Interaction between Major Histocompatibility Complex Antigens and Epidermal Growth Factor Receptors on Human Cells

ALAIN B. SCHREIBER, JOSEPH SCHLESSINGER, and MICHAEL EDIDIN
Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel. Dr. Schreiber's present address is Department of Immunology, Syntex Research, Palo Alto, California 94304; and Dr. Edidin's present address is Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218.

ABSTRACT It has been suggested that products of the major histocompatibility complex, the MHC, of vertebrates function in many processes of recognition and ligand binding at the cell surface. Here we show that binding of polyclonal and monoclonal antibodies against human MHC antigens, HLA, reduced the binding of epidermal growth factor (EGF) to its membrane receptors on A-431 tumor cells and on normal human fibroblasts. Binding of EGF at 37°C similarly inhibited the binding of Fab fragments and intact Ig anti-HLA to human cells. The inhibitory effect of anti-HLA antibodies was rapid and dependent upon temperature and antibody concentration and valence.

Fluorescence microscopy qualitatively confirmed the binding data and showed that MHC antigens and EGF-receptors do not co-cluster in the membrane.

A major histocompatibility complex, MHC,[1] was first identified in mice as one of many segregating histocompatibility loci whose products provoke allograft rejection. The antigenic products of the mouse MHC, H-2, not only provoked vigorous rejection, they also stimulated production of circulating antibodies, reactive with tissues bearing graft donor alloantigens. These antibodies allowed a fine serological analysis of H-2 transplantation antigens, and such analysis, together with results on grafting, indicated that H-2 is highly polymorphic. Similar approaches in human transplantation, as well as work with teleost fish, chickens, cattle, and dogs, led to similar results. Each species possesses a single complex genetic region, highly polymorphic, whose products are major allograft antigens as well as being detectable by circulating antibodies (12, 33). Hence, the general term major histocompatibility complex, MHC, denotes such a region, in addition to a particular species name. Genes of the MHC specify various cell surface glycoproteins, some of which (the so-called class II products) are represented only in immunocytes, while others (class I products) are present in virtually all nucleated cells.

The MHC plays a pivotal role in immune processes. Its products function in immune responses as targets, as contexts for recognition of other cell surface antigens, or as sites of cell-to-cell interactions (reviews in 2, 34). MHC products may also function in general as contexts for ligand binding and cell-cell interactions. The MHC functions in immune responses would be a special case of this more general function (11, 24, 30). MHC haplotype is associated with the number and affinity of receptors for glucagon and insulin in liver membranes (18, 19), and influences cell contact and adhesion in several different systems (1, 10, 38). These associations are likely to be due to known class I antigens since antibodies to these antigens inhibit hormone binding (18, 32) and cell adhesion (1).

Here we use a monoclonal antibody to human class I MHC antigens, HLA-A, B, C, to probe the interaction of these antigens with the receptor for epidermal growth factor (EGF) in intact cells. We show that in two different cell types, A-431 human tumor cells, and normal human fibroblasts, the binding of antibody to HLA antigens alters the display of EGF receptors, while binding of EGF to its receptors affects the binding of antibody to HLA. The interaction between MHC antigens and the EGF receptor system is rapid and sensitive to temperature and to antibody valence.

[1] *Abbreviations used in this paper:* DME, Dulbecco's modified Eagle's medium; EGF, epidermal growth factor; HFF, human foreskin fibroblasts; MHC, major histocompatibility complex.

THE JOURNAL OF CELL BIOLOGY · VOLUME 98 FEBRUARY 1984 725–731
 · 0021-9525/84/02/0725/07 $1.00

MATERIALS AND METHODS

Reagents: EGF was isolated from the submaxillary glands of adult male mice according to the procedure of Savage and Cohen (27). EGF was purified to homogeneity as judged by isoelectric focusing, amino terminal, and total amino acid analysis. EGF was iodinated by the chloramine-T method (15) to a specific activity of 10^5–2×10^5 cpm/ng protein. Rhodamine conjugated EGF was prepared as described by Schlessinger et al. (28). Human growth hormone was a generous gift from Dr. Z. Laron (Weizmann Institute) and was iodinated by a modification of the lactoperoxidase method (5) to a specific activity of 4–8×10^4 cpm/ng protein. Affinity-purified goat anti-mouse Ig was the gift of Dr. J. Haimovich (Weizmann Institute). Bovine pituitary fibroblast growth factor was a kind gift of Dr. I. Vlodavski (Weizmann Institute). Concanavalin A and wheat germ agglutinin were purchased from Miles-Yeda (Rehovot, Israel) and iodinated according to published procedures (9). A murine cell line producing a monoclonal IgG2a, denoted KE-2, was the generous gift of Dr. Roger Kennett (University of Pennsylvania). KE-2 cells were grown in Eagle's Minimal Essential Medium, with 10% fetal calf serum (Reheis Chemical Co.). The IgG was purified from spent medium by adsorption to protein A-Sepharose at pH 8.5 and elution from the washed column at pH 4.5. The antibody appears to be directed to a monomorphic HLA antigenic specificity. It precipitates material of M_r 44,000 and 11,000 from detergent extracts of a number of cell lines, including ^{35}S-labeled A431, but does not react with Daudi, a line lacking surface HLA. The KE-2 preparation was free of EGF contamination as judged by a sensitive radioimmunoassay performed with rabbit anti-EGF antibodies.

