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Monoclonal Antibodies to Receptors for Insulin and Somatomedin-C*

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Three monoclonal antibodies, designated αIR-1, αIR-2, and αIR-3, were prepared by fusing FO myeloma cells with spleen cells from a mouse immunized with a partially purified preparation of insulin receptors from human placenta. These antibodies were characterized by their ability to immunoprecipitate solubilized receptors labeled with ^{125}I-insulin or ^{125}I-somatomedin-C in the presence or absence of various concentrations of unlabeled insulin or somatomedin-C. αIR-1 preferentially immunoprecipitates insulin receptors and also less effectively imnoprecipitates somatomedin-C receptors, while αIR-2 and αIR-3 preferentially immunoprecipitate somatomedin-C receptors, but may also weakly immunoprecipitate insulin receptors.

These three monoclonal antibodies, as well as A410, a rabbit polyclonal antibody, were used to immunoprecipitate insulin and somatomedin-C receptors from solubilized human lymphoid (IM-9) cells and human placenta membranes that had been ^{125}I-labeled with lactoperoxidase. Analysis of the immunoprecipitates by sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicates that both receptors are composed of α and β subunits. The β subunit of the insulin receptor (immunoprecipitated by αIR-1 and A410) has a slightly more rapid mobility than the corresponding subunit of the somatomedin-C receptor (immunoprecipitated by αIR-2 and αIR-3). Interestingly, the α subunit of the placenta somatomedin-C receptor has a slightly faster mobility than its counterpart from IM-9 cells.

Immunoprecipitation of receptor that had been reduced and denatured to generate isolated subunits indicates that αIR-2 and αIR-3 interact with the α subunit of the somatomedin-C receptor while A410 interacts with both subunits of the insulin receptor. αIR-1 failed to react with reduced and denatured receptors.

Insulin and somatomedin-C[1] are structurally related peptide hormones with overlapping biological activities (1). Each binds with high affinity to its own receptor,[2] but each can also bind with considerably lower affinity to the other's receptor (2–5). This cross-reactivity is attributable to structural similarities between the receptors as well as between the peptides themselves. Both receptors are composed of two types of subunits, which have approximate molecular weights of about 135,000 and 90,000 (3, 4, 6–12). These are thought to form disulfide-linked heterotetramers containing two copies of each type of subunit (3, 4, 6–8, 10–12). Antibodies from a patient with insulin resistance and acanthosis nigricans have been shown to inhibit the binding of both insulin and somatomedin-C to their respective receptors, suggesting that the receptors are also immunochemically similar (13). Somatomedin-C also binds with relatively high affinity to insulin-like growth factor II receptors. This receptor is structurally different from somatomedin-C and insulin receptors, and has little or no affinity for insulin (3, 4, 14).

The present studies describe three monoclonal antibodies to insulin and somatomedin-C receptors. These are used to investigate the immunochemical cross-reactivity of the two receptors and to identify their subunits in human placenta and IM-9 cells. Some properties of αIR-1 have been described previously (15).

MATERIALS AND METHODS

Receptor Purification—Human placenta membranes were solubilized with 2% Triton X-100, and insulin receptor was purified by sequential chromatography on concanavalin A-Sepharose, insulin-Sepharose, and wheat germ agglutinin-Sepharose (16–18). As previously reported by others (10, 19), we found that somatomedin-C receptors could be quantitatively recovered in the eluate of the concanavalin A column, and that about 30–50% was adsorbed to the insulin-Sepharose column. However, no somatomedin-C binding activity was detected in the urea eluate of the insulin-Sepharose column or at later stages of purification, while insulin-binding activity could be followed throughout the purification procedure (data not shown). In view of results to be presented later, somatomedin-C receptors may have been present but in a denatured form incapable of binding hormone. To assess the degree of purity and the amount of protein present, a small aliquot of the wheat germ agglutinin eluate was reduced and analyzed by SDS[3]-polyacrylamide gel electrophoresis followed by silver staining (20). A 135,000 molecular weight band and faint minor 90,000- and 45,000-molecular weight bands were present. About 5–10 μg of receptor protein was obtained per placenta.

