In vitro modulation of antigen-primed T cells by a glycosylation-inhibiting factor that regulates the formation of antigen-specific suppressive factors

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ABSTRACT BDF1 [C57BL/6 × DBA/2]F1 mice were primed with alum-absorbed ovalbumin, and their spleen cells were cultured with ovalbumin to activate antigen-primed T cells. The activated T cells were then propagated in interleukin 2-containing medium in the presence or absence of affinity-purified glycosylation-inhibiting factor (GIF). Upon incubation with ovalbumin-pulsed macrophages, T cells propagated in the presence of GIF produced IgE-suppressive factor and GIF. The ovalbumin-primed T cells propagated in the presence of GIF constitutively produced the 13-kDa GIF that lacked affinity for ovalbumin. However, stimulation of the same T cells with ovalbumin-pulsed macrophages resulted in the production of 80- and 35-kDa GIF that had affinity for ovalbumin. Both the antigen-specific GIF and nonspecific GIF from the activated BDF1 T cells had I-J\(^+\) determinants. Since the ovalbumin-specific GIF is derived from Lyt-2\(^+\), I-J\(^+\) ovalbumin-specific suppressor T cells and suppresses the anti-IgE antibody response to dinitrophenyl-coupled ovalbumin, the results strongly suggest that the presence of GIF during the propagation of antigen-primed T cells facilitates the generation of antigen-specific suppressor T cells.

We have described two types of T-cell factors that have affinity for IgE and selectively regulate the IgE response (1). One of the IgE-binding factors selectively enhances the IgE response, i.e., IgE-potentiating factors, while the IgE-binding factor selectively suppresses the response, i.e., IgE-suppressive factors (1). The IgE-potentiating factors have affinity for lentil lectin and concanavalin A, while IgE-suppressive factors fail to bind to the lectins. Subsequent experiments showed that IgE-potentiating factors and IgE-suppressive factors shared a common structural gene, and thus a common polypeptide chain (2, 3), and that their biological activities are determined by their carbohydrate moieties. Under physiological conditions, the carbohydrate moieties of IgE-binding factor are controlled by two T-cell factors, i.e., glycosylation-enhancing factor (GEF) and glycosylation-inhibiting factor (GIF), which either enhance or inhibit the attachment of N-linked oligosaccharides to the peptide (1). It was found that GIF had a molecular weight of \(\approx 15,000\), bound to monoclonal antibodies against lipocortin, a phospholipase inhibitory protein, and exerted phospholipase inhibitory activity upon dephosphorylation (4). However, subsequent studies in BDF1 mice revealed (5) that antigen-specific suppressor T cells released a GIF that had a molecular weight of \(\approx 35,000\) and had affinity for the antigen. GIF from BDF1 mouse T cells had I-J\(^+\) determinants. The results suggested that the antigen-specific GIF is related to antigen-specific suppressor factor, and GIF has been proven to have immunosuppressive effects (6). Repeated injections of the 13-kDa nonantigen-specific GIF from a rat-mouse T-cell hybridoma into antigen-primed BDF1 mice resulted in the suppression of both IgE and IgG antibody responses. Further studies on the mechanisms for the immunosuppression suggested that GIF injected into ovalbumin-primed mice facilitated the generation of antigen-specific suppressor T cells, which produced their own GIF that had affinity for ovalbumin (6), and that the ovalbumin-specific GIF suppressed the in vivo anti-IgE antibody response to dinitrophenyl-coupled ovalbumin (7). The present experiments were undertaken to reproduce this phenomenon in vitro. The results show that ovalbumin-primed T cells propagated in the presence of nonantigen-specific GIF produced antigen-specific GIF upon stimulation with ovalbumin-pulsed syngeneic macrophages.

MATERIALS AND METHODS

Animals, Antigen, and Antibodies. Female C57BL/6 × DBA/2F1 (BDF1) mice were purchased from Charles River Breeding Laboratories. Lewis strain rats were obtained from M. A. Bioproducts (Walkersville, MD). BDF1 mice were immunized by an intraperitoneal injection of 1 \(\mu\)g of crystalline ovalbumin (ICN) adsorbed to 1 mg of aluminum hydroxide gel, and their spleen cells were obtained 2 weeks after priming. At the time of sacrifice, the IgE anti-ovalbumin antibody titer of their serum was from 1:320 to 1:640 as determined by passive cutaneous anaphylaxis. Lewis strain rats were infected with 3000 third-stage Nipponstrongylus brasiliensis larvae (8).

