Molecular mass analysis of murine immunosuppressive immunoglobulin G-binding factors (IgG-BFs) produced by T-cell hybrids

Catherine Néauport-Sautès, Maria-José Gelabert, Janine Moncuit and Wolf Herman Fridman

Unité INSERM 255, Institut Curie, 26, rue d'Ulm, 75231 Paris Cédex 05, France

Received 6 April 1984

Induced and constitutive murine IgG-binding factors (IgG-BFs) have been purified by affinity chromatography from supernatants of T-cells preincubated with or without murine monoclonal IgG1 and IgG2b, respectively. IgG-BF M_r values have been studied by SDS-polyacrylamide gel electrophoresis (PAGE) after treatment with SDS under conditions which do not noticeably alter their immunosuppressive activities on the secondary in vitro IgG antibody response. Suppression was recovered at M_r values of 80000, 40000 and 20000. When induced IgG-BF was tested, the isotype-specific suppressive activity was found only at 40 kDa. The 20-kDa moiety appeared to derive from the 40-kDa component and the material found at 80 kDa exerted non-specific immunosuppressive effects. We conclude therefore that isotype-specific IgG-BF has an apparent M_r of 40000.

Molecular mass *T-cell hybrid* *T-cell factor* *Antibody production* *Isotype* *Polyacrylamide gel electrophoresis*

1. INTRODUCTION

The production of immunoglobulin isotypes is specifically regulated by T-cell factors which are induced by a given isotype and selectively bind to this isotype. These factors have been called immunoglobulin-binding factors (IBF) [1] and found for IgG (IgG-BF) [2], IgE (IgE-BF) [3] and IgA (IgA-BF) [4]. For instance, murine IgG-binding factor produced spontaneously during the incubation of activated T-cells and of T-cell hybrids in serum-free medium at 37°C binds specifically to the Fc portion of IgG [1] and suppresses secondary in vitro antibody responses of IgG1, IgG2a and IgG2b subclasses [1,2]. This factor is called constitutive IgG-BF. Selective induction of the production of subclass isotype-specific IgG-BF is obtained by treatment of T-cell hybrids (T2D4) with monoclonal murine IgG [2]. Incubation of T2D4 cells with IgG1 leads to the production of a factor binding to IgG1 and suppressing IgG1 antibody synthesis. This factor is called IgG1-BF. Conversely, the incubation of T2D4 with IgG2a and IgG2b monoclonal proteins induces the production of IgG2-BF binding to IgG2a and IgG2b and suppresses both IgG2a and IgG2b antibody responses.

In previous experiments, radiolabeled constitutive IgG-BF purified by affinity chromatography was found, by SDS–PAGE, to be composed of subunits of 38–40 kDa and 18–20 kDa [5,6]. We present here an analysis by SDS–PAGE of the M_r of constitutive and induced IgG-BF produced by the same cells and pretreated under mild denaturing conditions. The data, based on the suppression of the secondary in vitro IgG anti-sheep red blood cell (SRBC) response, show that (i) isotype-specific IgG-BF appears to have an M_r of 40000, (ii) the 20-kDa subunit, although biologically active, corresponds to degradation products of the 40-kDa molecule and (iii) induced and constitutive IgG-BFs have similar apparent M_r values.

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

2. MATERIALS AND METHODS

2.1. *Hybridoma cells*

T2D4 T-cell hybrids were prepared as in [7] by fusion of alloantigen-activated T-cells with BW-5147 T-lymphoma cells.

2.2. *IgG-BF preparation*

Constitutive IgG-BF was isolated from supernatants of T2D4 cells incubated at 2×10^6 cells/ml for 2 h at 37°C in serum-free balanced salt solution (BSS) under sterile conditions. When indicated, *p*-methylsulfonylfluoride (PMSF; Sigma, St Louis, MO) at 0.001 M final concentration and aprotinin (Sigma) at 0.1 units/ml were added to cell-free supernatants. Supernatants were 10 times concentrated on Amicon (YM 10) filters, and applied to affinity chromatography columns of Sepharose 4B (Pharmacia, France) coupled with rabbit IgG as in [8]. Material eluting with 0.2 M glycine–HCl buffer (pH 2.8), concentrated to half of the volume of the 10× supernatant was used as constitutive IgG-BF.