Production of Monoclonal Antibodies—Three SJL mice (Jackson Laboratories, Bar Harbor, ME) were injected subcutaneously, with wheat germ agglutinin-Sepharose eluate containing 3 μg of receptor, emulsified in an equal volume of complete Freund's adjuvant, and boosted three times at 3 week intervals with a similar amount of purified receptor emulsified in incomplete Freund's adjuvant. All mice developed antiserum that immunoprecipitated receptors labeled with ^{125}I-insulin and ^{125}I-somatomedin-C (see Table I for details of assay). The mouse with the highest titer of antibodies to insulin receptors received an i.v. boost of 10 μg of receptor. Three days later, it was sacrificed and its lymph node and spleen cells were fused with FO myeloma cells (Cell Distribution Center, Salk Institute, La Jolla, CA) (21). To achieve an initial cloning stage, hybrids were seeded at a low

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[1] Somatomedin-C has been sequenced and is identical to insulin-like growth factor I (27).

[2] The receptor which is referred to here as the somatomedin-C receptor has also been called the insulin-like growth factor, type I receptor (4). The receptor which is referred to here as the insulin-like growth factor II receptor has also been called the insulin-like growth factor, type II receptor (4).

[3] The abbreviation used is: SDS, sodium dodecyl sulfate.

density using peritoneal exudate cells from SJL mice as feeders (21). Hybridomas grew to numbers exceeding 1000 cells/cm^2/ml of culture medium in 26 out of 1000 wells. Supernatants were screened for antibodies that immunoprecipitated insulin or somatomedin-C receptors as described in the legend to Table I. Six wells were initially positive for antibodies to insulin receptors. These and only these were also positive for antibodies to somatomedin-C receptors. Cell lines from three of these wells eventually died out or stopped producing antibody. Hybridomas from the remaining three wells were serially subcloned by limiting dilution four times. The resulting clones and the antibodies they produce have been designated αIR-1, αIR-2, and αIR-3. Antibodies used in this paper were harvested from ascites fluid of Balb/C × SJL F_1 hybrids (Jackson Laboratories) inoculated with these cell lines. αIR-1 and αIR-3 were further purified on DEAE-cellulose equilibrated with 10 mM potassium phosphate, pH 8.0. αIR-2 was retained on DEAE-cellulose under these conditions, and ascites fluid was used directly without further purification.

All three antibodies are $IgG_1(\kappa)$ (determined by Mono AB-ID EIA Kit, Zymed Laboratories, Burlingame, CA).

Iodination of Cells and Membranes—IM-9 cells were labeled with ^{125}I by using lactoperoxidase (22). The labeled cells were washed with phosphate-buffered saline, solubilized by vortexing with 1% Triton X-100, and the labeled glycoproteins purified by wheat germ agglutinin-Sepharose as described for placenta. Placenta membranes were iodinated with lactoperoxidase as follows: 1 mg of placenta membrane was suspended in 5 ml of phosphate-buffered saline. 100 μg of lactoperoxidase was added followed by 2 mCi of [^{125}I]NaI. A 20-μl aliquot of 10^{-3} M H_2O_2 was added every 4 min for 12 min. The membranes were then washed 3 times by centrifugation at 50,000 × *g* for 30 min with 8 ml of phosphate-buffered saline. The membrane pellet was solubilized with 2% Triton X-100 in 50 mM Tris·HCl, pH 7.7, containing 1 mg/ml of bacitracin and 20 μg/ml of phenylmethylsulphonyl fluoride. After 30 min, the solubilized placenta membranes were centrifuged at 100,000 × *g* for 1 h. The supernatant was diluted with three volumes of Tris·HCl, pH 7.7, containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$ and applied to a 0.5-ml wheat germ agglutinin-Sepharose column equilibrated with this buffer containing 0.2% Triton X-100. The column was washed with 20 ml of the Triton-containing buffer, and the labeled glycoproteins were eluted with 0.5 M *N*-acetyl glucosamine in 50 mM Tris·HCl containing 0.2% Triton X-100, 1 mg/ml of bacitracin, and 20 μg/ml of phenylmethylsulfonyl fluoride.

RESULTS

Table I illustrates the ability of the three monoclonal antibodies to immunoprecipitate receptor-bound ^{125}I-insulin and ^{125}I-somatomedin-C. αIR-1 immunoprecipitates considerably more bound ^{125}I-insulin and ^{125}I-somatomedin-C than does normal mouse serum. If solubilized placenta is omitted from the assay (or if it is heated to 70 °C for 10 min (data not shown)), there is no specific immunoprecipitation of either labeled hormone by αIR-1. This indicates that the antibody is not directly reacting with the hormone (or in the case of somatomedin-C, a binding protein in serum or ascites fluid), but with hormone binding proteins present in placenta membranes. The ability of insulin to inhibit the immunoprecipitation of the labeled hormones indicates that these binding proteins are saturable and have a relatively high affinity for insulin. αIR-2 and αIR-3 both immunoprecipitate more receptor-bound ^{125}I-insulin than normal serum but considerably less than αIR-1. Both antibodies immunoprecipitate similar amounts of bound ^{125}I-somatomedin-C. As with αIR-1, specific immunoprecipitation of both labeled hormones by αIR-2 and αIR-3 is dependent on the presence of solubilized placenta and is inhibited by native insulin, or by heat treating the solubilized placenta (data not shown).

