membranes were pelleted after a 30-min centrifugation at 45,000 rev/min. The membranes were resuspended in the same buffer at a concentration of 5 to 10 mg/ml and frozen at −70°C or used immediately. NGF was iodinated by lactoperioxidase treatment to 2800 to 3800 cpm/fmol (*22*). Equilibrium binding to ^{125}I-labeled NGF was carried out in a 100-μl reaction containing 10 to 40 μg of membranes in 50 m*M* Hepes, *p*H 8, BSA (1 mg/ml Sigma) for 60 min at 30°C. Binding was concluded with 1.5 ml of wash buffer [20 m*M* $NaHPO_4$, *p*H 7.4, 50 m*M* NaCl, BSA (1 mg/ml), protamine sulfate (1 mg/ml), 37°C] and each reaction was filtered under vacuum through Millipore HVLP filters (0.45 μm). After three 10-ml rinses in wash buffer, the filters were counted. Nonspecific binding was assessed by including unlabeled NGF to a final concentration of 800 n*M*. Specific binding ranged from 60 to 90% of total binding.

17. Psi2 fibroblast cells were infected with a recombinant retrovirus from PAE1c (*12*) and a neo$^+$, NGF receptor-expressing clone was isolated. Equilibrium binding of ^{125}I-labeled NGF to membranes isolated from this cell line indicated that only low affinity receptors were present.
18. The minor differences noted in K_d of the low affinity sites measured on intact cells ($K_d = 1 \times 10^{-9} M$) and cell membranes ($K_d = 2$ to $5 \times 10^{-8} M$) may be related to a loss of cytoskeletal association in membrane preparations, as described by S. Buxser *et al.*, *J. Biol. Chem.* **260**, 1917 (1985).
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Increased Expression of DNA Cointroduced with Nuclear Protein in Adult Rat Liver

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DNA and nuclear proteins were transferred into cells simultaneously at more than 95% efficiency by means of vesicle complexes. The DNA was rapidly transported into the nuclei of cultured cells, and its expression reached a maximum within 6 to 8 hours after its introduction. Moreover, when the plasmid DNA and nuclear protein were cointroduced into nondividing cells in rat liver by injection into the portal veins of adult rats, the plasmid DNA was carried into liver cell nuclei efficiently by nuclear protein. The expression of the DNA in adult rat liver, on introduction of the DNA with nuclear protein, was more than five times as great as with nonnuclear protein.

INTRODUCTION OF FOREIGN GENES into cultured cells by methods such as calcium-phosphate precipitation, electroporation, virus vector, and liposome fusion has provided new insights into the functions of many types of cells and macromolecules (*1*). These methods introduce genes into the cell cytoplasm; however, the genes cannot be expressed unless the DNA reaches the nucleus.

Recently, the migration of nuclear proteins into the nucleus has been investigated (*2, 3*), but the mechanism of transport of exogenous DNA into the nucleus remains to be elucidated (*4*). Sendai virus (HVJ) was used to fuse DNA-loaded ganglioside liposomes with red blood cell (RBC) membrane vesicles that contain proteins, so that DNA and proteins can be simultaneously introduced into the same cells efficiently. The procedure for constructing the vesicle complexes is shown in Fig. 1. HVJ-liposomes (*5*) containing a thymidine kinase (TK) gene were mixed with RBC membrane (*5, 6*) containing the fluorescein protein phycoerythrin (*7*) to form vesicle complexes. When these complexes were added to cultured mouse Ltk$^-$ cells, more than 95% of the cells showed red fluorescence in their cytoplasm and incorporated [^{3}H]thymidine into their nuclei (*8*).

