expressing low class I antigens (Tera II and EP2102) with little or no significant effect on high class I expressing lines (T24 and RT112). In one bladder line (Wil) IFN-γ, whilst failing to alter monomorphic class I, upregulated polymorphic HLA-A2 and A3 antigens. None of the 17 lines expressed class II antigens, but could all be induced by IFN-γ except T24, TccSup, Tera II and Lan lines. This defect was not due to the absence of IFN-γ receptor, since under the same conditions intracellular adhesion molecule 1 was upregulated. IFN-α, whilst failing to have any effect on class II, induced class I antigens. IFN-β showed no activity on either class I or II antigens when used alone. However, in combination, it inhibited IFN-γ induced class II antigens. Thus, it may be possible to study cells from fresh tumours to preselect the minority of patients who might benefit from cytokine therapy.
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INTRODUCTION

DURING THE past decade it has become clear that a small minority of durable complete remissions occur after the use of cytokines [interferon (IFN) α and interleukin (IL) 2] for treating human cancer [1]. *In vivo* studies with cloned neomycin-resistant gene-labelled tumour infiltrating lymphocytes from some of these patients provide the most compelling evidence supporting the concept that these tumour rejections are mediated by immune T lymphocytes. There is a pressing need for methods of preselecting this subgroup of patients to reduce the unnecessary use of expensive and toxic drugs for patients who will not benefit.

The recognition and response to foreign antigens requires the participation of major histocompatibility complex (MHC) class I and II antigens acting as associative molecules for presentation of antigens to T cells [2]. This stimulated interest as to whether abnormality in expression of these antigens may be a factor for tumour escape from cytolytic T cell attack.

It has become apparent that a wide variety of aberrant expression of MHC antigens can be demonstrated on both experimental and human tumour cells. This and the demonstration that transfection of the missing class I gene into a mouse lymphoma led to loss of tumorogenicity and induced protection against untransfected cells [3], has stimulated speculation as to whether these changes may be major factors in tumour escape from immune surveillance.

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