Biochem. J. (1990) 266, 737–742 (Printed in Great Britain)

Insulin-like growth factor binding to the atypical insulin receptors of a human lymphoid-derived cell line (IM-9)

Helen A. JONAS* and Alison J. COX
Department of Medicine, University of Melbourne, Royal Melbourne Hospital, Parkville, Victoria 3050, Australia

The cells of the IM-9 human lymphocyte-derived line contain a sub-population of insulin-binding sites whose immunological and hormone-binding characteristics closely resemble those of the atypical insulin-binding sites of human placenta. These binding sites, which have moderately high affinity for multiplication-stimulating activity [MSA, the rat homologue of insulin-like growth factor (IGF) II] and IGF-I, are identified on IM-9 cells by ^{125}I-MSA binding. They account for ~ 30% of the total insulin-receptor population, and do not react with a monoclonal antibody to the type I IGF receptor (αIR-3). The relative concentrations of unlabelled insulin, MSA and IGF-I required to displace 50% of ^{125}I-MSA from these binding sites (1:4.7:29 respectively) are maintained for cells, particulate membranes, Triton-solubilized membranes precipitated either by poly(ethylene glycol) or a polyclonal antibody (B-10) to the insulin receptor, and receptors purified by insulin affinity chromatography. Because the atypical insulin/MSA-binding sites outnumber the type I IGF receptors in IM-9 cells by ~ 10-fold, they also compete with the latter receptors for ^{125}I-IGF-I binding. Thus ^{125}I-IGF-I binding to IM-9 cells is inhibited by moderately low concentrations of insulin (relative potency ratios for insulin compared with IGF-I are approx. 1/14 to 1/4) and is partially displaced (65–80%) by αIR-3. When type I IGF receptors are blocked by αIR-3 or removed by B-10 immunoprecipitation or insulin affinity chromatography, the hormone-displacement patterns for ^{125}I-IGF-I binding resemble those of the atypical insulin/MSA-binding sites.

INTRODUCTION

The biological actions of the insulin-like growth factors, IGF-I and IGF-II, are mediated via interaction with IGF receptors (Van Wyk *et al.*, 1985; Conover *et al.*, 1987; Hari *et al.*, 1987), and to a lesser extent, with insulin receptors (King *et al.*, 1980). Two major forms of IGF receptors have been reported. The type I IGF receptor, though distinct from the insulin receptor, contains many similar amino acid sequences (Ullrich *et al.*, 1986), and is composed of similarly sized M_r-135000 (α) and M_r-95000 (β) subunits linked by disulphide bonds into a heterotetrameric complex (Massague & Czech, 1982; Kull *et al.*, 1983). The type II IGF receptor is a monomer (M_r 260000) (Massague & Czech, 1982) closely resembling or identical with the mannose 6-phosphate receptor (Morgan *et al.*, 1987). The type I receptor has a higher affinity for IGF-I than for IGF-II and a low affinity for insulin; the type II receptor has a higher affinity for IGF-II than for IGF-I and no affinity for insulin. Both IGFs cross-react to a small degree with the insulin receptor (Massague & Czech, 1982).

However, we (Jonas *et al.*, 1986, 1989) and others (Hintz *et al.*, 1984; Tollefson *et al.*, 1987) have also described another type of receptor in human placenta which binds IGFs and insulin with high affinity. These atypical insulin receptors, which co-purify with insulin receptors during insulin affinity chromatography (Jonas *et al.*, 1986, 1989; Tollefson *et al.*, 1987) have the same subunit structure and immunological properties as insulin receptors and do not cross-react with a monoclonal antibody to the type I IGF receptor (αIR-3) (Jonas *et al.*, 1986, 1989). They differ from classical insulin receptors in their unusually high affinity for multiplication-stimulating activity (MSA, the rat homologue of IGF-II) and IGF-I, account for 10–20% of the total insulin-receptor population, and can be detected by precipitation with a polyclonal insulin-receptor antibody (B-10) after labelling with ^{125}I-MSA (Jonas *et al.*, 1986, 1989).

