Class I histocompatibility antigens and insulin receptors: Evidence for interactions

(H-2 antigens/cell-surface receptors)

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ABSTRACT We provide evidence for an interaction between mouse class I major histocompatibility complex antigens and insulin receptors. Antibodies against class I but not class II major histocompatibility complex antigens immunoprecipitate photoaffinity-labeled hepatic insulin receptors. Haplotypic specificity is demonstrated by reciprocal precipitation using anti-class I antibodies and three strains of mice. Antibodies against the 45-kDa products of either the H-2K or H-2D locus and rabbit anti-mouse β2-microglobulin antibodies were shown to precipitate insulin receptors. We also demonstrate the specific binding of 125I-labeled insulin and 125I-labeled epidermal growth factor, but not 125I-labeled glucagon or 125I-labeled atrial natriuretic factor, to solubilized plasma membranes immunoprecipitated with anti-H-2K antibody. These observations suggest a specific interaction between class I major histocompatibility complex antigens and certain hormone receptors.

Insulin receptors (IRs) of several tissues consist of two major glycoprotein subunits of 125-130 kDa and 90-95 kDa (1-5). A glycoprotein of 40-45 kDa (3, 6, 7) and, under certain experimental conditions, an 85-kDa component have also been detected (8). Czech and coworkers (2, 9) have concluded that the IR is a heterodimer of two a subunits of 125 kDa and two β subunits of 95 kDa, considering the 40- to 45-kDa component to be a proteolytic fragment of the 95-kDa subunit (2, 3, 7). In contrast, Yip and Moule (8) proposed that the IR is composed of one 130-kDa, one 90-kDa, one 85-kDa, and two 40- to 45-kDa subunits. The 125- to 130-kDa subunit binds insulin, whereas the 90- to 95-kDa subunit is a protein kinase (10-14). The functional roles of the remaining putative IR subunits are not known.

Binding of glucagon and insulin to mouse liver plasma membranes (LPM) was reported to differ among H-2 haplotypes (15). Meruelo and Eidin (16) suggested that the major histocompatibility complex (MHC) antigens (Ags) modify specific cell surface receptors, notably epidermal growth factor (EGF) receptors. Since the putative 40- to 45-kDa IR subunits are similar in molecular size to the heavy chain of class I MHC, it is possible that class I Ags may interact with IRs and thus modify receptor binding activity. Simonsen et al. (17) reported that capping of class I MHC Ags with anti-class I antibody (Ab) inhibits binding of insulin to a leukemia cell line. Chvatchko et al. (18) reported that photoaffinity-labeled mouse hepatic IRs were precipitable by Abs against H-2K class I Ags. In the present study, we confirmed and extended their findings using a panel of class I and class II specific Abs, different photoaffinity insulin derivatives, and mice of several H-2 haplotypes. We present evidence that the 45-kDa heavy chains of class I MHCs interact with IRs and EGF receptors but not with glucagon or atrial natriuretic factor (ANF) receptors.

MATERIALS AND METHODS

Mice. Mice were bred in our colony. Strains and their haplotypes are given in the text. Male mice were used at 8-16 wk of age.

Antibodies. The 15-5-5S (C3H.SW anti-C3H, IgG2a, D<sub>B</sub>G<sub>1</sub>, f<sup>a</sup>), 34-1-2 (C3H anti-BDF1, IgG<sub>2a</sub>, K<sub>B</sub>, T<sub>B</sub>, P<sub>B</sub>, Q<sub>A</sub>, Qa), 20-8-4 (C3H anti-C3H.SW, IgG<sub>2a</sub>, K<sub>D</sub>, D<sup>B</sup>, D<sup>K</sup>, D<sup>B</sup>, r<sup>1</sup>, Qa), 28-13-3 (C3H anti-C3H.SW, IgM, K<sub>r</sub>, f), and 28-14-8 (C3H anti-C3H.SW, IgG<sub>2a</sub>, D<sub>B</sub>, L<sub>B</sub>, q) monoclonal Abs (mAb) (19, 20) were used as ascites fluids and were the kind gift of D. Sachs (National Cancer Institute, Bethesda, MD). 11.4 (BALB/c anti-CKB, IgG<sub>2a</sub>, K<sup>B</sup>) mAb (21) was prepared as ascites fluid in syngeneic mice injected with 10<sup>7</sup> hybridoma cells obtained from the American Type Culture Collection. For some experiments, isotype specific immunoglobulin was purified from ascites (22). Normal rabbit serum and normal mouse serum (NMS) derived from C3H or BALB/c mice were used as controls. Anti-human IR immunoglobulin, from the autoimmune serum of a patient with acanthosis nigricans, was the kind gift of B. Posner, McGill University, Montreal. Y-9 B-4 rabbit anti-mouse β2-microglobulin (β2m) serum (23) was generously supplied by H. Grey (National Jewish Hospital, Denver, CO). Rabbit anti-mouse immunoglobulin and rabbit anti-mouse brain serum (24) were purchased from Cedarlane Laboratories (Hornby, ON).

