Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 8634–8637, December 1985
Immunology

Molecular association between major histocompatibility complex class I antigens and insulin receptors in mouse liver membranes

(H-2 antigen/monoclonal antibody/photoreactive insulin)

MAX FEHLMANN*, JEAN-FRANÇOIS PEYRON*, MICHAEL SAMSON*, EMMANUEL VAN OBBERGHEN*, DIETRICH BRANDENBURG†, AND NICOLE BROSSETTE*

*Institut National de la Santé et de la Recherche Medicale U 145, Faculté de Médecine (Pasteur) 06034, Nice Cédex, France; and †Deutsches Wollforschungsinstitut, D-5100 Aachen, Federal Republic of Germany

Communicated by Jacques Oudin, August 6, 1985

ABSTRACT Molecular association between major histocompatibility complex (MHC) antigens and cellular proteins are thought to be involved in various immunological and nonimmunological functions of MHC antigens, including hormone signaling. The existence of physical interactions between insulin receptors and MHC class I antigens was investigated in liver plasma membranes from congenic H-2^k mice. Insulin receptors were specifically labeled with a ^{125}I-labeled photoreactive insulin analogue, and cellular proteins were solubilized and incubated with various monoclonal antibodies. Immunoprecipitates were analyzed by polyacrylamide gel electrophoresis followed by autoradiography. Antibodies reacting with distinct epitopes on H-2^k class I antigens were all able to precipitate up to 25% of the labeled insulin receptors in H-2^k mouse liver membranes, whereas no insulin receptors were precipitated in H-2^b mouse liver membranes. Sequential immunoprecipitations showed that insulin receptors and H-2 antigens were coprecipitated and that no cross-reactivity occurred. The specificity of the interaction between insulin receptors and H-2 antigens was demonstrated after double labeling of membrane proteins by photoreactive insulin and lactoperoxidase-catalyzed iodination. These results thus show that, in mouse liver membranes, insulin receptors are physically associated to class I antigens of the MHC.

In the immune system, major histocompatibility complex (MHC) antigens are co-recognized with foreign antigens by T lymphocytes. This co-recognition process, called MHC restriction, is involved in the lysis of viral-infected cells by cytotoxic T lymphocytes (class I restriction) and in the stimulation of helper T lymphocytes by antigen-presenting cells (class II restriction). One possible molecular basis for these mechanisms resides in a physical interaction between MHC proteins and antigens occurring at the surface of target cells (1–5). Similarly, molecular associations between MHC antigens and other cellular proteins or extracellular ligands are thought to be involved in the various nonimmunological functions of MHC antigens (for a review, see ref. 6), including their participation in cellular interactions (7) and in hormone signaling (8–13). Nevertheless, for both these immunological and nonimmunological functions, physical interactions between MHC antigens and other cellular components have not been unequivocally demonstrated. We report here that, in mouse liver plasma membranes, class I MHC antigens are closely associated with insulin receptors, suggesting an important role of MHC antigens in the mechanism of insulin action.

MATERIALS AND METHODS

Liver plasma membranes were purified from C3H (H-2^k) mice by the method of Neville (14). Insulin receptors were specifically labeled using a ^{125}I-labeled photoreactive insulin analogue, B29 (2-nitro-4-azidophenylacetyl) insulin (15). Purified membranes (0.5 mg of protein) were incubated in 200 μl of Krebs–Ringer bicarbonate buffer containing 1% bovine serum albumin, 0.8 mg of bacitracin per ml, 2 mM phenylmethylsulfonyl fluoride, 10,000 units of aprotinin per ml, and 30 × 10^6 cpm of ^{125}I-labeled photoreactive insulin for 3 hr at 15°C in the dark. Photoactivation was induced by a 5-min exposure of the membrane suspension to ultraviolet light given by a high-pressure mercury lamp (Philips HPK 125 W/L) and was filtered through a black glass filter (UVW 55, Hanau AG, F.R.G.). In some experiments, membrane proteins were subsequently iodinated by a lactoperoxidase-catalyzed reaction. In those cases, membranes were collected by centrifugation (18,000 × *g* for 5 min) and resuspended in 500 μl of Krebs–Ringer bicarbonate buffer containing 7 units of lactoperoxidase per ml, 160 mM glucose, and 600 × 10^6 cpm of Na^{125}I. The reaction was initiated by the addition of 25 units of glucose oxidase per ml. After 30 min at room temperature, membranes were collected by centrifugation (18,000 × *g* for 5 min), washed five times with Krebs–Ringer bicarbonate buffer, and finally resuspended in 100 μl of 50 mM Hepes, pH 7.4/150 mM NaCl containing protease inhibitors and 1% Nonidet P-40. Membranes were solubilized for 90 min at 4°C by continuous stirring and were centrifuged at 130,000 × *g* for 15 min at 4°C. The supernatant was incubated for 16 hr at 4°C with different antibodies, and immunoprecipitations with protein A were achieved by addition of *Staphylococcus aureus* cells (Pansorbin). After 4 hr at 4°C, the immunoprecipitates were collected by centrifugation (18,000 × *g* for 1 min) and washed twice with Hepes/NaCl. The labeled proteins were separated by NaDodSO$_4$/PAGE (16) under reducing conditions and were revealed by autoradiography.