The cells of the IM-9 human lymphoid-derived line also contain an unusual population of IGF-II-binding sites (Hintz *et al.*, 1984), whose immunological and hormone binding characteristics (Misra *et al.*, 1986) resemble those of the atypical insulin receptors of human placenta. However, the studies by Misra *et al.* (1986) were conducted on whole IM-9 cells and particulate membranes, whereas our placental studies were performed on solubilized receptors separated from type I IGF receptors by B-10 precipitation or insulin affinity chromatography (Jonas *et al.*, 1986, 1989). Furthermore, MSA bound with higher affinity (about 10-fold) to the solubilized atypical receptors from placenta than did IGF-II to the surface of intact IM-9 cells. Thus it is possible that the unusual IGF-II-binding properties of IM-9 cells might not reflect the presence of atypical insulin receptors: they may, instead, represent membrane interactions between type I IGF receptors, insulin receptors and neighbouring non-receptor proteins.

Therefore, to determine whether the IGF-II/MSA-binding properties of IM-9 cells were maintained after membrane disruption and removal of type I IGF receptors, we examined binding of insulin, MSA and IGF-I to

Abbreviations used: IGF, insulin-like growth factor; MSA, multiplication-stimulating activity; PMSF, phenylmethanesulphonyl fluoride; CDI-agarose, 1,1'-carbonyldi-imidazole-activated agarose; PEG, poly(ethylene glycol) 6000.

* To whom correspondence and reprint requests should be addressed.

IM-9 receptors on whole cells, particulate membranes and solubilized membranes precipitated with B-10 or purified by insulin affinity chromatography. To compare accurately the MSA-binding properties of IM-9 cells with those of the placental atypical insulin receptors, affinity-purified IM-9 receptors were assayed under the same conditions as for their placental counterparts.

MATERIALS AND METHODS

Materials

The reagents used in this study, including unlabelled and ^{125}I-labelled insulin, MSA and IGF-I, and antisera B-10 and αIR-3, have been described previously (Jonas *et al.*, 1989).

IM-9 cells, membrane preparations and affinity-purified receptors

IM-9 cells, an established human lymphoid-derived cell line obtained from Dr. R. Hintz (Stanford, CA, U.S.A.), were grown in RPMI-1640 medium (Flow Laboratories, Sydney, Australia) supplemented with 2 g of $NaHCO_3$/l, 2 mM-L-glutamine and 10% (v/v) fetal-calf serum (Flow Laboratories, Australia). Cells in late-exponential to early stationary phase were harvested by centrifugation, then washed in phosphate-buffered saline (8.0 g of NaCl/l, 1.15 g of Na_2HPO_4/l, 0.2 g of KH_2PO_4/l and 0.2 g of KCl/l, pH 7.4) or 0.15 M-NaCl.

Membranes were prepared from washed IM-9 cells [$(1–3.5) \times 10^9$] by using the homogenization and centrifugation procedures described by Misra *et al.* (1986). The 20000 *g* pellet was washed with lymphocyte-binding buffer (0.1 M-Hepes, 0.12 M-NaCl, 1.2 mM-$MgSO_4$, 5 mM-KCl, 15 mM-sodium acetate, 1 mM-EDTA and 10 mM-glucose, pH 7.5) containing no added BSA, then resuspended to a final volume equivalent to 175×10^6 cells/ml, or 1 mg of membrane protein/ml. Portions of the resuspended membranes were solubilized with an additional 0.25 vol. of Triton X-100 (50 g/l) containing Trasylol (5000 Kallikrein units/ml), 10 mM-phenylmethanesulphonyl fluoride (PMSF) and bacitracin (2500 units/ml) for 1 h at 4 °C, then centrifuged at 100000 *g* for 1.5 h at 4 °C. Particulate and solubilized membranes were stored at −70 °C until assay.

To purify the insulin receptors, washed IM-9 cells (810×10^6) were solubilized in 12 ml of 0.1 M-sodium phosphate, pH 7.5, containing Triton X-100 (10 g/l), Trasylol (1000 Kallikrein units/ml), PMSF (2 mM) and bacitracin (500 units/ml) for 2 h at 4 °C. After centrifugation at 100000 *g* (1.5 h at 4 °C), the supernatant was subjected to sequential affinity chromatography on wheat-germ-lectin–Sepharose 6MB and insulin–1,1′-carbonyldi-imidazole-activated agarose (CDI-agarose) under the conditions described by Newman & Harrison (1985). Purified receptors were eluted from the insulin–CDI-agarose column with 0.05 M-sodium acetate, pH 5.0, containing 1 M-NaCl, Triton X-100 (1 g/l) and 0.1 mM-PMSF, and neutralized with 0.25 vol. of 1 M-Tris/HCl, pH 7.4.