Monovalent Fab fragments were prepared from KE-2 Ig by brief papain digestion, following the procedure of Guyer et al. (13). The Fab were isolated on Sephadex G-100 and freed from contamination by Fc and intact IgG by passage over protein A-Sepharose. The KE-2 IgG_2a and its monovalent Fab fragments were iodinated by a modification of the chloramine-T method as described by Mason and Williams (22) to a specific activity of 2–5×10^6 cpm/μg protein. The labeled proteins were >90% as active as native unlabeled proteins.

Tetramethylrhodamine and fluorescein conjugates of the KE-2 IgG_2a and its Fab fragments were prepared as described by Brandtzaeg (4) with tetramethylrhodamine isohthiocyanate and fluorescein isothiocyanate (Research Organics, Inc., Cleveland, OH). The molar fluorophore to protein ratio, determined by absorbance measurements, ranged between 1.5 and 3. A polyclonal anti-HLA antiserum, Stoltzfus, reacting with >90% of all individuals was the gift of Dr. Wilma Bias (The Johns Hopkins Medical School). An immunoglobulin fraction of this antiserum was prepared by ammonium sulfate precipitation followed by DE-52 (Whatman Laboratory Products, Inc., Clifton, NJ) anion exchange chromatography. The murine monoclonal IgG_2a 4B3, and 2D6, directed against cell surface components of A-431 cells, were prepared from hybridomas established in our laboratory by fusion of NS-1 myeloma cells and spleen cells of mice immunized with fixed A-431 cells (29). They react with A-431, human foreskin fibroblasts (HFF), and mouse 3T3 cells, but not with lymphocytes. They do not precipitate material of the size or with the kinase activity of EGF receptor from detergent extracts of A431 cells.

Cells: Human epidermoid carcinoma cells (A-431 cell line) were kindly provided by Dr. G. Todaro (Frederick Cancer Research Center, Frederick, MD). HFF from primary cultures were a gift from Dr. M. Gabbay (Weizmann Institute). All cells were grown in Dulbecco's modified Eagle's medium (DME) containing 10% heat-inactivated fetal calf serum.

Binding Assays: Cells were grown to confluent monolayers in 24-well Costar trays (Costar. Data Packaging, Cambridge, MA) and incubated with iodinated ligands following various time and temperature protocols in a final volume of 0.25 ml of either DME containing 0.1% BSA and buffered at pH 7.4 with HEPES (DME-BSA) or 10 mM phosphate-buffered saline pH 7.4 containing 0.1% BSA (PBS-BSA). In some experiments cells were prefixed with neutralized 3% paraformaldehyde at room temperature. Binding of EGF to these fixed cells was similar in magnitude to binding to unfixed cells, in agreement with published work (14). At the end of the incubation, cells were washed four times with cold buffer, lysed with 0.1 N NaOH, and the cell-associated radioactivity was determined. Nonspecific binding was assessed in each assay. Radioactivity bound in the presence of 100-fold excess of unlabeled over labeled ligand did not exceed 15% of specific binding in any assay.

Fluorescence Microscopy: Cells were plated at low density on coverslips in 35-mm culture dishes and incubated with fluorescent derivatives of EGF or of anti-HLA antibodies in DME-BSA. Cells were observed with a Zeiss inverted microscope equipped with filters for selective observation of either fluorescein or rhodamine fluorescence. Photographs were taken on Kodak Tri-X film.

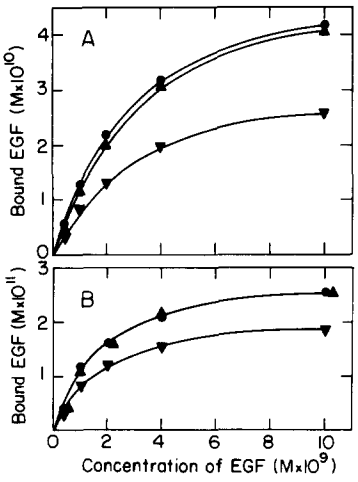

FIGURE 1 Effect of anti-HLA IgG and its Fab fragment on EGF binding to A-431 and HFF cells. A-431 cells (*A*) or HFF cells (*B*) were preincubated at 37°C for 30 min with either 1.5×10^{-7} M anti-HLA IgG (▼), 10^{-6} M anti-HLA Fab (▲), or buffer (●), before incubation at 4°C for 1 h with various concentrations of ^{125}I-EGF.

RESULTS

Anti-HLA Antibodies Reduce Binding of EGF to A-431 and HFF Cells

Incubation of confluent monolayers of live A-431 and HFF cells at 37°C with 1.5×10^{-7} M monoclonal IgG anti-HLA, KE-2, reduced the subsequent level of binding of radiolabeled EGF at 4°C (Fig. 1). A Scatchard plot analysis indicates that the A-431 cells bear an apparently homogeneous population of 1.5×10^6 EGF receptors per cell and the HFF bear ~10^5 EGF receptors per cell. K_{app} for both receptors is 10^9 M^{-1}. Incubation at 37°C with 1.5×10^{-7} M KE-2 IgG reduced the binding of EGF to A-431 cells by ~40% and the binding to HFF cells by ~30% over the whole EGF concentration range assayed. (Fig. 1). The inhibition of EGF binding was dependent on the concentration of KE-2 IgG (Fig. 2) and was proportional to the equilibrium binding of MHC antigens by the antibody (Fig. 3). The inhibitory effect reached a plateau value at 3×10^{-7} M KE-2 IgG and decreased somewhat at higher antibody concentrations (Fig. 2). Inhibition was most effective if cells were not washed after binding KE-2 and before adding EGF. However, significant inhibition of binding (80% of maximum inhibition) was seen in washed cells.

