Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 2824–2828, March 1994
Immunology

Antigen-binding glycosylation inhibiting factor from a human T-cell hybridoma specific for bee venom phospholipase A_2

(suppressor T-cell factor/epitope specificity)

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ABSTRACT **We obtained human T-cell hybridomas that are specific for bee venom phospholipase A_2 (PLA_2) and constitutively secrete glycosylation inhibiting factor (GIF). Upon crosslinking of CD3, the hybridoma produced GIF having affinity for PLA_2. When affinity-purified PLA_2-binding GIF was used as an immunogen, monoclonal antibodies specific for the antigen-binding GIF were obtained. Monoclonal antibody 110BH3 bound the antigen-binding GIF but failed to bind the 13-kDa nonspecific GIF, as determined by both bioassay and ELISA. In contrast, 388F1, a monoclonal antibody against nonspecific GIF, gave ELISA signals with both the nonspecific GIF and the antigen-binding GIF. Gel filtration of affinity-purified antigen-binding GIF revealed the presence of a 72- to 80-kDa protein which gave ELISA signals with both 110BH3 and 388F1 and contained GIF bioactivity. Upon reduction and alkylation, the antigen-binding GIF dissociated into a 62- to 64-kDa protein which gave positive ELISA with antibody 110BH3 but no signal with antibody 388F1, and a 15-kDa protein, which gave ELISA signal with the 388F1 but not with 110BH3. Immunoblotting of a PLA_2-binding GIF preparation revealed that under reducing conditions, the antigen-binding GIF dissociated a 13-kDa peptide which reacted with polyclonal antibodies against recombinant GIF. The results indicate that the 13-kDa nonspecific GIF is a subunit of antigen-binding GIF. The PLA_2-binding GIF has affinity for an epitope, representing amino acid residues 19–28 in PLA_2 which appears to be an external structure in the antigen.**

Existence of T-cell factors having affinity for nominal antigens is a controversial issue in cellular immunology. The majority of antigen-specific suppressor T-cell factors (TsFs), having affinity for a protein antigen or synthetic hapten, were obtained from murine T cells and T-cell hybridomas (1). Although the presence of similar factors from human T cells was predicted (2), no biochemical studies of such factors has been carried out. In our previous studies on the generation of antigen-specific suppressor T (Ts) cells, we constructed human T-cell hybridomas from peripheral blood lymphocytes of a patient sensitive to honeybee venom and obtained T-cell hybridomas specific for bee venom phospholipase A_2 (PLA_2) (3). These hybridomas expressed CD3 and $\alpha\beta$ T-cell receptors (TCRs) and secreted glycosylation inhibiting factor (GIF), a 13-kDa lymphokine that switches normal T cells from the formation of glycosylated IgE-binding factor (IgE-BF) to the formation of unglycosylated IgE-BF. Upon crosslinking of CD3 or TCR, however, the majority of the GIF-producing hybridomas formed GIF having affinity for PLA_2 (3). These findings were in agreement with previous observations on ovalbumin-specific murine Ts hybridomas, which constitutively secrete GIF having no affinity for ovalbumin but produce ovalbumin-specific GIF upon stimulation with ovalbumin-pulsed antigen-presenting cells (4) or by crosslinking of either CD3 or $\alpha\beta$ TCR (5). The ovalbumin-binding murine GIF suppressed anti-hapten antibody responses of syngeneic mice to dinitrophenyl derivatives of ovalbumin in a carrier-specific manner (4), and shared several antigenic determinants with antigen-specific TsFs (5). Furthermore, evidence was obtained that a nitrophenyl-specific TsF described by Okuda *et al.* (6) had GIF bioactivity and bound to an anti-GIF monoclonal antibody (mAb) (7). These findings indicated that antigen-binding GIF may represent an antigen-specific TsF and suggested that the human GIF having affinity for homologous antigen may represent the human counterpart of murine TsF (3).

