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Immunology

Association of the "major histocompatibility complex subregion" I-J determinant with bioactive glycosylation-inhibiting factor

(suppressor T-cell factor/macrophage migration-inhibitory factor/posttranslational modification)

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ABSTRACT **Murine suppressor T-cell hybridoma cells (231F1) secrete not only bioactive glycosylation-inhibiting factor (GIF) but also an inactive peptide comparable to bioactive GIF peptide in its molecular size and reactivity with anti-GIF; the amino acid sequence of the inactive peptide is identical to that of the bioactive homologue. The inactive GIF peptide in culture supernatant of both the 231F1 cells and a stable transfectant of human GIF cDNA in the murine suppressor T hybridoma selectively bound to Affi-Gel 10, whereas bioactive GIF peptides from the same sources failed to bind to the gel. The inactive cytosolic human GIF from the stable transfectant and *Escherichia coli*-derived recombinant human GIF also had affinity for Affi-Gel 10. Both the bioactive murine GIF peptide from the suppressor T hybridoma and bioactive recombinant human GIF from the stable transfectant bound to the anti-I-J^b monoclonal antibody H6 coupled to Affi-Gel. However, bioactive hGIF produced by a stable transfectant of human GIF cDNA in BMT10 cells failed to be retained in H6-coupled Affi-Gel. These results indicate that the I-J specificity is determined by the cell source of the GIF peptide and that the I-J determinant recognized by monoclonal antibody H6 does not represent a part of the primary amino acid sequence of GIF. It appears that the epitope is generated by a posttranslational modification of the peptide.**

We previously described glycosylation-inhibiting factor (GIF), a 13-kDa cytokine involved in selective formation of IgE-suppressive factors (1). GIF inhibits N-glycosylation of IgE-binding factors (IgE-BF), and unglycosylated IgE-BF then selectively suppresses IgE synthesis. Furthermore, GIF facilitates the generation of antigen-specific suppressor T (Ts) cells (2) and appears to be a subunit of antigen-specific Ts cell factors (3, 4). We anticipated that GIF formation is specific to Ts cells. After molecular cloning of the cytokine, however, we realized that various murine and human cell line cells contained 0.6-kb mRNA that hybridized with murine GIF cDNA and human GIF cDNA, respectively (5). Both Ts cells and non-Ts cells secrete the 13-kDa peptide that reacts with polyclonal antibodies against recombinant GIF, but only the peptide secreted by Ts cells demonstrated GIF bioactivity (6). However, nucleotide sequences of GIF cDNA from the Ts and non-Ts cells were identical, indicating that bioactive and inactive GIFs are identical in amino acid sequences (6). Thus, we speculated that bioactivity of GIF is generated by post-translational modification of the peptide. This idea was supported by the fact that BMT10 cells transfected with human GIF (hGIF) cDNA secreted inactive GIF, whereas transfection of a chimeric cDNA encoding a fusion protein consisting of the N-terminal pro region of calcitonin precursor and hGIF into the same cells resulted in the secretion of bioactive GIF (6). Evidence was obtained that the fusion protein is translocated into the endoplasmic reticulum and cleaved at the Golgi apparatus by a furin-like enzyme to form the mature 13-kDa peptide, which was then secreted through the classical secretory pathway. Because GIF has no signal peptide sequence (5), the inactive 13-kDa peptide synthesized by the former transfectant does not go through the endoplasmic reticulum but was secreted through unknown mechanisms. However, transfection of hGIF cDNA into mouse Ts hybridoma cells (231F1) resulted in hGIF secretion, for which bioactivity was comparable to that produced by human Ts hybridomas (6). It was also found that the stable transfectants, as well as the original murine Ts hybridoma cells, contained a substantial quantity of inactive GIF peptide in their cytosols. In view of these findings, we speculated that Ts cells may contain a machinery that converts a fraction of inactive cytosolic GIF peptide to bioactive GIF during the secretory process.