Binding assays

Cells (4×10^6) were incubated with 15000–20000 c.p.m. of ^{125}I-insulin, ^{125}I-MSA or ^{125}I-IGF-I and increasing concentrations of unlabelled insulin, MSA and IGF-I in a total of 0.2 ml of lymphocyte-binding buffer, pH 8.0, containing BSA (10 g/l) and bacitracin (45 units/ml). Incubations were performed in 12 mm × 75 mm plastic tubes in a shaking water bath for 90–120 min at 15 °C. To separate cells from unbound hormones, the cells were mixed with 0.5 ml of ice-cold buffer containing BSA (10 g/l), centrifuged for 5 min at 4000 *g*, then washed and centrifuged again. In some experiments, cells were assayed after preincubation for 30 min at 15 °C with saturating concentrations of αIR-3 [10-fold dilution of serum-free RPMI 1640 conditioned medium from the αIR-3 hybridoma clone, concentrated 20-fold against an Amicon PM-30 (M_r 30000) membrane]. Unbound αIR-3 was removed by washing with lymphocyte-binding buffer, before addition of hormones.

IM-9 membranes (20–40 μg of protein) were similarly incubated with ^{125}I-labelled and unlabelled hormones in a final assay volume of 0.2 ml. To separate membranes from unbound hormones, 1.0 ml of ice-cold buffer containing BSA (10 g/l) was added to each tube, and the membranes were centrifuged for 20 min at 4000 *g*.

Neutralized eluates or 'flow-through' fractions from the insulin–CDI-agarose column or solubilized membranes (40 μg of protein) in neutralized eluent buffer containing 1–2 g of Triton X-100/l (0.1 ml) were incubated with ^{125}I-labelled and unlabelled hormones (0.1 ml; in 0.1 M-sodium phosphate buffer, pH 7.5, containing 10 g of BSA/l) for 18–24 h at 4 °C. Receptor-bound hormone was precipitated by poly(ethylene glycol) 6000 (PEG) or immunoprecipitated with B-10 IgG (final dilution 14 μg/ml) and *Staphylococcus aureus* as previously described (Jonas *et al.*, 1982).

Total binding was expressed as a percentage of the total radioactivity added per assay tube. To determine specific binding (B%) of ^{125}I-insulin, ^{125}I-MSA or ^{125}I-IGF-I to their receptors, the non-specific binding of radioactivity in the presence of unlabelled insulin (5 μg) or unlabelled IGF (2 μl of 0.05% pure preparation) was subtracted from total binding. The incubations used to determine non-specific binding and specific binding in the absence of unlabelled hormone were performed in quadruplicate. All other incubations were performed in duplicate. Data from the competition-binding studies were analysed by the method of Scatchard (1949).

RESULTS

Binding studies to whole cells

Specific binding of ^{125}I-insulin, ^{125}I-MSA and ^{125}I-IGF-I to IM-9 cells at cell concentrations of 20×10^6/ml averaged 64±11% (mean±S.D.; $n = 5$), 18±4% (mean±S.D.; $n = 5$) and 21±5% (mean±S.D.; $n = 7$) respectively. Like Misra *et al.* (1986), we found that the binding of ^{125}I-insulin and ^{125}I-MSA was displaced by 50% by low concentrations of unlabelled insulin (14–28 ng/ml and 7–14 ng/ml respectively), but not by αIR-3. Moreover, the relative concentrations of unlabelled insulin, MSA and IGF-I required to displace 50% of bound ^{125}I-insulin and ^{125}I-MSA (2:80:≥290 and 1:4.7:29 respectively; mean proportions from five experiments) were very similar to those reported for the classical and atypical insulin receptors of human placenta (Jonas *et al.*, 1989). Data from a representative experiment are displayed in Table 1.