Only limited information has been available on the biochemical properties and molecular basis of murine TsFs. However, several studies have strongly suggested a close relationship between murine TsFs and TCR α chain (8–10). Furthermore, we recently succeeded in molecular cloning of cDNAs encoding murine and human GIF and showed that polyclonal antibodies against recombinant mouse GIF reacted with hybridoma-derived mouse and human GIF (11). In view of such progress, the present experiments were undertaken to establish mAbs specific for human antigen-binding GIF and to obtain biochemical evidence for the presence of antigen-specific human T-cell factors having affinity for nominal antigen. The results show that the 13-kDa GIF is a subunit of the antigen-binding T-cell factor.

MATERIAL AND METHODS

Antigens and Antibodies. Lyophilized bee venom PLA_2 was purchased from Sigma. Crystalline ovalbumin was purchased from Nutritional Biochemicals. Synthetic peptides corresponding to aa 7–22, 13–28, and 25–40 in PLA_2 molecules were supplied by Howard Grey, Cytel (San Diego). The peptide corresponding to aa 19–35 in PLA_2 was synthesized by Kirin Pharmaceutical Laboratory (Maebashi, Japan). All of the synthetic peptides were purified by HPLC, and their amino acid sequences were confirmed. Anti-human GIF mAb 388F1 has been described (3). Anti-human CD3 antibody was obtained from the mouse hybridoma SPV-T3b (12), which was kindly supplied by J. E. deVries, DNAX. The mAbs were purified from ascitic fluid of hybridoma-injected BALB/c mice by protein A-Sepharose. Specifically purified goat antibodies against mouse IgG (13) and the IgG fraction of rabbit polyclonal antiserum against recombinant mouse GIF (11) were previously described.

mAb 388F1 was coupled to Affi-Gel 10 (Bio-Rad) according to the manufacturer's protocol. Two to 3 mg of IgG was coupled to 1 ml Affi-Gel. Bee venom PLA_2, synthetic peptide

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Abbreviations: GIF, glycosylation inhibiting factor; IgE-BF, IgE-binding factor; PLA_2, bee venom phospholipase A_2; Ts cell, suppressor T cell; TsF, Ts factor; BSA, bovine serum albumin; mAb, monoclonal antibody.

19–35, and ovalbumin were coupled to CNBr-treated CL-Sepharose 4B. One to 2 mg of PLA_2 or peptide or 5 mg of ovalbumin was coupled to 1 ml of Sepharose.

Cell Lines. Human T-cell hybridoma AC5 cells (3) were adapted to and cultured in protein-free ABC medium (Cell Biotechnology, Rockville, MD). Mouse T-cell hybridoma 12H5 cells (14) were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 10% NCTC 135 medium (GIBCO), 10 mM Hepes buffer, bovine insulin (0.2 unit/ml; Sigma), sodium pyruvate (50 μg/ml), oxaloacetic acid (150 μg/ml), and antibiotics. B-cell hybridomas were constructed from spleen cells of BALB/c mice (The Jackson Laboratory) that had been immunized with affinity-purified antigen-binding GIF (see below). One week after the last booster immunization, their spleen cells were fused with hypoxanthine phosphoribosyltransferase-deficient Sp2/0 Ag14 cells (15). Subcloning of the hybridomas was carried out by limiting dilution. The hybridomas were maintained in the supplemented DMEM described above.

Detection of GIF. GIF was detected by the capacity of this cytokine to switch 12H5 cells from the formation of glycosylated IgE-BF to the formation of unglycosylated IgE-BF (16). In brief, aliquots of the 12H5 cells were cultured for 24 hr with mouse IgE in the presence or absence of a sample to be tested, and culture filtrates were fractionated on lentil lectin-Sepharose. IgE-BF in the effluent fraction and eluate with methyl α-D-mannoside was determined by rosette inhibition of Fc_ε receptor-positive cells with IgE-coated ox erythrocytes. When the 12H5 cells were cultured with IgE alone, essentially all IgE-BF formed by the cells bound to lentil lectin-Sepharose and was recovered by elution with methyl α-D-mannoside. When a sufficient amount of GIF was added to the culture of 12H5 cells together with IgE, the majority of IgE-BF formed by the cells failed to be retained in the lentil lectin-Sepharose and was recovered in the effluent fraction (16). A test sample was taken as GIF-positive if the ratio of the percent rosette inhibition between the effluent and eluate fractions was 2.0 or higher.

