CELL SURFACE EXPRESSION OF MURINE, RAT, AND HUMAN Fc RECEPTORS BY XENOPUS OOCYTES

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Xenopus laevis oocytes provide an efficient translation system for microinjected, heterologous mRNAs. They offer several advantages over conventional in vitro translation systems since oocytes can perform posttranslational processes such as proteolytic cleavage, glycosylation, sequestration, and secretion of products (1–3). Translation products have been detected in whole oocyte extracts and secretory proteins have been found in the culture medium of the oocytes (4–7). The acetylcholine receptor, an integral membrane protein, was found associated with a membrane fraction of oocyte extracts (4). Oocyte translation products have been detected by their biologic activity (6), enzymatic activity (8), and by biosynthetic labeling with radioactive precursors followed by immunoprecipitation (4, 7). We are interested in the expression of Fc receptors and their genetic organization. Fc receptors (FcR) are a group of plasma membrane proteins that bind the Fc domain of immunoglobulin (Ig). These receptors are expressed by many cells of the immune system (9), are in many instances inducible (10), and differ in their specificity for different isotypes of Ig. We report here that translated mammalian plasma membrane receptors can be detected by binding of labeled ligands or monoclonal anti-receptor antibodies on the oocyte plasma membrane. This assay affords a sensitive and rapid method for the detection of rare mRNA species that code for plasma membrane proteins.

Materials and Methods

Cell Lines. The murine macrophage-like cell line J774 and the T cell lymphoma L5178Y were grown in Dulbecco's medium containing 5% fetal bovine serum (FBS); the rat basophilic cell line RBL-1 was cultured in alpha-modified minimum essential medium and 10% FBS; the human promonocytic cell line U937 was grown in RPMI 1640 with 10% FBS. All lines were grown in spinner culture in media supplemented with 2 mM glutamine, 5 µg/ml gentamycin, 100 U/ml penicillin, and 10 µg/ml streptomycin.

Immunoglobulins. The IgG of 2E2A and the Fab fragment of the monoclonal anti-Fc,2/3,2R IgG, 2.4G2, were prepared as described (11, 12). Rat IgE from the IR-162 myeloma was kindly provided by Dr. H. Metzger (National Institutes of Health) and the mouse IgE was prepared as described (13). Mouse IgG2a was purified from the ascites fluid of mice bearing the LPC-1 myeloma by ammonium sulfate precipitation and DE-52 ion exchange chromatography. The F(ab')2 fragment of mouse IgG2a was prepared by pepsin digestion and isolated by high performance liquid chromatography gel filtration. Proteins were iodinated using iodogen (Pierce Chemical Co., Rockford, IL) (12).

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Isolation of mRNA. RNA was isolated by extraction with guanidine thiocyanate (14) from cells grown in spinner culture. Poly(A)^+ mRNA was selected by affinity chromatography on oligo-dT cellulose.

Xenopus Oocyte Assays. Isolated oocytes (15) were incubated for 2 h at room temperature with gentle agitation in Ca^{2+}-free OR-2 saline containing 2 mg/ml collagenase (Type 1A; Sigma Chemical Co., St. Louis, MO) and were then rinsed with half-strength L-15 medium (Gibco Laboratories, Grand Island, NY) supplemented with 100 U/ml of penicillin, 5 μg/ml of gentamycin, and 10 mM Hepes, pH 7.4. The oocytes were injected with 60 nl of mRNA (usually 1 mg/ml) in water and incubated for 2 d in half strength L-15.

For binding assays, the oocytes were incubated for 1 h at room temperature in medium containing ¹²⁵I-ligand (1 μg/ml, 10^6 cpm/ng protein) and [¹⁴C]sucrose (0.67 mM sucrose, 3.7 mCi/m mole; New England Nuclear, Boston, MA). After washing, oocytes showing intact animal/vegetal pole morphology were individually assayed for ¹²⁵I in a gamma counter, and the oocytes were then prepared for scintillation counting by the addition of 0.1 ml of 0.5 M NH₄OH and 2 ml of Hydroflour (New England Nuclear). The high end of the ¹⁴C spectrum was assayed, and the results corrected for 1% spillover of ¹²⁵I into the ¹⁴C channel. Oocytes that took up more [¹⁴C]sucrose than the mean plus 3 SD incorporated by control oocytes were considered nonviable, and were disregarded. Binding of ligand was scored as positive if the amount of ¹²⁵I-ligand bound was >3 SD above the mean bound by controls. The stimulation index was calculated as: experimental cpm bound/average control cpm bound.

