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ISOLATION AND PARTIAL CHARACTERIZATION OF MESSENGER RNA, FROM MURINE T CELL HYBRIDS, CODING FOR SUPPRESSIVE IMMUNOGLOBULIN G-BINDING FACTOR[1]

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Poly A RNA has been isolated from a murine T cell hybridoma (T_2D_4) that spontaneously secretes suppressive immunoglobulin G-binding factor (IgGBF). Translation products, obtained from a rabbit reticulocyte lysate translation system and after injection into *Xenopus laevis* oocytes, contain material with the biologic activity, the affinity, and the m.w. of murine IgGBF; it suppresses secondary *in vitro* IgG antibody production in a dose-dependent fashion. The suppressive factor binds to IgG but not to IgM immunoadsorbents and, after mild $NaDodSO_4$ treatment, dissociates in $NaDodSO_4$ polyacrylamide gels into two peaks at 78 and 40 kD. Translation products from two non-IgGBF-secreting cell lines (BW-5147, a T lymphoma line, and A_9, a fibroblast cell line) fail to exert any suppressive activity. On sucrose gradients, the RNA responsible for the biologic activity was found in one major peak located at 11S. IgGBF synthesized in a cellfree translation system by using poly A RNA and sucrose gradient fractions was also characterized by immunoprecipitation with Fc fragments of [^{35}S]methionine-labeled proteins. On $NaDodSO_4$ polyacrylamide gels, it migrates in one peak located at 37 kD. We conclude that IgGBF is coded for by 11S poly A RNA and that no post-translational modifications (other than proteolytic cleavage) are necessary to obtain a biologically active factor with Ig-binding properties.

The production of Ig classes and subclasses by B cells is regulated by T cell factors (1), among which immunoglobulin-binding factors (IBF)[3] may play a major role (2). Produced by Fc receptor (FcR)-positive T cells, these factors bind specifically to the Fc portion of Ig and modulate the *in vitro* production of the corresponding Ig isotype. Three different isotype-specific T cell factors have been successively described. First, the IgG-binding factor (IgGBF) produced by Fcγ receptor-positive T cells (3) binds specifically to the Fc portion of IgG (4) and suppresses the production of IgG antibodies in secondary *in vitro* antibody responses (5). Second, the IgE-binding factors (IgEBF) produced by Fcϵ receptor-positive T cells bind specifically to IgE and, depending on their glycosylation, enhance (6) or inhibit (7) production of IgE antibodies. More recently, an IgA-binding factor (IgABF) that binds specifically to IgA and suppresses the IgA *in vitro* antibody response has been described (8). Thus, these factors share common properties: they bind specifically to a given class of antibody and regulate the production of antibodies of the corresponding class.

During these studies, it became apparent that the production of isotype-specific IBF could be specifically induced by the corresponding Ig isotype. For instance, incubation of T cells with IgE leads to the production of IgEBF (9). In an attempt to obtain a homogeneous and unlimited source of IgGBF, T cell hybrids were prepared between alloantigen-activated T cells and BW-5147 T lymphoma cells (10). These hybrids express FcγR and FcαR and produce a factor(s) that, by all criteria tested (biologic activity, affinity, and m.w.), appear constitutively similar to T cell-secreted IgGBF (2, 10). Recent experiments have shown that incubation of these T cell hybrids (T_2D_4) in the presence of IgG1, IgG2, or IgA monoclonal proteins induces the production of high amounts of isotype-specific IBF that suppress IgG1, IgG2, or IgA antibody production, respectively (8, 11).

In this paper, we present the isolation and characterization of mRNA coding for a suppressor IBF. We show that the poly A RNA extracted from T_2D_4 T cell hybrids can be specifically translated, in reticulocyte lysates and in oocytes, into polypeptides having a biologic activity, an affinity, and a m.w. that are similar to cell-secreted IgGBF. After separation on sucrose gradient, IgGBF mRNA was found in one major peak located at 11S.

MATERIALS AND METHODS

Cell lines. Three different cell lines were used to prepare mRNA: an IgGBF-producing T hybridoma line T_2D_4 of H-2^k haplotype (10), and two nonproducing lines, BW-5147 used as fusion partner for hybridization (10) and A_9, a fibroblast cell line of H-2^k (C3H) haplotype (kindly provided by Dr. R. Cassingena, Villejuif). To prepare T cell hybrids, spleen cells (95% Thy-1.2-positive, 30% FcγR-positive) from BALB/c (H-2^d) irradiated mice (850 R) inoculated 5 days earlier with thymocytes from a B10-BR (H-2^k) strain were fused, in the presence of polyethylene glycol, with hypoxanthine-aminopterin-thymidine (HAT)-sensitive T lymphoma cells, BW-5147 (H-2^k, Thy-1.1, FcγR-negative), as described (10). After selection in the presence of HAT, FcγR-positive T cell hybrids were grown in RPMI 1640

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[2] Address correspondence and reprints requests to: Dr. C. Neauport-Sautes, Unité INSERM U 255, Institut CURIE, 26, rue d'Ulm, 75005-PARIS
[3] Abbreviations used in this paper: IBF, immunoglobulin-binding factor; FcR, receptor for the Fc portion of Ig; β-ME, β-mercaptoethanol; kD, kilodaltons; IgGBF, IgEBF, IgABF, immunoglobulin-binding factors for IgG, IgE, and IgA.

medium (GIBCO, France) containing streptomycin and penicillin (100 U/ml) and supplemented with 10% fetal calf serum (FCS). The T_2D_4 cell line expresses FcγR and produces suppressive IgGBF (10). T_2D_4 and BW-5147 cells were grown in ascitic form in pristane-treated Swiss Nu/Nu mice (Biotem, France).