Fractionation and Purification of GIF. Nonspecific GIF in culture supernatants of unstimulated AC5 cells was partially purified by chromatography on a DEAE-Sepharose column equilibrated with 10 mM Tris·HCl, pH 8.0/50 mM NaCl (3). The flowthrough fraction and buffer wash of the column were concentrated. Nonspecific GIF in the original culture supernatant was affinity-purified by using 388F1-coupled Affi-Gel as described (3).

To produce antigen-binding GIF, AC5 cells were stimulated by crosslinking of CD3 (3). The cells (5×10^6 per ml) were treated with anti-CD3 mAb (5 μg/ml) for 30 min at 4°C, and the antibody-treated cells were incubated for 30 min at 4°C with anti-mouse IgG (10 μg/ml). After washing, the cells were suspended in ABC medium at 2×10^6 cells per ml and cultured for 24 hr. Antigen-binding GIF was purified by adsorption of culture supernatant with PLA_2-coupled Sepharose in the presence of 10 mM EDTA and recovered by elution with 0.1 M glycine/HCl buffer at pH 3.0 (3). Affinity-purified GIF was fractionated by gel filtration on a Superose 12 column (1.6 cm × 50 cm; Pharmacia) connected to an HPLC instrument (Beckman System Gold, Fullerton, CA), as described (3). The column was calibrated with bovine serum albumin (BSA) (M_r 67,000), ovalbumin (M_r 43,000), soybean trypsin inhibitor (M_r 20,100) and cytochrome *c* (M_r 12,500). In some experiments, an affinity-purified GIF preparation was reduced and alkylated. The GIF preparation in 0.05 M Tris·HCl, pH 8.5/0.15 M NaCl was incubated for 1 hr at room temperature with 10 mM dithiothreitol and then alkylated with a 30% molar excess of iodoacetamide.

ELISAs. Each well of a Nunc F plate (Max Sorp; Nunc) was coated with 50 μl of serial dilutions of a GIF preparation overnight at 4°C in duplicate or triplicate. Plates were washed five times with phosphate-buffered saline containing 0.05% Tween 20 (Sigma) (Tween/PBS) between each of the following steps except the step prior to substrate. The plates were blocked with 2% BSA in Tween/PBS for 1 hr at 37°C. Binding of mAb 388F1 to GIF-coated wells was determined with an amplification system (17). Fifty microliters of PBS containing biotinylated mAb 388F1 at 150 ng/ml was added to each well. After a 2-hr incubation at 37°C followed by washing, 50 μl of an appropriate dilution (1:6000) of streptavidin-coupled alkaline phosphatase (Zymed) was added to each well and the plate was incubated for 2 hr at 37°C. The plate was washed with 0.05% Tween 20 in 0.05 M Tris·HCl, pH 7.5/0.15 M NaCl, and an ELISA signal was developed by 30 min of incubation with 50 μl of alkaline phosphatase substrate followed by amplifier solution (GIBCO/BRL). A_{490} was determined in a Dynatek MRC 5000 ELISA reader.

An ELISA was also set up with mAb against antigen-binding GIF. After Max-Sorp plates were coated with a GIF preparation and blocked with BSA, 50 μl of the mAb (200 ng/ml) in PBS was added to each well and the plate was incubated for 2 hr at 37°C. Depending on the isotype of the mAb, a 1:3000 dilution of horseradish peroxidase-coupled goat anti-mouse IgM (Bio-Rad) or anti-mouse IgG (Zymed) or a 1:2000 dilution of horseradish peroxidase-coupled anti-mouse IgG/A/M (Zymed) was added to each well. The ELISA signal was developed by peroxidase substrate (Zymed) and determined by A_{405}.