TABLE I

Immunoprecipitation of receptors labeled with ^{125}I-insulin and ^{125}I-somatomedin-C

Solubilized placenta membranes were incubated at 4 °C with 50,000 cpm of ^{125}I-insulin or 15,000 cpm of ^{125}I-somatomedin-C in 0.12 ml of 50 mM Tris·HCl, pH 7.7, containing 0.1% bovine serum albumin and 0.1% Triton X-100. In one set of control tubes (+ insulin) 20 μg/ml of unlabeled insulin was added with the labeled hormones. In a second set of control tubes (− receptor), solubilized placenta was omitted. After 18 h, 20 μl of normal mouse serum diluted 1:50 was added alone or with αIR-1 (final concentration 19 μg/ml), αIR-2 (final dilution of ascites 1:420), or αIR-3 (final concentration 11 μg/ml). After an additional 6 h, 7 μl of anti-mouse serum (Cappel, Cochraneville, PA) were added. After 18 h at 4 °C, the immunoprecipitates were washed twice with 4 ml of the Tris buffer by centrifugation at 3,000 × *g*.

	cpm Immunoprecipitated (± S.E.)	
	^{125}I-Insulin	^{125}I-Somatomedin-C
Normal mouse serum	112 ± 8	74 ± 11
αIR-1	20,333 ± 362	864 ± 57
αIR-1 + insulin	124 ± 6	117 ± 3
αIR-1 − receptor	115 ± 12	78 ± 6
αIR-2	189 ± 36	1,145 ± 37
αIR-2 + insulin	115 ± 2	402 ± 16
αIR-2 − receptor	121 ± 3	67 ± 8
αIR-3	371 ± 11	816 ± 15
αIR-3 + insulin	128 ± 9	164 ± 15
αIR-3 − receptor	116 ± 6	63 ± 10

Receptor Specificity—Since in these studies, ^{125}I-insulin and ^{125}I-somatomedin-C are immunoprecipitated as labeled hormone-receptor complexes, the potency of unlabeled hormones to compete for receptor binding, and thereby inhibit immunoprecipitation of labeled hormone, reflects their specificity for the receptor. This can be used to identify the receptor to which the labeled hormone is bound when it is immunoprecipitated.

The concentrations of unlabeled insulin and somatomedin-C that inhibit the immunoprecipitation of ^{125}I-insulin by αIR-1 (Fig. 1*A*) are similar to those previously reported to inhibit the binding of ^{125}I-insulin to the insulin receptor (2, 4). This suggests that the ^{125}I-insulin that is immunoprecipitated by αIR-1 (Fig. 1*A*) is bound mainly to the insulin receptor, and that αIR-1, therefore, recognizes insulin receptors. Similarly, the concentrations of unlabeled insulin and somatomedin-C that inhibit the immunoprecipitation of ^{125}I-somatomedin-C by αIR-2 and αIR-3 (Fig. 1, *E* and *F*) are similar to those previously reported to inhibit the binding of ^{125}I-somatomedin-C to the somatomedin-C receptor (2–5). This suggests that αIR-2 and αIR-3 recognize the somatomedin-C receptor.

The competition binding curves in Fig. 1, *B*, *C*, and *D* are more complex. Since ^{125}I-insulin will bind weakly to the somatomedin-C receptor and since αIR-2 and αIR-3 immunoprecipitate the somatomedin-C receptor, it is possible that the relatively small amounts of ^{125}I-insulin immunoprecipitated by these antibodies are bound entirely to somatomedin-C receptors. However, the data (Fig. 1, *B* and *C*) are not consistent with this interpretation. The potency of native insulin to inhibit the immunoprecipitation of ^{125}I-insulin by αIR-2 and αIR-3 is too high, and the potency of unlabeled somatomedin-C is too low (Fig. 1, *B* and *C*) for all the immunoprecipitated ^{125}I-insulin to be bound to the somatomedin-C receptor. Similarly, the potency of unlabeled insulin is too low and the potency of unlabeled somatomedin-C is too high for the ^{125}I-insulin immunoprecipitated by these antibodies to be bound entirely to insulin receptors. The simplest explanation for these data is that ^{125}I-insulin immunoprecipitated by αIR-2 and αIR-3 is bound to a combination of insulin receptors and somatomedin-C receptors. The flat slopes of the competition curves (Fig. 1, *B* and *C*) are consistent with the presence of more than one type of receptor. This reasoning suggests that αIR-2 and αIR-3 do immunoprecipitate insulin receptors, although at the concentration of antibody used, considerably less effectively than αIR-1. Similarly, the ^{125}I-somatomedin-C immunoprecipitated by αIR-1 (Fig. 1*D*) appears to be bound to a mixture of insulin and somatomedin-C receptors, suggesting that αIR-1 weakly recognizes