Preparation of IgG, Fc fragments, and immunoadsorbents. Rabbit IgG was isolated from normal serum by precipitation in the presence of 40% $(NH_4)_2SO_4$ followed by ion exchange chromatography on DEAE cellulose (DE 52 Whatman) in 0.02 M phosphate buffer, pH 7.2.

Fc and Fab fragments were obtained by digestion of rabbit IgG in the presence of preactivated papain (enzyme to protein ratio of 1%) for 2 hr at 37°C in 0.1 M phosphate buffer, pH 8. They were separated from undigested IgG by chromatography on Sephadex G-100 columns and then purified by affinity chromatography on protein A-coupled Sepharose (Pharmacia, France) as described (12). Immunoadsorbents of rabbit IgG and Fab fragments were prepared by coupling cyanogen bromide-activated Sepharose 4B (Pharmacia) with rabbit IgG, 7 mg/ml, or Fab fragments, 3 mg/ml, as described (4).

Isolation and sucrose gradient analysis of mRNA. Total RNA was isolated from T_2D_4, BW-5147, and A_9 cells by lysis in 4 M guanidium thiocyanate and centrifugation of the homogenate through a 5.7 M cesium chloride cushion as described (13). Poly A RNA was purified on an oligo-dT column (type 7; PL Biochemical) Milwaukee, WI, USA) (14). Poly A RNA, 250 μg, from T_2D_4 cells was fractionated on a 15 to 30% sucrose gradient following the procedure described (13). Each of the 18 fractions were ethanol-precipitated, washed, dissolved in 20 μl of sterile triple-distilled water, and kept frozen in liquid nitrogen. Ribosomal RNA, tRNA, and globin mRNA were sedimented in parallel tubes and were used as size markers.

Translation of mRNA. Total poly A RNA and poly A RNA sucrose-gradient fractions were translated in a micrococcal nuclease-treated reticulocyte lysate (15). Incubation was performed with 0.5 μl (0.25 μg) RNA in a final volume of 12.5 μl, in the presence of 2 to 5 μCi of [^{35}S]methionine (specific activity, 1000 Ci/mM, Amersham, France). Total incorporation into proteins was determined in 2-μl samples. Ten microliters of the radioactive peptides were analyzed on a 0.1% $NaDodSO_4$ linear (10 to 20% polyacrylamide gradient) gel under reducing conditions (16). When reticulocyte lysate translation products were prepared for biological tests, incubation was performed with 4 μl (2 μg) of RNA in a final volume of 100 μl in the absence of [^{35}S]methionine, and the translation products were assayed directly or after passage on IgG immunoadsorbents.

Total and fractionated poly A RNA were also microinjected into *Xenopus laevis* oocytes as described (13). Approximately 50 ng of RNA were microinjected into each of 20 oocytes. Oocytes were incubated for 40 hr at 20°C in Barth's solution (10 μl/oocyte). The incubation medium was finally withdrawn and stored frozen at −70°C in 1% bovine serum albumin (BSA) until it was assayed for suppressive activity.

Immunoprecipitation. Twenty microliters of [^{35}S]methionine-labeled reticulocyte translation products were diluted in the same volume of 1 M KCl, 0.1 M Tris, pH 8.8, 2% Triton X-100 with 2 μl of phenylmethylsulfonyl fluoride (4.5 mg/ml in ethanol) and were incubated for 2 hr at 25°C in the presence of 4 μl of Fc fragment (400 μg/ml) with occasional agitation. Then 45 μl of protein A-Sepharose (Pharmacia CL-4B) (160 mg/ml) pre-equilibrated in the same buffer were added and incubated overnight at 4°C. The sediments were washed five times with 0.5 M KCl, 0.01 M Tris, pH 8.8, 0.5% Triton X-100 and twice with 0.01 M Tris, pH 8.8. Occasionally, samples were submitted to a protein A-Sepharose or Fab-Sepharose clearance for 2 hr at 25°C before the addition of Fc fragments (4 μl) and were then processed following the same procedure. Finally, sediments were suspended in 40 μl of electrophoresis sample buffer (1% $NaDodSO_4$, 250 mM β-mercaptoethanol (β-ME)) and boiled for 5 min. All supernatants were then applied to $NaDodSO_4$ polyacrylamide (10 to 20%) slab gels. After electrophoresis, the gels were subjected to fluorography, dried, and autoradiographed.