This delivery system was then used to cointroduce pBR-SV40 DNA with either nonhistone chromosomal protein [high-mobility group–1 (HMG-1)] or bovine serum albumin (BSA) into Ltk$^-$ cells. Within 6 hours of treatment of cells with the vesicle

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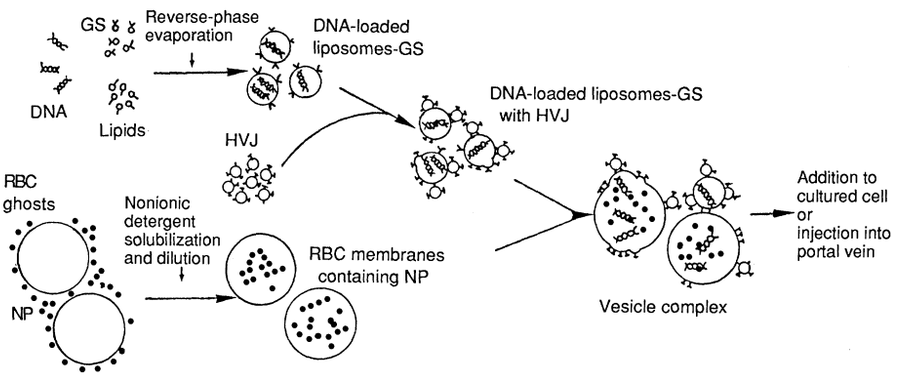

Fig. 1. The procedure for simultaneous introduction of plasmid DNA and nuclear proteins into cultured cells or adult rat liver. GS, gangliosides; NP, nuclear protein. The first step was the interaction of DNA-loaded liposomes with HVJ. Liposomes containing DNA and gangliosides were prepared by the reverse-phase evaporation method (*5, 17*), which traps about 30% of the plasmid DNA in liposomes (*5*). Proteins cannot be incorporated because of the use of organic solvents. About 50 μg of plasmid DNA (about 20 μg in the case of pTK4) was entrapped in 2 ml of liposome suspension (10 mg of lipids). Liposomes suspended in 2 ml of balanced salt solution were mixed with 2 ml of HVJ (about 15,000 HAU hemagglutination units per 0.5 ml) and 1 m*M* $CaCl_2$ and incubated at 4°C for 20 min and then at 37°C for 40 min. Next, 600 μl of packed volume of human RBC membranes (RBC ghosts) prepared as described (*5, 6*) was mixed with 300 μl of HMG-1 (10 mg/ml), BSA (Sigma) (30 mg/ml), rabbit IgG (CPL) (60 mg/ml), or IgG-Tpep (*14*) (8 mg/ml). Then RBC membrane vesicles containing proteins were prepared by the detergent solubilization and dilution method (*5, 6*). These vesicles (about 320 μl) were incubated with 4 ml of HVJ-liposomes at 4°C for 20 min and then at 37°C for 3.5 hours with shaking. Cultured cells (10^6 to 10^7 in suspension and 10^5 in monolayer) were incubated with a 2-ml suspension of the vesicle complexes (about 4×10^8) at 4°C for 10 min and then at 37°C for 60 to 120 min. For in vivo introduction of genes, 3 ml of the vesicle complexes (about 6×10^8) was injected into the portal vein of each Sprague-Dawley rat (6 to 8 weeks old).

complex containing DNA and HMG-1, the DNA was concentrated in the cell nuclei, as determined by in situ hybridization (Fig. 2A). This location was unchanged after 24 hours (Fig. 2D). In contrast, when BSA was used instead of HMG-1, the grains were mainly located in the cytoplasm after 6 hours (Fig. 2B) and were seen in both the cytoplasm and the nucleus after 24 hours (Fig. 2E). When the vesicle complex without HVJ was used, no specific grains were detected in the cells (Fig. 2, C and F).

When pAct-CAT was introduced with HMG-1, chloramphenicol acetyltransferase (CAT) activity (*9*) reached a maximum within 6 hours and remained at this level until at least 24 hours after introduction of the gene. Similar results were obtained when HMG-1 was replaced by the DNA binding proteins of extracts (*10*) of *Xenopus laevis* oocytes (*11*). When nonimmune immunoglobulin G (IgG) was transferred with pAct-CAT, the CAT activity was about 14% of that observed with HMG-1 at 6 hours and then gradually increased to almost the same level as that with HMG-1.

