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Immunology

Biochemical characterization of murine glycosylation-inhibiting factor

(suppressor T-cell factor/lipocortin/IgE-binding factors)

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ABSTRACT **The glycosylation-inhibiting factor (GIF) was isolated from serum-free culture supernatants of the murine T-cell hybridoma, 231F1 cells, by using an immunosorbent coupled with the monoclonal anti-lipomodulin antibody. The isolated lymphokine is a 14-kDa protein with a pI of 5.5, as determined by SDS/PAGE and two-dimensional gel electrophoresis. Fractionation of a mixture of radiolabeled GIF with culture supernatant of the 231F1 cells on ion-exchange and reverse-phase columns and by gel filtration demonstrated homogeneity of the 14-kDa GIF and confirmed that the bioactivity of GIF and the antigenic determinant recognized by the monoclonal anti-GIF antibody are associated with the 14-kDa protein. The ^{125}I-labeled 14-kDa protein binds to the murine T-cell hybridoma 12H5 cells, which have been used for bioassay of GIF, and the murine B-cell line A20.3 cells, but the binding of the protein to resting murine splenic lymphocytes was barely detectable. Under the same experimental conditions, binding of the ^{125}I-labeled recombinant human lipocortin I to the 12H5 cells was not detectable. In contrast, the ^{125}I-labeled lipocortin, but not the 14-kDa GIF, bound to phosphatidylserine vesicles. The results indicate that GIF does not belong to the anexin family.**

In our previous experiments on isotype-specific regulation of the IgE antibody response, we described T-cell factors that either enhance or suppress IgE synthesis in an isotype-specific manner (1). Both IgE-potentiating factors and IgE-suppressive factors have affinity for IgE. The two types of IgE-binding factors (IgE-BF) share a common structural gene but are different in carbohydrate moieties in the molecules (2). It was found that the carbohydrate moieties and biologic activities of IgE-BF are controlled by two T-cell factors that either enhance or inhibit the posttranslational glycosylation process of IgE-binding peptide during their biosynthesis (1). The glycosylation-inhibiting factor (GIF) inhibits N-glycosylation of IgE-BF and renders the latter factors to selectively suppress IgE synthesis. GIF exerted phospholipase inhibitory activity after treatment with alkaline phosphatase and bound to monoclonal antibodies against rat lipomodulin or rabbit lipomodulin (3, 4). Subsequent experiments showed that the ovalbumin (Ova)-specific suppressor T-cell (Ts) hybridoma 231F1 cells constitutively secrete the 14-kDa GIF having no affinity for Ova. However, the same cells produce the 80-kDa GIF molecules having affinity for Ova and IgE-suppressive factors upon stimulation with Ova-pulsed syngeneic macrophages (5). The Ova-binding GIF appears to consist of an antigen-binding polypeptide chain and a non-specific GIF chain (6) and can suppress the *in vivo* antibody response of syngeneic mice in a carrier-specific manner (5). Thus, Ova-binding GIF is similar to the antigen-specific suppressor factors (TsF) described by other investigators (7–9). The hypothesis is supported by the fact that representative TsF from hapten-specific Ts hybridomas bind to monoclonal anti-lipomodulin antibody and share various antigenic determinants with antigen-binding GIF (10).

We expected that biochemical characterization of GIF may provide a clue to solve some controversial issues on antigen-specific TsF. In this report, we describe the purification of GIF to homogeneity and biochemical properties of the lymphokine. The results also show that purified GIF has high affinity for its target cells and suggest that the lymphokine may not belong to the lipocortin family.

MATERIALS AND METHODS

Cell Lines and Cell Cultures. The murine T-cell hybridoma 231F1 cells (11) and 12H5 cells (12) have been described. They were maintained in high glucose Dulbecco's modified Eagle's medium (DMEM) (11). The 231F1 cells were propagated to 10^6 cells per ml in DMEM containing 10% Nu-Serum (Collaborative Research). The cells were washed twice with and resuspended in serum-free DMEM at a concentration of 1.5×10^6 cells per ml and were cultured for 48 hr to recover culture supernatants. The murine B-lymphoblastoid cell line A20.3 cells were obtained from the American Type Culture Collection. The GIF-producing human T-cell hybridoma CL3 has been described (13). Both the A20.3 cells and CL3 cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (GIBCO). Normal mouse spleen cells were obtained from BALB/c mice (The Jackson Laboratory) and human peripheral blood mononuclear cells were obtained from a normal individual. The cell suspensions were passed through a Sephadex G10 column (Pharmacia) to remove adherent cells.