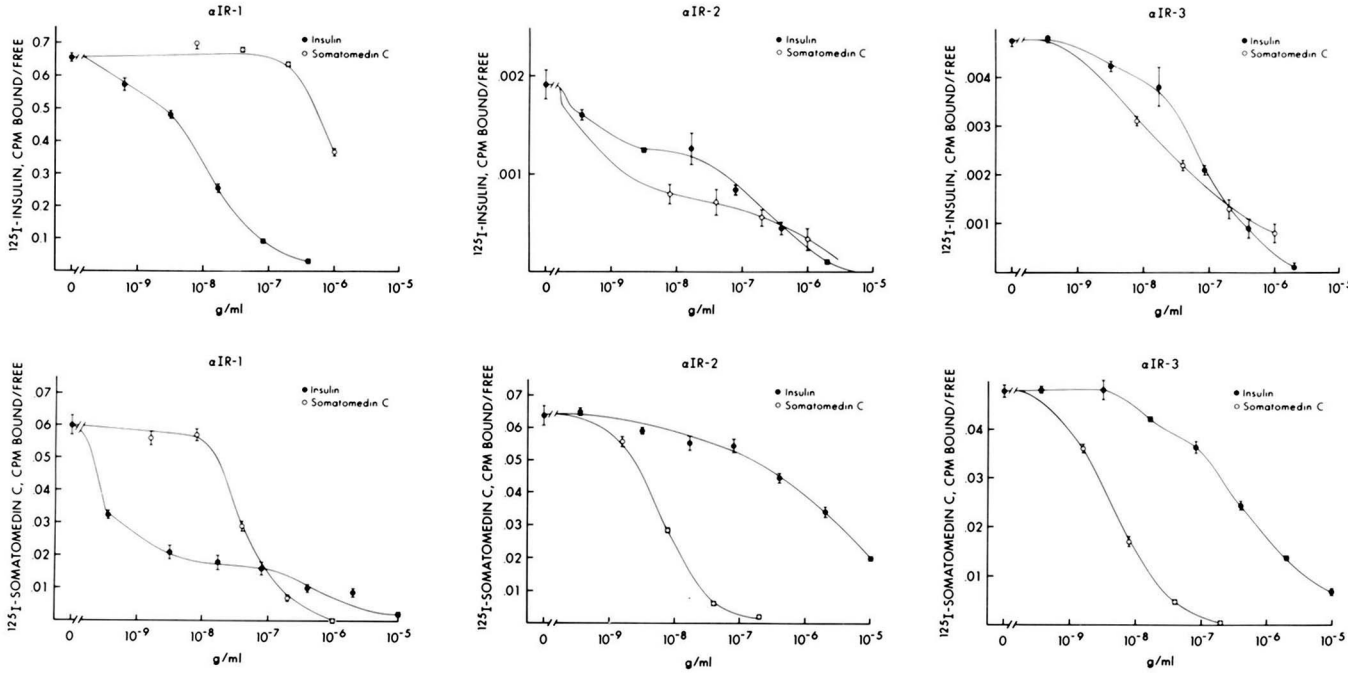

FIG. 1. **Specificity of immunoprecipitated receptors.** Solubilized placenta membranes were incubated as indicated in Table I with 50,000 cpm of ^{125}I-insulin (*A*, *B*, and *C*) or 15,000 cpm ^{125}I-somatomedin-C (*D*, *E*, and *F*) in the absence or presence of various concentrations of unlabeled insulin (●) or somatomedin-C (○). The labeled receptors were then immunoprecipitated as described in the legend to Table I by: *A* and *D*, αIR-1 (19 μg of IgG/ml); *B*, and *E*, αIR-2 (1:420 dilution of ascites); *C* and *F*, αIR-3 (11 μg of IgG/ml). Nonspecific counts (counts immunoprecipitated by normal mouse serum without added monoclonal antibody) have been subtracted in the calculations.

somatomedin-C receptors.