Preincubation with Ke-2 IgG at 37°C also decreased EGF binding to cells at 37°C. At this temperature, EGF induces a rapid clustering of its membrane receptor (39) followed by

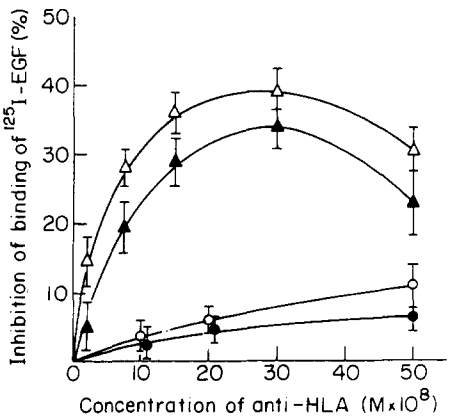

FIGURE 2 Concentration dependence of the effect of anti-HLA IgG and its Fab fragment on EGF binding to A-431 and HFF Cells. A-431 cells (open symbols) or HFF cells (closed symbols) were preincubated at 37°C for 30 min with either anti-HLA IgG (△) or anti-HLA Fab (○) before incubation at 4°C for 1 h with 2×10^{-9} M ^{125}I-EGF. Data points represent the mean ± standard deviation of two independent experiments performed in triplicate.

internalization and degradation (at different rates for different target cells) of the hormone-receptor complexes (7). The anti-HLA antibody decreased both the cell surface bound EGF, assayed by release after mild acetic acid treatment (37), and the internalized hormone (data not shown).

The effect of the anti-HLA antibody on EGF binding was almost immediate. Varying preincubation times from 5 min to 4 h at 37°C with the KE-2 IgG did not significantly alter the reduction in EGF binding (Table I). Moreover, the anti-HLA antibody affected the initial rate of binding of ^{125}I-EGF to cells at 4°C as well as equilibrium binding. The effect of KE-2 IgG on EGF binding was not peculiar to this anti-HLA reagent. A polyclonal monospecific anti-HLA reagent, Stoltzfus, inhibited EGF binding to A-431 and HFF cells to about the same extent as KE-2 IgG (Table I).

Specificity of the Inhibition

Two murine monoclonal IgG$_2$a antibodies (denoted 4B3 and 2D6) directed against abundant cell surface components of A-431 and HFF cells but not against EGF receptors did not affect the binding of EGF to these cells (Table I). The KE-2 IgG did not change the number or apparent affinity of membrane lectin receptors on A-431 and HFF cells (~5 × 10^7 receptors per cell), or the number or apparent affinity of human growth hormone receptors (~2 × 10^4 per cell).

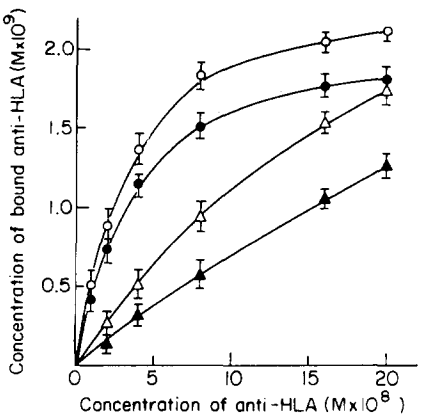

FIGURE 3 Binding of anti-HLA IgG and its Fab fragment to A-431 cells and the effect of EGF. A-431 cells were preincubated at 37°C for 30 min either with buffer (open symbols) or 5 × 10^{-9} M EGF (closed symbols) before incubation at 4°C for 1 h with either various concentrations of anti-HLA IgG (○) or anti-HLA Fab (△).

Requirements for Inhibition of EGF Binding by Anti-HLA Antibodies

Fab fragments of KE-2 IgG bound readily to A-431 (and HFF) cells, although with a lower apparent affinity than the intact antibody (Fig. 3). The Fab fragments did not significantly reduce binding of EGF to its receptors on either A-431 or HFF cells (Fig. 1), even when relatively high concentrations were used (Fig. 2). EGF binding was inhibited when the cell-bound KE-2 Fab fragments were cross-linked at 37°C with a second layer of goat anti-mouse Ig antibodies.

Binding anti-HLA at 4°C or to cells previously fixed in 3% paraformaldehyde had no effect on subsequent EGF binding. Though cross-linking of HLA antigens appears to be required, EGF binding was inhibited by anti-HLA antibodies even in the presence of 10^{-5} M cytochalasin D 10^{-7} M colchicine, or 0.1% NaN_3. Thus, although cell metabolism appears to be required for an effect of anti-HLA on EGF receptors, aggregation of receptors into caps or large patches is not required.

EGF Reduces Binding of Anti-HLA Antibodies to A-431 and HFF Cells

Binding of KE-2 IgG and Fab to A431 and HFF cells was measured with iodinated Ig and Fab (Fig. 3). There were ~2 × 10^6 HLA molecules per A-431 cell and approximately one-tenth this number on HFF. K_{app} of the Ig was ~2 × 10^7 M^{-1} and that of the Fab ~3 × 10^6 M^{-1}. Scatchard plot analysis of the data of Fig. 3 showed that about 1.5-fold more Fab bound to A431 cells than did IgG. Thus, about one-half of the Ig was bound to two HLA molecules and half was bound to one HLA molecule. The ratio of Fab bound/IgG bound is expected to range between 1, when all IgG is bound by one combining site only, to 2, when all IgG is bound divalently, by both combining sites (22). Incubation of cells at 37°C with EGF reduced the number of sites occupied by the Ig or its Fab but did not alter their apparent affinities for HLA. The ratio of Fab bound to IgG bound in the presence of EGF was ~2. Hence all of the IgG bound to HLA in the presence of EGF was bound divalently.