Ltk^- cells have a generation time of 16 to 18 hours as measured by counting cell number when grown in Dulbecco's modified Eagle's minimum essential medium supplemented with 100% fetal bovine serum at 37°C. TK activity in these cells was measured as incorporation of [^{3}H]thymidine into DNA (Fig. 3B). The incorporation reached a plateau within 8 hours when pTK4 DNA was cointroduced with HMG-1, but took more than 20 hours to reach a plateau when nonimmune IgG was used instead of HMG-1. Similar results were obtained when a mixture of nucleoplasmin (*10*) of frog nuclear protein and histones was used instead of HMG-1. When nonimmune IgG was used, the maximum expression was about 80% of that obtained with nuclear proteins.

These results suggest that when DNA is introduced into cells with nuclear proteins, it can reach the nuclei of interphase cells before mitosis. To confirm this in vivo and to determine whether this could be used for efficient expression of genes in adult liver, which consists of G_0 cells, we carried out the following experiments. First, a vesicle complex containing conjugates (*12*) of IgG and synthetic peptides corresponding to the nuclear location signal (*13*) of SV40 large T antigen (Tpep) was injected into the portal veins of adult rats (*14*). Immunohistochemical analysis showed that in this way IgG-Tpep was transported into about 10% of the nuclei of rat liver cells (mainly hepatocytes). Also, nuclear protein could migrate into the nuclei of nondividing cells without degradation.

Next, we injected vesicle complexes containing pBR-SV40 DNA and HMG-1 or BSA into the portal veins of adult rats. DNA from isolated nuclei of the livers of rats killed 3 days after injection was analyzed by electrophoresis. Injection of vesicle com-

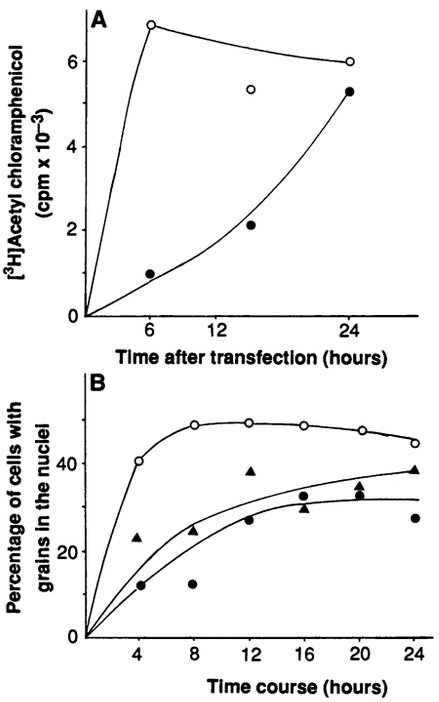

Fig. 3. Influence of HMG-1 on rapid expression of the CAT gene (A) and TK gene (B) introduced into mouse Ltk^- cells. (**A**) CAT activity was expressed as the radioactivity of [^{3}H]acetyl chloramphenicol formed from [^{3}H]acetyl-CoA (*9*). Ltk^- cells (10^7) were incubated in suspension with 2 ml of the vesicle complex containing about 25 μg of pAct-CAT, originally constructed by N. Davidson (*19*), and HMG-1 (○) or rabbit IgG (●) at 37°C for 1 hour and cultured for 6, 12, or 24 hours. Then the cells were collected, and the cell extract was prepared by repeated freezing and thawing of cells (*20*). Reaction mixtures [150 μl of cellular extract, 25 μl of 1*M* tris-HCl (*p*H 7.8), 50 μl of 5 m*M* chloramphenicol, and 5 μCi of [^{3}H]acetyl-CoA (Amersham)] were layered gently onto 0.5 ml of scintillation fluid (Econofluor, Du Pont), incubated at 37°C for 60 min and then counted in a liquid scintillation counter (*9*). Values for radioactivity were corrected for the background level (3824 cpm), which represents incorporation in the absence of pAct-CAT. (**B**) TK gene expression is shown as the number of cells containing grains (more than 30 per nucleus) in their nuclei as a percentage of the total cells (about 500 cells). About 10^5 Ltk^- cells cultured on a cover slip were incubated (37°C, 20 min) with 2 ml of the vesicle complex containing pTK4 (*5*) (about 10 μg) and HMG-1 (○), rabbit IgG (▲), or no protein (●). After removal of the mixture, the Ltk^- cells were labeled for 4 hours in culture medium containing 20 μCi of [*methyl*-^{3}H]thymidine (Amersham) per milliliter. Then the cells were fixed and dipped as described (*5*). After 48 hours, they were developed and stained with Giemsa. In both cases [(A) and (B)], experiments were performed three times (in duplicate) with almost identical results.