Isolation of IgGBF. Isolation of IgGBF was performed by affinity chromatography by using a batch procedure. Translation products, 100 μl, were incubated with 50 μl of Sepharose 4B coupled with rabbit IgG for 16 hr at 4°C. Effluents were recovered, and the immunoadsorbent was then washed five times with 20 vol of sodium phosphate buffer, 0.02 M, pH 7.0. Material bound was eluted by using three successive incubations with 100 μl of 0.2 M glycine-HCl buffer, pH 2.8, at 4°C, and was then neutralized with 1 M KH_2PO_4. Effluents and eluates were dialyzed in phosphate-buffered saline (PBS), pH 7.2. The dilution factor due to the elution procedures was taken into account to evaluate the final dilution of products added to *in vitro* cultures. To prepare cell-secreted IgGBF, T_2D_4 cells cultured *in vitro* were incubated at 2×10^6 cells/ml at 37°C in balanced salt solution (BSS) for 2 hr.

Supernatants were collected and passed on IgG immunoadsorbents as described (4). Acid eluates were concentrated 100-fold (as compared with the starting material) on centriflow CF 25 membranes (Amicon, France), dialyzed in PBS, sterilized by passage through 0.22-μm Millipore filters, and used as source of purified IgGBF.

Secondary in vitro antibody response. Spleen cells from mice, inoculated i.p. 10 days before with 2×10^8 sheep red blood cells (SRBC), were cultivated in Falcon plastic tubes at 7×10^6 cells/ml in RPMI 1640 medium (GIBCO, France) containing 100 U/ml penicillin, streptomycin, and 10% fetal calf serum (FCS). Twenty microliters of SRBC, 2×10^8 cells/ml, were added to the 1-ml cultures at day 0 as described (5). After five days of incubation in a 5% CO_2, humidified atmosphere, indirect and direct plaque-forming cells (PFC) were measured by using the local hemolysis technique, as described (17), that makes use of guinea pig serum as a source of complement. Indirect plaques were revealed by using a rabbit anti-mouse IgG antiserum. Translation products, effluents, and acid eluates were added to cell cultures at different dilutions at day 0. Reticulocyte lysates, incubated in the absence of mRNA, and supernatants from oocytes injected with triple distilled water were used as negative controls.

One-dimensional $NaDodSO_4$ polyacrylamide gel electrophoresis (PAGE). Electrophoresis was performed on slab gels (1.2 mm thick) in 0.1% $NaDodSO_4$ by using the Laemmli buffer system (18). One-hundred microliters of T_2D_4 poly A mRNA translation products (obtained from reticulocyte lysates) and 100-times concentrated, purified IgGBF from T_2D_4 cells were treated with 0.1% $NaDodSO_4$ for 20 min at 0°C. After addition of glycerol (1/3 final dilution), samples were applied onto 10-cm high 12.5% polyacrylamide gels and were run at 3 mA/cm of gel in parallel with a mixture of ^{125}I-labeled BSA, IgG heavy and light chains, and phenol red. After migration, vertical bands corresponding to each sample were cut and then sliced into 0.45-cm slices. Each slice was homogenized and incubated for 16 hr at 0°C with 0.5 ml of RPMI supplemented with 10% FCS. After centrifugation, the incubation medium was recovered and sterilized through 0.22-μm Millipore filters before addition to cell cultures. Bands corresponding to the labeled markers were cut similarly and were directly counted in a gamma counter (LKB, France).

RESULTS

Presence of biologically active IgGBF in translation products of poly A RNA from T_2D_4 cells. Poly A RNA was isolated from an IgGBF-producing T hybridoma line T_2D_4 and from two nonproducing lines, BW-5147, the T lymphoma line that was used as a fusion partner for hybridoma preparation, and A_9, a fibroblast cell line. The suppressive activity of the translation products was tested in secondary *in vitro* anti-SRBC responses. Translation products were added at day 0 of the spleen cell cultures, and the IgG indirect plaque response was measured at day 5.

In the first set of experiments, rabbit reticulocyte lysates were used as an *in vitro* translation system, and incubation products were tested for suppressive activity in a 1/100 final dilution. As shown in Figure 1*A*, proteins encoded by mRNA from T_2D_4 cells exerted a strong inhibitory effect on the secondary *in vitro* antibody response (90% inhibition of the IgG indirect plaque response). In contrast, proteins encoded by mRNA from BW-5147 T lymphoma cells and A_9 cells did not significantly modify IgG antibody production. Reticulocyte lysates, when used alone in a 1/100 final dilution, had no effect on the response.