Induced IgG-BFs were prepared from 18-h culture supernatants of T2D4 cells preincubated for 2 h at 37°C with murine monoclonal IgG1 or IgG2b. Supernatants were applied on immunoadsorbents coupled with IgG1 or IgG2b monoclonal proteins. Immunoadsorbents were washed and treated at acid pH [8]. Acid eluates, concentrated to the starting volume, were used as induced IgG-BFs.

2.3. *SDS–polyacrylamide gel electrophoresis*

Ninety μl of 10× concentrated constitutive IgG-BF or of induced IgG-BFs were incubated for 10 min at 70°C with 10 μl of 1% SDS, 30 μl of glycerol were then added and the mixture was applied and run on to polyacrylamide slab gels [9] as in [10]. ^{125}I-labeled BSA and IgG heavy and light chains boiled at 100°C in sample buffer [(1% SDS, 250 mM β-mercaptoethanol (βME)] were run in parallel and used as size markers. Gel bands corresponding to IgG-BF slots were cut in 0.42-mm slices; slices were incubated for 16 h at 37°C in 0.5 ml RPMI 10% FCS – eluted material was then tested for biological activity at 1/20 final dilution. In some experiments incubation was performed in 0.5 mM Tris–HCl buffer (pH 6.8). The eluate of the slice located at 40 kDa was then treated with 0.1% SDS for 10 min at 70°C and applied on a second gel.

2.4. *Biological assays*

One ml cell suspension containing $6–8 \times 10^6$ spleen cells from mice injected 7 days before with 10^8 SRBC were restimulated with SRBC and cultured in RPMI 10% FCS supplemented with 1% horse serum. Five days later, indirect plaque-forming cell (PFC) numbers were evaluated [11]. Total IgG plaques were developed by using a rabbit anti-mouse IgG antiserum at 1/20 final dilution; IgG1 and IgG2b plaques were revealed by using rabbit anti-mouse IgG1 or IgG2b antisera (Litton Bionetics, Kensington, MD) at 1/10 final dilution. IgG-BF-containing samples were added to cell cultures at day 0.

3. RESULTS

Preliminary experiments were performed to determine temperatures at which IgG-BF could be treated with SDS without losing its biological activity. IgG-BF was incubated for 10 min in the presence of 0.1% SDS at temperatures ranging from 0 to 100°C, and then added, at 1:100 final dilution, to secondary in vitro cultures of spleen cells stimulated with SRBC. The results indicated that the suppressive activity of IgG-BF was not significantly modified when it was treated at temperatures up to 70°C (not shown). When incubated under these conditions, size markers such as albumin, ovalbumin and chymotrypsinogen migrated 10–20% less compared to the usual denaturing and reducing conditions (1% SDS, 250 mM βME) (not shown).

In a second set of experiments, constitutive IgG-BF was run on 12.5% acrylamide gels. Material eluting from each slice was then tested for biological activity on secondary in vitro anti-SRBC responses. Results of a typical experiment are given in fig.1. Suppressive activity was found in the first slice of the gel and into slices corresponding to M_r 42000 and 20000. The suppressive material present at the beginning of the gel migrated on 10% acrylamide gels at M_r ~75000–80000 (not shown).