To further evaluate which receptors are immunoprecipitated by each antibody, placenta membranes were incubated with ^{125}I-somatomedin-C in the absence of unlabeled peptides (Fig. 2, *lanes 1–4*), with 100 ng/ml of somatomedin-C (Fig. 2, *lanes 5–8*), with 100 ng/ml of insulin (Fig. 2, *lanes 9–12*), or with both 100 ng/ml of somatomedin-C and insulin (Fig. 2, *lanes 13–16*). ^{125}I-somatomedin-C was then covalently cross-linked to the receptor to which it was bound with disuccinimidyl suberate. The membranes were solubilized with Triton X-100, immunoprecipitated with normal mouse serum, αIR-1, αIR-2 or αIR-3, and analyzed by SDS-polyacrylamide gel electrophoresis.

αIR-2 and αIR-3 immunoprecipitated a 132,000-M_r band that was heavily labeled in the absence of somatomedin-C (Fig. 2, *lanes 3* and *4*). Labeling of this band was readily inhibited by 100 ng/ml of somatomedin-C (Fig. 2, *lanes 7* and *8*), but not inhibited by 100 ng/ml of insulin (Fig. 2, *lanes 11* and *12*). Because of its relative affinity for insulin and somatomedin-C and its electrophoretic mobility, this band appears to be the α subunit of the somatomedin-C receptor.

In the absence of unlabeled peptides, the band immunoprecipitated by αIR-1 (Fig. 2, *lane 2*) is less heavily labeled than those immunoprecipitated by αIR-2 or αIR-3. In addition, it is broader and has a portion with a slightly slower electrophoretic mobility. Furthermore, its labeling is only partially inhibited by unlabeled somatomedin-C (Fig. 2, *lane 6*) and is also partially inhibited by unlabeled insulin (Fig. 2, *lane 10*), suggesting that this band is composed of α subunits of both insulin and somatomedin-C receptors.

When similar studies are carried out using ^{125}I-insulin as the labeled peptide instead of ^{125}I-somatomedin-C, αIR-1 specifically immunoprecipitates a labeled band with a molecular weight of 135,000 (Fig. 2, *lane 17*). Labeling of this band is readily inhibited by 100 ng/ml of insulin (data not shown). When ^{125}I-insulin is used as the labeled peptide, αIR-2 and

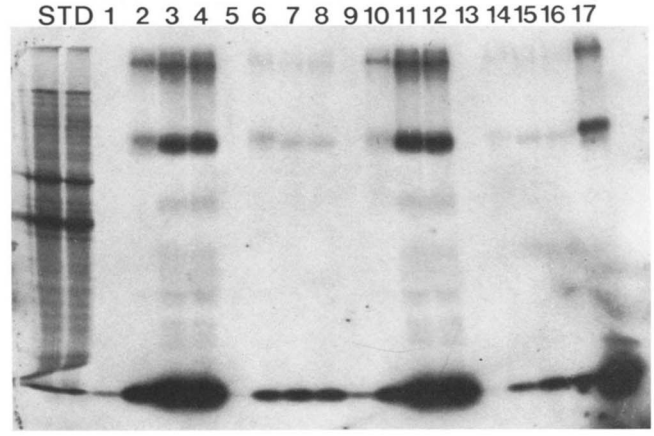

FIG. 2. **Immunoprecipitation of affinity cross-linked receptor.** Placenta membranes (0.4 mg of protein) were incubated for 90 min at 15 °C in 1.0 ml of 20 mM $NaPO_4$, pH 7.4, containing 0.05% albumin with 10^6 cpm of ^{125}I-somatomedin-C (*lanes 1–16*) or ^{125}I-insulin (*lane 17*) and no unlabeled hormone (*lanes 1–4* and *17*), 100 ng/ml of somatomedin-C (*lanes 5–8*), 100 ng/ml of insulin (*lanes 9–12*), or both 100 ng/ml of somatomedin-C and 100 ng/ml of insulin (*lanes 13–16*). Then 0.1 mg of disuccinimidyl suberate was added. After 30 min, the disuccinimidyl suberate was quenched with 20 μl of 1 M NH_4Cl. 4 ml of 50 mM Tris·HCl, pH 7.7, containing 0.2% albumin was added and the membranes pelleted. The membrane pellet was dissolved in 50 mM Tris·HCl containing 2% Triton X-100, with bacitracin (1 mg/ml) and phenylmethylsulfonyl fluoride and centrifuged for 30 min at 200,000 × *g*. The supernatant was diluted 1:4 with Tris·HCl containing bacitracin and immunoprecipitated as described in the legend to Fig. 1 with normal mouse serum (*lanes 1, 5, 9,* and *13*), αIR-1 (*lanes 2, 6, 10, 14,* and *17*), αIR-2 (*lanes 3, 7, 11,* and *15*), or αIR-3 (*lanes 4, 8, 12,* and *16*). The immunoprecipitates were washed twice with 4 ml of Tris·HCl containing 0.2% Triton X-100 and once with 4 ml of H_2O. They were then lyophilized and subjected to SDS-polyacrylamide gel electrophoresis on a 6.5% gel. The standards in the *left lanes* are myosin heavy chain, phosphorylase, and albumin.