The inhibition of anti-HLA ligand binding mediated by

TABLE I

Influence of Various Inhibitors and Incubation Conditions on the Binding of ^{125}I-EGF to A-431 Cells

Inhibitor	Preincubation			Inhibition of ^{125}I-EGF-binding
	°C	h		%
IgG$_2$a 2D6 (5 × 10^{-7} M)	37	4	DME-BSA	3 ± 5
IgG$_2$a KE-2 (2 × 10^{-7} M)	37	5	DME-BSA	37 ± 6
	37	4	DME-BSA	46 ± 6
	4	4	DME-BSA	6 ± 4
	37	4	DME-BSA + 10^{-5} M cytochalasin D	34 ± 8
	37	4	DME-BSA + 10^{-5} M colchicin	42 ± 5
	37	4	PBS-BSA + 0.1% NaN_3	38 ± 6
Prefixed cells	37	4	DME-BSA	7 ± 5
IgG fraction of polyclonal Sto anti-HLA antiserum (5 × 10^{-6} M)	37	4	DME-BSA	32 ± 7
	4	4	DME-BSA	11 ± 5

Confluent monolayers of A-431 in 24-well Costar trays were preincubated under various conditions before addition of 10 ng/ml ^{125}I-EGF at 4°C for 1 h and determination of the cell-associated radioactivity. Results are the mean ± standard deviation of at least two independent experiments performed in triplicate.

EGF was rapid and temperature dependent. Preincubation of the cells with EGF at 37°C for 5 min was sufficient to obtain its full effect, while saturation of the EGF receptors at 4°C had no influence on subsequent anti-HLA antibody binding. The inhibition of anti-HLA antibody binding was affected at concentrations of EGF that are approximately half-saturating at equilibrium.

Distribution of Fluorescently Labeled Anti-HLA Antibodies and EGF on A-431 Cells

When fluorescently labeled EGF and KE-2 were bound to cells at 4°C, the fluorescence of both appeared continuous around the cell periphery. KE-2 bound at 4°C and aggregated into coarse patches by a fluorescent anti-Ig at 37°C did not co-patch EGF receptors (Fig. 4, *A–C*). Incubation of cells with fluorescent EGF and KE-2 IgG at 37°C produced cells with patchy internal EGF fluorescence which bound fluorescent anti-mouse Ig (detecting HLA) at the cell surface (Fig. 4, *D–F*).

Though Fab KE-2 bound well to A-431 to 37°C (not shown) it was displaced by EGF when fluorescein Fab and rhodamine-EGF were incubated at 37°C (compare the intensities in Fig. 5, *A* and *B* with *C* and *D*). Co-incubation of cells with rhodamine EGF and fluorescein KE-2 Ig at 37°C yielded cells that fluoresced strongly for fluorescein but weakly for rhodamine (Fig. 5, *E* and *F*).

DISCUSSION

The binding of anti-HLA antibodies to A431 cells or to normal fibroblasts reduced the number of receptors available for binding by epidermal growth factor, EGF. Conversely, EGF bound to the A-431 cells reduced the number of anti-HLA antibodies bound. Although there are other treatments that alter the display of EGF receptors, these generally affect the affinity of the receptors (3, 21, 26, 31) and not their number. Interferon-treated cells express increased levels of MHC antigens (for example, reference 35), but this requires long exposure, in contrast to the immediate effect of EGF on HLA display.

The inhibitory effect of anti-HLA antibodies was specific. Several monoclonal antibodies directed against cell surface components of human fibroblasts other than the EGF receptor did not affect the binding of EGF to its receptors. The anti-HLA antibodies do not induce gross changes in cell morphology. They did not affect the binding of radiolabeled human growth hormone, Concanavalin A, or wheat germ agglutinin to their respective membrane receptors. Our anti-HLA antibody preparation was free of EGF contaminants and in immunoprecipitation experiments brought down components of 44,000 and 11,000 mol wt, of the size of HLA heavy, light chains and significantly smaller than the size of the EGF receptor on A431 cells (6, 17).

Binding of MHC antigens by anti-HLA antibodies or Fab fragments is not sufficient to inhibit EGF binding to its receptor. The anti-HLA antibodies must be divalent and brief preincubation at 37°C was required. Indeed, a 5-min incubation at 37°C was more effective than a 4-h incubation with KE-2 at 4°C. Prefixation of the cells abolished the effect of the antibodies. The inhibition of EGF binding by the anti-HLA antibodies was not influenced by the presence or absence of Ca^{2++} ions or azide, nor by the treatment of cells with microfilament (cytochalasin D) or microtubule (colchicin) disrupting drugs.

Only a fraction of HLA or EGF receptors must be bound for the binding of one ligand to affect the display of receptors for the other. A 5-min incubation at 37°C was sufficient to alter receptor display, and a 4-h incubation with anti-HLA antibody had no further effect on EGF binding. Fixed cells did not alter EGF receptor number in response in anti-HLA, and there was no effect of either EGF or anti-HLA if cells are maintained at 4°C. Thus, the cell response to the ligand binding may require metabolism. Sodium azide did not inhibit the effect of anti-HLA, but this may merely have indicated that anaerobic metabolism is sufficient for modification of receptor display, or that cell ATP levels are reduced too slowly to affect the response.