Results and Discussion

Expression of Murine Fcγ2b/γ1R on Oocytes. The J774 mouse macrophage cell line expresses a receptor that binds mouse IgG2b and IgG1 aggregates (Fcγ2b/γ1R) (11). Poly(A)^+ mRNA isolated from the Fcγ2b/γ1R-positive J774 and Fcγ2b/γ1R-negative L5178Y cell lines was injected into Xenopus oocytes (Fig. 1) and 2 d later the binding to oocytes of ¹²⁵I-2.4G2 Fab, a monoclonal antibody that binds to Fcγ2b/γ1R (11), was measured. To control for nonviable oocytes, which trap ligand nonspecifically, [¹⁴C]sucrose, an impermeant tracer, was added to the incubation mixture with the ¹²⁵I-ligand. Only oocytes that were injected with

![Figure 1. Expression of Fcγ2b/γ1R by oocytes injected with J774 mRNA. The oocytes were injected with 60 nl of poly(A)^+ mRNA (1 mg/ml, dissolved in water) isolated from J774 (●) or L5178Y cells (○), incubated for 2 d, and assayed as described in Materials and Methods.](image_url)
mRNA from the Fcγb/h1R-positive cell line bound $^{125}$I-2.4G2 Fab specifically (Fig. 1). Those oocytes injected with mRNA from L5178Y (an FcR-negative T cell line) that bound $^{125}$I-2.4G2 Fab also took up [¹⁴C]sucrose, demonstrating that these oocytes were nonviable and that uptake of ligand was thus nonspecific. Binding of $^{125}$I-2.4G2 Fab was detected after the injection of as little as 120 pg of mRNA per oocyte and was dependent on the amount of mRNA injected (Fig. 2A). The percentage of oocytes binding $^{125}$I-2.4G2 Fab over background levels reached a plateau at 70% with 6 ng of injected mRNA (data not shown), and the amount of receptor per oocyte generally began to plateau at 30–60 ng of mRNA (Fig. 2A). Receptor expression was detectable 24 h after injection and increased until day 3 (Fig. 2B). The binding of $^{125}$I-2.4G2 Fab was inhibited by the presence of an excess of unlabeled 2.4G2 Fab (data not shown).

We investigated whether radiolabeled monoclonal antibodies specific for J774 cell surface proteins other than FcR were bound by oocytes injected with J774 mRNA. $^{125}$I-2E2A, a monoclonal IgG directed against a major 82,000 Mr plasma membrane protein (12) found on J774 cells (6 × 10⁵ sites per cell), binds to oocytes injected with J774 mRNA. The percentage of oocytes binding $^{125}$I-2.4G2 Fab and $^{125}$I-2E2A over background levels was 80 and 65%, respectively. The average stimulation index was 5.4 ± 3.2 for $^{125}$I-2E2A Fab and 20 ± 11 for $^{125}$I-2.4G2 Fab. These data indicate that binding of monoclonal antibodies to oocyte plasma membranes is likely to be a general method for the study of cell surface components.

Expression of Functional Rat FcR on Oocytes. We next investigated the applicability of the oocyte system to translation of other Fc receptors for which we had no monoclonal antibody probe. The RBL-1 rat basophilic leukemia line (2H3 subline) expresses a high avidity receptor for IgE (FcR) (16). Individual oocytes injected with RBL-1 mRNA bound as much as 22,000 cpm of $^{125}$I-rat IgE, compared with an average of 215 ± 36 cpm bound to water-injected oocytes (Fig. 3A and B). Comparable data were obtained with a mouse IgE hybridoma (13) (data not shown), indicating that the FcR bound the IgE by the Fc domain and not the antigen-combining site. Furthermore, as might be predicted by the absence of high avidity FcR on J774 cells, $^{125}$I-rat IgE did not
bind to J774 mRNA–injected oocytes (data not shown). It is apparent that the FcR on the oocyte plasma membrane was in a conformation that fulfilled the requirements for ligand binding.