The present experiment was undertaken to distinguish bioactive GIF from inactive GIF. We discovered that the inactive GIF peptide has affinity for Affi-Gel 10 (Affi; Bio-Rad) and can be separated from bioactive GIF peptide by absorption on Affi. Accumulating evidence indicated that anti-I-J antibodies recognize murine Ts cell factor (7, 8). It was also found that both polyclonal anti-I-J^b antibodies and anti-I-J^b monoclonal antibody (mAb), H6, bound GIF activity in the culture supernatant of the BDF1 mouse T-cell-derived Ts hybridoma 231F1 cells, but polyclonal anti-I-J^k antibodies failed to do so (3). Because the amino acid sequence of murine GIF peptide from various murine cell line cells appears identical, irrespective of the major histocompatibility complex phenotype of cell sources (6), we anticipated that the anti-I-J^b mAb may recognize conformational structures of the peptide generated by posttranslational modifications. The present experiments show that the anti-I-J^b mAb binds not only bioactive mouse GIF peptide from 231F1 cells but also bioactive hGIF peptide formed by the stable transfectant of hGIF cDNA in a murine Ts hybridoma.

MATERIALS AND METHODS

Cell Line Cells. Murine Ts hybridoma 231F1 cells have been described (9). The 2FH2 cell line is a stable transfectant of hGIF cDNA in 231F1 cells (6). The BTCH clone is a stable transfectant of a chimeric cDNA encoding a fusion protein consisting of the N-terminal region of procalcitonin and hGIF in BMT10 cells (6). These cells were cultured in high-glucose Dulbecco's modified Eagle's medium/10% Nu-Serum V (Collaborative Biomedical Products, Bedford, MA). The 2FH2

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Abbreviations: GIF, glycosylation-inhibiting factor; hGIF, human GIF; rhGIF, recombinant GIF; IgE-BF, IgE-binding factor; mAb, monoclonal antibody; Ts, suppressor T; MIF, macrophage migration-inhibitory factor; Affi, Affi-Gel 10; rIgG, normal rabbit IgG.

cells produce 10- to ≈20-fold more GIF peptide than the 231F1 cells.

Antibodies. Anti-hGIF mAb 388F1 (10) and anti-I-J^b mAb H6 (11) were obtained from culture supernatants of respective hybridomas. These mAbs, as well as the IgG fraction of rabbit polyclonal anti-mouse GIF antiserum (5) and normal rabbit IgG (rIgG), were purified with protein A–Sepharose (Pharmacia). Each of the mAbs or rIgGs was coupled to Affi according to the manufacturer's protocol. Two to 3 mg of the mAb or 5 mg of rIgG was coupled to 1 ml of Affi. In some experiments, Affi was treated with ethanolamine to block the reactive sites and used as an adsorbent.

Purification and Fractionation of GIF. Culture supernatant (0.6 to ≈1 liter) of GIF-producing cell line cells was concentrated 20-fold, and a concentrated sample was mixed overnight at 4°C with 10 ml of rIgG-coupled Affi (rIgG–Affi). The flow-through fraction was circulated overnight through 2–3 ml of either 388F1-coupled Affi (388F1–Affi) or anti-GIF-coupled Affi. After washing with 30 to ≈40 column vol of phosphate-buffered saline (PBS), proteins retained in the column were eluted with 0.1 M glycine·HCl buffer, pH 3.0. The cytosolic fraction of the 2FH2 cells was obtained by a described method (6), and inactive GIF peptide in the fraction was purified by using the 388F1–Affi. Recombinant hGIF (rhGIF) expressed in *Escherichia coli* was purified from a soluble fraction of the cells. The fraction was adjusted to pH 6.0, applied to a CM–Sepharose column equilibrated with 20 mM acetate buffer, pH 6.0, and proteins were eluted by NaCl gradient. The GIF preparation recovered by elution with the acetate buffer/0.5 M NaCl gave a single 13-kDa band in SDS/PAGE analysis and silver staining.

To determine the binding of GIF peptide to an appropriate Affi-Gel-immunosorbent, an affinity-purified GIF sample in 2.0 ml of PBS was mixed overnight with 0.5 ml of immunosorbent in the presence of bovine serum albumin at 0.5 mg/ml. The suspension was packed into a small column to obtain the flow-through fraction. After being washed with 10 ml of PBS, proteins retained in the column were recovered by elution with 1.5 ml of glycine·HCl buffer, pH 3.0.