Plasma Membrane Preparations. LPM were prepared by the method of Neville (25) up to step 11, and brain plasma membranes were prepared according to Posner et al. (26).

Preparation and Iodination of Photoactive Insulins. The photoreactive derivative of beef insulin, N<sub>B</sub>α<sup>2</sup>-monoaizido- benzoyl-insulin (B<sub>2α</sub>-MABI) was prepared and iodinated as described (1).

Photoaffinity Labeling of Plasma Membranes with 125I-Labeled B<sub>2α</sub>-MABI. Binding was done in Krebs-Ringer bicarbonate buffer supplemented as described (18). Plasma membranes (1.5 mg of protein per ml) were incubated with insulin derivative (3 x 10<sup>-8</sup> M) for either 1 hr or 6 hr at 4°C in the dark, photolysed for 30 sec (8), pelleted by centrifugation for 20 min at 16,000 x g, solubilized either by boiling for 20 min in 3% (wt/vol) NaDodSO<sub>4</sub> sample buffer or by resuspending in a 1% Triton solubilization buffer (18) for 90 min at 4°C with stirring, and recentrifuged for 30 min at 18,000 x g to yield the supernatant for precipitation.

Immunoprecipitation. The supernatant (200 µl) was incubated with either 8-10 µl of ascites, 15-20 µl of serum, or 12

Abbreviations: MHC, major histocompatibility complex; Ag, antigen; β<sub>2m</sub>, β<sub>2</sub>-microglobulin; IR, insulin receptor(s); Ab, antibody; LPM, liver plasma membranes; EGF, epidermal growth factor; ANF, atrial natriuretic factor; mAb, monoclonal antibody; NMS, normal mouse serum; B<sub>2α</sub>-MABI, N<sub>B</sub>α<sup>2</sup>-monoaizido-benzoyl-insulin; SpA, Sepharose-protein A.
μg of purified immunoglobulin, for 16 hr at 4°C. When IgM was used, 12 μl of rabbit anti-mouse immunoglobulin serum was added, and the incubation was continued for 1.5 hr. Twenty percent glutaraldehyde-fixed Staphylococcus aureus cells (100 μl) were incubated with each sample 1.5 hr. The samples were centrifuged at 8000 × g, and supernatants were removed. For sequential precipitations, supernatants were retained and reprecipitated with an additional Ab. Ag-Ab-S. aureus cell complexes were washed three times in buffer containing 150 mM NaCl, 50 mM Hepes (pH 7.4), and 1 mM phenylmethylsulfonyl fluoride. Samples were boiled and analyzed by NaDodSO4/polyacrylamide gel electrophoresis on 5–10% gradient slab gels (7, 9). Autoradiograms of dried gels were obtained on Kodak X-Omat AR film using a DuPont Cronex Lightning Plus intensifying screen. In some experiments, individual IR subunit bands were cut from gels and counted. NMS control values were subtracted from those of samples exhaustively precipitated with anti-IR serum and 11.4. Percent association was calculated as the ratio of cpm from 11.4 to anti-IR for the 130-kDa subunit of the IR. Molecular size markers used were thyroglobulin (669 kDa), thyroglobulin intermediates (475 kDa, 280 kDa, and 255 kDa), catalase dimer (120 kDa), and catalase monomer (60 kDa).