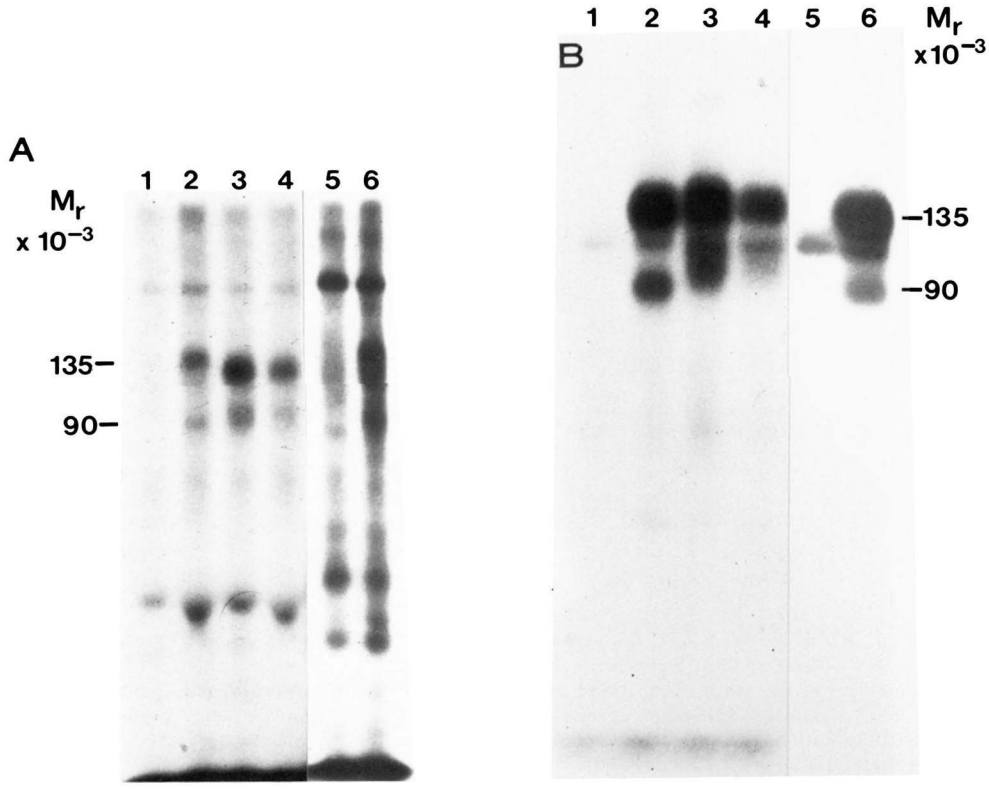

FIG. 3. **Immunoprecipitation of labeled receptor from iodinated human placenta membranes and IM-9 cells.** *A*, ^{125}I-labeled placenta membrane glycoproteins (1.2×10^6 cpm) were incubated in 50 mM Tris·HCl, pH 7.7, containing 0.2% Triton X-100, 0.1% bovine albumin, 1 mg/ml of bacitracin, and 20 μg/ml of phenylmethylsulfonyl fluoride with: *lane 1*, normal mouse serum diluted 1:300; *lane 2*, normal mouse serum diluted 1:300 plus αIR-1 (19 μg of IgG/ml); *lane 3*, normal mouse serum diluted 1:300 plus αIR-2 (ascites fluid 1:420); *lane 4*, normal mouse serum diluted 1:300 plus αIR-3 (11 μg of IgG/ml); *lane 5*, 100 μg/ml of preimmune rabbit IgG; *lane 6*, 85 μg/ml of A410. After 8 h at 4 °C, 20 μl of anti-mouse serum (Cappel) diluted 1:3 was added to the tubes containing mouse immunoglobulin, and 20 μl of fixed staphylococci bearing protein A (Pansorbin) was added to tubes containing rabbit immunoglobulin, and the incubation was continued overnight at 4 °C. The immunoprecipitates were then washed three times with 4 ml of Tris·HCl, containing 0.2% Triton X-100, and once with distilled water. The immunoprecipitates were lyophilized and electrophoresed on a 6.5% SDS-polyacrylamide gel. Shown is an autoradiogram of the dried gel. *B*, ^{125}I-labeled IM-9 cell membrane glycoproteins (3.2×10^5 cpm) immunoprecipitated as described above. *Lane 1*, normal mouse serum; *lane 2*, αIR-1; *lane 3*, αIR-2; *lane 4*, αIR-3; *lane 5*, preimmune rabbit IgG; *lane 6*, A410.