Electrophoresis and Immunoblotting. Affinity-purified GIF preparations were analyzed by SDS/PAGE in a 15% polyacrylamide slab gel under reducing conditions (12), along with serial 2-fold dilutions of *E. coli*-derived rhGIF of known concentrations. After silver staining, the concentration of the 13-kDa peptide in the sample was estimated from the band intensity (5). Immunoblotting was done with the enhanced chemiluminescence immunoblot detection system (Amersham), using polyclonal anti-GIF at 1 μg/ml and horseradish peroxidase-coupled $F(ab')_2$ of donkey anti-rIgG (Amersham).

Detection of GIF Bioactivity. Bioactive GIF was detected by its ability to switch mouse T-cell hybridoma 12H5 cells from the formation of glycosylated IgE-BF to the formation of unglycosylated IgE-BF. Procedures for the assay have been detailed (2). IgE-BF in culture filtrates was fractionated on lentil lectin–Sepharose. Without GIF, essentially all IgE-BF formed by the cells bound to the lectin and was recovered by elution with 0.2 M methyl α-D-mannoside. With sufficient GIF, the most IgE-BF formed by the cells was not retained in the column. The GIF titer represents the highest dilution of a sample that is sufficient to switch the nature of IgE-BF in this assay.

ELISA Assays. Detailed procedures have been described (13). Briefly, microplates (Maxi Sorp, Nunc) were coated with serial 2-fold dilutions of an affinity-purified GIF. After blocking, plates were incubated for 2 hr with polyclonal anti-GIF at 2 μg/ml, followed by incubation for 2 hr with horseradish peroxidase-coupled $F(ab')_2$ of donkey anti-rIgG. ELISA signals were developed by adding substrate and determined by absorption at 405 nm.

Amino Acid Sequencing. The method of sequencing has been described (5). Briefly, the 13-kDa peptide immobilized on poly(vinylidene difluoride) membrane was reduced, S-carboxymethylated *in situ*, and digested with *Achromobacter* protease and then by endoproteinase Asp-N (14). Peptides released from the membrane after each digestion were fractionated by reversed-phase HPLC, and the amino acid sequence of each peptide was determined with a gas-phase sequencer (Applied Biosystems, model 492). The N-terminal sequence was determined by injecting poly(vinylidene difluoride)-immobilized protein into the sequencer.

RESULTS

Binding of Inactive GIF to Affi. Culture supernatants of the 231F1 cells were preabsorbed with rIgG-Affi, before affinity-purification of GIF with anti-GIF-coupled Affi-Gel. To investigate the biochemical properties of the proteins retained in rIgG-Affi, the absorbent was washed extensively with PBS, and proteins retained in the column were eluted at an acid pH. Analysis of the acid eluate fraction by SDS/PAGE and silver staining showed a single band of 12–13 kDa (Fig. 1*A*). The peptide was transferred to a poly(vinylidene difluoride) membrane and probed with anti-GIF antibodies or normal rIgG. The results clearly showed that anti-GIF, but not normal rIgG, bound to the 13-kDa peptide (data not shown). However, the 13-kDa peptide failed to show GIF bioactivity at 1 μg/ml. Thus, the peptide immobilized on a poly(vinylidene difluoride) membrane was used for the determination of amino acid sequence. Surprisingly, the N-terminal 15 amino acids of the peptide were PMFIVNTNVPRASVP, which were identical to the deduced sequence of murine GIF, except for the N-terminal methionine (5). We have also obtained the peptides DRVYINYY and DMNAANVGWNGSTFA (C terminus), which together correspond to the C-terminal 23 amino acids of murine GIF (5). Lack of the N-terminal methionine is not unexpected because 85% of affinity-purified 231F1-derived murine GIF used for molecular cloning (5) also lacked N-terminal methionine. The results indicate that the 13-kDa peptide has an amino acid sequence identical to GIF. Direct binding of the inactive GIF peptide to Affi was confirmed with an *E. coli*-derived rhGIF preparation that required 1 μg/ml to detect GIF activity. When 2-μg samples of the peptide were mixed with 0.5 ml of rIgG-Affi or Affi treated with ethanolamine, essentially all peptide bound to the adsorbent, as determined by SDS/PAGE analysis and immunoblotting with anti-GIF (data not shown). Apparently culture supernatants of