In the second set of experiments, *X. laevis* oocytes were used as a translation system and were injected with poly A RNA from T_2D_4 or BW-5147 cells. Supernatants obtained after a 40 hr incubation period were added at a 1/100 final dilution to spleen cell cultures. As shown in Figure 1*B*, supernatants of oocytes injected with BW-5147 mRNA did not modify the secondary anti-SRBC response, whereas supernatants of oocytes injected with

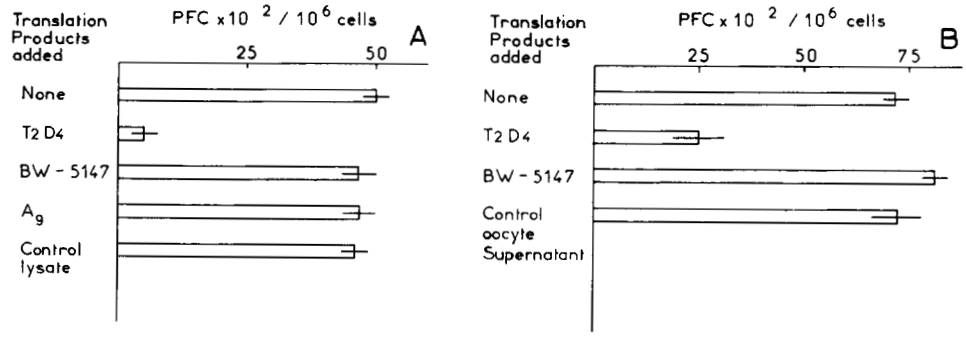


Figure 1. Effect, on the secondary *in vitro* anti-SRBC response of translation products obtained from reticulocyte lysates (panel A) and in oocytes supernatants (panel B). Products added to cell cultures at 1/100 final dilution at day 0. Indirect IgG PFC measured at day 5 (direct PFC response: panel A, 250 ± 20 PFC/10^6 cells; panel B, 280 ± 32 PFC/10^6 cells). Control lysate, reticulocyte lysate incubated in the absence of mRNA; control oocyte supernatant, supernatant of water-injected oocytes.

T_2D_4 mRNA exerted a significant inhibition of the IgG anti-SRBC plaque response (57% inhibition of the indirect PFC). Indeed, supernatants of water-injected oocytes did not exert any inhibitory effect (Fig. 1*B*).

Therefore these results show that the *in vitro* and *in vivo* translation products of mRNA, isolated from an IgGBF-producing T lymphoma line but not those from two nonproducing cell lines, exert suppressive effects on secondary IgG *in vitro* antibody responses.

To investigate whether IgGBF was, at least in part, responsible for this inhibition, translation products were incubated with immunoadsorbents of Sepharose 4B coupled with rabbit IgG, and the suppressive activities of the acid eluate, effluent, and starting material were compared. As shown in Figure 2, acid eluates, which contain translation products binding to IgG, were found to have enriched suppressive activity when compared with the starting material. At 1/1000 final dilution, the acid eluate and the starting material inhibited 100 and 46% of the response, respectively, whereas the effluent exerted no significant suppressive effect (25% inhibition of the response). These data show that the suppressive activity present in *in vitro* translation products is dose dependent and can be enriched after passage on IgG immunoadsorbents. When tested at the same dilutions, acid eluates of control lysates (incubated without mRNA) passed on IgG immunoadsorbents did not significantly modify the response (5 to 15% inhibition). In addition, when translation products were passed on Sepharose 4B coupled with IgM, the suppressive activity was found in the effluent (54% inhibition of the response at a 1/500 final dilution) but not in the eluate (no inhibition of the response at the same dilution); these results indicate that, like cell-secreted IgGBF, the translated suppressive factor(s) does not bind to IgM (data not shown).

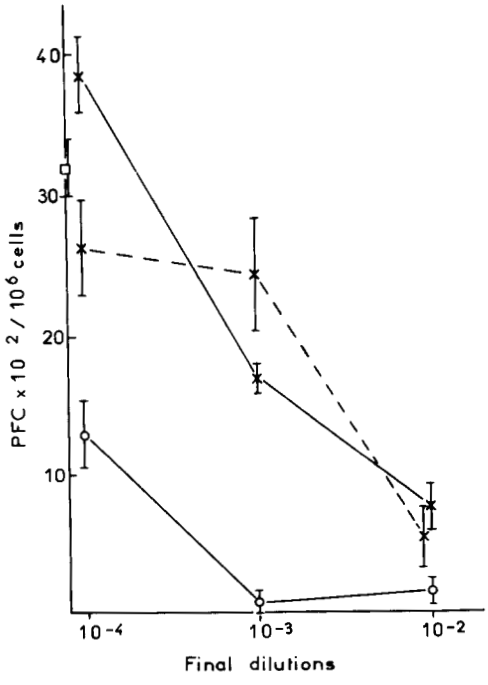


Figure 2. Dose-response curve and retention, on IgG immunoadsorbent of the suppressive activity of T_2D_4 poly A RNA translation products synthesized in reticulocyte lysates and tested on the secondary *in vitro* anti-SRBC response. Products added to cell cultures at day 0. Indirect IgG PFC measured at day 5. x——x, starting material; O——O, acid eluate; x----x, effluent; □, control response. (Direct PFC response: 450 ± 48 PFC/10^6 cells).