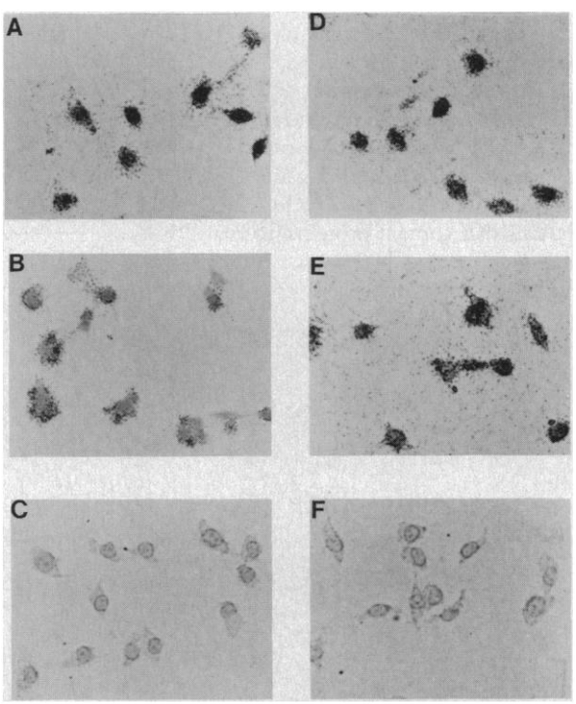

Fig. 2. In situ detection of plasmid DNA introduced into Ltk^- cells. The pBR-SV40 DNA was introduced into mouse Ltk^- cells by means of a vesicle complex containing HMG-1 (**A** and **D**) or BSA (**B** and **E**). L cells were cultured on a glass slide for 6 hours (A, B, and C) or 24 hours (D, E, and F), fixed, and hybridized with a ^{35}S-labeled probe. As a control, Ltk^- cells were treated with a vesicle complex lacking pBR-SV40 DNA (**C** and **F**). The pBR-SV40 was constructed by insertion of the whole SV40 genome (5.2 kb) into the Bam HI site of the pBR322 plasmid (4.4 kb). After incubation with 2 ml of vesicle complexes containing pBR-SV40 and HMG-1 or BSA, about 5×10^6 Ltk^- cells were placed on glass slides and cultured at 37°C in a CO_2 chamber. After 6 or 24 hours, the cells were fixed in cold methanol-acetic acid (3:1), dried, treated with 2× standard saline citrate (SSC) (*p*H 7.0) for 30 min at 70°C, and incubated with ribonuclease (RNase) A (100 μg/ml) at room temperature for 2 hours. They were then incubated in 0.1*M* triethanolamine (*p*H 8.0) containing 0.5% acetic acid, dehydrated in succesive ethanol washes, and air-dried. The dried cells were hybridized overnight at 42°C in hybridization buffer [4× SSC, 50% deionized formamide, and sonicated salmon sperm DNA (250 μg/ml)] containing 10% dextran sulfate (*18*) and a Pst I–Bam HI fragment (671 bp) of SV40 DNA labeled with [^{35}S]dCTP (Amersham). The cells were washed six times in 2× SSC at 37°C for 10 min each time. They were then exposed under Kodak NTB-3 emulsion for 1 to 6 days before development with D19 (Kodak) and fixation (Fuji).

plexes resulted in the appearance of DNA bands that hybridized to a DNA probe for T antigen (Fig. 4A). No bands were detected after injection of vesicle complexes that lacked either HVJ or DNA. On the basis of the density ratio of hybridized bands in each lane, DNA was carried into the nucleus from three to five times as efficiently by HMG-1 as by the nonnuclear protein BSA. The amount of DNA introduced into nuclei with HMG-1 remained unchanged for at least 7 days and then decreased rapidly between 7 and 12 days (*15*).