αIR-3 fail to produce detectable specific immunoprecipitation of affinity labeled bands (data not shown). This is consistent with the relatively weak ability of αIR-2 or αIR-3 to immunoprecipitate receptor labeled with ^{125}I-insulin as is indicated by Table I and Fig. 1.

Immunoprecipitation of Lactoperoxidase-labeled Receptors—To further demonstrate that these antibodies interact directly with receptors for insulin and somatomedin-C, and to establish their specificity, we examined their ability to immunoprecipitate ^{125}I-labeled membrane glycoproteins from human placenta and IM-9 cells. As previously described (15), αIR-1 specifically immunoprecipitated two polypeptides with apparent molecular weights of 135,000 and 90,000 from both human placenta and IM-9 cells (Fig. 3*A*, *lane 2* and Fig. 3*B*, *lane 2*). Polypeptides with similar molecular weights were immunoprecipitated by A410 (Fig. 3*A*, *lane 6* and Fig. 3*B*, *lane 6*), a rabbit antiserum to rat liver insulin receptor (23). These bands correspond to the α and β subunits of the insulin receptor described previously by several laboratories (6–9, 22).

αIR-2 and αIR-3 also specifically immunoprecipitate two polypeptides with apparent molecular weights of approximately 135,000 and 90,000 (Fig. 3*A*, *lanes 3* and *4*, and Fig. 3*B*, *lanes 3* and *4*). Because of the specificity of αIR-2 and αIR-3, these presumably are subunits of the somatomedin-C receptor. In both placenta and IM-9 cells, the broad band corresponding to the β subunit has a slightly slower mobility (apparent M_r 92,000–98,000) than the corresponding subunit of the insulin receptor. In some gels, this band appears as a doublet, the faint lower component having a mobility similar to the corresponding subunit of the insulin receptor. Interestingly, in human placenta, the α subunit of the somatomedin-C receptor (immunoprecipitated by αIR-2 or αIR-3) has a slightly faster mobility (apparent M_r 132,000) than the corresponding subunit of the somatomedin-C receptor from IM-9 cells (apparent M_r 136,000) or of the insulin receptor (immunoprecipitated by αIR-1 or A410) from either tissue (apparent M_r 135,000).

In order to determine with which subunit these antibodies interact, immunoprecipitation studies were performed with iodinated placenta membrane glycoproteins that had been treated with dithiothreitol and SDS to dissociate receptor subunits (Fig. 4). After this treatment, neither subunit is immunoprecipitated by αIR-1, perhaps indicating that this antibody recognizes an epitope that is destroyed by reduction and denaturation. αIR-2 and αIR-3 specifically immunoprecipitate the α subunit of the somatomedin-C receptor. A410 immunoprecipitates both the α and β subunits of the insulin receptor. Since A410 is polyclonal, this does not necessarily

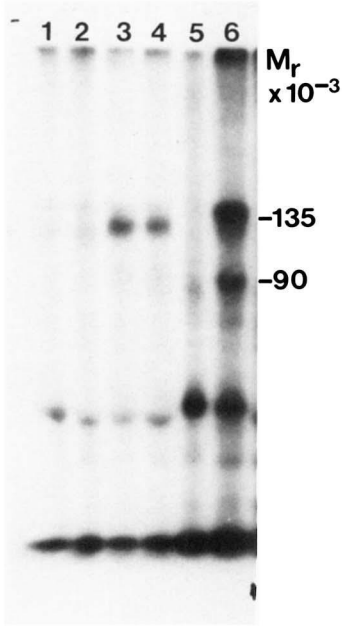

FIG. 4. **Immunoprecipitation of reduced and denatured receptor.** ^{125}I-labeled placenta glycoproteins were reduced and denatured by incubation with 1% SDS and 5 mM dithiothreitol for 5 min at room temperature. The dithiothreitol was then quenched with 12 mM *N*-ethylmaleimide and the denatured proteins diluted 20-fold with 1% albumin in 50 mM Tris·HCl, pH 7.7, containing 0.2% Triton X-100, 1 mg/ml of bacitracin, and 20 μg/ml of phenylmethylsulfonyl fluoride. The reduced and denatured labeled receptor was then immunoprecipitated as described in Fig. 3. *Lane 1*, normal mouse serum; *lane 2*, αIR-1; *lane 3*, αIR-2; *lane 4*, αIR-3; *lane 5*, preimmune rabbit serum; *lane 6*, A410.