In conclusion, these results provide evidence that poly A RNA from T_2D_4 cells specifically encodes for protein(s) having biologic and affinity properties similar to IgGBF.

The m.w. of suppressive IgGBF synthesized in reticulocytes is similar to that of cell-secreted IgGBF. The M_r of the suppressive material encoded by T_2D_4 mRNA was compared with that of IgGBF secreted by T_2D_4 cells. For this purpose, acid eluates from IgG immunoadsorbents incubated with serum-free supernatants of T_2D_4 cells were prepared and used as a source of cell-secreted IgGBF. Before electrophoresis, purified IgGBF and *in vitro* translation products of T_2D_4 poly A RNA were treated by mild $NaDodSO_4$ conditions (0.1% $NaDodSO_4$) that did not noticeably alter their biologic activity (Fig. 3*A* and *B*, right lane).

After gel electrophoresis of translation products, proteins with suppressive activity dissociated into two peaks, corresponding to M_r 78 kD and 40 kD (Fig. 3*A*). Similarly, IgGBF from T_2D_4 cells gave two peaks of suppressive activity located at 75 kD and 40 kD (Fig. 3*B*). Indeed, no significant suppressive activity was found at M_r between 50 and 35 kD, if translation products of BW-5147 mRNA were run on gels and were tested under the same conditions (11% inhibition of the indirect PFC response).

Characterization of mRNA encoding for IgGBF. To better characterize the IgGBF mRNA, T_2D_4 poly A RNA was fractionated by sucrose gradient centrifugation under nondenaturing conditions, and the biologic activities of each fraction were evaluated after translation into oocytes (Fig. 4) and reticulocyte lysates (Fig. 5).

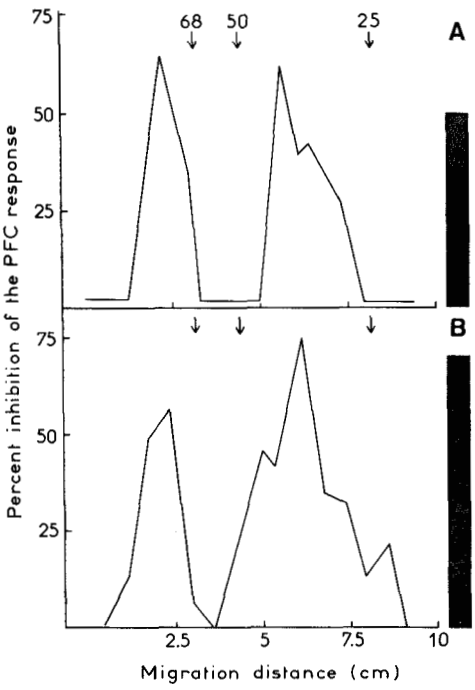


Figure 3. $NaDodSO_4$ PAGE analysis under mild denaturing conditions of the suppressive activity of T_2D_4 poly A RNA translation products (obtained in reticulocyte lysates) (Fig. 3*A*) and of purified IgGBF (prepared from supernatants of T_2D_4 cells) (Fig. 3*B*) on the secondary *in vitro* anti-SRBC response. Translation products and IgGBF were run on 12.5% acrylamide slab gels. Material eluted from each of the 20 slices was added to cell cultures at 1/20 final dilution at day 0. Results are expressed as percent inhibition of the indirect IgG PFC response measured at day 5. Indirect responses: panel A, 2700 ± 540 PFC/10^6 cells; panel B, 3220 ± 108 PFC/10^6 cells. Direct responses: panel A, 480 ± 60 PFC/10^6 cells; panel B, 200 ± 25 PFC/10^6 cells. Right lanes, ■ percent inhibition of the indirect IgG PFC response obtained with 0.1% $NaDodSO_4$-treated starting material (scale shown on the left of the figure). Molecular weight marker proteins are indicated on the top of the figure.

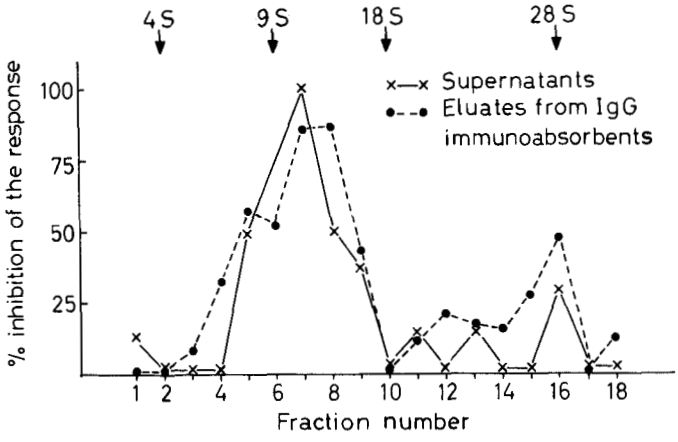


Figure 4. Immunosuppressive activity associated with the secondary *in vitro* anti-SRBC response of oocyte translation products of T_2D_4 poly A RNA sucrose gradient fractions. Supernatants from oocytes microinjected with each sucrose gradient fraction (x——x) or acid eluates from supernatants passed on IgG immunoadsorbents (●– –●) were added at 1/50 dilution at day 0 to cell cultures. Results are expressed as percent inhibition of the indirect IgG PFC response measured at day 5.