RESULTS AND DISCUSSION

Liver membranes were purified from congenic H-2^k mice. Insulin receptors were specifically labeled with a ^{125}I-labeled photoreactive insulin analogue, and membranes were solubilized in 1% Nonidet P-40. Immunoprecipitation of insulin receptors by anti-insulin receptor antibodies (17) (Fig. 1, lane A) revealed the presence of a major 130-kDa labeled polypeptide corresponding to the α subunit of the insulin receptor identified in a variety of tissues and cell types (21). Quantification of the radioactivity associated with the immunopre-

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Abbreviations: MHC, major histocompatibility complex; EGF, epidermal growth factor.

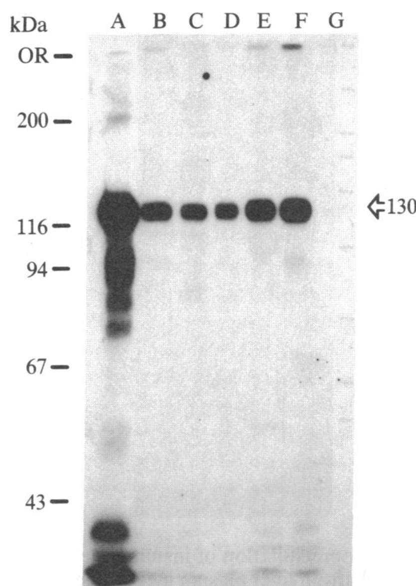

FIG. 1. Immunoprecipitation of insulin receptors by anti-H-2 monoclonal antibodies. Liver plasma membranes were labeled with ^{125}I-labeled photoreactive insulin and solubilized in 1% Nonidet P-40. Immunoprecipitations with the following antibodies were performed: lane A, serum containing antibodies to the insulin receptor at a 1:300 dilution; lanes B and C, monoclonal antibodies (at 20 μg/ml) reacting with K^k, D^k [H-100-30/23 (18) and 3-83 (19), respectively]; lanes D–F, monoclonal antibodies reacting with K^k [H-100-5/28 (18), 11-4-1 (20), and 16-3-1 (19), respectively]; and lane G, normal mouse IgGs at 20 μg/ml. Precipitates were analyzed by $NaDodSO_4$/PAGE on a 7.5% acrylamide gel under reducing conditions followed by autoradiography. OR, origin; arrow, α subunit of the insulin receptor.

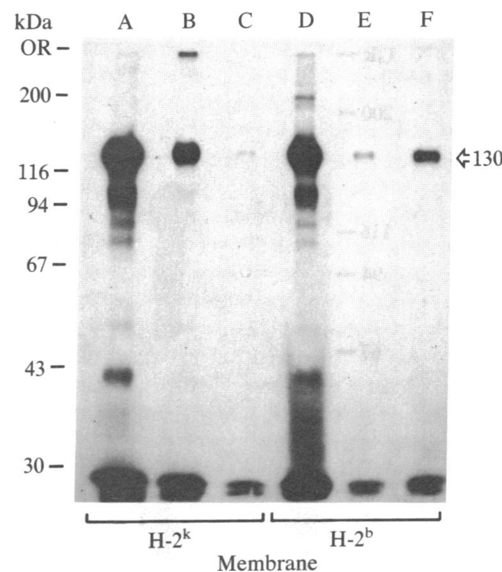

FIG. 2. Immunoprecipitation of insulin receptors by appropriate anti-H-2 monoclonal antibodies in H-2^k and H-2^b mouse liver membranes. Liver plasma membranes were prepared from C3H (H-2^k) and C57BL/6J (H-2^b) mice. Immunoprecipitations with the following antibodies were performed: lanes A and D, serum containing antibodies to insulin receptor at a 1:300 dilution; lanes B and E, monoclonal antibody to K^k, D^k (H-100-30/23 at 20 μg/ml); lanes C and F, monoclonal antibody to K^b, D^b [20-8-4 (22) at 20 μg/ml]. Precipitates were analyzed by $NaDodSO_4$/PAGE on a 7.5% acrylamide gel under reducing conditions followed by autoradiography. OR, origin; arrow, α subunit of the insulin receptor.