SDS/PAGE and Immunoblotting. Affinity-purified GIF was dialyzed against 0.01% SDS and lyophilized. Samples were then analyzed by SDS/PAGE in a 15% polyacrylamide slab gel with the Laemmli system (18). Purified recombinant GIF from *Escherichia coli* (11) was electrophoresed in parallel as a standard. Immunoblotting was carried out with enhanced chemiluminescence (ECL) Western blot detection reagents (Amersham). Detailed procedures were described in a previous article (11).

RESULTS

Preparation of mAbs Specific for Antigen-Binding GIF. The PLA_2-binding GIF from AC5 cells was affinity purified by using PLA_2-coupled Sepharose. The preparation could switch 12H5 cells from the formation of glycosylated IgE-BF to the formation of unglycosylated IgE-BF at a dilution of 1:30 to 1:60. BALB/c mice were immunized by an intraperitoneal injection of 0.1 ml of the preparation in complete Freund's adjuvant and received five booster injections of the same antigen in incomplete Freund's adjuvant. Two weeks after the last booster injection, their spleen cells were fused with the B-cell line Sp2/0 Ag14. Culture supernatants of hybridomas were tested for the presence of anti-GIF by ELISA. Maxi-Sorp wells were coated with either PLA_2-binding GIF or nonspecific GIF, and binding of mouse immunoglobulin in the culture supernatant was determined by using horseradish peroxidase-coupled anti-mouse IgG/A/M. Culture supernatants of 10 hybridomas gave significant ELISA signals with PLA_2-binding GIF, but only 2 of them gave substantially higher ELISA signal with PLA_2-binding GIF than with nonspecific GIF. After repeated subclonings, we obtained subclones of the two hybridomas, 110BH3 and 205AD2, whose culture supernatants gave ELISA signals with antigen-binding GIF but not with nonspecific GIF. mAb 110BH3 was μκ isotype, while 205AD2 was $\gamma_1\kappa$ isotype.

mAb 110BH3 from the culture supernatant of the hybridoma was enriched by precipitation with ammonium sulfate (33% saturation), and 5 mg of IgM was coupled to 1.5 ml of CL-Sepharose 4B. The PLA_2-binding GIF and nonspecific GIF from the AC5 cells were then fractionated on the antibody-coupled Sepharose. PLA_2-binding GIF bound to

the immunosorbent and was recovered by elution at acid pH, whereas nonspecific GIF failed to be retained.

Attempts were made to detect antigen-binding GIF and nonspecific GIF by ELISA. The antigen-binding factors in the culture supernatants of anti-CD3-stimulated cells were partially purified by using PLA_2-coupled Sepharose, and the factors in acid eluates were further purified by 110BH3-coupled Sepharose. mAb 388F1 gave ELISA signals with both nonspecific GIF and antigen-binding GIF preparations, whereas mAb 110BH3 reacted with antigen-binding factor but not with the nonspecific GIF (Fig. 1).

The preparation of the antigen-binding factor was analyzed by SDS/PAGE (Fig. 2*A*). Under nonreducing conditions, the preparation gave three major bands of 85, 66, and 58 kDa and a minor band of 13 kDa. Under reducing conditions, the 85-kDa band disappeared and several new bands were detected. Since one of the major bands detected under reducing conditions was at 13 kDa, corresponding in mobility to nonspecific GIF (3), a partially purified PLA_2-binding GIF was analyzed by SDS/PAGE under reducing conditions followed by immunoblotting with polyclonal antibodies against recombinant GIF. The antibodies recognized the 13-kDa band (Fig. 2*B*, lane 1). The relationship between the 13-kDa band and nonspecific GIF was confirmed by immunoblotting of nonspecific GIF, which was purified from culture supernatant of unstimulated AC5 cells by affinity chromatography on 388F1-coupled Affi-Gel (lane 2).