Antibodies and Immunosorbents. The monoclonal anti-rabbit lipomodulin antibody 141B9 (4) was enriched from ascitic fluid by ammonium sulfate precipitation followed by gel filtration. The preparation contained not only IgG1 antibody but also free light chains and other serum proteins. For further purification, buffer for the protein fraction was replaced by 1.5 M glycine/3 M NaCl, pH 8.9, and the fraction was applied to a protein A-Sepharose column. After washing, IgG1 was eluted with 0.1 M citrate buffer (pH 6.0). Analysis of the fraction by SDS/PAGE showed that essentially all IgG1 was recovered in the eluate, which did not contain free light chain. The anti-murine GIF rat monoclonal antibody 32B10 has been described (14). Since the antibody is rat IgA dimer, it was enriched by ammonium sulfate precipitation followed by gel filtration. The fractions containing rat IgA were pooled and concentrated. The proteins were then biotinylated by the method of Bayer and Wilcheck (15). The monoclonal antibodies and human IgG were coupled to

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Abbreviations: GIF, glycosylation-inhibiting factor; IgE-BF, IgE-binding factor; Ova, ovalbumin; TsF, antigen-specific suppressor factor; BSA, bovine serum albumin; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine.

Affi-Gel-10 (Bio-Rad). Bovine serum albumin (BSA; Sigma) was coupled to Affi-Gel-15 (Bio-Rad). In all immunosorbents, 10 mg of protein in 0.1 M Mops (pH 7.5) (Sigma) was coupled to 1 ml of beads.

Detection of GIF. GIF was detected by its ability to switch the mouse T-cell hybridoma 12H5 cells from the formation of glycosylated IgE-BF to the formation of unglycosylated IgE-BF (16). Briefly, the 12H5 cells were cultured for 24 hr with a sample to be tested in the presence of mouse IgE. Culture supernatants were filtered through CF50A membranes (Amicon), and filtrates containing IgE-BF were fractionated on lentil lectin Sepharose (Pharmacia). Both the effluent and eluates with α-methylmannoside were assessed for the presence of IgE-BF by inhibition of rosette formation between $Fc_\varepsilon R^+$ lymphocytes and IgE-coated erythrocytes. The proportion of rosette-forming cells in 300 lymphocytes was determined in triplicate. When the 12H5 cells were cultured with IgE alone, essentially all IgE-BF bound to lentil lectin Sepharose and were recovered by elution with α-methyl mannoside (12). If a sufficient amount of GIF was added to the culture of the 12H5 cells, a majority of IgE-BF formed by the cells did not retain in the column and were recovered in the effluent fraction (16). The presence of GIF in a test sample was concluded when the percentage rosette inhibition by the effluent fraction was more than twice that obtained by the eluate fraction.

GIF was also detected by ELISA with an amplification system (17). Each well of a Nunc F plate (Max Sorp, Nunc) was coated with a 50-μl sample for 2 hr at 37°C. Plates were washed five times with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (Sigma) between each of the following steps except the step prior to substrate. The plates were blocked with 3% BSA in Tween/PBS overnight at 4°C. One hundred microliters of PBS containing 200 ng of biotinylated anti-GIF monoclonal antibody per ml was then added to each well. After 2 hr of incubation at 37°C, followed by washing, 100 μl of an appropriate dilution (1:2000) of avidin-conjugated alkaline phosphatase (Calbiochem) was added to each well, and the plate was incubated for 2 hr at 37°C. An ELISA signal was developed by 50 μl of alkaline phosphatase substrate followed by amplifier solution (GIBCO/BRL). Absorbance at 490 nm was determined in an ELISA reader MR 5000 (Dynatech).