Binding of Hormones to Immunoprecipitates. LPM from C3H mice were solubilized in 1% Triton X-100 buffer, and 100-μl aliquots were incubated with Ab followed by 6 mg of Sepharose-protein A (SpA) for 5 hr at 4°C. LPM-Ab-SpA beads were washed and incubated with either 125I-labeled insulin. 125I-labeled glucagon, 125I-labeled EGF, or 125I-labeled ANF. 125I-labeled TyrA14-insulin (350 μCi/μg; 1 Ci = 37 GBq) was obtained from Amersham. 125I-labeled glucagon (300 μCi/μg) was a gift from G. Kakis, University of Toronto. 125I-labeled EGF (100 μCi/μg) and EGF were gifts from J. Kudlow, University of Toronto. 125I-labeled ANF, with a maximal theoretical specific activity of 600 μCi/μg was prepared as described (27). Binding conditions for each ligand are given in the figure legends.

RESULTS

Precipitation of Insulin Receptor with Anti-MHC Abs. Initial experiments were designed to determine whether IR could be precipitated by Abs to class I MHC Ags. LPM were prepared from three inbred mouse strains, C3H (H-2k), BALB.B (H-2d), and B10.S (H-2s), and photoaffinity labeled with 125I-labeled B29-MABI. We observed specific labeling of two major IR subunits, 125-130 kDa and 90-95 kDa, and a third 40–45 kDa component (Fig. 1, lane 23), in agreement with previous observations (6, 8). Anti-H-2Kb mAbs (lane 1) precipitated the photolabeled LPM IRs from C3H but not BALB.B (lane 10) and B10.S (lane 17), respectively. Positive precipitation was indicated by detection of the 130-kDa band and, with varying levels of intensity, the 90- and 45-kDa receptor bands. Since insulin binding proteins are the only labeled proteins in these preparations, any nonspecific binding of LPM proteins to SpA results in detection of a background 130-kDa band in control lanes. This value is subtracted from all points before determination of positive binding. Haploype-specific precipitation of IRs was also obtained using anti-H-2Kb mAb with BALB.B (not shown) and B10.S (lane 19) but not C3H (lane 4) LPM. Anti-H-2Kb mAbs precipitated IRs from BALB.B (lane 11) but not C3H (lane 3) LPM. In addition to the 130- and 90-kDa subunits, two smaller bands at 40–45 kDa and 50–55 kDa were also detected. However, of these two bands, only the labeling of the 40–45 kDa band was specifically inhibited by an excess of native insulin (lanes 2, 12, 14, 19, 23, and 24).

Anti-H-2D Abs also precipitated IRs in a haplotype-specific manner. Anti-H-2Dk mAbs precipitated IRs from BALB.B (Fig. 1, lane 13) but not C3H (lane 6), and anti-H-2Dk mAbs (lane 5) specifically precipitated C3H IRs.

Unlike anti-class 1 mAbs, 10 anti-class II (I-A and I-E) Abs tested did not precipitate IRs from either H-2d or H-2k mice (not shown). However, IRs were precipitated from C3H (Fig. 2), B10.S, and B10 mice by a species-specific but not
haplotype-specific rabbit anti-mouse β₂m serum. Mouse brain tissue expresses little, if any, class I MHC Ag but does express Thy-1 (24). Accordingly, C3H mouse brain IRs were not precipitated with anti-H-2Kk, anti-H-2Dk, anti-I-Ak, and rabbit anti-mouse brain Abs.

To determine whether the interaction between class I MHC Ag and IR might have been artifically induced during solubilization of the LPM, we performed haplotype mixing experiments. C3H (H-2b) LPM was photoaffinity labeled with B₂₉-MABI and then mixed with an equal amount (wt/wt) of B10 (H-2b) or C3H unlabeled LPM. The mixed preparation was solubilized and incubated either anti-H-2Kk or anti-H-2Kb mAbs. Labeled IR was precipitated by anti-H-2Kk mAb but not by anti-H-2Kb, indicating that an interaction between the H-2b class I Ags and labeled C3H-derived IRs did not occur during solubilization. Homogenization of the mixed LPM before solubilization also did not induce mixed haplotype association.