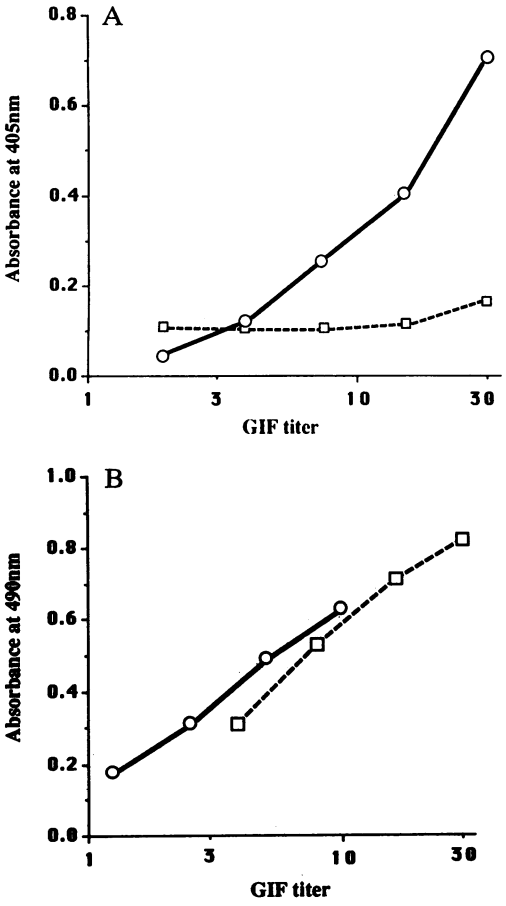

FIG. 1. ELISAs of GIF preparations. Maxi-Sorp wells were coated with serial dilutions of affinity-purified nonspecific GIF (□) or antigen-binding GIF (○). Abscissa indicates titer of a GIF preparation for coating as determined by bioassay. (*A*) Binding of mAb 110BH3 to the wells was detected using horseradish peroxidase-coupled goat anti-mouse μ chain. A_{405} was determined. (*B*) Binding of mAb 388F1 was detected by using biotin-coupled mAb 388F1, followed by alkaline phosphatase-coupled goat anti-mouse IgG. The amplification system was employed, and A_{490} was determined. Irrelevant mouse IgM (*A*) or IgG2a (*B*) was added to control wells and absorbance of the control well was subtracted.

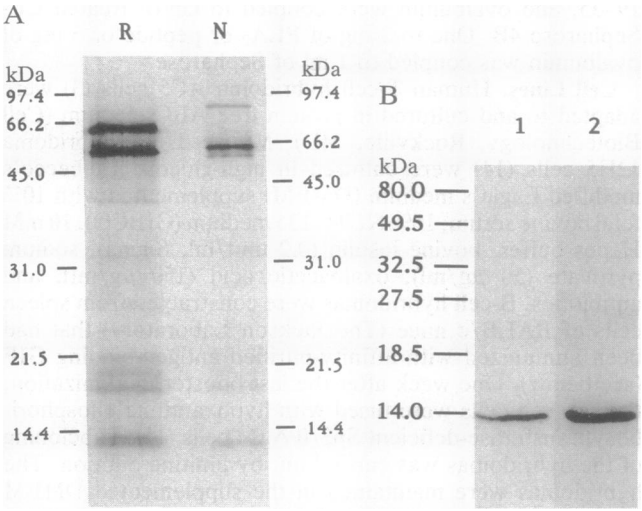

FIG. 2. SDS/PAGE analysis of affinity-purified GIF. (*A*) An affinity-purified antigen-binding GIF preparation was analyzed by SDS/PAGE under reducing (R) and nonreducing (N) conditions and proteins were visualized by silver staining. (*B*) Antigen-binding GIF obtained by using 110BH3-Sepharose (lane 1) and nonspecific GIF (lane 2) were analyzed by SDS/PAGE under reducing conditions. Proteins in the gels were transferred to poly(vinylidene difluoride) membranes and detected with anti-GIF. Recombinant mouse GIF was employed as the 13-kDa marker.