Monoclonal rat IgE from immunocytoma IR 162 was the same preparation as that described (5). Monoclonal antibody against rabbit lipomodulin (lipocortin) was obtained from the mouse hybridoma 141B9 (9). Anti-Lyt-1.1 and anti-Lyt-2.1 monoclonal antibodies were purchased from New England Nuclear. Fluorescein-coupled monoclonal antibodies, anti-Lyt-1 (53-7.3) and anti-Lyt-2 (53-6.7), were purchased from Becton, Dickinson. Allantosinsera specific for I-J\(^+\) or I-J\(^+\) determinants were obtained from the National Institute of Allergy and Infectious Diseases. Goat antibodies against mouse IgG (polyvalent anti-MGG) were described (5). Crystalline bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH) were purchased from Sigma and Calbiochem–Behring, respectively. Rat IgE, monoclonal anti-lipomodulin antibodies, anti-MGG, ovalbumin, BSA, and

Abbreviations: GIF, glycosylation-inhibiting factor; GEF, glycosylation-enhancing factor; BSA, bovine serum albumin; KLH, keyhole limpet hemocyanin; MLN, mesenteric lymph nodes; IL-2, interleukin 2; MGG, goat anti-mouse immunoglobulin antiserum; Fc\(_R\), receptor for the immunoglobin e-chain Fc region.

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K.L. were coupled to Sepharose CL-4B (Pharmacia) (7) at 5–10 mg of protein per 1 ml of Sepharose.

**Purified GIF.** GIF was obtained from culture supernatants of the rat-mouse T-cell hybridoma 23A4 as described (6). Briefly, 1–1.5 liters of culture supernatant was filtered through XM50 Diaflo membranes (Amicon), and the filtrate was concentrated to 10 ml by using YM5 membranes. GIF in the filtrates was adsorbed to 4 ml of anti-lipomodulin-Sepharose and eluted with 0.1 M glycine hydrochloride, pH 3.0. The final volume of the affinity-purified GIF was 0.5% of the volume of the original culture supernatant. A 1:300 to 1:1000 dilution of the GIF preparation was sufficient to modulate the normal rat mesenteric lymph node (MLN) cells to form unglycosylated IgE-binding factors (see below).

**Cell Culture.** The culture medium was RPMI 1640 medium (M. A. Bioproducts) supplemented with 5% (vol/vol) fetal calf serum, 3 mM L-glutamine, 50 μM 2-mercaptoethanol, and antibiotics. Conditioned medium containing interleukin 2 (IL-2) was prepared by cultured spleen cells (5 x 10^6 nucleated cells per ml) for 2 days with concanavalin A at 4 μg/ml (Pharmacia), and the culture medium were passed through Sephadex G-100 to remove concanavalin A. The eluate was incubated for 1 hr at room temperature with 1 mM phenylmethylsulfonyl fluoride to inactivate GEF (10). After dialysis, 10–20 ml of the conditioned medium was incubated with 2 ml of anti-lipomodulin-Sepharose to adsorb GIF, and the GIF-free conditioned medium was stored at –70°C.

Antigen-primed T cells were activated by incubation of ovalbumin-primed spleen cells (5 x 10^6 cells per ml) for 3 days with ovalbumin at 10 μg/ml. To propagate the activated T-cell blasts, nonadherent cells recovered from the culture were resuspended in RPMI culture medium (5 x 10^6 cells per ml) and an equal volume of IL-2-containing medium was added to the cell suspension. When the activated T cells were propagated in the presence of GIF, a 2–4% volume of the affinity-purified GIF was added, and the cells were cultured for 4 days.

T cells propagated in IL-2-containing medium were stimulated with ovalbumin-pulsed syngeneic macrophages that were prepared as described (11). The lymphoblasts were suspended in fresh culture medium (1.5 x 10^6 cells per ml), and 5–6 ml of the cell suspension was cultured for 24 hr with 8 x 10^5 ovalbumin-pulsed macrophages.

**Cell Fractionation.** Antigen-activated T cells were enriched by passing the cell suspension through a nylon wool column. Subsets of T cells in the activated T-cell blasts were depleted by using anti-Lyt-1.1 and anti-Lyt-2.1 monoclonal antibody together with rabbit complement (Accurate Chemicals, Westbury, NY) as described (5).