The translation products secreted in supernatants of injected oocytes were assayed for biologic activity at a 1/50 final dilution. As shown in Figure 4, two peaks of suppressive activity could be found: a) a major peak at fraction 7 (11S), which exerts 100% inhibition of the IgG anti-SRBC secondary response at a 1/50 dilution and 70% inhibition at a 1/500 dilution (data not shown), and b) a minor peak at fraction 16, which exerts only 30%

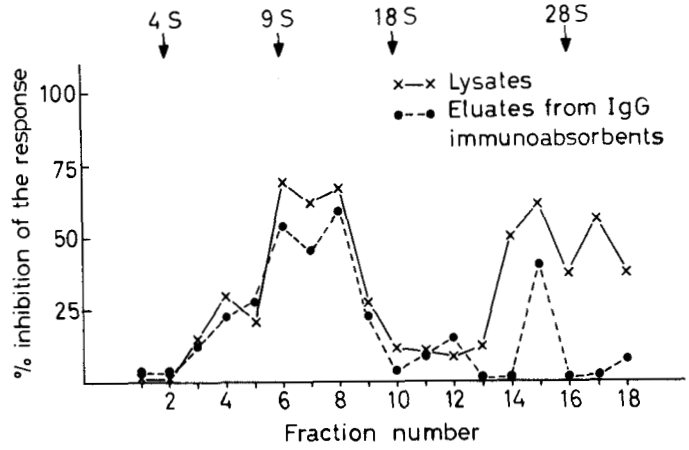


Figure 5. Immunosuppressive activity associated with the secondary *in vitro* anti-SRBC response of reticulocyte translation products of T_2D_4 poly A RNA sucrose gradient fractions. Reticulocyte products obtained with each sucrose gradient fraction (x——x) and acid eluates (●–●) from reticulocyte products passed on IgG immunoadsorbents were added to cell cultures at 1/250 final dilution at day 0. Results are expressed as percent inhibition of the indirect IgG PFC response measured at day 5.

inhibition and had no effect at higher dilutions (1/100 and 1/500; data not shown).

To test whether IgGBF was responsible for suppressive activity, translation products obtained from each fraction of mRNA were passed on IgG immunoadsorbents. When acid eluates were tested for biologic activity at a 1/50 final dilution, the two peaks corresponding to fractions 5 to 9 and 16 were recovered. Again, the inhibition was more pronounced in the first peak (80% inhibition with fraction 7) when compared with the second (45% inhibition with fraction 16).

Translation products obtained from reticulocyte lysates, as well as from their corresponding acid eluates from IgG immunoadsorbents, were also tested for suppressive activity, at a 1/250 final dilution, in secondary *in vitro* anti-SRBC responses. As shown in Figure 5, two peaks of suppressive activity were found, located in fractions 6 to 9 and 14 to 18. When acid eluates were tested at a 1/1000 final dilution, the first peak still inhibited 30 to 50% of the IgG anti-SRBC response, whereas the second peak no longer exerted suppressive activity (data not shown).

When considered together, these results suggested that mRNA coding for biologically active IgG-binding factor is located principally in fractions 5 to 9 that peak at 11S. Some biologic activity that might be due to aggregates of mRNA was also found in a lesser extent in heavier mRNA fractions.

Immunochemical identification of the translation products connected to IgGBF immunosuppressive activity. Because IgGBF binds to the Fc portion of IgG, attempts were made to identify translation products of total and fractionated T_2D_4 mRNA by using immunoprecipitation. For this purpose, the radioactive translation products of reticulocyte lysates incubated in the presence of poly A RNA from IgG-producing cells (T_2D_4) and non-producing cells (BW-5147 and A_9) were immunoprecipitated with Fc fragments of IgG followed by protein A-Sepharose. The preparations were then boiled and run on gels. When these methods were used, the products of the three different messengers gave bands located mainly at M_r around 45, 37 to 35, and 20 to 16 kD (Fig. 6). The

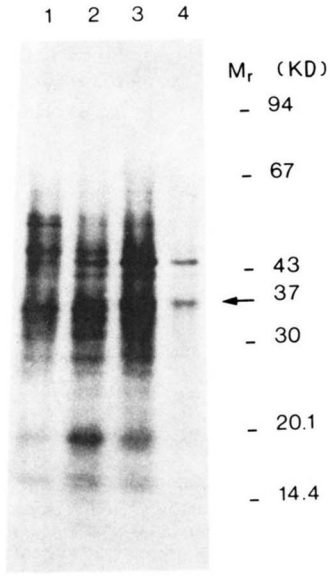


Figure 6. Immunoprecipitation of reticulocytes translation products of T_2D_4 poly A RNA with Fc fragments as analyzed by $NaDodSO_4$ PAGE. [^{35}S]methionine-labeled translation products of poly A RNA from A_9 (lane 1), BW-5147 (lane 2), and T_2D_4 cells (lanes 3 and 4) were immunoprecipitated with Fc fragments followed by protein A-Sepharose, before (lanes 1–3) or after (lane 4) clearance with Fab-Sepharose and run on 10 to 20% acrylamide slab gels. Molecular weight marker proteins are shown on the right of the figure. Arrow indicates the 37-kD polypeptide.