We suspected that the 13-kDa GIF and the antigen-binding polypeptide chain were associated with each other to form antigen-binding GIF. If this is the case, molecular size of antigen-binding GIF should be larger than that of nonspecific GIF. To test this possibility, PLA_2-binding GIF from AC5 cells was partially purified by using 110BH3-Sepharose, and the preparation was fractionated by Superose 12 gel filtration. Each fraction was assessed for the presence of antigen-binding GIF by ELISA. The majority of GIF, as detected by mAb 388F1, was eluted from the Superose column between 55.5 and 60.5 min (Fig. 3*A*). The size of the molecule, estimated from its elution time, was 72–80 kDa. As expected, the fractions contained GIF activity as determined by bioassay. The same fractions gave ELISA signal with mAb 110BH3. The results strongly suggest that the two distinct antigenic determinants recognized by mAbs 388F1 and 110BH3 are associated with the same molecules.

If the antigen-specific GIF actually consists of antigen-binding chain and nonspecific GIF, one might expect that the antigen-binding GIF may be dissociated into separate polypeptides by reduction and alkylation. Thus, the affinity-purified antigen-binding GIF was reduced and alkylated, and the sample was applied to the same Superose 12 column. Distribution of 388F1 antigen and 110BH3 antigen, determined by ELISA, indicated that approximately half of the GIF in the reduced and alkylated material was recovered in a fraction whose elution time corresponded to 15 Da (Fig. 3*B*). The experiment also showed two peaks of molecules recognized by 110BH3; the first peak corresponded to the original antigen-binding GIF, while the elution time of the second peak corresponded to 62–64 kDa. Since the latter fraction did not contain the GIF determinant recognized by 388F1, the 110BH3-positive protein in the fraction probably represented a cleavage product of antigen-binding GIF.

Epitope Specificity of Antigen-Binding GIF. Experiments were carried out to confirm that the PLA_2-binding GIF was specific for bee venom PLA_2. As expected, PLA_2-binding GIF from AC5 cells failed to be retained by ovalbumin-

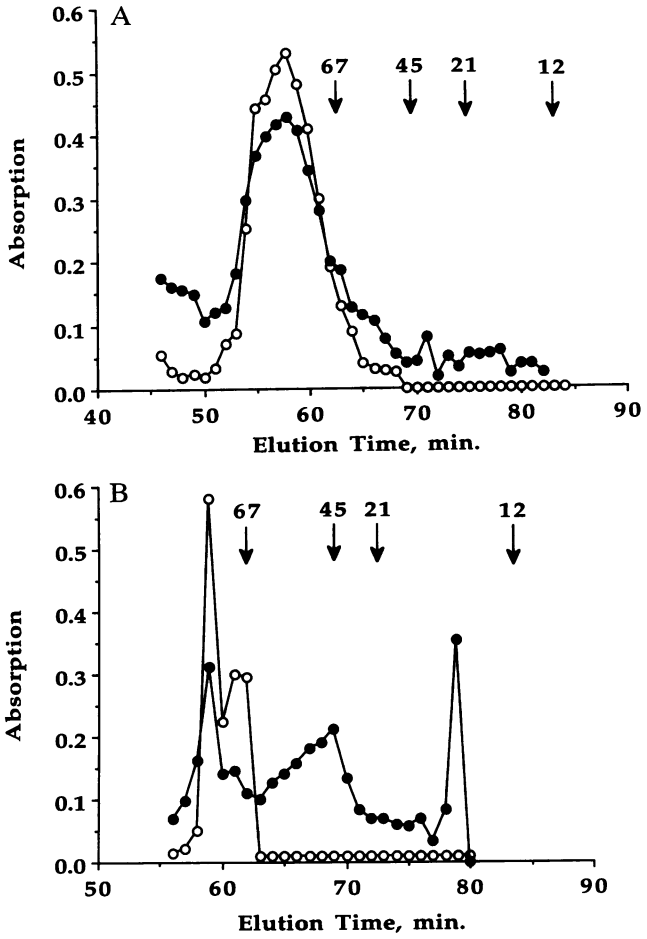

FIG. 3. Gel filtration of antigen-binding GIF. (*A*) Partially purified antigen-binding GIF was applied to a Superose 12 column and 1-min fractions were recovered. Each fraction was assessed for the presence of 110BH3 antigen (○) and 388F1 antigen (●) by ELISA. (*B*) Antigen-binding GIF preparation was reduced with 10 mM dithiothreitol and alkylated. The sample was applied to the same column and each 1-min fraction was assessed for 110BH3 antigen (○) and 388F1 antigen (●) by ELISA. Numbers and arrows indicate molecular mass (kDa) and elution volumes of standards.