Like Misra *et al.* (1986) and Jacobs *et al.* (1986), we found that ^{125}I-IGF-I binding to IM-9 cells was only partially inhibited (65–80%) by αIR-3. Further studies showed that ^{125}I-IGF-I binding was displaced to different

Table 1. Competition between ^{125}I-insulin, ^{125}I-MSA or ^{125}I-IGF-I and unlabelled hormones for binding to IM-9 cells, membranes and solubilized membranes

IM-9 cells (20×10^6 cells/ml), membranes (0.2 mg of protein/ml) and Triton-solubilized membranes (0.2 mg of protein/ml) were incubated with ^{125}I-insulin, ^{125}I-MSA or ^{125}I-IGF-I in the presence of increasing concentrations of unlabelled insulin, MSA or IGF-I under incubation conditions I (lymphocyte-binding buffer, pH 8.0, containing 10 g of BSA/l, for 1.5 h at pH 8.0) or II (0.05 M-sodium phosphate/0.1 M-Tris/HCl/0.02 M-sodium acetate/0.4 M-NaCl, pH 8.0, containing 10 g of BSA/l, for 18 h at 4 °C). Cells and membranes were precipitated by centrifugation; solubilized membranes were precipitated by PEG or B-10 IgG and *Staph. aureus*. The membrane preparations were prepared from the same batch of cells used in the binding assays. The data are expressed as percentage of labelled hormone specifically bound in the absence of unlabelled hormone (B_0 %), and the concentrations of unlabelled hormone (ng/ml) required to displace 50 % of specifically bound labelled hormone.

		Labelled hormone											
		^{125}I-insulin				^{125}I-MSA				^{125}I-IGF-I			
			Concn. of unlabelled hormone causing 50 % displacement (ng/ml)				Concn. of unlabelled hormone causing 50 % displacement (ng/ml)				Concn. of unlabelled hormone causing 50 % displacement (ng/ml)		
Sample	Incubation conditions	B_0 %	Insulin	MSA	IGF-I	B_0 %	Insulin	MSA	IGF-I	B_0 %	Insulin	MSA	IGF-I
Cells	I	75 %	22	700	> 800	22 %	10	30	330	26 %	80	20	20
Membranes	I	62 %	4.0	160	> 800	10 %	1.5	13	38	10 %	90	10	7
Membranes	II	64 %	3.0	32	95	28 %	1.0	4.6	9.5	32 %	< 10	5.0	6.7
Solubilized membranes (PEG)	II	52 %	0.8	22	52	17 %	0.6	5.9	9.0	19 %	< 10	2.5	5.7
Solubilized membranes (B-10)	II	43 %	1.6	29	70	9.2 %	0.7	2.2	4.4	6.7 %	0.6	1.8	4.3

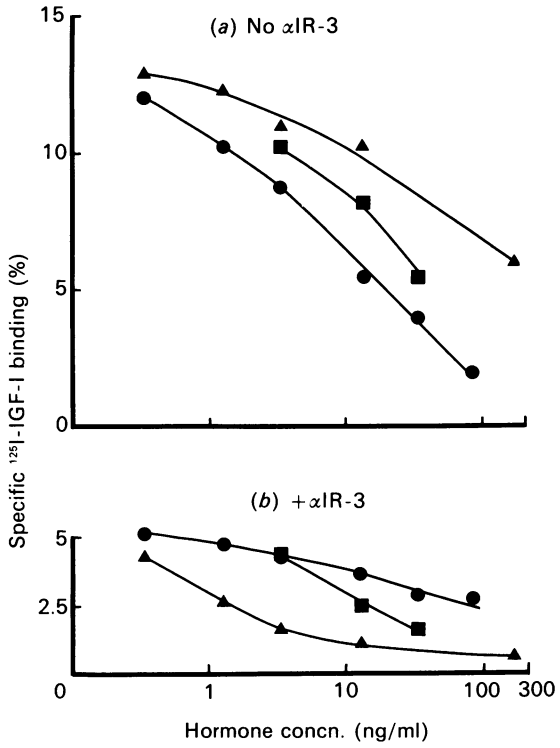

Fig. 1. Competition between ^{125}I-IGF-I and unlabelled hormones for binding to IM-9 cells: effect of preincubation with αIR-3