45-kD band probably corresponded to actin, because this band was also present in immunoprecipitates performed with anti-actin antiserum (data not shown).

The 45-, 35-, 20-, and 16-kD polypeptides were present in immunoprecipitates from T_2D_4, BW-5147, and A_9 cells as well; their presence probably reflects nonspecific binding to the immunoadsorbent. The 37-kD polypeptide, however, was only found in the products of T_2D_4 messengers. After clearance with Fab-Sepharose, only two major bands were observed in T_2D_4 immunoprecipitates. They were located at M_r 45 kD and 37 kD and thus presumably correspond to actin and IgGBF, respectively (Fig. 6).

Subsequently, each fraction from the mRNA gradient was translated in reticulocyte lysates, and the translation products were analyzed by using $NaDodSO_4$ PAGE before and after immunoprecipitation with Fc fragments. As shown in Figure 7*A*, the m.w. of the synthesized products increases from the top to the bottom through the gradient showing that even large polypeptides could be efficiently encoded by the heavy mRNA fractions. As illustrated in Figure 7*B*, after immunoprecipitation with the Fc fragments, various polypeptides were found the M_r of which are similar to those observed after Fc binding of the T_2D_4 poly A RNA products. The 37-kD polypeptide was seen mostly in fractions 7 and 8, faintly in fraction 6, and it was absent in the other fractions. Of note, these fractions corresponded to those encoding for maximal immunosuppressive activity (Fig. 5). Because the 37-kD polypeptide was a) specifically present in immunoprecipitates of T_2D_4 translation products and b) found only in immunoprecipitates corresponding to the biologically active fractions of the gradient, these data may indicate that it corresponds to *in vitro*-translated IgGBF.

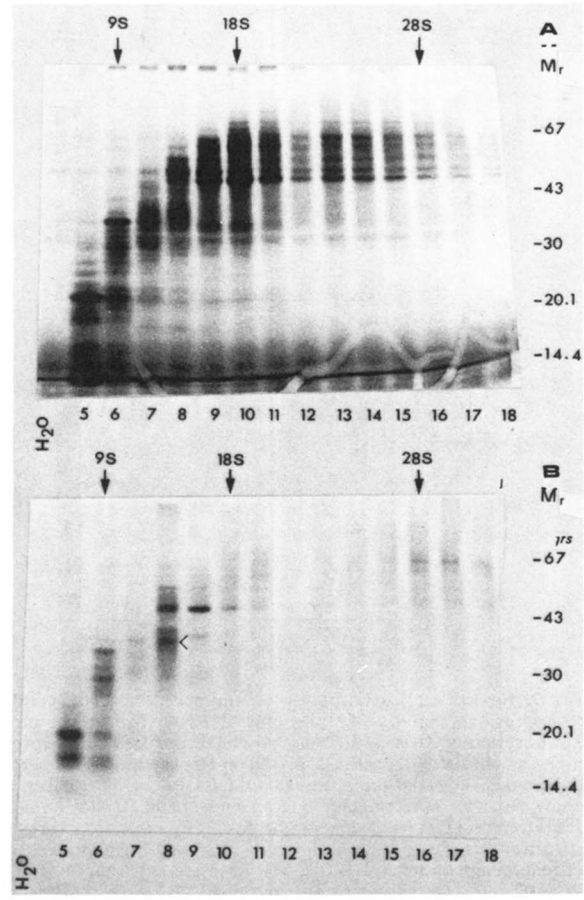


Figure 7. $NaDodSO_4$ PAGE analysis (10 to 20% acrylamide) of [^{35}S] methionine-labeled reticulocyte translation products of sucrose gradient fractions of T_2D_4 poly A RNA, before (*A*) or after (*B*) immunoprecipitation with Fc fragments. The first lane on the left (H_2O) represents incubation without mRNA, lanes 5 to 18 represent translation products from the corresponding sucrose gradient mRNA fractions (top to bottom). Translation products from sucrose gradient fractions 1 to 4 showing very low template activity are not presented. Molecular weight markers are indicated on the right of the figure. <, indicates the 37-kD polypeptide.

