Clin. exp. Immunol. (1992) **87**, 208–214

Increased levels of soluble low-affinity Fcγ receptors (IgG-binding factors) in the sera of tumour-bearing mice

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(Accepted for publication 9 September 1991)

SUMMARY

Soluble forms of low affinity Fcγ receptors (FcγR), also called IgG-binding factors (IgG-BF), have been shown to play a regulatory role in immune responses. By using an immunodot assay with the anti-mouse FcγR MoAb, 2.4G2, the levels of IgG-BF have been measured in the sera of mice bearing syngeneic tumours of lymphoid or non-lymphoid origin or in mice injected with high doses of murine IgG. These sera contained large amounts of IgG-BF as compared with controls. In the case of mice bearing IgG2a- or IgG2b-secreting hybridomas or lymphomas, serum IgG-BF increased progressively with tumour size and serum monoclonal IgG concentration, reaching 4–12 times the normal levels. A less than three-fold increase was found in mice bearing an IgG1-secreting hybridoma or tumours which do not secrete IgG (IgA-secreting hybridoma, non-immunoglobulin-secreting lymphoid tumours or melanoma) or in mice injected with 9 mg of monoclonal IgG2a. The enhancement of serum IgG-BF levels was independent of the expression of FcγR by the tumour cells, suggesting that the majority of IgG-BF secreted in response to tumours was produced by the host rather than by the tumour. The increased production of IgG-BF may participate in the control of tumour growth and in the modulation of the host immune responses in tumour-bearing animals.

Keywords soluble Fcγ receptors tumours serum isotypic regulation

INTRODUCTION

IgG-binding factors (IgG-BF) are molecules which bind the Fc portion of IgG (reviewed by Fridman & Sautès [1]). First described in the supernatants of murine Fcγ-receptor (FcγR)-positive T cells [2], IgG-BF were then detected in the supernatants of B cells [3] and macrophages [4] and in mouse [3,5] and human sera [6,7]. IgG-BF may exert important regulatory roles *in vivo* since these molecules were shown to interfere with IgG-mediated effector functions such as complement fixation [2,8,9], and antibody-dependent cell-mediated cytotoxicity [10], and participate in the regulation of antibody production *in vitro* [1,11]. Immunoglobulin-binding factors (IBF) specific for other immunoglobulin isotypes such as IgE (IgE-BF) [12,13], IgA (IgA-BF) [14,15], and IgD (IgD-BF) [16], have been described in the supernatants of activated lymphocytes, some of which, like IgE-BF, are also found in serum [17]. These factors may also participate in the isotypic regulation of antibody production [1].

Several lines of evidence have shown that IgG-BF represents soluble forms of low affinity FcγR [18,19]. In the mouse, the two types of low affinity FcγR, called FcγRII and III, are 50–70-kD glycoproteins encoded by two genes, β and α respectively (reviewed by Ravetch & Anderson [20]). The proteins display almost identical extracellular domains of 180 amino acids long coupled to divergent transmembrane and intracytoplasmic domains. Two transcripts of the β gene, β1 and β2, have been identified in lymphocytes and in macrophages, respectively. FcγRIII are expressed in macrophages, natural killer cells and mast cells. Murine IgG-BF produced by activated T cells incubated *in vitro* are 38–40-kD glycoproteins [21] which react with 2.4G2 [22], a MoAb directed against FcγRII and FcγRIII. Recent structural and biological data indicate that an IgG-BF corresponding to the two extracellular domains of murine FcγRII is generated by proteolytic cleavage of the membrane receptor [23]. Like membrane FcγRII and III, this molecule binds murine IgG1, IgG2a and IgG2b but not IgG3 [11,23].

The expression of FcR and the production of IBF are highly dependent upon cell activation and environmental factors. Murine IgG-BF have been detected in supernatants of murine alloantigen-activated T cells [2] and of lipopolysaccharide (LPS)-stimulated B lymphocytes [3]. Cytokines such as interferons (IFN-α, -β) or IL-2 up-regulate FcγR expression and IgG-BF production [24–26]. In addition to cytokines, immunoglobulins provide another regulatory stimulus by enhancing the expression of receptors for the corresponding isotype. For example, incubation of murine T cell hybrids with IgG leads to increased expression of membrane FcγR [27] and to the production of IgG-BF [28].

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These regulatory phenomena, which have been observed *in vitro*, may also occur *in vivo*. It has been known from some time that the growth of tumours in humans and in mice causes a significant increase in FcR-positive T cells. In man, it has been reported that B-CLL and multiple myeloma are accompanied by an expansion of FcR-expressing T cells [29,30]. In patients or in mice with monoclonal immunoglobulin-secreting tumours, increased numbers of T cells with receptors specific for the isotype of the myeloma immunoglobulin have been described [30]. Such cells may participate in the control of tumour growth and immunoglobulin secretion by tumour cells, either directly or indirectly via the release of immunoregulatory IBF. Mathur *et al.* [31] have shown that FcεR-positive T cells induced in mice bearing certain IgE-secreting hybridomas down-regulate IgE secretion by the tumour cells. In addition, IgG-BF-producing FcγRII-positive T cells as well as semi-purified IgG-BF have been shown to inhibit IgG secretion by hybridoma B cells as well as their *in vitro* growth [32–34]. The FcγR-positive T cells may also participate in the modulation of the immune responses of the tumour-bearing animals.