Sequential Precipitations of IRs with Anti-Class I and Anti-IR Abs. To determine whether all IRs are associated with class I Ags, sequential precipitations were performed. Photolabeled C3H LPM were precipitated with either 11.4, 15-5-5S, or anti-IR serum. Supernatants from these precipitations were retained and incubated with either the same or an alternate Ab. Anti-IR serum precipitated more photolabeled IRs than did anti-H-2Kk. Reprecipitation of an anti-IR-cleared supernatant with anti-IR serum precipitated more IR, while reprecipitation with anti-H-2Kk was not different from that observed with NMS controls. Reprecipitation of an anti-H-2Kk-cleared sample with anti-H-2Kk also did not precipitate any significant amount of additional IR while reprecipitation of this supernatant with anti-IR serum yielded a large amount of the 130-kDa subunit and detectable amounts of the 95- and 45-kDa proteins. When exhaustive precipitation was performed to determine the total amount of precipitable receptor, 15–30% of the IRs precipitable by anti-IR serum were found to be precipitated by 11.4 mAb after subtraction of NMS controls. This percentage was not altered when the Triton-solubilized LPM were centrifuged at higher speed (18 min at 100,000 × g). This suggests that the precipitation of IRs by anti-class I MHC Abs is not the result of vesicle formation in the membrane preparation. Also this ratio was not affected when the time of photoaffinity labeling was increased from 1 to 6 hr at 4°C, suggesting that the observed interaction between class I Ags and IRs was not induced during incubation of the LPM.

When photoaffinity-labeled C3H LPM were precleared by precipitation with anti-H-2Kk (Fig. 3, lane 1) and reprecipitated with anti-H-2Dk (lane 3), or vice versa (lanes 2 and 4), no additional IR material was precipitated. This suggests that all of the receptors associated with H-2D molecules are also associated with H-2K.

Binding of Hormones to Immunoprecipitates. To determine whether an interaction between IRs and class I MHC Ags might be caused by crosslinking of the insulin photoprobe to IR, we first immunoprecipitated unlabeled IR from C3H LPM using Abs and SpA beads and then photoaffinity labeled the IR while bound to the beads. Both anti-IR serum (Fig. 4, lane 5) and anti-Kk (lane 1) precipitated the IR that was subsequently photoaffinity labeled by B₂₉-MABI. Thus, the interaction between IR and class I MHC Ag occurs in the absence of B₂₉-MABI binding.

Precipitation of IR by anti-class I MHC Abs could result from nonspecific coprecipitation of LPM proteins. We, therefore, compared the binding of four radiolabeled hormones to LPM-Ab-SpA. LPM-Ab-SpA was incubated with 125I-labeled insulin or 125I-labeled EGF at 4°C for 17 hr. LPM-11.4-SpA bound about 3.5 fmol of insulin and LPM-anti-IR serum–SpA bound about 1.5 fmol of insulin while no binding was seen with LPM–NMS–SpA (Fig. 5). The lower binding of insulin to LPM-anti-IR serum–SpA than to LPM-11.4-SpA was expected since anti-IR serum but not anti-H-2Kk inhibits insulin binding to LPM. LPM-11.4-SpA bound about 1 fmol of EGF, whereas no EGF binding to LPM-anti-IR serum–SpA was observed. Preclearing of solubilized LPM with anti-IR serum prior to the formation of LPM-11.4-SpA substantially reduced the binding of insulin to the beads whereas the binding of EGF to the beads was not significantly affected. These observations show that anti-H-IR serum could reduce the binding of insulin to the LPM.

![Fig. 2. C3H LPM were labeled with B₂₉-MABI. Samples were precipitated with the following antibodies: lanes 1 and 2, Y-9 B-4 (rabbit anti-β₂m); lanes 3 and 4, anti-IR serum (ARS); lane 5, NRS (normal rabbit serum). Black arrow, 125- to 130-kDa IR subunit; black arrowhead, 90- to 95-kDa IR subunit; white arrow, 40- to 45-kDa IR subunit; white arrowhead, 50- to 55-kDa non-specific band.](image-url1)