cipitated 130-kDa subunit has indicated that 70% of labeled insulin receptors on intact membranes were recovered after solubilization and immunoprecipitation (11). Up to 15–25% of these recoverable insulin receptors were immunoprecipitated with various anti-H2 monoclonal antibodies (Fig. 1, lanes B–F), whereas no labeled material could be precipitated with equivalent amounts of normal mouse IgGs (lane G). This precipitation of insulin receptors by anti-H-2 antibodies could be due either to the presence of common epitopes on insulin receptors and H-2 antigens (cross-reactivity), or to the formation of a molecular complex between the two antigens (coprecipitation). The demonstration that different monoclonal antibodies (lanes B, D, and F), known to react with distinct antigenic determinants (18–20), are all able to precipitate the insulin receptor strongly supports the coprecipitation possibility.

Further experimental evidence that precipitation of insulin receptors is not due to cross-reactions is presented in Fig. 2. Insulin receptors were labeled on liver membranes from mice of two different haplotypes (*H-2^k* and *H-2^b*). Membranes were solubilized and exposed to monoclonal antibodies reacting specifically with H-2^k antigens and H-2^b antigens. In the case of cross-reactions, the precipitation of insulin receptors by anti-H-2 antibodies should not depend on the presence of the appropriate antigen. Fig. 2 shows that, in fact, insulin receptors were only precipitated by anti-H-2 antibodies corresponding to appropriate haplotypes.

Finally, to exclude cross-reactions, we have tested the precipitation of insulin receptors by anti-H-2 antibodies in a sequential immunoprecipitation analysis. If the precipitation of insulin receptors by anti-H-2 antibodies is due to immunological cross-reaction, then removal of H-2 antigens from the extract should not markedly affect the subsequent immunoprecipitation of insulin receptors by anti-H-2 antibodies. In contrast, if the precipitation of insulin receptors is due to a physical association between insulin receptors and H-2 antigens, it should require the presence of H-2 antigens in the extract. When membrane extracts were first exposed to anti-H-2 antibodies to deplete H-2 antigens, and then further exposed to anti-H-2 antibodies, no labeled insulin receptors could be precipitated (Fig. 3, lane B). Preclearing by normal mouse IgGs did not prevent the precipitation of insulin receptors by anti-H-2 antibodies (lane E). By contrast, immunoprecipitation of insulin receptors by anti-insulin receptor antibodies was not significantly affected by a first precipitation by anti-H-2 antibodies (lane A).

The selectivity of the interaction between insulin receptors and H-2 antigens was investigated after labeling liver membranes with photoreactive insulin and by lactoperoxidase-catalyzed iodination prior to solubilization. With this double-labeling approach, it was possible to visualize the coprecipitation of H-2 antigens (a doublet at 45 kDa and a band at 12 kDa) and insulin-receptor-binding subunit (130 kDa) by anti-H-2 antibodies (Fig. 4, lane B). No other major bands were detectable, indicating that this association was specific. Moreover, when another major membrane protein is precipitated [e.g., the epidermal growth factor (EGF) receptor; $\approx 2 \times 10^6$ receptors per hepatocyte] by an antibody raised against a synthetic peptide corresponding to a fragment of the EGF receptor (23), no insulin receptors were precipitated together with EGF receptors (170 kDa) (lane D). In addition, the coprecipitation of insulin receptors with H-2 antigens was not affected by a variety of sugars that could have competed for this interaction if it was only due to the glycoprotein nature of the two proteins (data not shown). We can therefore exclude the possibility that (*i*) insulin receptors are sticking to any antigen–antibody complex, and (*ii*) all membrane proteins in our experimental conditions are crosslinked as a result of the photoactivation process.

These results thus demonstrate that insulin receptors and class I H-2 antigens form molecular complexes. The amount