**Detection of IgE-Binding Factor.** IgE-binding factor was assessed by inhibition of rosette formation of FcεR^+^ cells (cells with the receptor for the immunoglobulin e-chain Fc region) with rat IgE-coated ox erythrocytes (12). MLN cells of a rat infected with *N. brasiliensis* were used as a source of FcεR^+^ cells. The procedures for rosette inhibition have been described (13). The relative quantity of IgE-binding factor was expressed by the percentage of rosette inhibition, determined in duplicate. The experimental variation between duplicate tubes was less than ±10% of the average value.

**RESULTS**

Spleen cells of ovalbumin-primed BDF1 mice were cultured for 3 days with ovalbumin at 10 μg/ml or ovalbumin-pulsed syngeneic macrophages. The culture supernatant was incubated with IgE-Sepharose, and IgE-binding factor was recovered by elution at acid pH. Fractionation of the factors on lentil lectin-Sepharose showed that essentially all IgE-binding factor in the conditioned medium had affinity for the lectin (Table 1). Aliquots of the antigen-activated cells were cultured in IL-2-containing medium in the presence or absence of affinity-purified GIF from 23A4 cells. After 4 days of culture, the cells were recovered, resuspended in fresh culture medium, and incubated for 24 hr with ovalbumin-pulsed syngeneic macrophages. Culture supernatant from both cultures contained IgE-binding factor. However, fractionation of the factors on lentil lectin-Sepharose showed that GIF added during propagation of T cells affected the nature of IgE-binding factor formed. When T cells were propagated in the absence of GIF, the IgE-binding factor formed by the cells had affinity for lentil lectin. In contrast, the same T cells

<table>
<thead>
<tr>
<th>Cell</th>
<th>BDF1 cells</th>
<th>Lewis MLN cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original spleen cells</td>
<td>0/30</td>
<td>6/40 (GEF)</td>
</tr>
<tr>
<td>Cells propagated in IL-2</td>
<td>4/24</td>
<td>3/35 (GEF)</td>
</tr>
<tr>
<td>Cells propagated in IL-2/GIF</td>
<td>31/0</td>
<td>36/2 (GIF)</td>
</tr>
<tr>
<td>Medium control</td>
<td>27/30</td>
<td></td>
</tr>
</tbody>
</table>

*IgE-binding factors in culture filtrates were fractionated on lentil lectin-Sepharose. Numbers are the percentage of rosette inhibition by the unbound and bound fractions from lentil lectin-Sepharose. The percentage of rosette forming cells in the absence of IgE-binding factor was 2%.^1^ Culture filtrates were fractionated on IgE-Sepharose in the presence of 50 mM D-galactose, and the unbound material was added to normal Lewis MLN cells. The mixtures were cultured with rat IgE for 24 hr. IgE-binding factors formed by Lewis MLN cells were fractionated on lentil lectin-Sepharose to determine their distribution between the unbound and bound fractions. Based on this distribution, the culture supernatants had either GEF or GIF activity indicated in parentheses. The percentage of rosette forming cells in the absence of IgE-binding factor was 22.0 ± 0.5% in this assay.

^1^In the medium control, Lewis MLN cells were incubated with IgE alone.
propagated in the presence of GIF formed an IgE-binding factor that lacked affinity for the lectin (Table 1). The material that did not bind IgE-Sepharose and did not contain a detectable amount of IgE-binding factor was assessed for the presence of either GEF or GIF. As shown in Table 1, both the original spleen cells and antigen-stimulated T cells propagated in IL-2 alone formed GEF, whereas the activated T cells propagated in the presence of GIF formed GIF.

We next determined whether the GIF from the antigen-stimulated T cells had affinity for ovalbumin-Sepharose. Spleen cells of ovalbumin-primed mice were cultured with ovalbumin, and antigen-stimulated T cells were propagated in the presence of GIF, as described above. Aliquots of the cells were incubated for 24 hr with ovalbumin-pulsed syngeneic macrophages, with unpulsed macrophages, or with ovalbumin at 10 μg/ml. The culture supernatants were incubated with IgE-Sepharose, and unadsorbed material was fractionated on ovalbumin-Sepharose to determine the distribution of GIF between bound and unbound fractions. The results are summarized in Table 2. When the activated T cells were cultured with ovalbumin-pulsed macrophages, more of the GIF formed by the cells bound to ovalbumin-Sepharose and was eluted at acid pH. When the same cells were incubated either with syngeneic macrophages alone or with ovalbumin alone, GIF present in culture supernatants lacked affinity for ovalbumin-Sepharose. The same activated T cells incubated in culture medium also released GIF that lacked affinity for ovalbumin-Sepharose.