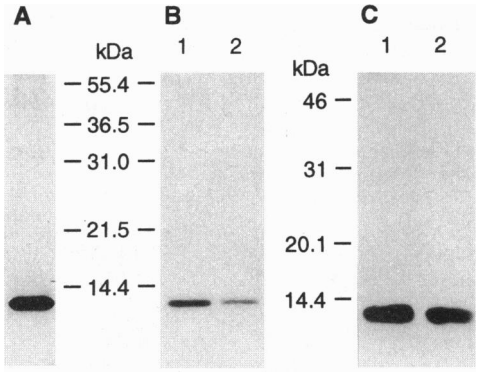

FIG. 1. Isolation of inactive and bioactive GIF. (*A*) Culture supernatant of 231F1 cells was absorbed with rIgG–Affi, and the acid-eluate fraction was analyzed by SDS/PAGE and silver staining. (*B* and *C*) rIgG–Affi eluate fraction (lane 1) and 388F1–Affi eluate fraction (lane 2) of culture supernatant of 2FH2 cells were analyzed by SDS/PAGE. The 13-kDa peptide was detected by silver staining (*B*) and by immunoblotting (*C*). The GIF titer of the 388F1–Affi eluate was 1:80, whereas the GIF bioactivity was not detectable in the rIgG–Affi eluate.

231F1 cells contained inactive GIF peptide together with bioactive GIF, and both species were copurified by absorption with anti-GIF–Affi.

To extend the above findings to a stable transfectant, culture supernatant of the 2FH2 cells was preabsorbed with rIgG–Affi, and bioactive GIF in the flow-through fraction was affinity-purified by using 388F1–Affi. Both the acid-eluate fractions from rIgG–Affi and 388F1–Affi gave a single 13-kDa band in SDS/PAGE analysis and silver staining (Fig. 1*B*), and the peptide bound anti-GIF in immunoblotting (Fig. 1*C*). Repeated experiments have shown that the quantity of the 13-kDa peptide recovered from the rIgG–Affi was 1.5- to 5-fold more than that recovered from 388F1–Affi. As expected, the 13-kDa peptide in the rIgG–Affi eluate failed to show GIF bioactivity at 1 μg/ml, whereas peptide at 3 to 5 ng/ml in the eluate of 388F1–Affi could switch 12H5 cells to the formation of unglycosylated IgE-BF. Refractionation of the affinity-purified bioactive GIF on 388F1–Affi or rIgG–Affi confirmed that all bioactivity and the 13-kDa peptide bound to 388F1–Affi, but the majority of the peptide failed to bind to rIgG–Affi (Table 1). A small fraction (≈20%) of the 13-kDa peptide recovered from rIgG–Affi did not have GIF bioactivity at 100 ng/ml. Table 1 shows that inactive GIF bound to both 388F1–Affi and rIgG–Affi.

We determined whether cytosolic GIF may or may not have affinity for Affi. The peptide was purified from the cytosol of 2FH2 cells using 388F1–Affi. The acid-eluate fraction from the immunosorbent gave a single band of 13 kDa in SDS/PAGE analysis and silver staining but failed to show GIF bioactivity at 0.5 μg/ml. Aliquots of the cytosolic GIF peptide were fractionated on either 388F1–Affi or rIgG–Affi. Table 1 shows that ≈70–75% of the peptide was recovered in the acid-eluate fraction from both 388F1–Affi and rIgG–Affi. It was also found that Affi treated with ethanolamine had the same capacity as rIgG–Affi for binding the cytosolic 13-kDa peptide. These results collectively indicate that inactive GIF in culture supernatant and in cytosol have affinity for Affi.

Because the 388F1–Affi and rIgG–Affi could bind comparable amounts of inactive 13-kDa peptide in the experiments of Table 1, we determined the reactivity of anti-GIF antibodies with the inactive GIF by ELISA. Fig. 2 shows that inactive GIF peptides recovered from the culture supernatant and the cytosol of 2FH2 cells gave comparable ELISA signals with bioactive GIF. None of the GIF preparations gave the signal when normal rIgG was used instead of anti-GIF (data not shown).