Only divalent anti-HLA affected a reduction in number of EGF receptors. The Fab fragments of our monoclonal antibody, which bound to cells with an affinity about 10-fold less than the intact antibody, were ineffective even when used at concentrations 10-fold higher than those of the intact IgG. Fab fragments cross-linked by anti-Fab were nearly as effective as intact Ig in altering EGF binding, while high concentrations of intact IgG (800 μg/ml) were not so effective as lower concentrations (80 μg/ml). Increasing the concentrations of antibody results in increasing amounts of Ig molecules bound monovalently. Thus, cross-linking of HLA antigens appears to be required to affect the EGF receptor. The Scatchard analysis of binding data shows that whereas 50% of anti-HLA is bound divalently to otherwise untreated cells, 100% of anti-HLA is bound divalently in EGF-treated cells. In this case, then, the apparent change in number of anti-HLA binding sites induced by EGF may be a change in the affinity of HLA antigens for the antibody. It is unlikely to be the result of cell shape changes induced in A431 by EGF. Such changes should equally reduce binding of IgG and Fab.

EGF binding is reduced 30–40% when A431 or normal fibroblasts (HFF) were treated with anti-HLA antibody. Though the numbers of EGF receptors and MHC antigens are 20-fold higher on A431 than on HFF, the ratio of EGF receptors and MHC antigens is about the same on both cell types. The magnitude of inhibition is about the same as that seen for inhibition of insulin and glucagon binding or cell adhesion by anti-H-2 (1, 18) or anti-HLA (Olsson, L., C. J. Honsik, and B. Diamant, submitted manuscript) antibodies. One monoclonal antibody against all HLA class I heavy chains has been an effective blocker of insulin binding (Olsson, L., C. J. Honsik, and B. Diamant, submitted manuscript) (32); but even the binding of this antibody is only partly blocked by preincubating cells with insulin.

The effects reported here and in other studies on MHC and peptide hormone binding are small when compared with the effects of MHC haplotype in immune responses. However, in such responses MHC affects an amplifying system of interacting cells. The magnitude of its effect depends upon the point at which MHC antigens function in the cellular cascade. Thus, when class I antigens serve as targets in the final steps of a cellular immune response, haplotype differences alter the response by factors of 2–4. Differences in class II products which act early, for example in antigen presentation to amplifying T cell subsets, may alter responses by a factor of 100. In contrast, our measurements here deal with the primary event of hormone binding to a receptor. There is no biological amplification of the primary event.