Experiments were carried out to confirm the antigenic specificity of GIF. Ovalbumin-primed T cells activated by the antigen were propagated in the presence of GIF, and the cells were incubated with ovalbumin-pulsed syngeneic macrophages. When conditioned medium was fractionated on ovalbumin-Sepharose, BSA-Sepharose, or KLH-Sepharose, GIF activity bound to ovalbumin-Sepharose but did not bind to BSA-Sepharose or KLH-Sepharose. The results indicate that ovalbumin-primed T cells propagated in the presence of GIF produce ovalbumin-specific GIF upon antigenic stimulation.

The molecular weights of antigen-specific GIF and non-specific GIF were estimated by gel filtration. Antigen-stimulated ovalbumin-primed T cells were propagated in IL-2-containing medium in the presence of GIF, and activated T cells were cultured for 24 hr with ovalbumin-pulsed macrophages or untreated macrophages. The culture supernatants were incubated with an anti-lipomodulin-Sepharose column. After washing with DPBS, the columns were eluted at acid pH, and the eluate fraction was applied to a TSK-G3000SW column. The distribution of GIF activity in the

Table 2. Formation of antigen-specific GIF by antigen stimulation

<table>
<thead>
<tr>
<th>Culture filtrate</th>
<th>Ovalbumin-pulsed macrophages</th>
<th>Macrophages</th>
<th>Ovalbumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfractionated</td>
<td>34/2(+)</td>
<td>38/6(+)</td>
<td>36/2(+)</td>
</tr>
<tr>
<td>Ovalbumin-Sepharose-unbound material</td>
<td>20/24</td>
<td>30/7(+)</td>
<td>32/4(+)</td>
</tr>
<tr>
<td>Ovalbumin-Sepharose-bound material</td>
<td>36/3(+)</td>
<td>23/24</td>
<td>22/24</td>
</tr>
<tr>
<td>Medium control*</td>
<td>25/24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Lewis MLN cells were incubated with IgE alone.

Activated T cells were incubated with ovalbumin-pulsed macrophages, macrophages, or ovalbumin alone, and GIF in culture filtrate was assessed by using Lewis MLN cells. Numbers represent the percentage of rosette inhibition by the unbound and bound fractions from lentil lectin-Sepharose. The proportion of rosette forming cells in the absence of IgE-binding factor was 23.0 ± 0.7%.

eluate fractions was determined by the ability of the fractions to inhibit the IgE-induced expression of FcR. As shown in Fig. 1, GIF activity in the antigen-stimulated cultures was eluted in fractions 26, 27, and 32–34, while GIF activity in unstimulated cultures was recovered in fractions 39–41. The fractions with GIF activity were pooled separately, and the pooled fractions were fractionated on ovalbumin-Sepharose. GIF activity in the two pooled fractions from antigen-stimulated cultures bound to ovalbumin-Sepharose and was recovered by acid elution. In contrast, GIF activity in fractions 39–41 from unstimulated cultures failed to bind to ovalbumin-Sepharose. The results indicate that antigen-specific GIF consists of two molecular weight species, 80–90 kDa and 35–40 kDa, while nonantigen-specific GIF from unstimulated cultures has a molecular weight of 13 kDa.

We then determined whether GIF from activated T cells of BDF1 mice had Jβ determinants. Antigen-activated T cells propagated in the presence of GIF were cultured with either ovalbumin-pulsed macrophages or untreated macrophages. The culture supernatants were incubated with the gammaglobulin fraction of either anti-I-Jβ and anti-I-Jδ alloantiserum, and the mixtures were incubated with anti-MGG-Sepharose. The results, shown in Table 3, indicate that both antigen-specific GIF and non-specific GIF bound to anti-I-Jβ antibodies but did not bind to anti-I-Jδ antibodies. We also determined whether the affinity-purified GIF from 23A4 cells, which was added to cultures of activated cells, might bind to anti-I-J antibodies. The GIF from 23A4 cells bound to anti-I-Jδ antibodies but not to anti-I-Jβ antibodies.