Binding of Bioactive GIF to Anti-I-J^b. To confirm that the anti-I-J^b mAb H6 can bind the bioactive murine GIF from 231F1 cells, culture filtrate of the cells was pre-adsorbed with rIgG–Affi to remove most of the inactive peptide, and bioactive GIF was purified by using anti-GIF–Affi. The affinity-purified bioactive GIF was then fractionated on H6-coupled

Table 1. Fractionation of bioactive and inactive GIF from 2FH2 cells on 388F1–Affi or rIgG–Affi

13-kDa peptide fractionated*	Bioactivity,† ng/ml	Fractionation 388F1–Affi FT/EL‡	Fractionation rIgG–Affi FT/EL‡
Bioactive GIF	5	0/100	85/15
rIgG–Affi eluate	>1000	10/90	20/80
Cytosolic GIF	>1000	25/75	25/75

*Bioactive GIF (0.5 μg), 1.8 μg of inactive GIF peptide rIgG–Affi eluate, or 3.0 μg of cytosolic GIF were fractionated with 0.5 ml of adsorbent.

†Minimum concentration of the 13-kDa peptide required for detection of GIF bioactivity.

‡Distribution of the 13-kDa peptide between flow-through (FT) and acid-eluate (EL) fractions. Numbers represent percentages of peptide recovery in the fractions.

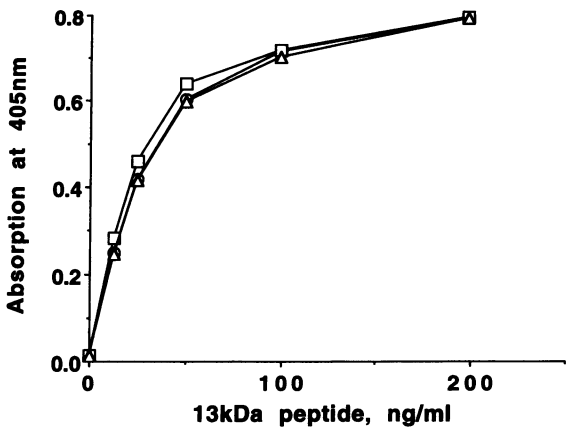

FIG. 2. ELISA assays of the 13-kDa peptide. Nunc plate was coated with the 388F1–Affi eluate (○) or rIgG–Affi eluate (□) shown in Fig. 1*B* or affinity-purified cytosolic 13-kDa peptide (△). The concentration of the peptide in each preparation was estimated by SDS/PAGE and silver staining.

Affi. As expected, essentially all GIF bioactivity in the affinity-purified material bound to H6–Affi and was recovered by acid elution. Analysis of the fractions from H6–Affi by SDS/PAGE and immunoblotting with anti-GIF clearly showed the 13-kDa GIF peptide in the acid-eluate fraction but not in the flow-through fraction (Fig. 3*A*). To confirm that the peptide represents bioactive GIF, an aliquot of the fraction was further fractionated on rIgG–Affi. All GIF bioactivity was recovered in the flow-through fraction, and the acid eluate of rIgG–Affi did not contain either the 13-kDa peptide or GIF bioactivity.

We then determined whether the bioactive hGIF from the 2FH2 cells binds to mAb H6. Bioactive GIF from the stable transfectant, prepared by using 388F1–Affi, gave a single band of 13 kDa in SDS/PAGE, and the peptide at 5 ng/ml was sufficient for bioactivity detection. Fractionation of the affinity-purified GIF on H6–Affi revealed that all GIF bioactivity in the preparation bound to H6–Affi and was recovered by acid elution. Analysis of the fractions by SDS/PAGE and immunoblotting (Fig. 3*B*) indicated that the 13-kDa GIF peptide bound to H6–Affi and was not detectable in the flow-through fraction. When the same sample was fractionated on rIgG–Affi, most of the peptide was recovered in the flow-through