Chromatography. All column chromatography was carried out by using a high-performance liquid chromatography (HPLC) system (System Gold, Beckman). For ion-exchange chromatography, a 1-ml sample in 10 mM Tris·HCl (pH 8.1) was applied to a TSK-3SW column (Toyo Soda, Tokyo), and proteins were eluted by a linear gradient of NaCl from 0 to 450 mM in 10 mM Tris·HCl (pH 8.1) at a flow rate of 0.5 ml/min. Every 1 ml, fractions were concentrated to 0.1 ml by using Centricon 10 (Amicon). Reverse-phase chromatography was performed by using an ultrasphere ODS (C_{18}) column (Beckman). The mobile phase is 0.1% trifluoroacetic acid (Sigma). Proteins were eluted with a linear gradient of 2-propanol (Aldrich) from 0% to 60% in 45 min at a flow rate of 1 ml/min. Every 1.0 ml, fractions were lyophilized in a Speed-Vac (Savant) and reconstituted in 50 μl of H_2O. Gel filtration was carried out by using a Superose 12 column (Pharmacia). Proteins were eluted with PBS at a flow rate of 1 ml/min and, every 1 ml, fractions were concentrated by using Centricon 10. The column was calibrated with IgE (185 kDa), BSA (67 kDa), Ova (43 kDa), soybean trypsin inhibitor (20.1 kDa), and cytochrome *c* (12 kDa).

Electrophoresis and Silver Staining. Affinity-purified GIF preparations were analyzed by SDS/PAGE in 15% polyacrylamide slab gels by using the Laemmli system (18). Unless otherwise stated, analysis was carried out under reduced conditions using low molecular weight prestained markers (Bio-Rad). Two-dimensional electrophoresis (19) was carried out, using ampholines of pH 3–10 and marker proteins from Bio-Rad. The second dimension SDS/PAGE was carried out in 15% polyacrylamide slab gel, using a low molecular weight electrophoresis calibration kit (Pharmacia). Proteins in the gel were visualized by silver staining (20).

Estimation of Protein Content and Radiolabeling. Affinity-purified GIF was mixed with a 1/10th vol of 100% (wt/vol) trichloroacetic acid (Sigma). The mixture was kept at −20°C for 15 min and centrifuged at 15,000 × g for 5 min. Pellets were rinsed twice with cold (−20°C) acetone and dissolved in 50 μl of 50 mM phosphate buffer (pH 7.5). To estimate protein content of the sample, an aliquot was analyzed by SDS/PAGE along with serial dilutions of BSA. Protein concentration of GIF was estimated by the intensity of the 14-kDa band in silver staining, in comparison with BSA bands. Approximately 100 ng of purified GIF was labeled with 0.1 mCi of ^{125}I (1 Ci = 37 GBq) using chloramine T (0.5 mg/ml). The specific activity of radiolabeled GIF was ≈10,000 dpm/ng. Recombinant human lipocortin I, which was kindly provided by J. Browning and R. B. Pepinsky (Biogen), was labeled with ^{125}I by the same procedures. The specific activity of the preparation was 74,000 dpm/ng.

Binding Assays. Binding of GIF or lipocortin to lymphocytes was determined by using ^{125}I-labeled protein. Lymphocytes or hybridoma cells were suspended in Dulbecco's PBS at a concentration of 1×10^7 cells per ml. One hundred-microliter aliquots of the cell suspension and 100 μl of Dulbecco's PBS containing an appropriate amount of ^{125}I-labeled protein were mixed in an Eppendorf tube in triplicate. A 500-fold excess of unlabeled protein was present in the mixtures in control tubes. After incubation for 20 min at 37°C, the mixtures were layered on 20% olive oil/di-*n*-butyl phthalate (Sigma) and centrifuged at 4000 × *g* for 2 min. After the water phase was removed, the tips of the tubes were cut off to determine cell-associated radioactivity.