In view of previous findings that the major cell source of GIF was Lyt-2+ suppressor T cells (5), we determined whether depletion of Lyt-1+ cells in the activated T-cell population might affect the formation of ovalbumin-specific GIF. Ovalbumin-primed spleen cells were cultured with ovalbumin for 5 days, and antigen-activated T cells were propagated in the presence or absence of GIF. A portion of the activated T-cell blasts was depleted of Lyt-1+ cells, and both the unfractionated T cells and Lyt-1+ -depleted population were incubated with ovalbumin-pulsed macrophages. The culture supernatants were then assessed for IgE-binding factor and GIF. As shown in Table 4, the unfractionated T cells formed IgE-binding factor, while the Lyt-1+ -depleted population failed to form IgE-binding factor. The unbound fraction from IgE-Sepharose was applied to ovalbumin-Sepharose, and the bound and unbound material from both columns was assessed for GIF activity. It is apparent in Table 4 that the Lyt-1+ -depleted population of T cells propagated in the presence of GIF formed ovalbumin-specific GIF upon antigenic stimulation, while the same fraction of T cells propagated in the absence of GIF failed to do so. The results indicate that nonspecific GIF facilitated the generation of Lyt-2+ T cells that formed ovalbumin-specific GIF upon antigenic stimulation.

**DISCUSSION**

Data presented in this paper show that addition of nonantigen-specific GIF to antigen-primed T cells during proliferation facilitated the generation of antigen-specific T-cell populations that produce their own GIF. Affinity-purified GIF added to the cultures possessed I-Jβ determinant(s), while GIF detected in the culture of antigen-activated cells had I-Jβ determinants. The results exclude the possibility that GIF detected in the latter culture is a carry-over of added GIF. Furthermore, ovalbumin-activated T cells cultured with GIF formed GIF molecules having affinity for ovalbumin when the cells were cultured with ovalbumin-pulsed syngeneic macrophages. Neither ovalbumin alone nor macrophages alone induced the formation of ovalbumin-specific GIF. It was also found that ovalbumin-
pulsed semisyngeneic macrophages from BALB/c or C57BL/6 mice, but not ovalbumin-pulsed allogeneic macrophages from CBA mice, could induce the formation of ovalbumin-specific GIF (results not shown). These findings indicate that major histocompatibility complex-restricted antigen-primed T cells are involved in the formation of ovalbumin-specific GIF.

Studies on BDF1 mice have shown (5) that antigen-specific Lyt-2+, I-J+ suppressor T cells are the major cell source of antigen (ovalbumin)-specific GIF. The present experiments show that the depletion of Lyt-1+ cells in the antigen-activated T-cell population removed the cell source of IgE-binding factor but failed to affect the formation of ovalbumin-specific GIF (Table 4). Since the Lyt-1+ -depleted population of T cells from GIF(−) cultures failed to form a detectable amount of GIF, it appears that GIF added during propagation of antigen-activated T cells facilitated the selective expansion of antigen-specific, Lyt-2−-suppressor T cells that produce GIF.

Table 3. Association of I-J+ determinant(s) with GIF

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IgE-binding factors, % rosette inhibition</th>
<th>Ovalbumin-pulsed macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unabsorbed</td>
<td>36/5 (+)</td>
<td>40/3 (+)</td>
</tr>
<tr>
<td>Anti-I-J+</td>
<td>23/25</td>
<td>22/24</td>
</tr>
<tr>
<td>Anti-I-J-</td>
<td>38/5 (+)</td>
<td>37/0 (+)</td>
</tr>
<tr>
<td>Medium control</td>
<td>25/28</td>
<td>25/24</td>
</tr>
</tbody>
</table>

Activated T cells were incubated with either macrophages or ovalbumin-pulsed macrophages. Aliquots of culture filtrates were incubated with either anti-I-J+ or anti-I-J− alloantiserum, and the mixtures were adsorbed to anti-MGG-Sepharose. The concentration of the antiserum in the mixtures was 2% of that of the original antiserum. The unbound material from anti-MGG-Sepharose was assessed for GIF by using Lewis MLN cells. Numbers represent the percentage of rosette inhibition by the unbound and bound fractions from lentil lectin-Sepharose. The proportion of rosette forming cells in the absence of IgE-binding factor was 25.2 ± 1.2%. (+), the presence of GIF.