IM-9 cells (20×10^6 cells/ml) were incubated with ^{125}I-IGF-I and increasing concentrations of IGF-I (●), MSA (■) or insulin (▲) in lymphocyte-binding buffer at pH 7.5, before (*a*) and after (*b*) preincubation with αIR-3.

extents by unlabelled IGF-I, MSA and insulin, the relative concentrations for different batches of cells ranging from 1:4:70 to 1:2.7:10 (Fig. 1*a*) to 1:1:4 (Table 1). When the cells were preincubated with saturating concentrations of αIR-3, the competition-binding data obtained with unlabelled insulin, MSA and IGF-I (Fig. 1*b*) resembled those obtained with ^{125}I-MSA as tracer (Table 1), suggesting that IGF-I was binding to two types of receptors on the surface of IM-9 lymphocytes: the IGF-II/MSA-binding sites (i.e. atypical insulin-binding sites) and classical type I IGF receptors.

Binding studies to IM-9 receptors purified by insulin affinity chromatography

The IM-9 receptors purified by insulin affinity chromatography contained a sub-population of IGF-binding sites (identified by ^{125}I-MSA and ^{125}I-IGF-I binding) which closely resembled the placental affinity-purified atypical insulin receptors (Jonas *et al.*, 1986) in that they bound insulin, MSA and IGF-I with high affinity (Figs. 2*b* and 2*c*) and were quantitatively precipitated by the polyclonal insulin-receptor antibody B-10. The type I IGF receptors were largely excluded from the insulin–CDI-agarose column, because the ^{125}I-MSA- and ^{125}I-IGF-I-binding sites appearing in the 'flow-through' fractions (1.4 pmol compared with 12 pmol eluted) were not immunoprecipitated by antibody B-10 and showed the same patterns of hormone displacement (Figs. 2*e* and 2*f*) characteristic of type I IGF receptors (IGF-I > MSA > insulin) (Jonas *et al.*, 1986).

Binding studies to particulate and solubilized IM-9 membranes

The affinity-purifed IM-9 receptors, like their placental counterparts, bound insulin, MSA and IGF-I with much higher affinity than did intact IM-9 cells ($K_a = 24 \times 10^9$ M^{-1}, 2.7×10^9 M^{-1} and 2.4×10^9 M^{-1}, versus 1.6×10^9 M^{-1}, 0.2×10^9 M^{-1} and 0.4×10^9 M^{-1} respectively). To determine whether the higher binding affinities could be ascribed to the different assay conditions, we measured labelled hormone binding to particulate IM-9 membranes (20 μg of protein) in lymphocyte binding buffer (15 °C for 1.5 h, pH 8) or in 0.1 M-sodium phosphate buffer diluted 1:1 with neutralized eluent buffer (0.2 M-Tris/HCl/0.04 M-sodium acetate/0.8 M-NaCl) (4 °C for 18 h, pH 8). Under the latter conditions of incubation, the binding values for ^{125}I-insulin, ^{125}I-MSA and ^{125}I-IGF-I were higher (48%, 21% and 20%, compared with 31%, 4% and 3% respectively; see also Table 1), and lower concentrations of unlabelled insulin and MSA were required to displace 50% of bound ^{125}I-insulin and ^{125}I-MSA (Table 1).

However, the relative binding displacement patterns for ^{125}I-insulin and ^{125}I-MSA did not change substantially, and were maintained for solubilized membranes precipitated by PEG or by antibody B-10 (Table 1). When ^{125}I-IGF-I was substituted for ^{125}I-MSA as tracer, the displacement patterns obtained for solubilized membranes precipitated by antibody B-10 were the same (Table 1). We also noted that the proportion of MSA-binding sites compared with total insulin-binding sites in solubilized membranes precipitated by antibody B-10 was very similar to that obtained for intact cells (~ 30%) (Fig. 3).