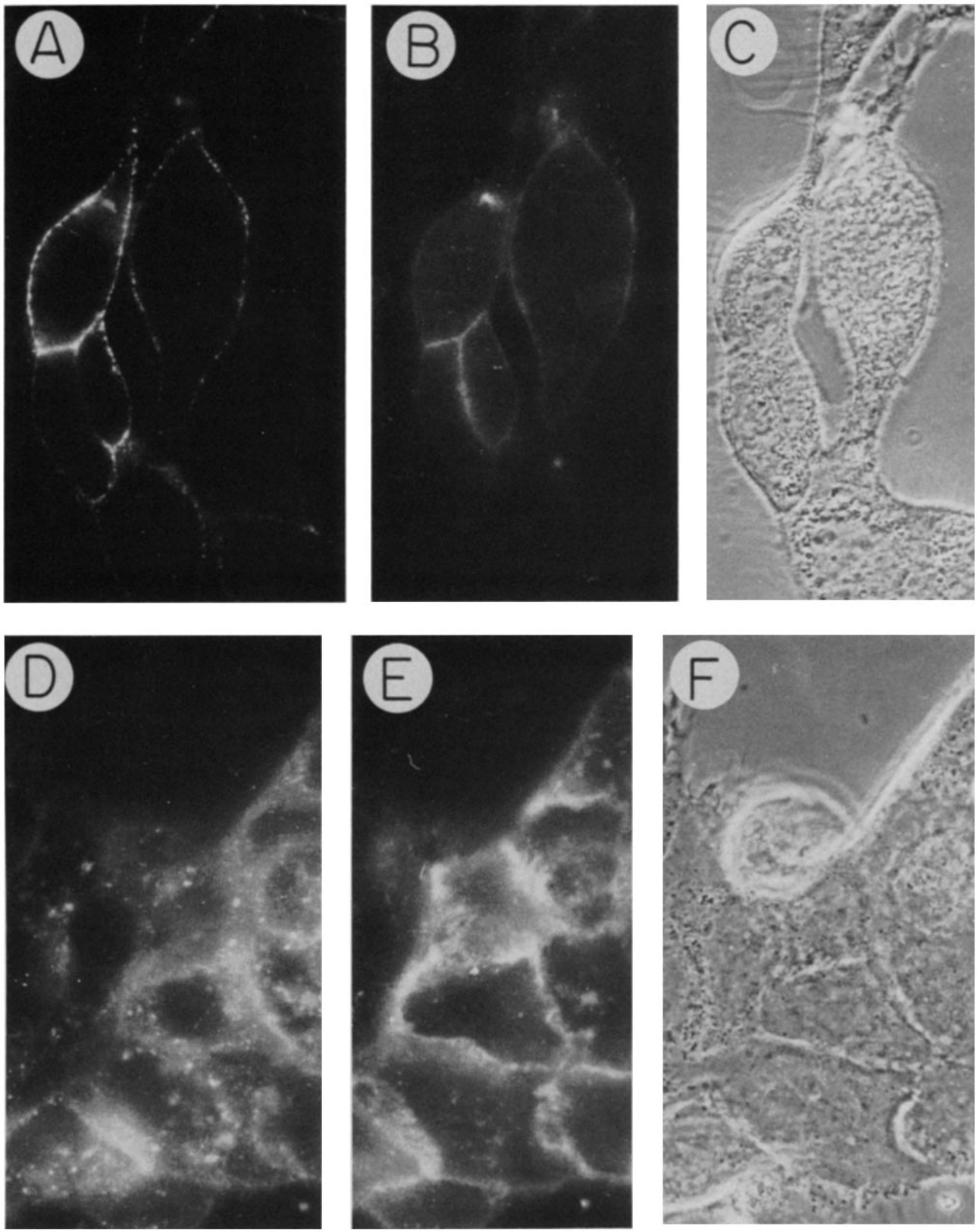

FIGURE 4 A431 cells were incubated with KE-2 IgG at 4°C followed by fluorescein-goat anti-mouse Ig at 37°C to patch the HLA antigens. After this incubation the cells were labeled with rhodamine EGF at 4°C. *A*, fluorescein anti-Ig forms coarse patches after incubation at 37°C; *B*, rhodamine EGF gives nearly continuous, finely speckled staining on a cell with patched HLA antigens. C, phase-contrast microscopy. Cells were labeled with rhodamine EGF and KE-2 at 4°C IgG and incubated at 37° to internalize EGF. After incubation the cells were labeled with fluorescein-goat anti-mouse Ig and examined. *D*, internalized rhodamine-EGF appears patchy and cell surfaces are not well outlined. EGF bound to cells as shown is not extractable by acetic acid and hence is internalized. *E*, cells as in *D* but viewed for fluorescein label, detecting HLA antigens. Significant amounts of HLA remain at the cell surface, *F*, phase-contrast microscopy. The shape of these cells is affected by EGF at 37°C.

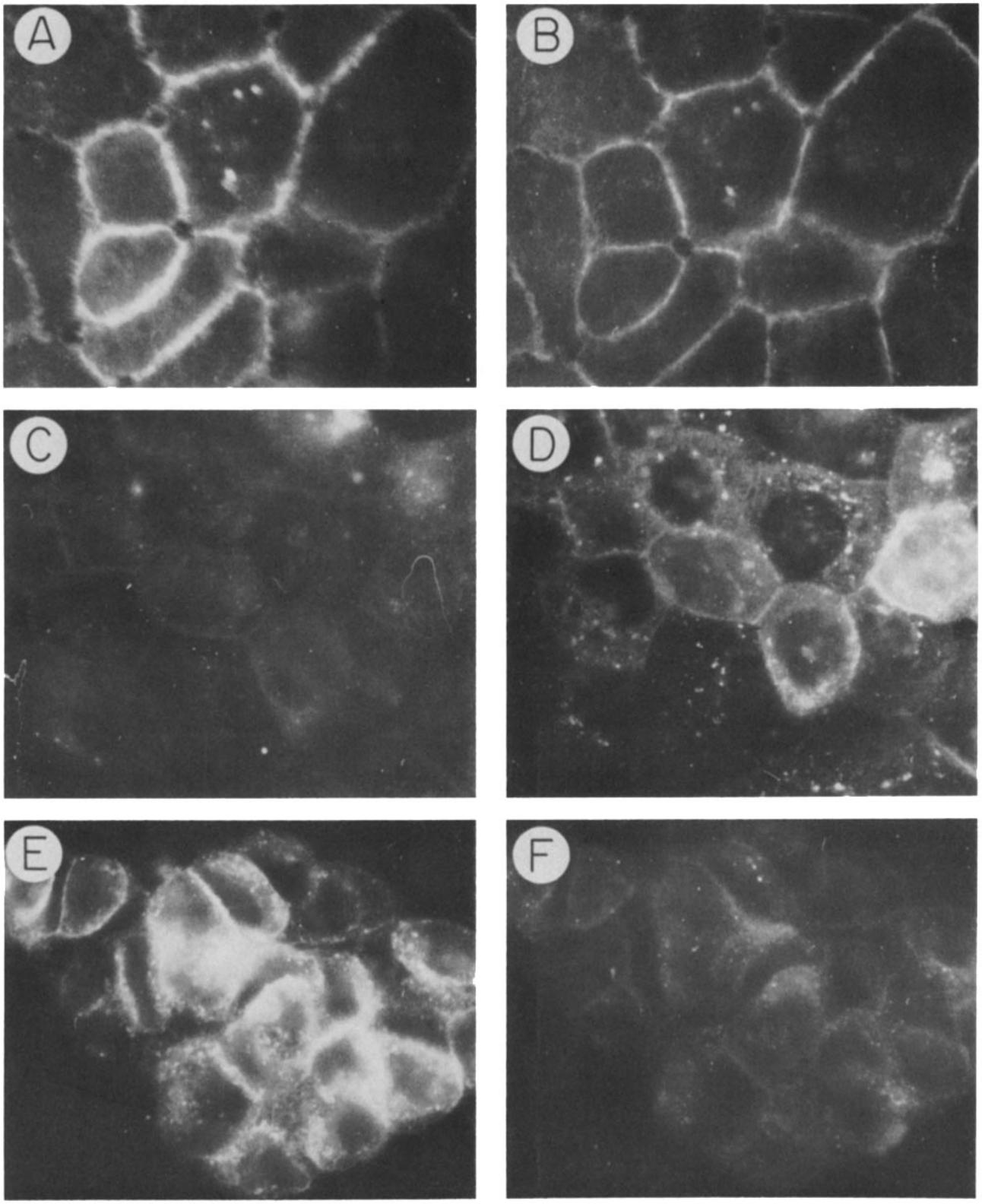

FIGURE 5 A431 cells were incubated with mixtures of reagents of 4°C or at 37°C. (*A* and *B*) Fluorescein Fab anti-HLA + rhodamine EGF at 4°C. *A*, fluorescein fluorescence; *B*, rhodamine fluorescence. (*C* and *D*) Fluorescein Fab (anti-HLA + rhodamine EGF at 37°C. *C*, fluorescein fluorescence; *D*, rhodamine fluorescence. (*E* and *F*) Fluorescein IgG anti-HLA + rhodamine EGF at 37°C. *E*, fluorescein fluorescence; *F*, rhodamine fluorescence.