Sepharose but did bind to PLA_2-Sepharose (results not shown). Since previous experiments on the PLA_2-binding GIF from murine Ts hybridomas had shown that the factor had affinity for the peptide representing aa 19–35 (p19–35) in bee venom PLA_2 (19), we determined whether the human PLA_2-binding GIF from AC5 cells might bind to the synthetic peptide coupled to Sepharose. Essentially all GIF bioactivity in the preparation was absorbed with the p19–35-Sepharose and was recovered by elution at acid pH (Table 1). To confirm the epitope specificity, aliquots of the PLA_2-binding GIF were incubated for 6 hr with one of the synthetic peptides (0.2 mg/ml) representing aa 7–22, 13–28, 19–35, or 25–40 in PLA_2, and each mixture was adsorbed with PLA_2-Sepharose. Determination of GIF activity in the flowthrough fraction and acid eluate fraction indicated that the binding of GIF to PLA_2-Sepharose was prevented by p13–28 and p19–35 but not by either p7–22 or p25–40. The results suggested that the sequence of aa 19–28 in PLA_2 contained the epitope which was recognized by the PLA_2-binding GIF.

DISCUSSION

In the present experiments, we obtained mAbs which bound antigen-binding GIF but failed to bind nonspecific GIF, as

Table 1. Epitope specificity of PLA_2-binding GIF

Exp.	Immunosorbent	Peptide added	GIF activity‡ Flow-through	GIF activity‡ Eluate
1*	PLA_2-Sepharose	None	2/28 (−)	23/6 (+)
	p19–35-Sepharose	None	3/30 (−)	28/4 (+)
	Medium control		4/28	
2†	PLA_2-Sepharose	None	0/28 (−)	27/3 (+)
		p7–22	0/26 (−)	25/4 (+)
		p13–28	22/0 (+)	0/23 (−)
		p19–35	20/5 (+)	0/28 (−)
		p25–40	2/25 (−)	23/0 (+)
	Medium control		3/24	

*The PLA_2-binding GIF was purified on PLA_2-Sepharose. Six milliliters of the preparation was fractionated on 1.5 ml of Sepharose coupled with p19–35 or PLA_2. Each of the flowthrough and acid eluate fractions was adjusted to 6.0 ml, and their GIF activity was determined by using 12H5 cells.

†A 0.5-ml aliquot of the acid eluate fraction from PLA_2-Sepharose was mixed overnight with 0.5 ml of PLA_2-Sepharose in the presence or absence of an appropriate peptide. Both flowthrough and acid eluate fractions from PLA_2-Sepharose were adjusted to 1.0 ml and assessed for GIF activity.

‡Numbers in this column represent percent rosette inhibition by the effluent/eluate fractions from lentil lectin Sepharose; (+) or (−) indicates the presence or absence of GIF. The proportion of rosette-forming cells in the absence of IgE-BF was 25.3 ± 0.7%.

determined by both bioassay and ELISA. Since the PLA_2-binding GIF preparations employed for ELISAs were not pure, the possibility may be considered that ELISA signals by 110BH3 were due to the binding of this antibody to an unknown protein that was nonspecifically bound to PLA_2-Sepharose. When culture supernatants of unstimulated AC5 cells were fractionated on PLA_2-Sepharose, however, the acid eluate fraction failed to give any ELISA signal with mAb 110BH3 (results not shown). It was also found by ELISA that the 110BH3 antigen in the affinity-purified PLA_2-binding GIF rebound to PLA_2-Sepharose, but the binding was inhibited by the synthetic peptide p19–35, but not by p25–40. The results collectively indicate that PLA_2-binding GIF is responsible for ELISA signals obtained with 110BH3.