Table 4. GIF enhances the generation of ovalbumin-specific Lyt-2+ cells that produce ovalbumin-specific GIF

<table>
<thead>
<tr>
<th>Addition to medium</th>
<th>IgE-binding factors, % rosette inhibition</th>
<th>Ovalbumin-Sepharose bound</th>
<th>Unbound</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIT/IL-2</td>
<td>Unfractionated</td>
<td>33</td>
<td>24/26</td>
</tr>
<tr>
<td>Lyt-1−-depleted</td>
<td>0</td>
<td>29/24</td>
<td>35/3 (+)</td>
</tr>
<tr>
<td>IL-2</td>
<td>Unfractionated</td>
<td>37</td>
<td>29/28</td>
</tr>
<tr>
<td>Lyt-1−-depleted</td>
<td>0</td>
<td>26/28</td>
<td>24/23</td>
</tr>
</tbody>
</table>

Ovalbumin-primed spleen cells were activated with ovalbumin, and activated T cells were propagated in the presence or absence of GIF. T cells were cultured with ovalbumin-pulsed macrophages, and the culture supernatants were incubated with IgE-Sepharose. The eluates were assessed for IgE-binding factor. Numbers represent the percentage of rosette inhibition by the eluates. Unbound material from IgE-Sepharose was further fractionated on ovalbumin-Sepharose, and modulators in the unbound and bound fractions were assessed. Numbers show the distribution of IgE-binding factor between the unbound and bound fractions from lentil lectin-Sepharose.

Fig. 1. Gel filtration of antigen-specific GIF (●) and nonspecific GIF (○) through a TSK-G3000SW column. GIF activity in each fraction was detected by the ability to inhibit the expression of FcγR. Aliquots of MLN cells from an N. brasiliensis-infected rat were incubated overnight with rat IgE in the presence of each fraction, and the proportion of FcγR+ cells in the cell suspensions were assessed. As controls for the detection of ovalbumin-specific GIF, MLN cells were incubated with IgE (●) or without IgE (○), and the proportion of FcγR+ cells was determined. Similar controls for the detection of nonspecific GIF were shown as (▲) and as (△). Molecular sizes of standards in kDa are shown. Appropriate fractions (I–IV) were pooled and tested for the presence of GIF by their ability to stimulate normal MLN cells to form unglycosylated IgE-binding factor. The results are shown (Lower). The presence (+) or absence (−) of GIF is indicated.
antigen-specific GIF. These findings would explain why GIF injections into ovalbumin-primed BDF1 mice induced the generation of ovalbumin-specific suppressor T cells in vivo (6).

The antigen-specific GIF obtained by antigenic stimulation of activated T cells has immunologic properties similar to antigen-specific suppressive factor (TsF). Jardieu et al. (16) established the ovalbumin-specific T-cell hybridoma clone 231F1, which produces IgE-suppressive factors and IgG-suppressive factors upon incubation with ovalbumin-pulsed syngeneic macrophages. Subsequent experiments by the same investigators showed that this T-cell hybridoma constitutively secreted the 15-kDa nonspecific GIF, but incubation of the cells with ovalbumin-pulsed syngeneic macrophages resulted in the formation of ovalbumin-specific GIF (7). It was found that the ovalbumin-specific GIF from the hybridoma was more effective than nonspecific GIF from the same cells in the suppression of in vivo anti-hapten antibody responses of BDF1 mice to dinitrophenol-coupled ovalbumin, but that the two GIF preparations had comparable and weak suppressive effects on the anti-hapten antibody response to dinitrophenol-coupled KLH. It was also found that the ovalbumin-specific GIF from the hybridoma was composed of two molecular weight species, 80–90 kDa and 35–40 kDa, and that the 80- to 90-kDa ovalbumin-specific GIF may be responsible for the carrier (ovalbumin)-specific suppressive effects (7). It should be noted that this species of antigen-specific GIF shares properties with antigen-specific suppressive factor. They both have similar molecular weights, I-J determinants, and affinity for antigen (17, 18). The present experiments show that the same species of ovalbumin-specific GIF is formed by antigen-specific T cells propagated in the presence of nonspecific GIF and strongly suggest that GIF facilitates the generation or selective proliferation of antigen-specific suppressor T cells.

We do not know the mechanism through which GIF facilitates the expansion of antigen-specific, Lyt-2+ T-cell populations. Nevertheless, this phenomenon may be related to a well-known suppressor T-cell circuit in which antigen-specific suppressive factor 1 (TsF1) and antigen induces the generation of second-order antigen-specific suppressor T cells (Ts2), which produce antigen-specific suppressive factor (TsF2) (19). The association of I-J determinants with lipocortin has been shown, which was induced by incubation of mouse T cells with glucocorticoids (15). Since the 15-kDa nonspecific GIF is a fragment of phosphorylated lipocortin (4), one might speculate that the phospholipase inhibitory activity of GIF plays an important role in the generation of antigen-specific suppressor T cells.

This work was supported by Research Grants AI-14784 and AI-11202 from the Department of Health and Human Services. This paper is Publication 665 from the O’Neill Laboratories of the Good Samaritan Hospital.