We have also measured the binding of ^{125}I-labeled proteins to phospholipids. Phophatidylserine (PS), phosphatidylinositol (PI), and phosphatidylcholine (PC) were sonicated in 10 mM Tris·HCl/50 mM NaCl for 3 min to prepare vesicles (21). ^{125}I-labeled protein was mixed with a 200-μl suspension of phospholipid vesicles containing 2 μg of phospholipid in 10 mM imidazole buffer containing 40 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, and 1 mg of BSA per ml. A 500-fold excess of unlabeled protein was added to control mixtures. After 10 min at room temperature, the mixtures were centrifuged for 15 min at 100,000 × *g*. Vesicles were washed twice with the same buffer, and radioactivity associated with the vesicles was determined.

RESULTS

Isolation of GIF to Biochemical Homogeneity. The 231F1 cells were cultured in serum-free DMEM for 48 hr to recover supernatants. In this culture medium, proliferation of the cells was limited; however, viability was well maintained. Ten-liter culture supernatants were concentrated 1000-fold. The concentrated supernatant was preabsorbed with BSA-coupled Affi-Gel and human IgG-coupled Affi-Gel and then mixed with 141 B9-coupled Affi-Gel for 6 hr at 4°C. The immunosorbent was washed extensively with 10 mM phosphate buffer (pH 7.5) containing 50 mM NaCl, followed by the same buffer containing 500 mM NaCl. Proteins retained in the immunosorbent were then eluted with 0.1 M citric acid/NaOH, pH 3.0. The effluent, washing, and acid eluate fractions were dialyzed against RPMI 1640 medium and assessed for GIF activity by using the 12H5 cells. As expected, GIF activity was detected only in the acid eluate fraction. The remainder of the fraction was dialyzed against distilled water and lyophilized. The pellet was dissolved in 100 μl of distilled water, and 20 μl of the sample was analyzed

by SDS/PAGE. As shown in Fig. 1, the acid eluates from the 141B9-Affi-Gel gave a single band of 14 kDa. An aliquot of the sample applied to SDS/PAGE was diluted with RPMI 1640 medium and assessed for GIF activity. It was found that a 1:1000 dilution of the sample could switch the 12H5 cells from formation of glycosylated IgE-BF to formation of unglycosylated IgE-BF.

In a separate experiment, GIF was purified by affinity chromatography on the 141B9-Affi-Gel. Proteins in the acid eluates were precipitated by 10% trichloroacetic acid and analyzed by two-dimensional electrophoresis. As shown in Fig. 2, a single spot of pI ≈5.5 and with a size of 14 kDa was demonstrated. The results indicate that the acid eluate from the 141B9-Affi-Gel contains only a single polypeptide in charge and in size.

Experiments were carried out to determine whether the 14-kDa protein represents the major species of GIF in the original culture supernatant. An aliquot of the purified GIF shown in Fig. 1 was radiolabeled with ^{125}I. Since the minimum concentration of this preparation for the detection of GIF bioactivity was 2 ng/ml, 1 ng of the radiolabeled protein was added to a 1000× concentrated culture supernatant and the mixture was analyzed by HPLC. As expected, the comigration of radioactivity and GIF bioactivity in ion-exchange column chromatography was demonstrated. The GIF activity was detected only in the fraction with the highest radioactivity (see Fig. 3 legend). Distribution of GIF in the chromatographic fractions was confirmed by ELISA. As shown in Fig. 3, only the fractions containing the radiolabeled protein bound biotinylated anti-GIF for significant ELISA signals.

A mixture of radiolabeled 14-kDa protein and the concentrated culture supernatant was fractionated on a reverse-phase column, and fractions were assessed for radioactivity and for the presence of the protein recognized by anti-GIF antibody. As shown in Fig. 4, comigration of the 14-kDa ^{125}I-labeled protein and GIF antigen was observed. Fractionation of the mixture by gel filtration through a Superose 12 column showed that both the radiolabeled protein and the GIF antigen were recovered in the same fraction containing proteins of 12–18 kDa (results not shown). The results