Experiments were then designed to investigate the relationships between these 3 different peaks. Pretreatment of IgG-BF by dithiothreitol

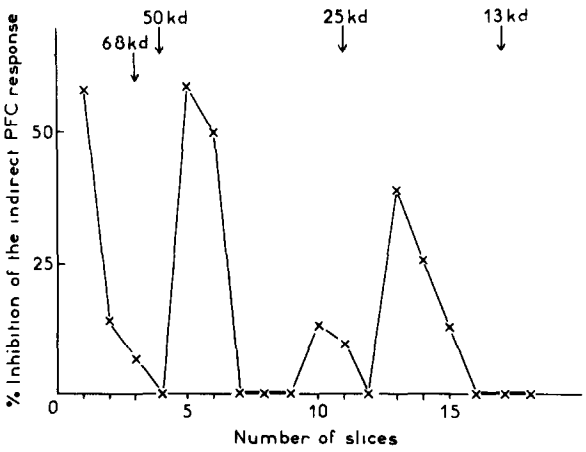

Fig.1. SDS–PAGE (12.5% polyacrylamide gel) of constitutive IgG-BF. Material eluted from slices was added at 1/20 final dilution in a secondary in vitro anti-SRBC response. Values indicate the percentage of inhibition of the indirect IgG PFC responses of duplicate cultures. Control response: 3220 ± 108 IgG PFC/10^6 cells. Arrows indicate positions of size markers.

(0.020 M) did not modify the suppression profile (not shown) suggesting that the higher-M_r molecules were probably not multimers of the 20-kDa chain(s). When proteolytic enzyme inhibitors were added to cell supernatants before IgG-BF purification, biological activity was present in only two peaks located respectively at 80 and 40 kDa (fig.2). No suppressive material was found at 20 kDa. These results indicate that the low-M_r component might correspond to degradation products of higher-M_r molecules.

To investigate further this possibility, suppressive factor migrating at M_r 40000 was eluted, treated with SDS, and rerun on a second gel. Suppressive activity was then found at M_r 18000 (fig.3) providing evidence that the 20-kDa peak corresponds to biologically active degradation products of the 40-kDa molecules. The same kind of experiment was performed with the 80-kDa peak. However, no significant suppressive activity was recovered after migration on a second gel.

In the last set of experiments, induced IgG1-BF was run on polyacrylamide gels under similar conditions. Material eluting from slices was then tested for suppressive activity on the indirect IgG1 anti-SRBC response. In the absence of proteolytic enzyme inhibitors, a suppression profile similar to that of constitutive IgG-BF was found (fig.4) sug-

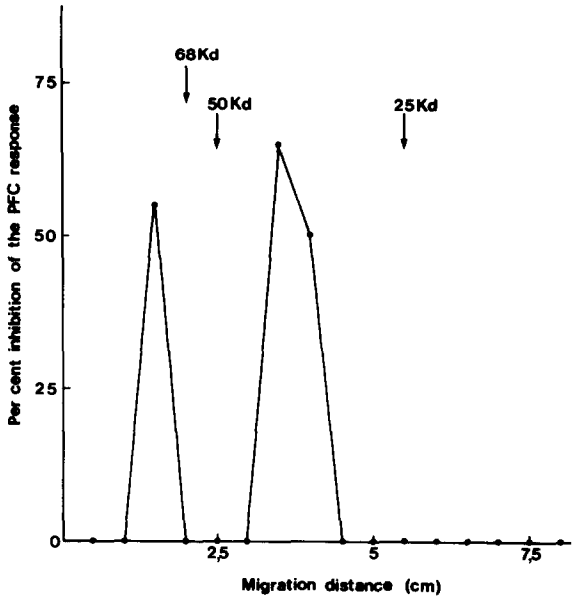

Fig.2. SDS–PAGE (12.5% acrylamide gel) of constitutive IgG-BF prepared in the presence of proteolytic enzyme inhibitors. For details see fig.1. Control response: 2896 ± 517 IgG PFC/10^6 cells.

gesting structural similarity between these factors. In previous experiments, induced IgG-BFs were found to exert, depending on their dilution of use, both isotype-specific and non-specific suppressive effects. The materials eluting from slices located at