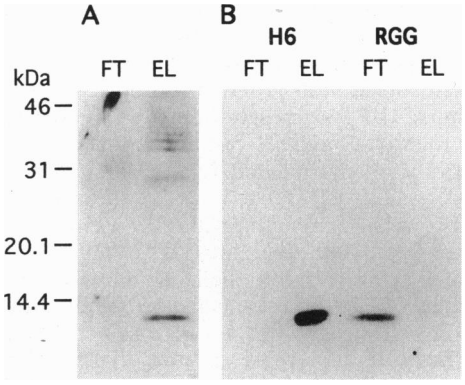

FIG. 3. Binding of bioactive GIF from 231F1 cells and 2FH2 cells to H6–Affi. (*A*) Bioactive GIF from 231F1 cells was fractionated on H6–Affi, and the flow-through (FT) and acid-eluate (FL) fractions were analyzed by immunoblotting with anti-GIF. GIF titers of both the original GIF preparation and EL were 1:60, whereas the activity was not detectable in FT. (*B*) Bioactive GIF from the 2FH2 cells was fractionated on H6–Affi or rIgG (RGG)–Affi. Flow-through (FT) and acid-eluate (EL) fractions were analyzed by immunoblotting. Both EL from H6–Affi and FT from RGG–Affi had GIF titer of 1:40, whereas the activity was not detectable in the other fractions.

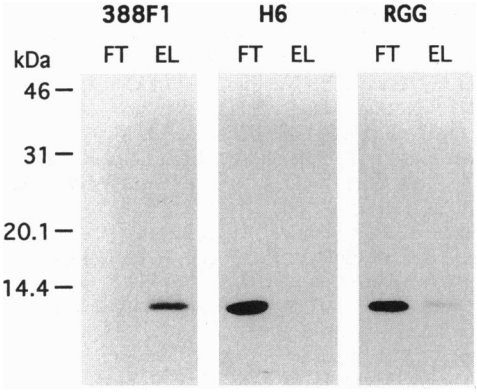

FIG. 4. Fractionation of bioactive GIF from BTCH cells. Samples of bioactive GIF preparation were fractionated on 388F1–Affi, H6–Affi, or rIgG (RGG)–Affi. Flow-through (FT) and acid-eluate (FL) fractions from each immunosorbent were analyzed by immunoblotting with anti-GIF. EL from 388F1–Affi and FT from H6–Affi had GIF titer of 1:80. FT from rIgG–Affi had GIF titer of 1:40. The activity was not detectable in the other fractions.

fraction, and GIF bioactivity failed to be retained in rIgG–Affi. The results indicate that bioactive GIF from the 2FH2 cells binds to H6–Affi.

Similar experiments were done by using bioactive hGIF from BTCH cells. The affinity-purified bioactive GIF preparation gave a single 13-kDa band in SDS/PAGE, and the minimum concentration of the peptide for bioactivity detection was 5 ng/ml. Aliquots of the purified sample were fractionated on 388F1–Affi, H6–Affi, or rIgG–Affi, and distribution of the GIF peptide between the flow-through and acid-eluate fractions was determined by immunoblotting. Fig. 4 shows that the 13-kDa peptide and the GIF bioactivity bound to 388F1–Affi and were recovered by acid elution. However, the peptide failed to bind to H6–Affi or rIgG–Affi. Both the 13-kDa peptide and all GIF bioactivity in the preparation were recovered in the flow-through fractions.

DISCUSSION

Previous attempts to detect biochemical differences between the bioactive GIF and inactive GIF by two-dimensional electrophoresis have identified three species of GIF peptides in the culture supernatant of 2FH2 cells (6). Because two of the three species were also detected in the cytosol, which contained only inactive GIF, we predicted that the remaining one species in the culture supernatant represents bioactive GIF. The present experiments actually show that inactive GIF peptide in both culture supernatant and cytosol bound to Affi (and rIgG–Affi), whereas bioactive GIF is not retained in the gel. Binding of the inactive GIF to Affi was quite selective. None of the serum proteins in the culture medium or cellular proteins other than GIF peptide was retained in the Affi column. However, affinity of the inactive peptide for Affi does not appear high. When culture supernatant was absorbed with rIgG–Affi, a small fraction of the inactive peptide frequently remained in the flow-through fraction (see Table 1). Under the same experimental conditions, neither the inactive GIF from mammalian cells nor *E. coli*-derived rGIF bound to agarose or CL-Sepharose. Affi is an *N*-hydroxysuccinimide derivative of agarose and negatively charged. However, heparin-coupled agarose, which is also negatively charged, failed to bind the inactive GIF peptide. At present, the biochemical basis for binding of the peptide to Affi is unknown. We suspect that the difference between the bioactive GIF and inactive GIF in their affinities for Affi would be due to the differences in their conformational structures because inactive GIF and bioactive GIF appear to have an identical amino acid sequence. Nevertheless, pre-absorption of culture supernatants of Ts hybridomas and stable transfectants with rIgG–Affi before affinity purification of GIF substantially increases specific bioactivity of the GIF preparations and provides definitive evidence that bioactive GIF and inactive GIF are distinct molecules.