![Fig. 3. C3H LPM were labeled with B₂₉-MABI. Samples were precipitated using the following antibodies: lane 1, 11.4 (Kk, anti-H-2Kk) or lane 2, 15-5-5S (Dp, anti-H-2Dp). Supernatants from these, or identical samples, were reprecipitated with the following antibodies: lane 3, 15-5-5S or lane 4, 11.4. Black arrow, 125- to 130-kDa IR subunit; black arrowhead, 90- to 95-kDa IR subunit; white arrowhead, 50- to 55-kDa non-specific band; white arrow, 40- to 45-kDa IR subunit.](image-url2)
FIG. 4. Solubilized C3H LPM were precipitated with 11.4, NMS, or anti-IR serum (ARS) followed by SpA (400 μg of membrane and 10 μg of SpA per lane). The LPM-Ab-SpA complex was washed and then 100 μl of 50 mM Hepes, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, and 0.1% bovine serum albumin at pH 7.4 was added containing 125I-labeled B29-MABI and 1 μM cold insulin where indicated (+). Binding occurred at 4°C for 17 hr, followed by photolysis, washing, and then solubilization of the SpA-bound protein in 3% (wt/vol) NaDodSO4 for NaDodSO4/PAGE. >, 125- to 130-kDa IR subunit.

2Kβ was able to precipitate insulin and EGF receptors independently; anti-IR serum precipitated only IRs.

Incubation of 125I-labeled glucagon with LPM-Ab-SpA at 4°C, caused no specific binding of the hormone to any of the immunobeads. Since binding of glucagon to its receptor is poor at 4°C (28), we repeated the binding at 37°C for 15 min, conditions known to be optimal for glucagon receptor binding but suboptimal for insulin binding. LPM-11.4–SpA bound more glucagon than did NMS or the no Ab control. When the binding of glucagon and insulin to LPM was studied, we found that six times as many femtomoles of glucagon as insulin were bound (Table 1). Since the ratio of glucagon to insulin bound to the immunobeads is less than that seen with unprecipitated LPM, we do not feel that the result is positive. LPM-anti-IR serum–SpA would also be expected to be negative since anti-IR serum does not precipitate the glucagon receptor. The ratio again was not increased. 11.4 mAb did not inhibit the binding of 125I-labeled glucagon to LPM glucagon receptors. ANF receptors have been identified on LPM (29). As shown in Table 2, while 125I-labeled insulin was bound by LPM-anti-IR serum–SpA and LPM-11.4–SpA, there was no specific binding of 125I-labeled ANF to these beads. We conclude that glucagon receptors and ANF receptors, unlike insulin and EGF receptors, were not precipitated by 11.4 mAbs.

**DISCUSSION**

We have demonstrated that Abs against polymorphic determinants on either H-2K or H-2D Ags were able to precipitate IRs that had been photoaffinity labeled. Haplo-type-specific precipitations of IR were obtained in mice of three MHC haplotypes, suggesting that this is not a unique property of a particular haplotype.

The observation that the anti-H-2 Abs did not quantitatively precipitate all of the photolabeled IRs suggests that precipitation by these Abs was not due to their cross-reactivity with the 125- to 130-kDa or 90- to 95-kDa subunits of the IR; rather, it is likely to be due to an interaction between the IR and the class I MHC Ag. There may be subpopulations of IRs, some of which are associated with a different class I Ag. As expected in this case, anti-H-2K Abs precipitated only a portion of the total IRs. The results of the reciprocal precipitation using anti-H-2K and anti-H-2D Abs (Fig. 3) suggest that there is a subpopulation of IR each of which is associated with one H-2K and one H-2D polypeptide. The possibilities that IR may also interact with membrane components other than class I Ags and that other class I MHC gene products—i.e., H-2L, Qa and Tla Ags—form similar associations with IR remain to be determined.