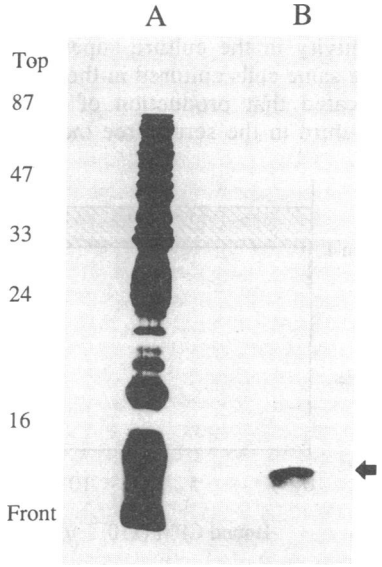

FIG. 1. Analysis of affinity-purified GIF in SDS/PAGE. The effluent (lane A) and acid eluate fraction (lane B) from the 141B9-Affi-Gel were lyophilized and analyzed under reduced conditions. Molecular mass markers: BSA, 87 kDa; Ova, 47 kDa; carbonic anhydrase, 33 kDa; soybean trypsin inhibitor, 24 kDa; lysozome, 16 kDa.

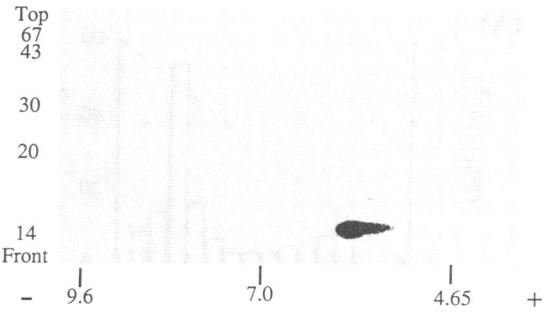

FIG. 2. Two-dimensional electrophoresis of affinity-purified GIF. The first dimension was separated in the pH gradient between 3 and 10 by the nonequilibrium pH gradient gel electrophoresis method. PI markers: phycocyanin, 4.65; equine myoglobin, 7.0; cytochome *c*, 9.6. The second dimension was conventional SDS/PAGE with the following molecular mass markers: BSA, 67 kDa; Ova, 43 kDa; carbonic anhydrase, 30 kDa; soybean trypsin inhibitor, 20.1 kDa; RNase A, 14.4 kDa.

collectively indicate that both the GIF bioactivity and the antigenic determinant recognized by anti-GIF antibody are associated with the 14-kDa protein.

Binding of Purified GIF to Lymphocytes. Since the bioactivity of the purified GIF could be detected by using 12H5 cells, attempts were made to detect the binding of ^{125}I-labeled GIF (^{125}I-GIF) to the cells. Approximately 100 ng of affinity-purified GIF, which gave a single 14-kDa band in SDS/PAGE, was labeled with ^{125}I. As a preliminary experiment, 0.1 ml-aliquots of a suspension of 12H5 cells (containing 2 × 10^6 cells) were incubated for 20 min with a serial dilution of ^{125}I-GIF, in the presence or absence of a 500-fold excess of