imply immunochemically similar sites on both subunits. Similar results were obtained with labeled membrane glycoproteins from IM-9 cells (data not shown).

DISCUSSION

The present studies describe three separate monoclonal antibodies which react predominantly with insulin receptors (αIR-1) or somatomedin-C receptors (αIR-2 and αIR-3). We have interpreted the data in Fig. 1, *B C*, and *D* as indicating that immunoprecipitated labeled ligand is bound to a combination of insulin and somatomedin-C receptors, and therefore, that each antibody can react with both receptors. The ability of αIR-1 to immunoprecipitate both insulin and somatomedin-C receptors is also suggested by affinity cross-linking studies (Fig. 2). However, other explanations for the data are also possible. For example, the antibodies may recognize a third type of receptor that is distinct from both insulin and somatomedin-C receptors and that binds both of these ligands with intermediate affinity. The insulin like growth factor II receptor is a possible candidate, but it probably can be ruled out since that receptor has little or no affinity for insulin (3, 4), while the receptors responsible for labeled ligand binding in Fig. 1, *B*, *C*, and *D* do. Furthermore, polyacrylamide gel electrophoresis of the immunoprecipitates of lactoperoxidase labeled cells and membranes reveals no labeled bands in the 220–260-kDa range (Fig. 3) which could correspond to the insulin-like growth factor II receptor (3, 4, 14).

αIR-2 and αIR-3 have many similar properties. Both are $IgG_1(\kappa)$, both have selectivity for somatomedin-C receptors, and both recognize the reduced and denatured 135,000-molecular weight subunit. However, they are clearly different antibodies. αIR-2 has more stringent specificity for somatomedin-C receptors. (At the antibody concentration used, αIR-2 immunoprecipitates more ^{125}I-somatomedin-C and less ^{125}I-insulin than does αIR-3 (Fig. 1 and Table I)). Furthermore, αIR-2, in contrast to αIR-3, is retained on DEAE-cellulose equilibrated with 10 mM potassium phosphate, pH 8.0 (data not shown).

The structure of insulin receptors has been extensively studied by a variety of techniques (7). It is clearly composed of α and β subunits with molecular weights of approximately 135,000 and 90,000, respectively. Other less well characterized subunits have also been identified by some laboratories (6, 24–26). Some of these may be precursors or degradation products of the receptor (24–26). In Fig. 3, the only detectable bands specifically immunoprecipitated by αIR-1 and A410, which react predominantly with insulin receptors, have molecular weights of approximately 135,000 and 90,000.

Information about the structure of the somatomedin-C receptor is more limited and has been obtained almost exclusively from affinity labeling studies (3, 4, 10, 11). In these studies, a 135,000-molecular weight α subunit has been clearly identified which is disulfide-linked to other subunits. Evidence for a β subunit has been directly inferred from similarities between partially reduced and unreduced forms of the somatomedin-C and insulin receptors (4, 11), although in some affinity labeling studies, a faintly labeled 90,000-molecular weight subunit of the somatomedin-C receptor has been observed (4). The somatomedin-C receptor immunoprecipitated with αIR-2 and αIR-3 (Fig. 3) clearly contains both subunits. The β subunit moves slower on SDS-polyacrylamide gels than the corresponding subunit of the insulin receptor. This is fortunate because it provides a distinct method of distinguishing the two receptors aside from their immunochemical and ligand-binding specificities. In some gels, the β subunit of the somatomedin-C receptor appears as a doublet, the faint lower component having a mobility similar to that of the corresponding subunit of the insulin receptor. The origin of this band is not clear. It may be due to proteolysis or to a small amount of insulin receptor co-immunoprecipitated by these antibodies, or it may be due to a microheterogeneity of somatomedin-C receptor. Interestingly, the α subunit of the somatomedin-C receptor from placenta has a slightly more rapid mobility than its counterpart from IM-9 cells. Here too, this difference may merely result from proteolysis of the receptor during preparation of the membranes, or it may indicate tissue specific differences in the receptors.

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