Expression of Functional Human Fc,Rh on Oocytes. The human monocyte-like cell line U937 has a high affinity receptor for IgG (Fc,Rh), the expression of which is amplified by the addition of γ-interferon (γ-IFN) (17) (Table I). We prepared mRNA from untreated and γ-IFN-treated U937 cells for injection into oocytes. The oocytes were able to express functional human Fc,Rh, as demonstrated by binding of 125I-IgG2a to oocytes injected with mRNA from γ-IFN-treated U937 cells (Table I and Fig. 3 C). Remarkably, the density per μm² of Fc,Rh is comparable on the γ-IFN-induced U937 cells and the oocytes injected with mRNA isolated from these cells (Table I). The binding of 1 μg/ml of 125I-IgG2a was inhibited 60% by 2 μg/ml of unlabeled IgG2a, but not by the F(ab')2 fragment (Fig. 3, D and E). However, 10-fold less binding of 125I-IgG2a was observed on oocytes injected with mRNA from untreated U937 cells compared with γ-IFN-treated U937 cells (Table I), which suggests that the treatment of the U937 cells with γ-IFN results in induction of new mRNA coding for Fc,Rh.

The results presented here demonstrate that oocytes efficiently synthesize heterologous plasma membrane proteins and are capable of expressing them in a functional conformation on the plasma membrane. Similar results have recently been reported by Gunderson et al. (18), who showed that injection of mRNA from human brain into oocytes induced the appearance of receptors and channels
### Table 1

**Effect of γ-IFN on the Expression of Fc,Rh~**

|          | U937 cells | Oocytes injected with mRNA from U937 cells |
|----------|------------|------------------------------------------|
|          | Untreated  | γ-IFN-induced | Untreated | γ-IFN-induced |
| Fc,Rh~ per cell* | 8 x 10⁴ | 7 x 10⁴ | 5 x 10⁴ | 6 x 10⁴ |
| Fc,Rh~ per µm² cell surface† | 8 | 69 | 9.7 | 113 |

U937 cells grown in spinner (3-4 x 10⁵ cells/ml) were treated with 100 U/ml of recombinant γ-IFN. Cells were assayed or mRNA prepared after 18 h. Binding studies on U937 cells were performed at 25°C as described (17) using iodinated IgG2a mouse myeloma protein LPC-1 (1 µg/ml; 8 x 10⁵ cpm/ng). Oocytes injected with mRNA were also assayed for binding of ¹²⁵I-LPC-1. In reticulocyte lysates, both mRNA preparations catalyzed the synthesis of high M, proteins, indicating that the mRNA preparations were of good quality.

* Data were corrected by subtraction of binding to the FcR-negative cell line MOLT-4 or L5178Y and uninjected oocytes.
† The approximate surface areas of the cells were calculated from their diameters.

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in the oocyte membrane. Many monoclonal antibodies (including 2.4G2 IgG) do not immunoprecipitate reticulocyte lysate translation products. Since the FcγR/γ1R message is rare, it is difficult to immunoprecipitate FcγR/γ1R from [³⁵S]-methionine-labeled oocyte lysates. The screen for ligand or monoclonal antibody binding to the oocyte surface is a rapid and sensitive assay for minor mRNA species, and the method promises to be of great utility for the molecular analysis of membrane receptors by both their ligand binding and antigenic properties.

**Summary**

We report that *Xenopus laevis* oocytes can efficiently translate and insert heterologous membrane receptors into the oocyte plasma membrane, where they can be detected by the binding of either monoclonal antibodies or ligands. Thus, oocytes injected with mRNA from the mouse J774 macrophage-like cell line, the rat RBL-1 basophilic leukemia, and the U937 promonocyte cell line, bound 2.4G2 Fab, rat IgE, and mouse IgG2a, respectively. The increase in the high avidity Fc,R observed after γ-interferon induction of U937 cells was also observed after injection of mRNA from γ-interferon-induced U937 cells into oocytes. This suggests either much greater message stability or a greater rate of transcription of Fc,Rh mRNA in the γ-interferon-induced cells. The assay affords a sensitive method for the detection of rare mRNA species that code for plasma membrane proteins.

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