Heterogeneity of GIF peptides may explain possible relationships between bioactive GIF and similar peptide with the other biologic activities. Although the recombinant human macrophage migration-inhibitory factor (MIF), described by Weiser *et al.* (15), should have almost the same primary structure as GIF, the MIF activity in the culture supernatant of COS-1 cells transfected with MIF cDNA failed to bind to monoclonal anti-GIF, and the bioactive rhGIF failed to show MIF activity (5). Because the transfected COS-1 cells produce inactive GIF peptide rather than bioactive GIF (5), the MIF activity could be associated with the inactive GIF peptide. An independent work by Bernhagen *et al.* (16) suggested that "murine MIF" secreted from pituitary cells is involved in endotoxin shock and indicated that *E. coli*-derived recombinant "murine MIF," which has an identical sequence to murine GIF, markedly increased lethality of mice when coinjected with lipopolysaccharide. Calandra *et al.* (17) also showed that the same recombinant peptide stimulated macrophages to form bioactive tumor necrosis factor α. Because most GIF/MIF peptide formed by the transformed *E. coli* is inactive GIF, which has affinity for Affi, it is quite likely that the biologic activities of the recombinant peptide are associated with the inactive GIF peptide.

Our experiments demonstrate that not only the bioactive GIF peptides formed by 231F1 cells but also the peptide formed by 2FH2 cells process I-J^b determinant recognized by mAb H6. Because the 2FH2 cells are a stable transfectant of hGIF cDNA in 231F1 cells, the transfectant should produce endogenous murine GIF. However, a substantial fraction, if not a majority, of the GIF peptide formed by the transfectant should be the product of hGIF cDNA (6). Binding of essentially all bioactive GIF peptide from the 2FH2 cells to H6–Affi (see Fig. 3*B*) indicates that the mAb binds not only bioactive murine GIF but also hGIF formed by the cells. In contrast, bioactive hGIF from the BTCH clone failed to bind to the mAb, although the primary structure of the rhGIF formed by this clone should be identical to that of 2FH2-derived rhGIF (6). Our results collectively indicate that the epitope recognized by H6 is generated during the process of posttranslational modification of the peptide in the H-2^{bd} hybridoma but does not represent a portion of amino acid sequence in the peptide.

These findings may help in solving controversies on I-J determinants. Initial genetic analysis located the genes encoding "I-J molecules" in a chromosomal segment that lies between I-A and I-E subregions of the major histocompatibility complex (18, 19). However, molecular cloning of the major histocompatibility complex showed that the nucleotide sequence in this region was identical in B10.A (3R) (I-J^b) and B10.A (I-J^k) mice, indicating that "I-J" gene does not exist in the region (20). Nakayama *et al.* (21) identified I-J molecules on T cells as a homodimer of the 42- to 46-kDa glycopeptide, but molecular cloning of the peptides has been unsuccessful. Tada *et al.* (22) indicated that I-J determinant associates with multiple heterogeneous molecules expressed on functionally different mature T cells. If the structures recognized by anti-I-J antibodies are those generated by posttranslational modifications, multiple peptides with different primary structures would be predicted to share a common I-J determinant. This hypothesis also agrees with recent findings by O'Hara *et al.* (23) on Ts cell factor activity obtained by transfection of T-cell receptor α chain cDNA that I-J restriction of the resulting Ts cell factor is determined by the recipient cells rather than by T-cell receptor α-chain gene. In any event, our findings, which imply that the I-J phenotype is determined by the cells involved

in posttranslational modification of a certain peptide rather than its amino acid sequence, clearly explain several unsolved problems of the I-J puzzle—particularly concerning the properties of the I-J determinant of Ts cell factor (7) and adaptive acquisition of I-J specificity in allogeneic chimera (24, 25)—and justify previous observations on the association of I-J determinant with Ts cell factor.

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