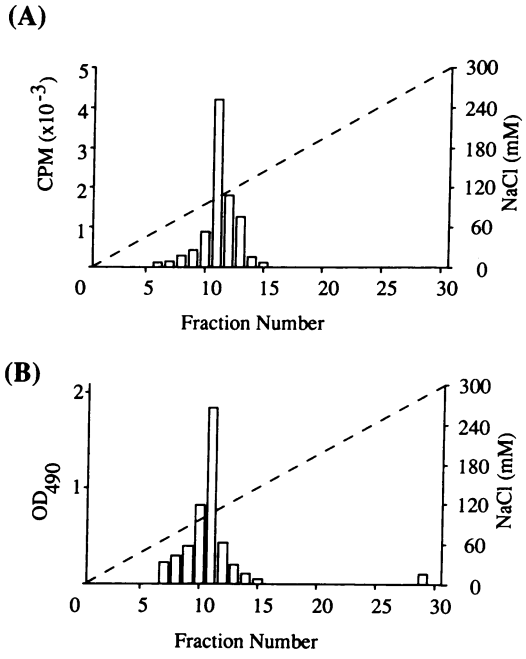

FIG. 3. Comigration of GIF activity and GIF antigen with the 14-kDa protein in ion-exchange chromatography. A mixture of ^{125}I-labeled 14-kDa protein (10,000 cpm) and crude GIF was applied to a TSK-3SW column and analyzed by HPLC with a linear gradient of NaCl (---) in 10 mM Tris·HCl (pH 8.1). Distributions of radioactivity (*A*) and GIF antigen determined by ELISA (*B*) are shown. Fractions were also assessed for GIF bioactivity by using the 12H5 cells. Distributions of IgE-BF between the effluent and eluate fractions from lentil lectin Sepharose were 22/19 for fraction 10, 27/8 for fraction 11, 32/33 for fraction 12, and 0/24 for the control. The results of the bioassay indicated that the peak fraction of GIF bioactivity was fraction 11.

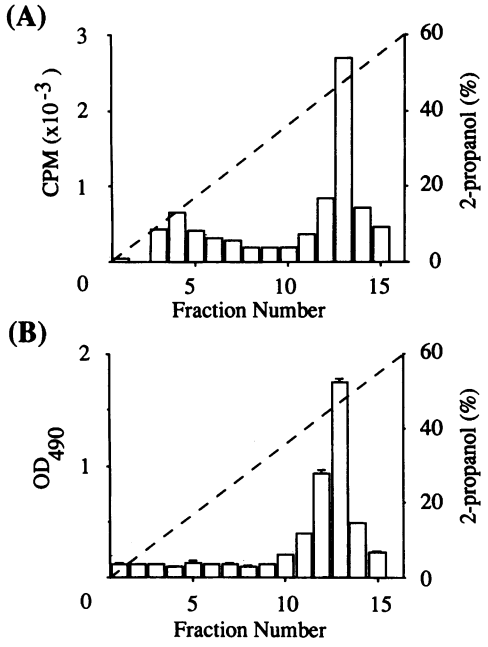

FIG. 4. Comigration of GIF antigen and the 14-kDa protein in reverse-phase chromatography. A mixture of ^{125}I-labeled 14-kDa protein (10,000 rpm) and crude GIF was analyzed by C_{18} reverse-phase HPLC with a linear gradient of 2-propanol (---) in 0.1% trifluoroacetic acid. Distributions of radioactivity (*A*) and of GIF antigen determined by ELISA (*B*) are shown.

unlabeled GIF. Determination of radioactivity associated with the cells and that remaining in the fluid phase have shown that 18–25% of the total radioactivity bound to the cells when they were incubated with 1–3 ng of ^{125}I-GIF and that the binding of ^{125}I-GIF was blocked by the presence of unlabeled GIF. To confirm the specificity of the binding, we determined the binding of ^{125}I-GIF to the 12H5 cells, A20.3 cells, normal mouse splenic lymphocytes, normal human peripheral blood lymphocytes, and the human T-cell hybridoma CL3. As shown in Fig. 5*A*, ^{125}I-GIF bound to the 12H5 cells and A20.3 cells, and the majority of the binding was inhibited by unlabeled GIF. Splenic lymphocytes of normal mice bound little, if any, GIF. It was also found that neither human T-cell hybridoma CL3 cells nor normal human lymphocytes bound ^{125}I-GIF.

Distinction of GIF from Lipocortin. Since both human lipocortin I and GIF are phospholipase inhibitory proteins and could switch the 12H5 cells from formation of glycosylated IgE-BF to the formation of unglycosylated IgE-BF (22), we determined possible binding of ^{125}I-labeled lipocortin to the 12H5 cells and A20.3 cells. Under the experimental conditions described above, specific binding of recombinant human lipocortin I to the cells was not detected (Fig. 5*B*). Thus, we sought to determine whether the radiolabeled lipocortin and GIF bind to phospholipid vesicles. ^{125}I-GIF did not bind to any of the PS, PI, or PC vesicles. As expected from a previous publication (23), ^{125}I-lipocortin bound to PS vesicles (Fig. 6).