We next did Northern blot analysis of polyadenylated [poly(A)$^+$] RNA extracted from the livers of rats that had been injected with vesicle complexes containing pBR-SV40 and HMG-1 or BSA (Fig. 4B). The amount of poly(A)$^+$ RNA encoding SV40 large T antigen was 5.2 times as great when HMG-1 was the added protein as observed when BSA was used (Fig. 4B, lanes 3 and 4) (*16*).

Four days after injection, the synthesis of protein directed by introduced SV40 DNA was examined in rat liver sections by means of indirect radioimmunostaining with a monoclonal antibody to SV40 large T antigen (Fig. 4, C and D). About 10% of the cells contained labeled nuclei; more than half of these were identified as hepatocytes (Fig. 4C). When BSA was used as the co-introduced protein, 1 to 3% of total nuclei were immunoreactive, and the number of grains per nucleus was much smaller than that observed with HMG-1 (Fig. 4D).

These in vivo experiments indicate that nuclear proteins can facilitate the migration of foreign DNA into the nucleus and consequently enhance its transcription and translation in nondividing cells. DNA can be transported to the nuclei of rat liver cells in the absence of nuclear proteins (Fig. 4A, lanes 2 and 8, and Fig. 4B, lane 2), although the amount of DNA transported is less than that observed when the two were co-introduced. This suggests that intrinsic nuclear proteins newly synthesized in rat liver cells participate in the transport of the DNA into the nucleus. We speculate that the complex of DNA and nuclear proteins is more resistant to nucleases or has conformational properties that allow it to pass through nuclear pores more easily than DNA alone.