DISCUSSION

Our results suggest that the unusual IGF-II/MSA-binding properties of human IM-9 cells are not due to interactions between type I IGF receptors and insulin receptors or adjacent non-receptor proteins. Rather, they reflect the presence of a unique insulin/IGF-binding species whose immunological and hormone-binding characteristics closely resemble those of the atypical insulin receptors of human placenta. The relative binding potencies of insulin, MSA and IGF-I in inhibiting ^{125}I-MSA binding to IM-9 cell preparations remain constant whether the binding is assayed on cells, particulate or solubilized membranes, or after elution from an insulin affinity column: the different binding affinities of the solubilized binding sites compared with those of intact cells can be attributed to the different assay conditions employed.

The MSA-binding sites/atypical insulin-binding sites account for a larger fraction of the total insulin-binding sites in IM-9 cells (~ 30%) than do the atypical insulin-binding sites in human placenta (10–20%). They also constitute a much greater proportion of the IGF-binding sites in IM-9 cells than in human placenta. In IM-9 cells, MSA binds only to atypical insulin-binding sites, whereas IGF-I binds to both atypical insulin-binding sites and type I IGF receptors, the former accounting for 20–35% of the total ^{125}I-IGF-I binding. In human placenta, 25–50% of the ^{125}I-MSA-binding activity to solubilized membranes can be ascribed to the atypical insulin-binding sites, and > 95% of the [^{125}I]IGF-I binding

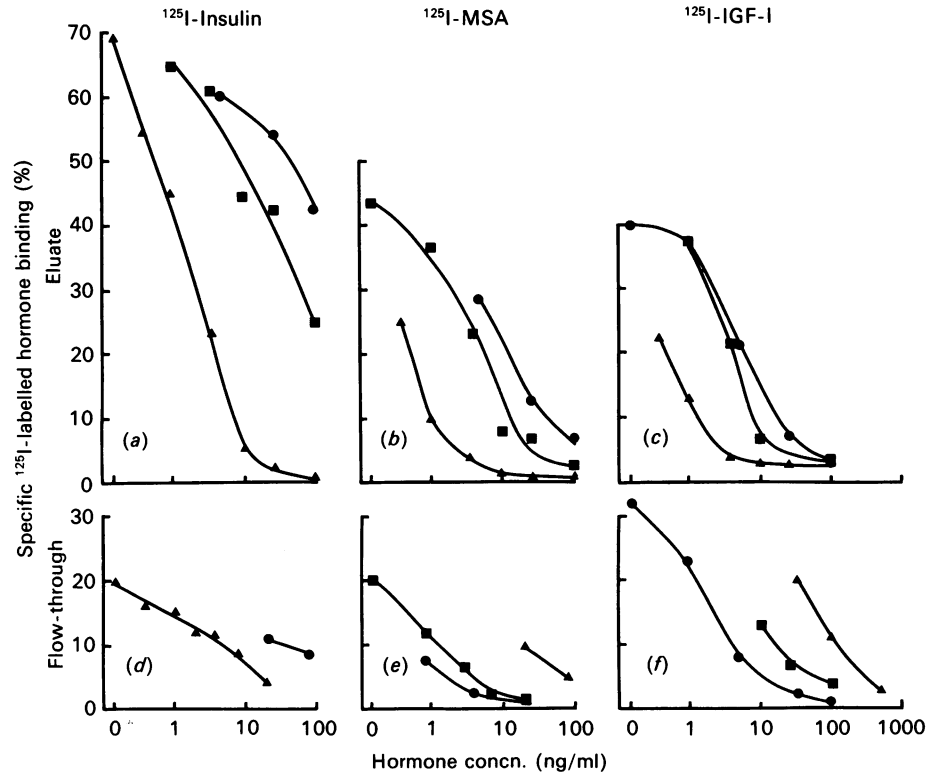

Fig. 2. Competition between ^{125}I-insulin, ^{125}I-MSA or ^{125}I-IGF-I and unlabelled hormones for binding to IM-9 affinity-purified receptors

Receptors eluted from the insulin–CDI-agarose column (0.1 ml of neutralized eluate) were incubated for 24 h at 4 °C with ^{125}I-insulin (*a*), ^{125}I-MSA (*b*) or ^{125}I-IGF-I (*c*) and increasing concentrations of insulin (▲), MSA (■) or IGF-I (●), made up in an additional 0.1 ml of 0.1 M-sodium phosphate containing 10 g of BSA/ml. The 'flow-through' fractions [0.1 ml, in 0.05 M-Hepes/0.3 M-*N*-acetyl-D-glucosamine/Triton X-100 (1 g/l)/0.15 M-NaCl, pH 7.6] were incubated as above with ^{125}I-insulin (*d*), ^{125}I-MSA (*e*) or ^{125}I-IGF-I (*f*) and unlabelled hormones. Receptor-bound hormone was precipitated with PEG.