DISCUSSION

In previous studies in our laboratory, GIF from 231F1 cells was identified by biosynthetic labeling of the cells with [^{35}S]methionine (14). SDS/PAGE analysis of the partially purified GIF demonstrated 14- and 41-kDa bands in the autoradiographs. Association of GIF activity with the two peptides was supported by the finding that the bioactivity of the lymphokine was recovered by extraction of gel slices followed by renaturation of proteins in the extracts. How-

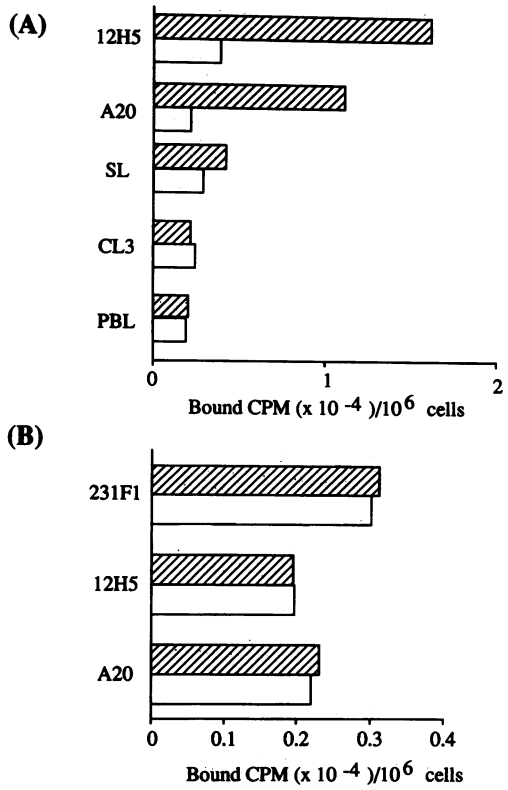

FIG. 5. Binding of ^{125}I-GIF and ^{125}I-lipocortin to lymphoid cells. ^{125}I-GIF (60,000 cpm) (*A*) or ^{125}I-lipocortin (400,000 cpm) (*B*) was mixed with 1 × 10^6 cells in the presence (□) or absence (□) of a 500-fold excess of unlabeled homologous protein, and the radioactivity associated with the cells was measured. Each bar represents the average of triplicate assays. 12H5, murine T-hybridoma 12H5 cells; A20, murine B-cell line A20.3 cells; 231F1, murine T-hybridoma 231F1; SL, normal mouse splenocytes; CL3, human T-hybridoma CL3; PBL, human peripheral blood lymphocytes.

ever, the biosynthetically labeled GIF preparations contained unlabeled proteins that are apparently derived from culture medium. In the present experiment, therefore, the 231F1 cells were cultured in serum-free medium. Comparisons of GIF activity in the culture supernatant with that produced by the same cells cultured in the presence of 10% Nu-Serum indicated that production of GIF declined to one-half to one-third in the serum-free medium. Neverthe-

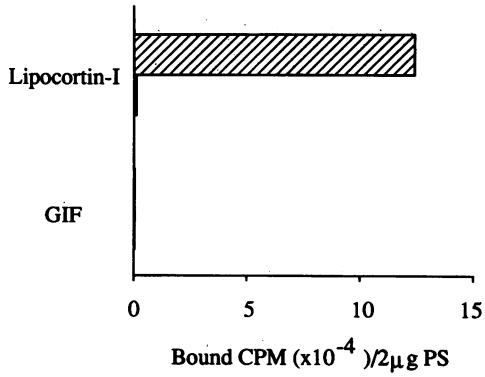

FIG. 6. Binding of ^{125}I-GIF and ^{125}I-lipocortin to phospholipid vesicles. ^{125}I-GIF (≈3 ng; 60,000 rpm) or ^{125}I-lipocortin (≈3 ng; 400,000 cpm) was mixed with 2 μg of PS vesicles in the presence (□) or absence (□) of a 500-fold excess of unlabeled homologous protein. After 10 min at room temperature, radioactivity associated with the vesicles was measured. Each bar represents the average of triplicate assays.