Modulation of EGF binding by anti-HLA antibody and modulation of antibody binding by EGF appeared to require some cell metabolism, since the effects are found only at 37°C in unfixed cells. Continued binding of HLA antigens by the antibodies is implied if the results of experiments in which antibody was present during incubation with EGF are compared with the results of the experiment in which cells were washed after binding antibody and before binding EGF. This

observation, and the fact that inhibitors of patching and capping do not block the effect of anti-HLA on EGF binding, tell against a mechanism in which cross-linking and internalization of HLA antigens lead to internalization of EGF receptors. Observations on fluorescently labeled cells also suggest that co-internalization is not a factor in inhibiting EGF or anti-HLA binding. Regions of internalized EGF did not fluoresce well for the fluorescein marker of Fab anti-HLA, while there was little sign of rhodamine EGF in cells simultaneously exposed to fluorescein IgG anti-HLA and rhodamine EGF.

A plausible mechanism for interaction of HLA antigens and EGF receptors involves association of HLA heavy chains with EGF receptor glycoproteins, and configurational changes in both molecules. It has been suggested that EGF receptors alter configuration after binding EGF so as to predispose them to association with other molecules on the cell surface (14). It is known that the antigenicity of HLA heavy chains is altered by association with β-2 microglobulin light chain (20) and that this association is not stable (16). Associations between MHC antigens and hormone receptors, similar to those between MHC antigens and viral membrane glycoproteins (8), could alter receptor or HLA antigens so that one or the other could no longer bind its ligand.

Our data imply that an HLA dimer is necessary to alter EGF receptors. Inhibition of EGF binding by anti-HLA antibody, then, would be due to stabilization of antigen dimers by the antibody. Failure to inhibit EGF binding at 4°C in fixed cells may be due to reduced associations between HLA antigens and EGF receptors, either because reduced lateral diffusion inhibits formation of receptor-antigen complexes, or because cell metabolism is required to stabilize the associations.

The data presented here are the latest in a series of observations indicating that the binding of peptide hormones by specific cell surface receptors is moduated by interaction with histocompatibility antigens. Thus MHC haplotype ought to affect the sensitivity of cells to hormones, either exacerbating or mitigating the effects of hormone imbalance. We suggest that biological relationships between peptide hormone receptors and MHC antigens will be found in diabetes and perhaps as well in host responses to tumors whose growth is driven by transforming growth factors.

This work was supported by grants from the National Institutes of Health (CA-25820 to J.S.; AI-14584 to M.E.), from the U.S.-Israel Binational Science Foundation (J.S.) and from the Israel Cancer Society. A.B.S. is a recipient from a European Molecular Biology Organization long-term fellowship. This is contribution number 1237 from the Department of Biology.

Received for publication 10 December 1982, and in revised form 6 October 1983.