The possibility may be considered that mAb 110BH3 is specific for an idiotype of PLA_2-specific factors. Since we have established GIF-producing human T-cell hybridomas specific for the Japanese cedar pollen allergen CryJ-I (20) by the same procedures as those employed for constructing AC5 cells (3), and some of the hybridomas produced antigen-binding GIF upon crosslinking of CD3 (unpublished results), we determined whether the antigen-binding GIF from a representative CryJ-I-specific hybridoma, 31E9, would bind to 110BH3-coupled Sepharose. Indeed, GIF from the anti-CD3-treated hybridoma cells bound to the immunosorbent, and essentially all GIF activity recovered from the immunosorbent bound to CryJ-I-coupled Sepharose. As expected, GIF from unstimulated 31E9 cells failed to be retained in the immunosorbent. The ability of mAb 110BH3 to bind not only PLA_2-binding GIF but also CryJ-I-binding GIF excluded the possibility that the mAb recognizes an idiotypic determinant.

The present experiments provided definitive evidence for our hypothesis that the 13-kDa GIF is a subunit of antigen-binding factors. The antigen-binding GIF appears to be a 72- to 80-kDa molecule that has two antigenic determinants recognized by 388F1 and 110BH3, respectively, and dissociates nonspecific GIF upon reduction and alkylation (Fig. 3). Association of the 13-kDa GIF with the antigen-binding factor was confirmed by immunoblot analysis with polyclonal antibodies against recombinant GIF (Fig. 2*B*). One might predict that the antigen-binding GIF consists of the 13-kDa GIF and an antigen-binding peptide(s). When a reduced and

alkylated antigen-binding GIF preparation was fractionated on a Superose 12 column, we detected 62- to 64-kDa molecules which had the 110BH3 determinant but lacked the 388F1 determinant. This molecule may represent an antigen-binding peptide; however, the peptide was not biochemically identified in the present experiment. In view of the accumulated evidence on a close relationship between murine TsF and TCR α chain (8–10, 21–23), it should be determined whether the 62- to 64-kDa polypeptide from AC5 cells may be related to the α chain. Although neither 110BH3 nor 205AD2 could stain human TCR in immunofluorescence, this does not exclude the possibility that the peptide is related to human TCR α chain. A typical mAb against mouse TCR α chain, H28-710, which binds mouse antigen-specific TsF (22, 23) and antigen-binding GIF (5), is specific for a hidden portion of α chain.

One of the fundamental questions on the antigen-specific TsF is why this molecule can bind to a nominal antigen. The binding is in conflict with the well-established fact that TCRs on helper T cells and cytotoxic T cells recognize processed antigen in the context of either class I or class II products of the major histocompatibility complex (24). However, previous studies on murine TsF indicated that the epitope recognized by the antigen-binding GIF represents an external structure of the antigen molecules and that TCRs on the cell source of the factors recognize the same epitope in the context of class II product (5, 25). All murine T-cell hybridomas that could produce PLA_2-binding GIF recognized PLA_2 synthetic peptide p19–34 on syngeneic antigen-presenting cells, and the PLA_2-binding factor had affinity for the same peptide (19). The present results provide evidence that PLA_2-binding GIF from human hybridoma AC5 cells has affinity for the same peptide and suggest that the sequence of aa 19–28 probably contains the epitope specific for the factor. The x-ray crystal structure of PLA_2 indicates that this sequence represents an external structure of PLA_2 (19, 26), and this explains why the factor could bind to the nominal antigen.

In the murine systems, evidence indicates that both the antigen-binding GIF and the effector-type TsF consist of multiple polypeptide chains (27–29). The ovalbumin-binding GIF from murine Ts hybridomas had a molecular mass of about 80 kDa as estimated by gel filtration (4). The present finding that the human PLA_2-binding GIF consists of multiple polypeptide chains and has a molecular mass of 72–80 kDa suggests that the T-cell factor is a counterpart of mouse TsF. Further studies are required to determine whether the antigen-binding human GIF can actually suppress antibody formation by human lymphocytes in an antigen-specific manner.

This work was supported by Research Grants AI32834, AI11202, and AI14784 from the U.S. Department of Health and Human Services. This paper is publication no. 69 from the La Jolla Institute for Allergy and Immunology.

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