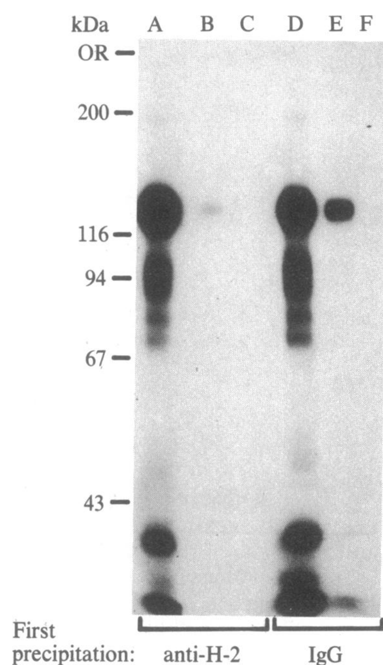

FIG. 3. Sequential immunoprecipitations of insulin receptors by anti-H-2 monoclonal antibodies. Insulin receptors from H-2^k mouse liver plasma membranes were labeled as described in Fig. 1. The solubilized proteins were first incubated with anti-H-2 antibodies (11-4-1 at 50 μg/ml) (lanes A–C) or with normal mouse IgGs (50 μg/ml) (lanes D–F). After immunoprecipitation with protein A, the supernatants were subsequently incubated with serum containing antibodies to insulin receptor at a 1:300 dilution (lanes A and D); monoclonal antibody to K^k (11-4-1) at 20 μg/ml (lanes B and E); and normal mouse IgGs at 20 μg/ml (lanes C and F). Precipitates were analyzed by $NaDodSO_4$/PAGE on a 7.5% acrylamide gel under reducing conditions followed by autoradiography. OR, origin.

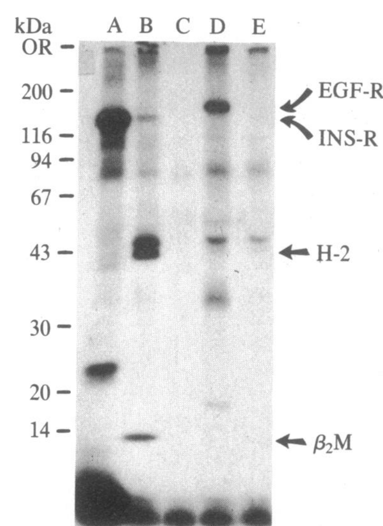

FIG. 4. Immunoprecipitation of insulin receptors, H-2 antigens, and EGF receptors from liver plasma membranes. Membranes were labeled with ^{125}I-labeled photoreactive insulin and lactoperoxidase-catalyzed iodination prior to solubilization in 1% Nonidet P-40. Immunoprecipitations with the following antibodies were performed: lane A, serum containing antibodies to the insulin receptor at a 1:300 dilution; lane B, monoclonal antibody reacting with K^k (11-4-1) at 20 μg/ml; lane C, normal mouse IgGs at 20 μg/ml; lane D, serum containing antibodies to the EGF receptor at a 1:10 dilution; lane E, preimmune serum from lane D at a 1:10 dilution. Precipitates were analyzed by $NaDodSO_4$/PAGE on a 7.5-17.5% acrylamide gradient gel followed by autoradiography. OR, origin; EGF-R, EGF receptor; INS-R, insulin receptor; β_2M, β_2-microglobulin.

of solubilized insulin receptors associated with H-2 antigens in different experiments represents 10–25% of recoverable insulin receptors precipitated with anti-insulin receptor antibodies. These values may be largely underestimated compared to the actual stoichiometry in native plasma membranes. Indeed, chemical cross-linking of membrane proteins prior to solubilization markedly increased the amount of insulin receptors precipitated by anti-H-2 antibodies (data not shown).

To our knowledge, a physical interaction between a hormone receptor and a histocompatibility antigen has never been reported so far. However, such interactions have been suggested by experiments showing that anti-HLA antibodies can inhibit EGF binding (9) and insulin binding (10) to cultured human cells and that antibodies against β_2-microglobulin, the light chain of class I MHC antigens, inhibit platelet aggregation in response to thrombin (13). The present results that insulin receptors are associated to H-2 antigens raise the possibility that H-2 proteins are involved in the transduction of the hormone signal triggered by insulin binding to its receptor. It has been shown recently that the insulin receptor β subunit contains a tyrosine kinase activity that may be responsible for signal transduction (for a review, see ref. 24). Since HLA antigens can be phosphorylated by an oncogene-associated tyrosine kinase (25), it is tempting to speculate that MHC class I antigens are preferential cellular substrates for receptor-associated kinases involved in cell growth and proliferation.

We thank Dr. A. M. Schmitt-Verhulst (Centre d'Immunologie de Luminy, Marseille, France) for the gift of most monoclonal antibodies used in this study. We are also grateful to Dr. C. R. Kahn (Joslin Diabetes Center, Boston) for anti-insulin receptor antibodies, to Drs. R. Kris and J. Schlessinger (Weizmann Institute, Rehovot, Israel) for anti-EGF receptor antibodies, and to Dr. D. Sachs (National Institutes of Health, Bethesda, MD) for the gift of several hybridomas. The illustration work of Mr. G. Visciano and the secretarial assistance of Mrs. J. Duch and L. Capolongo are gratefully acknowledged.

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