The 45-kDa polymorphic heavy chain of class I MHC Ag is an integral membrane protein that noncovalently associates with a 12-kDa nonpolymorphic light chain component β2m (30). Significant precipitation of IRs was seen with anti-β2m Abs (Fig. 2). This result differs from that of

**Table 1. Insulin and glucagon binding to immunoprecipitated membranes**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Insulin bound, fmol</th>
<th>Glucagon bound, fmol</th>
<th>Ratio, glucagon to insulin</th>
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<tbody>
<tr>
<td>Unprecipitated</td>
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<td></td>
</tr>
<tr>
<td>NMS</td>
<td>0.40</td>
<td>2.43</td>
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<tr>
<td>NMS</td>
<td>0.13</td>
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<tr>
<td>Anti-H-2Kβ</td>
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<tr>
<td>Anti-IR</td>
<td>0.77</td>
<td>0.80</td>
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Solubilized C3H LPM were precipitated with 11.4, NMS, anti-IR serum, and SpA (200 μg of membrane and 6 mg of SpA). The LPM–Ab–SpA complex was washed and then incubated with 20 mM Tris-HCl, 1% bovine serum albumin, 1 mM phenylmethylsulfonyl fluoride (pH 7.6), 125I-labeled insulin, or 125I-labeled glucagon at 32,000 cpm per sample for 15 min at 37°C. Unlabeled insulin or glucagon was added at 1 μM to measure nonspecific binding. The LPM–Ab–SpA was then washed and counted. C3H LPM (25 μg) were incubated with 125I-labeled insulin and 125I-labeled glucagon using the same conditions.
Table 2. Insulin and ANF binding to immunoprecipitated membranes

<table>
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<th>Preparation</th>
<th>Insulin bound, cpm</th>
<th>ANF bound, cpm</th>
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<td>334</td>
</tr>
<tr>
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</tr>
<tr>
<td>Anti-H-2Kk</td>
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<tr>
<td>Anti-IR</td>
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Solubilized C3H LPM were precipitated with 11.4, NMS, anti-IR serum, and SpA (200 μg of membrane and 6 mg of SpA). The LPM–Ab–SpA complex was washed and then incubated in buffer, pH 7.4, containing 50 mM Hepes, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, aprotinin at 70 μg/ml, bacitracin at 0.8 mg/ml, leupeptin at 10 μg/ml, 0.2% bovine serum albumin, and for ANF binding, 5 mM MgCl₂, 125I-labeled insulin or 125I-labeled ANF (55,000 cpm) were added to give a total volume of 100 μl. Unlabeled insulin or ANF was added at 1 μM to measure nonspecific binding. Incubation times were 60 min for ANF and 17 hr for insulin at 4°C. The LPM–Ab–SpA was then washed and counted.

Simonsen et al. (17) who observed that anti-βm Abs did not inhibit the binding of insulin to a human leukemia cell line and suggested that the light chain is lost as a consequence of class I–IR interaction.

We considered the possibility that class I Ags form artificial complexes with IRs during solubilization and that our results simply represent nonspecific coprecipitation of abundant cell surface proteins. The results of several different types of experiments argue against these possibilities. First, we mixed photoaffinity labeled LPM from one strain of mouse with unlabeled LPM from a mouse of a different H-2 haplotype prior to solubilization and found that haplotype mixing did not occur during solubilization. Second, we showed that the interaction of these molecules is not induced by the binding of the photoaffinity probe to IR because receptors could be photoaffinity labeled after precipitation in LPM–11.4–SpA complexes. Finally, we compared the ability of anti-class I mAbs to precipitate several abundant cell surface molecules. No significant levels of 5′-nucleotidase activity could be found to be associated with anti-class I precipitates (unpublished observation). Abs against Thy-1 could not precipitate IRs from mouse brain plasma membrane. Using 125I-labeled ligands we found that both EGF and insulin bound significantly to anti-H-2K precipitates while glucagon and ANF did not. In addition, ANF receptors photoaffinity labeled with ANF were not precipitated with appropriate anti-H-2K Abs (unpublished observation). These results indicate that there is a preferential association of insulin and EGF receptors with certain class I MHC Ags. The biological or physiological significance of the observed association between class I Ag and IR is unknown. Edidin (31) proposed that class I molecules may exist as subunits of a variety of cell surface receptors. Although these investigators (15) have observed the influence of the H-2 haplotype on the binding of glucagon and insulin to mouse LPM, we were unable to demonstrate an effect of anti-H-2K Abs on insulin binding to LPM or on a response to insulin in isolated mouse adipocytes. Nonetheless, the allelic polymorphism of class I Ags and the association of certain MHC alleles with diabetes (e.g., Type I diabetes mellitus), poses some interesting questions about the physiological role of class I Ags in cell surface receptor–ligand interactions.

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