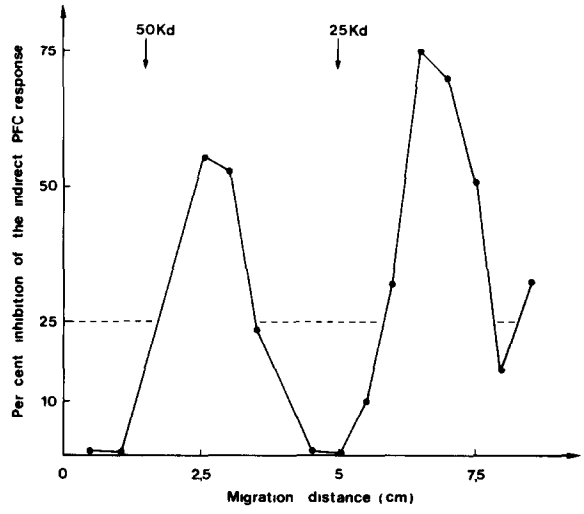

Fig.3. SDS–PAGE (15% polyacrylamide gel) of the 40-kDa component from constitutive IgG-BF. Control response: 4377 ± 417 IgG PFC/10^6 cells.

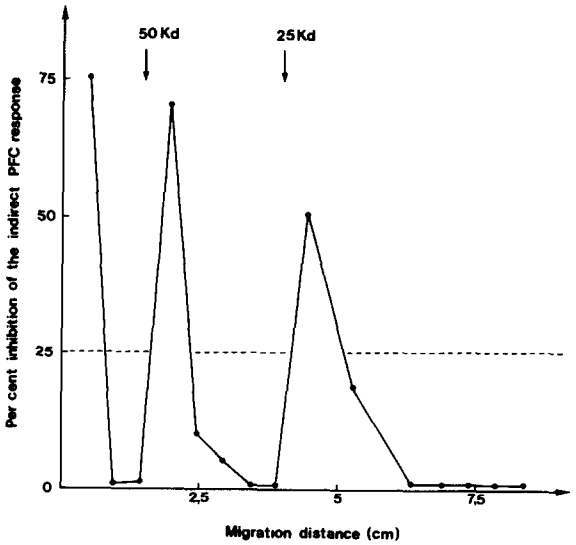

Fig.4. SDS–PAGE (15% polyacrylamide gel) of induced IgG1-BF. Values indicate the percentage of inhibition of the indirect IgG1 PFC response. Control response: 2670 ± 842 IgG PFC/10^6 cells.

80 and 40 kDa were thus tested for their effects on the IgG1 and IgG2b anti-SRBC responses. While the 80-kDa peak from both IgG1-BF and IgG2b-BF was found to suppress both responses, the 40-kDa peak of IgG1-BF inhibited only the IgG1 response and that of IgG2b-BF only the IgG2b

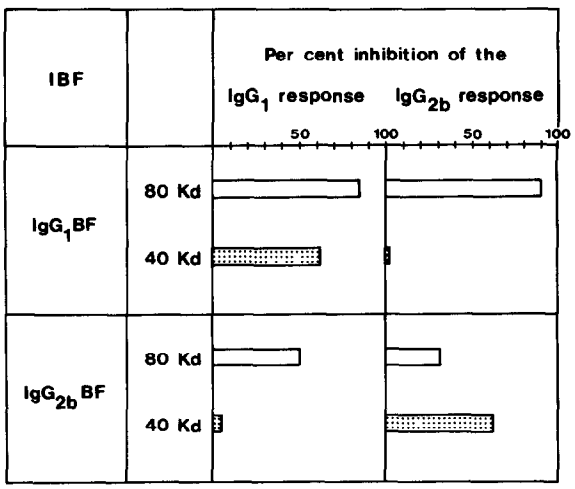

Fig.5. Isotypic specificity of the 80 and 40 kDa components from IgG1-BF and IgG2b-BF. Control anti-SRBC responses: 1253 ± 109 IgG1 PFC/10^6 cells, 523 ± 116 IgG2b PFC/10^6 cells.

response. In conclusion the present data provide evidence that isotype-specific IgG-BF has an M_r of 40000.