less, GIF in the culture supernatant could be isolated by one-step affinity chromatography on 141B9-Affi-Gel. The purified preparations gave a single band of 14 kDa in SDS/PAGE (Fig. 1) and a single spot with a pI of 5.5 in two-dimensional electrophoresis (Fig. 2). Fractionation of a mixture of a ^{125}I-GIF preparation with concentrated culture supernatant by ion-exchange chromatography, by reverse-phase chromatography, or by gel filtration confirmed biochemical homogeneity of the radiolabeled 14-kDa protein. The experiments showed comigration of the protein with GIF activity. It was also found that the GIF-containing fractions gave an ELISA signal with biotin-coupled anti-GIF antibody. In these experiments, the majority of GIF activity and the GIF antigen should be derived from the culture supernatant. The results indicate that the major species of GIF in the original culture supernatant is the 14-kDa protein. Since the molecular mass of the protein was comparable in reduced and nonreduced conditions (14), the 14-kDa GIF appears to be a single polypeptide chain.

It was noted that the 41-kDa GIF was not detected in SDS/PAGE in the present experiment. Distribution of GIF bioactivity after gel filtration of the culture supernatant suggests a lack of the 41-kDa GIF in the original culture supernatant. Previous experiments suggested that the 41-kDa peptide is susceptible to enzymatic degradation (14). Indeed, this molecular mass species was detected only when the 24-hr culture supernatant of a GIF-producing hybridoma was fractionated by gel filtration (14, 16). Since the original supernatant was recovered from a 48-hr culture in the present experiment, it is possible that the 41-kDa GIF had been degraded during culture.

In the past several years, we used 12H5 cells for the detection of GIF (16). The present experiments actually demonstrated the binding of ^{125}I-GIF to the T-cell hybridoma and a murine B-cell line A20.3. However, the same preparation of ^{125}I-GIF failed to bind to the human T-cell hybridoma CL3 or to normal human peripheral blood lymphocytes, indicating that the binding of murine GIF to 12H5 cells is specific. The present experiments are not sufficient for estimating the affinity of GIF for the target cells. However, specific binding of up to 20% of ^{125}I-GIF to the 12H5 cells and A20.3 cells (cf. Fig. 5) suggests that these cells may express receptors for GIF. Previous experiments have shown that GIF could change the nature of IgE-BF formed by normal murine T cells (5) and that comparable concentrations of GIF were required for switching the 12H5 cells and normal splenic lymphocytes for the selective formation of unglycosylated IgE-BF. However, the binding of ^{125}I-GIF to resting, normal splenic lymphocytes was barely detectable. These findings suggest that either a minor subset of lymphocytes or activated lymphocytes may express receptors for GIF. Identification of the subset would be important for elucidation of the mechanisms involved in the immunosuppressive effects of this lymphokine.

Finally, the present experiments provide evidence that GIF is distinct from lipocortin. Under the same experimental conditions in which the binding of GIF was demonstrated, binding of recombinant human lipocortin I to 12H5 cells or A20.3 cells could not be detected. Nevertheless, the recombinant lipocortin could switch the 12H5 cells for selective formation of unglycosylated IgE-BF at a concentration of 3 nM (22). It appears that GIF has a much higher affinity for 12H5 cells than human lipocortin I. Lipocortin I belongs to the family of Ca^{2+} phospholipid-binding protein—i.e., anexin—that binds acidic phospholipids in the presence of Ca^{2+} (23). Indeed, the present experiments confirmed the binding of lipocortin I to PS vesicles. In contrast, ^{125}I-GIF did not bind to any of the PS, PI, or PC vesicles under the same experimental conditions. It was also found that a monoclonal antibody against human lipocortin I does not bind GIF, while the monoclonal antibody 141B9, which was used for purification of GIF, has a weak affinity, if any, for lipocortin I (14). The possibility may be considered that the 14-kDa GIF is an enzymatic cleavage product of a larger molecule that has Ca^{2+}–phospholipid binding properties. However, this possibility is unlikely, because the binding to phospholipid is essential for lipocortin to exert phospholipase inhibitory activity (21), while GIF is a phospholipase inhibitory protein with no affinity for phospholipids. The conclusion on the possible relationship between GIF and lipocortin awaits gene cloning of the lymphokine.

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