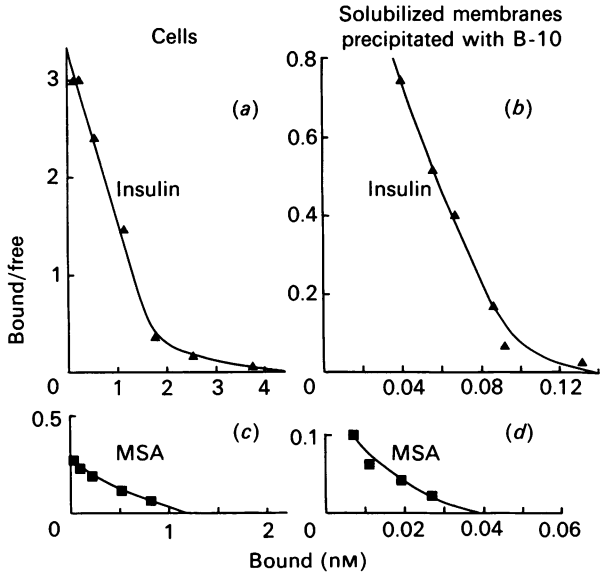

Fig. 3. Scatchard plots of insulin and MSA binding to IM-9 cells and solubilized IM-9 membranes immunoprecipitated with B-10 IgG

IM-9 cells and solubilized membranes immunoprecipitated with B-10 IgG were analysed for ^{125}I-insulin binding in the presence of increasing concentrations of unlabelled insulin (see Table 1), and the binding data were submitted to Scatchard analysis (*a* and *b* respectively). The same batches and concentrations of cells and immunoprecipitated membranes were also analysed for ^{125}I-MSA binding in the presence of increasing concentrations of unlabelled MSA (see Table 1), and the binding data were submitted to Scatchard analysis (*c* and *d* respectively).

activity to type I IGF receptors (Jonas *et al.*, 1989). These differences are best explained by the different proportions of type I IGF receptors and atypical insulin-binding sites in IM-9 cells and human placenta. When solubilized IM-9 cells were affinity-purified on insulin–CDI-agarose, the number of IGF-binding sites eluted from the insulin affinity column (atypical insulin-binding sites) exceeded the number of IGF-binding sites excluded from the column (type I IGF receptors) by about 8-fold. With similarly purified preparations of human placenta, the ratio of IGF-binding sites eluted from the column to those appearing in the 'flow-through' fractions was about 1:2 (Jonas *et al.*, 1986).

Other studies have shown that insulin is only 1% as potent as IGF-I in displacing [^{125}I]IGF-I from IM-9 cells (Rosenfeld & Hintz, 1980; Rosenfeld *et al.*, 1982; Morgan & Roth, 1986). However, we found that the relative potency of insulin compared with IGF-I was usually 1/14 to 1/4. These discrepancies suggest that the strains of IM-9 cells used in the above laboratories contained a smaller proportion of atypical insulin-binding sites compared with type I IGF receptors than did the strain used in our laboratory.

The recent immunological studies by Soos & Siddle

(1989) have provided strong evidence that IM-9 cells, human placental membranes and Hep G2 cells contain hybrid forms of the insulin and type I IGF receptors, i.e. receptors composed of the α and β subunits of the insulin receptor and the α and β subunits of the type I IGF receptor. These hybrid receptors are distinct from the atypical insulin receptors, because the former receptors are reactive with αIR-3, and bind IGF-I with more affinity than insulin (Soos & Siddle, 1989).