REFERENCES

1. Bartlett, P. F., and M. Edidin. 1978. Effect of the H-2 gene complex on rates of fibroblast intracellular adhesion. *J. Cell Biol.* 77:377–388.
2. Benacerraf, B. 1981. Role of MHC gene products in immune regulation. *Science (Wash. DC).* 212:1229–1238.
3. Bowen-Pope, D. F., P. E. DiCorleto, and R. E. Ross. 1983. Interactions between the receptors for platelet-derived growth factor and epidermal growth factor. *J. Cell Biol.* 96:679–683.
4. Brandtzaeg, P. 1973. Conjugates of immunoglobulin G with different fluorochromes. I. Characterization by anionic exchange chromatography. *Scand. J. Immunol.* 2:273–290.
5. Cadman, H. F., and M. Wallis. 1981. An investigation of sites that bind human somatotropin (growth hormone) in the lines of the pregnant rabbit. *Biochem. J.* 198:605–614.
6. Carlin, C. R., and B. B. Knowles. 1982. Identity of human epidermal growth factor (EGF) receptor with glycoprotein SA-7: evidence for differential phosphorylation of the two components of the EGF receptor from A431 cells. *Proc. Natl. Acad. Sci. USA.* 79:5026–5030.
7. Carpenter, G., and S. Cohen. 1979. Epidermal growth factor. *Annu. Rev. Biochem.* 48:193–216.
8. Ciavarra, R., and J. Forman. 1981. Cell membrane antigens recognized by anti-viral and anti-trinitrophenyl cytotoxic T lymphocyte. *Immunol. Rev.* 58:73–94.
9. Cuatrecasas, P. 1973. Interaction of wheat germ agglutinin and concanavalin A with isolated fat cells. *Biochemistry.* 12:1312–1323.
10. Curtis, A. S. G. 1980. Histocompatibility systems: cell recognition and cell adhesion. *In* Cell Adhesion and Motility. A. S. G. Curtis and J. D. Pitts, editors. Cambridge University Press, Cambridge. 273–292.
11. Dausset, J., and L. Contu. 1980. Is the MHC a general self-recognition system playing a major unifying role in an organism? *Hum. Immunol.* 1:5–17.
12. Gotze, D., editor. 1977. The Major Histocompatibility System in Man and Animals. Springer-Verlag, Berlin.
13. Guyer, R. L., M. E. Koshland, and P. Knoff. 1976. Immunoglobulin binding by mouse intestinal epithelial cell receptors. *J. Immunol.* 117:587–593.
14. Haigler, H. J., J. A. McKanna, and S. Cohen. 1979. Direct visualization of a ferritin conjugate of epidermal growth factor in human carcinoma cells. *J. Cell Biol.* 81:382–395.
15. Hunter, M. W., and F. C. Greenwood. 1962. Preparation of Iodine-131 labelled human growth hormone of high specific activity. *Nature (Lond.).* 194:495–496.
16. Hyafil, F., and J. L. Strominger. 1979. Dissociation and exchange of the β_2-microglobulin subunit of HLA-A and HLA-B antigens. *Proc. Natl. Acad. Sci. USA.* 76:5834–5838.
17. King, L. E., G. Carpenter, and S. Cohen. 1980. Characterization by electrophoresis of epidermal growth factor stimulated phosphorylation using A-431 membranes. *Biochemistry.* 19:1524–1528.
18. Lafuse, W. 1978. The role of the histocompatibility-2 locus in modifying cyclic AMP levels. Ph.D. Thesis, The Johns Hopkins University.
19. Lafuse, W., and M. Edidin. 1980. Influence of the mouse major histocompatibility complex, H-2, on liver adenylate cyclase activity and on glucagon binding to liver cell membranes. *Biochemistry.* 19:49–54.
20. Lancet, D., P. Parham, and J. L. Strominger. 1979. Heavy chain of HLA-A and HLA-B antigens is conformationally laibile: a possible role for β_2-microglobulin. *Proc. Natl. Acad. Sci. USA.* 76:3844–3848.
21. Lee, L., and I. B. Weinstein. 1978. Tumor-promoting phorbolesters inhibit binding of epidermal growth factor to cellular receptors. *Science (Wash. DC).* 202:313–315.
22. Mason, D. W., and A. F. Williams. 1980. The kinetics of antibody binding to membrane antigens in solution and at the cell surface. *Biochem. J.* 187:1–20.
23. Meruelo, D., and M. Edidin. 1975. Association of mouse liver adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels with histocompatibility-2 genotype. *Proc. Natl. Acad. Sci. USA.* 72:2644–2648.
24. Ohno, S. 1977. The original function of MHC antigens as the general plasma membrane anchorage site of organogenesis-directing proteins. *Immunol. Rev.* 33:59–69.
25. Rozengurt, E. 1980. Stimulation of DNA synthesis in quiescent cultured cells: exogenous agents, internal signals, and early events. *Curr. Top. Cell. Regul.* 17:59–88.
26. Rozengurt, E., K. D. Brown, and P. Pettican. 1981. Vasopressin inhibition of epidermal growth factor binding to cultured mouse cells. *J. Biol. Chem.* 256:716–722.
27. Savage, C. R., Jr., and S. Cohen. 1972. Epidermal growth factor and a new derivative. Rapid isolation procedures and biological and chemical characterization. *J. Biol. Chem.* 247:7609–7611.
28. Schlessinger, J., Y. Shechter, M. C. Willingham, and I. Pastan. 1978. Direct visualization of binding, aggregation, and internalization of insulin and epidermal growth factor on living fibroblastic cells. *Proc. Natl. Acad. Sci. USA.* 75:2659–2663.
29. Schreiber, A. B., I. Lax, Y. Yarden, Z. Eshhar, and J. Schlessinger. 1981. Monoclonal antibodies to epidermal growth factor receptors induce the early and delayed effects of epidermal growth factor. *Proc. Natl. Acad. Sci. USA.* 78:7535–7539.
30. Scofield, V. L., J. M. Schlumpberger, L. A. West, and I. L. Weissman. 1982. Protochordate allorecognition is controlled by a MHC-like gene system. *Nature (Lond.).* 295:499–502.
31. Shoyab, M., J. E. DeLarco, and G. J. Todaro. 1979. Biologically active phorbol esters specifically alter affinity of epidermal growth factor membrane receptors. *Nature (Lond.).* 279:387–391.
32. Simonsen, M., and L. Olssen. 1983. Possible roles of compound membrane receptors in the immune system. *Annales J. Immunol. (Institut Pasteur).* 134D:85–92.
33. Snell, G. C. 1968. The H-2 locus of the mouse: Observations and speculations concerning its comparative genetics and its polymorphism. *Folia Biol. (Prague).* 14:335–358.
34. Snell, G. D. 1981. Studies in histocompatibility. *Science (Wash. DC).* 213:172–178.
35. Vignaux, F., and I. Gresser. 1978. Enhanced expression of histocompatibility antigens on interferon-treated mouse embryonic fibroblasts (40076). *Proc. Soc. Exp. Biol. Med.* 157:456–460.
36. Wey, C.-L., R. A. Cone, and M. A. Edidin. 1981. Lateral diffusion of rhodopsin in photoreceptor cells measured by fluorescence photobleaching and recovery. *Biophys. J.* 33:225–232.
37. Yarden, Y., M. Gabbay, and J. Schlessinger. 1981. Primary amines do not prevent the endocytosis of epidermal growth factor into 3T3 fibroblasts. *Biochim. Biophys. Acta.* 674:188–203.
38. Zeleny, V., V. Matousek, and A. Lengerova. 1978. Intercellular adhesiveness of H-2 identical and H-2 disparate cells. *J. Immunogenet. (Oxf.).* 5:41–47.
39. Zidovetzki, R., Y. Yarden, J. Schlessinger, and T. M. Jovin. 1981. Rotational diffusion of epidermal growth factor complexed to cell surface receptors reflects rapid microaggregation and endocytosis of occupied receptors. *Proc. Natl. Acad. Sci. USA.* 78:6981–6985.