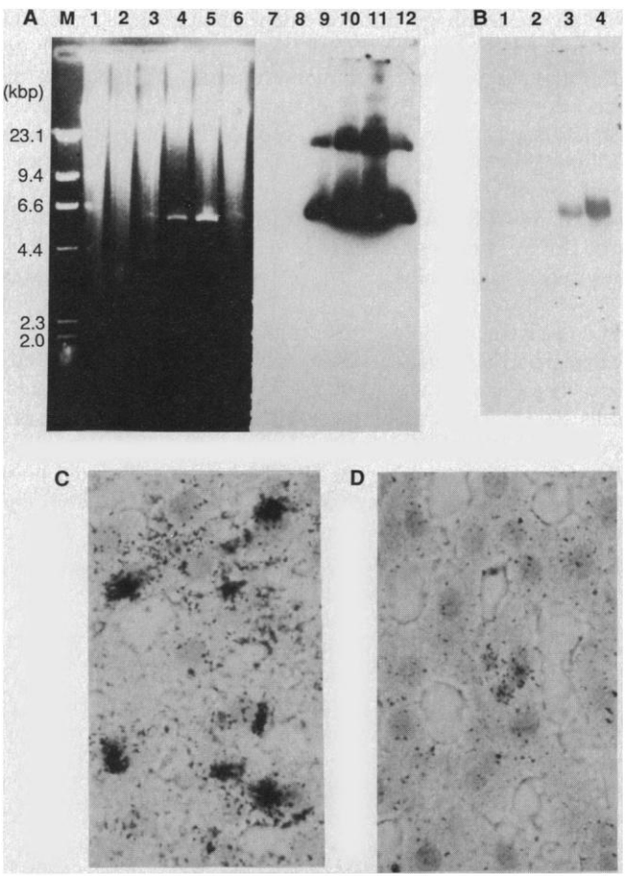

Fig. 4. Effects of HMG-1 on nuclear migration and expression of foreign DNA in adult rat liver. (**A**) Ethidium bromide staining (lanes 1 to 6) and Southern blot analysis (*21*) (lanes 7 to 12) of undigested DNAs (*22*) from rat liver nuclei (*23*). We separated 30 μg of undigested genomic DNA per lane by electrophoresis, stained the result with ethidium bromide, and transferred it to a nitrocellulose membrane, which was hybridized with ^{32}P-labeled SV40 large T probe (Pst I–Bam HI 671-bp fragment). Lanes 1 and 7: DNA from the untreated rat liver; lanes 2 and 8: DNA from the rat liver injected with the vesicle complex containing pBR-SV40 and HMG-1 without HVJ; lanes 3 and 9: DNA from the liver of a rat killed on day 3 after injection of the vesicle complex containing pBR-SV40 and BSA; lanes 4 to 6 and 10 to 12: DNA from the liver of a rat killed on day 3 (lanes 4 and 10), day 7 (lanes 5 and 11), and day 12 (lanes 6 and 12) after injection of the vesicle complex containing pBR-SV40 and HMG-1. The arrowheads indicate undigested closed circular forms of pBR-SV40. Lane M: Size marker. (**B**) Northern blot analysis of T antigen–specific poly(A)$^+$ RNA in rat liver. Total RNA was extracted from rat liver as described (*24*), and poly(A)$^+$ RNA was purified from the total RNA on an oligo(dT) column (*25*). Poly(A)$^+$ RNA (20 μg per lane) was hybridized with the same probe as for (A). Lane 1, poly(A)$^+$ RNA from untreated rat liver; lane 2, poly(A)$^+$ RNA from rat liver injected with the vesicle complex containing pBR-SV40 and HMG-1 without HVJ; lanes 3 and 4, poly(A)$^+$ RNA from the rat liver injected with the vesicle complex containing pBR-SV40 and BSA (lane 3) or HMG-1 (lane 4). The arrow indicates poly(A)$^+$ RNA of 2200 nucleotides of SV40 large T antigen. (**C** and **D**) Expression of SV40 large T antigen in adult rat liver on day 4 after injection of the vesicle complex. Rats were injected with pBR-SV40–loaded vesicle complexes containing HMG-1 (C) or BSA (D). T antigen in 6-μm-thick liver sections was detected by indirect immunostaining with a mouse monoclonal antibody to SV40 large T antigen (OSI) and ^{3}H-labeled rabbit antibody to mouse IgG (CPL). Nuclei were stained with hematoxylin after autoradiography.

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15. After 12 days, the amount of pBR-SV40 DNA was

about one-tenth as much as was detected 3 days after cointroduction with HMG-1. When the DNA was cointroduced with BSA, it was not detectable by Southern hybridization after 12 days.
16. To standardize the amount of poly(A)$^+$ RNA in each sample, the filter was hybridized with ^{32}P-labeled rat albumin cDNA probe. The density ratio of SV40 large T antigen poly(A)$^+$ RNA to rat albumin poly(A)$^+$ RNA was compared.
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Isolation and Expression of Functional High-Affinity Fc Receptor Complementary DNAs

JANET M. ALLEN AND BRIAN SEED

Human and murine mononuclear phagocytes express a high-affinity receptor for immunoglobulin G that plays a central role in macrophage antibody-dependent cellular cytotoxicity and clearance of immune complexes. The receptor (FcRI) may also be involved in CD4-independent infection of human macrophages by human immunodeficiency virus. This report describes the isolation of cDNA clones encoding the human FcRI by a ligand-mediated selection technique. Expression of the cDNAs in COS cells gave rise to immunoglobulin G binding of the expected affinity and subtype specificity. RNA blot analysis revealed expression of a 1.7-kilobase transcript in macrophages and in cells of the promonocytic cell line U937 induced with interferon-γ. The extracellular region of FcRI consists of three immunoglobulin-like domains, two of which share homology with low-affinity receptor domains.

THE MACROPHAGE-SPECIFIC RECEPTOR (FcRI) for the constant region (Fc) of immunoglobulin G (IgG) plays a central role in antibody-dependent cellular cytotoxicity and clearance of immune complexes (*1*). FcRI has been hypothesized to participate in antibody dependent infection of macrophages by dengue virus and human immunodeficiency virus (HIV) (*2*). Considerable recent success has attended the identification of cDNAs that encode low-affinity Fc receptors (*3, 4*). However, the cDNAs encoding the high-affinity receptor for IgG_1 have eluded detection. In this report, three independent cDNAs for FcRI were isolated by transient COS cell expression and affinity selection (*4, 5*). DNA sequence analysis revealed that the extracellular region comprised three immunoglobulin domains. The first two bear homology with the low-affinity Fc receptors, whereas the third domain is unique and may be responsible for the higher affinity of the receptor.