Thus atypical insulin receptors co-exist with classical insulin receptors, type I IGF receptors and insulin/IGF-I receptor hybrids in IM-9 cells and human placental membranes. So far, we have not been able to separate the atypical and classical insulin-binding sites by immunological means or by MSA affinity chromatography (Jonas *et al.*, 1989). Thus we do not know, at this stage, whether atypical insulin receptors form hybrids with classical insulin receptors and/or type I IGF receptors.

Because the atypical insulin/IGF-II-binding sites would account for a sizeable proportion of the insulin, IGF-II and IGF-I binding to IM-9 cells, they may mediate specific actions of insulin and IGFs on lymphoblast function and/or metabolism. Further work is required to determine the modes by which the atypical and classical insulin-binding sites may be differentially regulated, and whether they have evolved to subserve cell-specific functions.

This work was supported by the National Health and Medical Research Council of Australia. We thank Professor Leonard C. Harrison (Director, Burnet Clinical Research Unit, Walter and Eliza Hall Institute, Parkville) and Dr. John A. Hamilton (Principal Research Fellow, Department of Medicine, University of Melbourne) for helpful discussions and review of this manuscript.

REFERENCES

Conover, C. A., Rosenfeld, R. G. & Hintz, R. L. (1987) J. Cell. Physiol. **133**, 560–566

Hari, J., Pierce, S. B., Morgan, D. O., Sara, V., Smith, M. C. & Roth, R. A. (1987) EMBO J. **6**, 3367–3371

Hintz, R. L., Thorrson, A. V., Enberg, G. & Hall, K. (1984) Biochem. Biophys. Res. Commun. **118**, 774–782

Jacobs, S., Cook, S., Svoboda, M. E. & Van Wyk, J. J. (1986) Endocrinology (Baltimore) **118**, 223–226

Jonas, H. A., Baxter, R. C. & Harrison, L. C. (1982) Biochem. Biophys. Res. Commun. **109**, 463–470

Jonas, H. A., Newman, J. D. & Harrison, L. C. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 4124–4128

Jonas, H. A., Cox, A. J. & Harrison, L. C. (1989) Biochem. J. **257**, 101–107

King, G. L., Kahn, C. R., Rechler, M. M. & Nissley, S. P. (1980) J. Clin. Invest. **66**, 130–140

Kull, F. C., Jr., Jacobs, S., Su, Y.-F., Svoboda, M. E., Van Wyk, J. J. & Cuatrecasas, P. (1983) J. Biol. Chem. **255**, 6561–6566

Massague, J. & Czech, M. P. (1982) J. Biol. Chem. **257**, 5038–5045

Misra, P., Hintz, R. L. & Rosenfeld, R. G. (1986) J. Clin. Endocrinol. Metab. **63**, 1400–1405

Morgan, D. O. & Roth, R. A. (1986) Biochem. Biophys. Res. Commun. **138**, 1341–1347

Morgan, D. O., Edman, J. C., Standring, D. N., Fried, V. A., Smith, M. C., Roth, R. A. & Rutter, W. J. (1987) Nature (London) **329**, 301–307

Newman, J. D. & Harrison, L. C. (1985) Biochem. Biophys. Res. Commun. **132**, 1059–1065

Rosenfeld, R. G. & Hintz, R. L. (1980) Endocrinology (Baltimore) **107**, 1841–1848

Rosenfeld, R. G., Hintz, R. L. & Dollar, L. A. (1982) Diabetes **31**, 375–381

Scatchard, G. (1949) Ann. N.Y. Acad. Sci. **51**, 660–672

Soos, M. A. & Siddle, K. (1989) Biochem. J. **263**, 553–563

Tollefsen, S. E., Thompson, K. & Petersen, D. J. (1987) J. Biol. Chem. **262**, 16461–16469

Ullrich, A., Gray, A., Tam, A. W., Yang-Feng, T., Tsubokawa, M., Collins, C., Henzel, W., Le Bon, T., Kathuria, S., Chen, E., Jacobs, S., Francke, U., Ramachandran, J. & Fujita-Yamaguchi, Y. (1986) EMBO J. **5**, 2503–2512

Van Wyk, J. J., Graves, D. C., Casella, S. J. & Jacobs, S. (1985) J. Clin. Endocrinol. Metab. **61**, 639–643

Received 27 April 1989/31 October 1989; accepted 8 November 1989