DISCUSSION

In oocytes and reticulocyte lysates, poly A RNA extracted from the T_2D_4 T hybridoma line was found to direct the synthesis of proteins that, in terms of biologic activity, affinity, and m.w., are identical to those of IgGBF secreted by the same cells; namely, translation products were found to exert a profound inhibition of the secondary *in vitro* IgG antibody response. The inhibitiory activity was bound to IgG immunoadsorbents and was enriched in the corresponding acid eluates. In $NaDodSO_4$ PAGE, suppressive proteins synthesized by mRNA appeared to have the same M_r as those of cell-secreted IgGBF. Finally, the suppressive activity was specifically translated by mRNA isolated from T_2D_4 cells producing IgGBF but not from nonproducing cells, i.e., the BW-5147 fusion partner and the A_9 fibroblast line. Thus the conclusion determined from these findings is that poly A mRNA from T_2D_4 cells encodes for IgGBF.

IgGBF has been characterized as a glycoprotein in which the sugar moiety is apparently not involved in the IgG-binding site (19). The results, described herein, showing that IgG-binding suppressive molecules were synthesized in rabbit reticulocyte lysates, confirm this observation and suggest that, similar to other lymphokines, such as interferon-β (19a) and -γ (C. Vaquero, unpublished data) or interleukin 2 (20) and antigen-specific suppressor T cell factor (21), no post-translational modifications are necessary to obtain biologically active

IgGBF.

Translation of fractionated poly A RNA resulted in the biosynthesis of immunosuppressive proteins binding to IgG. In both translation systems, i.e., reticulocyte lysates and oocytes, the bulk of suppressive activity was encoded by 9 to 13S mRNA. In the oocyte translation system, a maximal suppressive activity was obtained with 11S mRNA. Some biologic activity was also encountered to a lesser extent around 26S mRNA. It cannot be excluded that this activity might be encoded by a distinct messenger coding for a larger polypeptide having biologic activity similar to IgGBF. More probably, however, it might correspond to aggregates of the smaller messengers, as has already been observed for other lymphokines (22, 23).

After mild treatment that does not significantly alter biologic activity, T_2D_4 mRNA translation products gave, in $NaDodSO_4$ PAGE, two peaks of suppressive activity corresponding to 78 and 40 kD. Peaks at identical m.w. were observed after electrophoresis of T_2D_4-secreted IgGBF treated with the same conditions. In another set of experiments, radioactive translation products of T_2D_4, BW-5147, and A_9 mRNA were immunoprecipitated with Fc fragments of IgG and were analyzed by $NaDodSO_4$ PAGE after denaturing treatment. The translation products of T_2D_4 messengers with immunosuppressive activity revealed the presence of a 37-kD polypeptide, which was absent in the products of BW-5147 and A_9 mRNA that were devoid of biologic activity, thus suggesting that this polypeptide may correspond to IgGBF. Other polypeptides were also immunoprecipitated by Fc fragments, but these were also present in the products of A_9 and BW-5147 messengers and disappeared after Fab-Sepharose clearance indicating that they may nonspecifically bind to immunoadsorbents. The fact that this 37-kD polypeptide is associated with IgGBF was further substantiated by the results from an analysis of immunoprecipitates obtained with fractionated mRNA. Fractionated in this manner, it was found only in fractions 6 to 8, which exert profound immunosuppressive effects on the *in vitro* antibody response.

Because, with $NaDodSO_4$ PAGE, suppressive activity dissociates at 78 kD and 40 kD under nondenaturing conditions and with Fc fragments, IgGBF immunoprecipitates at 37 kD after denaturing treatment, the present results suggest that the 78 kD may correspond to dimers of 37-kD polypeptides. These results are in accordance with our previous characterization of radio-labeled IgGBF produced by T lymphoma cells (24) and by alloantigen-activated T cells (19) indicating that it contained a major chain at 40 kD that associates, in the absence of β-ME, into 80-kD dimers. In the human, IgGBF was found to be composed of 43 kD and polymers with higher M_r of a 23-kD subunit (25).

Other isotype-specific immunoregulatory T cell factors have been described, namely IgEBF that regulate the IgE response (26) and the IgABF that suppresses IgA production (8). With respect to IgE regulators produced by T lymphocytes, the IgE-binding activity appears, after Sepharose G-75 filtration, around 40 and 15 kD, whereas the immunoregulatory activity (suppressive or potentiating), is found only at around 15 kD (27, 28). Moreover, IgE-specific suppressor substances produced by a T cell hybridoma were described as having M_r in the order of 45 kD (29).

T_2D_4 cells secrete IgGBF and express FcγR. Whether IBF and FcR do or do not represent distinct entities is still a matter of debate. The fact, however, that the M_r of murine T cell FcγR ranges between 47 and 70 kD (30), whereas the M_r of immunosuppressive IgGBF is around 37 kD, suggests that they might be different molecules or IgGBF represents part of T cell FcγR.

In conclusion, the present experiments provide evidence that mRNA coding for IgGBF has been isolated from murine T cell hybrids and the first basis for the cloning of this factor has been provided. Upon interaction with Ig classes and subclasses, the same hybrids can be induced to produce IgG_1BF, IgG_2BF, or IgABF (8, 11). Thus this model provides a useful tool to use in an attempt a) to clone the various IBF and b) to study, at the T cell level, the regulation by Ig isotypes of the expression of the genes coding for the different isotype-specific IBF.

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