4. DISCUSSION

In this study, our aim was to investigate the M_r of IgG-BFs under mild denaturing conditions. The suppressive activities related to constitutive and induced IgG-BFs produced by the same T-cell hybrids were found on polyacrylamide gels at 80, 40 and 20 kDa. The 20-kDa peak corresponds to degradation products of the 40-kDa molecule(s) since (i) rerunning of the latter on a second gel gives rise to 20-kDa suppressive molecules and (ii) the 20-kDa peak is absent when proteolytic enzyme inhibitors are added to cell supernatants before purification. These findings, together with the fact that isotype-specific suppressive activity of induced IgG-BFs is present only in the 40-kDa peak, provide evidence that IgG-BF has an M_r of 40000. They are compatible with our recent results showing that the M_r of IgG-BF present in translation products of mRNA isolated from the same T-cell hybrids is located at 37 kDa [12]. Radiolabeled IgG-BF produced by T-lymphoma cells and by alloantigen-activated T-cells was also found to be composed of a major chain located at 38–40 kDa which associates under non-reducing conditions into 80-kDa dimers [5,6]. Here, the 80-kDa peak could also correspond to dimers of molecules of smaller M_r which remain associated under our experimental conditions. However, it cannot be excluded that it corresponds to another immunosuppressive factor binding to IgG but having no isotypic specificity.

Human IgG-BF was found to be composed of a 43-kDa and of higher-M_r polymers of a 23-kDa subunit [13]. Other isotype-specific immunoregulatory T-cell factors, namely the IgE-BFs which regulate the IgE response, have an M_r around 40000 and 15000 as estimated by Sephadex G-75 gel filtration [14].

In conclusion our data provide evidence that isotype-specific IgG-BF has an apparent M_r of ~40000 when analysed under mild denaturing conditions. Other biochemical properties of this molecule are currently under investigation in our laboratory.

REFERENCES

[1] Fridman, W.H., Rabourdin-Combe, C., Néauport-Sautès, C. and Gisler, R. (1981) Immunol. Rev. 56, 51–88.

[2] Löwy, I., Brézin, C., Néauport-Sautès, C., Thèze, J. and Fridman, W.H. (1983) Proc. Natl. Acad. Sci. USA 86, 2323–2327.

[3] Ishizaka, K., Yodoï, J., Suemura, M. and Hirashima, M. (1983) Immunol. Today 4, 192–196.

[4] Yodoï, J., Adachi, M., Teshigawara, K., Miyama-Inaba, M., Masuda, T. and Fridman, W.H. (1983) J. Immunol. 131, 303–310.

[5] Néauport-Sautès, C. and Fridman, W.H. (1977) J. Immunol. 119, 1269–1274.

[6] Joskowicz, M., Rabourdin-Combe, C., Néauport-Sautès, C. and Fridman, W.H. (1968) J. Immunol. 121, 777–783.

[7] Néauport-Sautès, C., Rabourdin-Combe, C. and Fridman, W.H. (1979) Nature 277, 656–659.

[8] Guimezanes, A., Fridman, W.H., Gisler, R.H. and Kourilsky, F.M. (1976) Eur. J. Immunol. 6, 69–71.

[9] Laemmli, U.K. (1970) Nature 227, 681–683.

[10] Fridman, W.H., Gelabert, M.J. Daëron, M., Moncuit, J., Löwy, I., Thèze, J. and Néauport-Sautès, C. (1984) Methods Enzymol., in press.

[11] Pierce, C.W., Johnson, B.M., Gershon, H.E.and Hasofsky, R. (1971) J. Exp. Med. 134, 395–399.

[12] Vaquero, C., Fridman, W.H., Moncuit, J., Provost, M.A., Falcoff, E., Gelabert, M.J. and Néauport-Sautès, C. (1984) J. Immunol., in press.

[13] Caraux, J., Behrouz Chichenian, Serrou, B. and Weigle, W.O. (1983) J. Immunol. 130, 2295–2301.

[14] Yodoï, J., Hirashisma, M. and Ishizaka, K. (1980) J. Immunol. 125, 1436–1441.