A cDNA library was constructed from polyadenylated [poly(A)$^+$] RNA obtained from cells of a single patient undergoing extracorporeal interleukin-2 induction therapy. The library was introduced into COS cells by DEAE dextran transfection. Cells expressing Fc receptors were recovered 48 hours later by incubating them with mouse Ig and panning on dishes coated with affinity-purified goat antibody to mouse Ig (Cappel). Episomal DNA was recovered from adherent cells by the Hirt procedure (*6*), amplified in *Escherichia coli*, and reintroduced into COS cells by spheroplast fusion. After four rounds, DNA was prepared from individual colonies and transfected into COS cells. Expression of Fc receptors was determined by indirect immunofluorescence after 48 hours. Three clones, p135, p90, and p98/X2 were selected for further study.

Indirect immunofluorescence showed that COS cells transfected separately with the three clones bound human IgG, human IgG Fc fragments, murine IgG2a, and the FcRI-specific monoclonal antibodies (MAb), 32.2 (*7*) and 22.2 (*8*). Fluorescein-conjugated goat antibodies, human IgA, IgM, IgD, and $F(ab')_2$ fragments, as well as murine IgG_1, IgG_{2b}, IgM, and the FcRII-specific MAb, IV.3 (*9*) did not react. COS cells expressing one of the cDNA clones formed rosettes with bovine erythrocytes coated with rabbit IgG, but not with IgM (*10*).

IgG-agarose adsorption (*4*) of surface-labeled COS cells transfected with p135 and p90 showed a single species of relative molecular size 70 kD, whereas similar treatment of cells transfected with p98/X2 gave a band of 67 kD (Fig. 1). A slightly larger protein of 75 kD was adsorbed from untreated and interferon-γ (IFN-γ)–treated U937 cells. The smaller mass observed in COS cells is consistent with the reduced masses observed for other surface antigens expressed in COS cells (*5*).

Scatchard analysis (*11*) of the binding of radiolabeled IgG_1 to COS cells transfected with each of the three clones revealed 5×10^5 to 10×10^5 binding sites with similar association constants of $\approx 5 \times 10^7 M^{-1}$ (Fig. 2A). The specificity of the three clones, measured by displacement titrations of labeled IgG_1, showed the same rank order for human and murine subtypes in all three clones. In agreement with in vivo data (*1*), human IgG_1 and IgG_3 showed equipotent displacement of radiolabeled IgG_1, whereas human IgG_2 and IgG_4 were less effective (Fig. 2B). Among the murine IgG subtypes, the highest affinity was displayed by IgG_{2a} (Fig. 2C), consistent with previous studies. Murine IgG_3 also displaced binding of radiolabeled human IgG_1 to a lesser extent, whereas murine IgG_{2b} and IgG_1 were ineffectual (Fig. 2C).

RNA blot analysis (Fig. 3) with the p135 probe showed a single 1.7-kb transcript in RNA prepared from monocytes, placenta [a rich source of macrophage transcripts (*4*)], and the cells from which the library was generated. No transcripts were observed from RNA derived from similar cell preparations from two other patients. In the

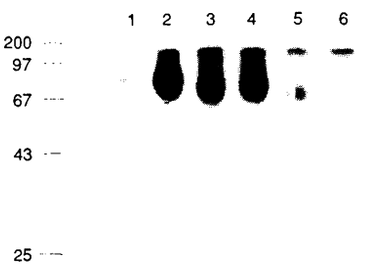

Fig. 1. IgG-agarose adsorption of surface ^{125}I-labeled cells. (Lane 1) U937 cells; (lane 2) U937 cells stimulated for 48 hours with recombinant IFN-γ (10 ng/ml) (Hoechst); (lanes 3 to 6) COS cells transfected with: p135 (lane 3), p90 (lane 4), p98/X2 (lane 5), and vector (lane 6). Cells were labeled by the lactoperoxidase method, lysed, and adsorbed to human IgG-agarose (Sigma) as described elsewhere (*4*). Markers in kilodaltons.

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