Little is known about serum IgG-BF levels in pathological situations. Elevated amounts have been detected in parasitic infections which modify the serum IgG concentrations [35]. A slight increase was observed in mice with autoimmune diseases [3]. We have previously shown that sera from alloimmunized mice contain increased amounts of IgG-BF [35]. In this study the serum levels of IgG-BF were investigated in tumour-bearing mice. Groups of 5–10 mice were injected with IgG-secreting or non-IgG-secreting tumours or with mouse monoclonal IgG, and the serum levels of IgG-BF were measured by using an immunodot assay with 2.4G2 MoAb. Our data show that the amount of circulating IgG-BF increases 2–12-fold in these animals.

MATERIALS AND METHODS

Cells

A20.2J, an IgG2a-secreting B cell lymphoma, was a gift from Dr B. Jones (Howard Hughes Medical Institute, New Haven, CT) and was cultured in RPMI 1640 medium (GIBCO, Paisley, UK), enriched with 10% fetal calf serum (FCS), 1% penicillin-streptomycin, 1% glutamine, 5% sodium pyruvate, and 0·01% mercaptoethanol (Sigma, St Louis, MO). IIA1.6 is a variant of A20.2J which is deleted in the FcγRII gene [36]. UN2.C3 is a subclone [37] of the hybridoma cell line UN2 (kindly provided by Prof. M. D. Scharff, Albert Einstein College of Medicine, NY) which produces IgG2a anti-sheep-erythrocyte antibodies. This cell line, as well as anti-arsonate hybridomas producing IgG2b: Ar13.4.9 (M. D. Scharff), IgG1: 9.3G7cl18.3 (kindly provided by D. Capra, University of Dallas, Dallas, TX), hybridoma and myeloma secreting anti-phosphorylcholine antibodies of IgG2a (RP93.9.1) or IgA (S.107.3) isotypes (M. D. Scharff) and IgG2a- and IgG2b-secreting myeloma of unknown specificity (respectively HOPC1 and 45TG1.7, a subclone of MPC11, M. D. Scharff) were used as immunoglobulin-secreting tumours. All tumour IgG were of K isotype. B16F10, a melanoma cell line, was a gift from Dr M. F. Poupon (IRSC, Villejuif, France). BW5147, a thymoma line originated from AKR mice, was obtained from Prof. L. Herzenberg (Stanford, CA). SP2/O is a hybridoma line which no longer secretes immunoglobulin. Cell lines were cultured in RPMI 1640 medium with 10% FCS, 1% penicillin-streptomycin, and 1% glutamine. The fibroblastic cell line Cu1B3 obtained by transfection of L cells with a cDNA encoding soluble murine FcγRII [11] was grown on hollow fibres in a perfusion cell culture system (Acusyst Jr, Endotronics Inc., MN).

Treatment of mice

Six-to-ten week-old BALB/c, C57BL/6, and AKR mice (IFFA-CREDO, L'Arbresle, France) were used. Mouse sera were obtained by eye-bleeding mice before tumour injection and at various times after tumour injection, depending on the kinetics of tumour growth. Tumour cells (2×10^6 cells in 0·5 ml buffered saline solution (BSS) per mouse) were injected either into the intraperitoneal cavity of the mice, 2–5 days after i.p. injection of 0·5 ml Pristane, or subcutaneously. Tumour sizes were measured in mm^2 with a caliper. In some experiments, mice were injected intraperitoneally (1 mg every 12 h) with a total dose of 6 mg (three mice) or 9 mg (two mice) of purified monoclonal IgG2a.

Antibodies

2.4G2 is a rat hybridoma cell line producing an IgG2b anti-mouse low affinity FcγR MoAb [38]. This antibody does not react with up to 5 mg/ml mouse IgG or IgM, as tested by immunodot assay (data not shown). For immunofluorescence analysis, $F(ab')_2$ fragments were prepared by digesting ion exchange-purified immunoglobulins with *Staphylococcus aureus* V_8 protease (ICN, Costa Mesa, CA) and were purified by Sephacryl S200 chromatography. For immunodot experiments, 2.4G2 IgG was iodinated using the chloramine T method and separated from free iodine on a G25 separation column. IgG2a was obtained by precipitation, with 18% sodium sulphate, of pools of ascites from BALB/c mice bearing UN2.C3 or RP93.9.1 tumours followed by chromatography on Protein A-Sepharose. All immunoglobulin isotypes were quantified by ELISA specific for mouse immunoglobulin subclasses.

Immunofluorescence

Cells (5×10^5), washed twice in BSS, were incubated for 1 h at 0°C with 50 μl of $F(ab')_2$ 2.4G2 antibody at a concentration of 10 μg/ml in BSS containing 5% FCS. All cell samples were then washed three times in BSS containing 5% FCS, and incubated for 1 h at 0°C with 50 μl FITC-labelled $F(ab')_2$ fragments of mouse anti-rat IgG (Southern Biotechnology, Birmingham, AL), diluted 1/30 in BSS containing 5% FCS. Cell samples were washed three times in BSS containing 5% FCS and then resuspended in 1 ml PBS before being analysed with a FACScan (Becton Dickinson, Mountain View, CA).

Immunodot assay

Serum samples were diluted to 1/10 in 0·01 M Tris, 0·15 M NaCl, pH 7·4, Western buffer (WB) and 500 μl were deposited on a nitrocellulose membrane (Schleicher and Schull, BA85, Dassel, Germany) using a dot blot apparatus (BRL, Cambridge, UK). In each experiment, various amounts of culture medium of Cu1B3 cells grown in bioreactors and containing 16 μg/ml of recombinant IgG-BF [19] were used as a positive control. After serum samples and Cu1B3 culture medium had been deposited, the membrane was saturated for 45 min at 40°C in 5% bovine serum albumin (BSA)/WB and incubated with [^{125}I]-labelled 2.4G2 in 5% BSA/WB (3×10^5 ct/min per 10 cm^2 of nitrocellu-

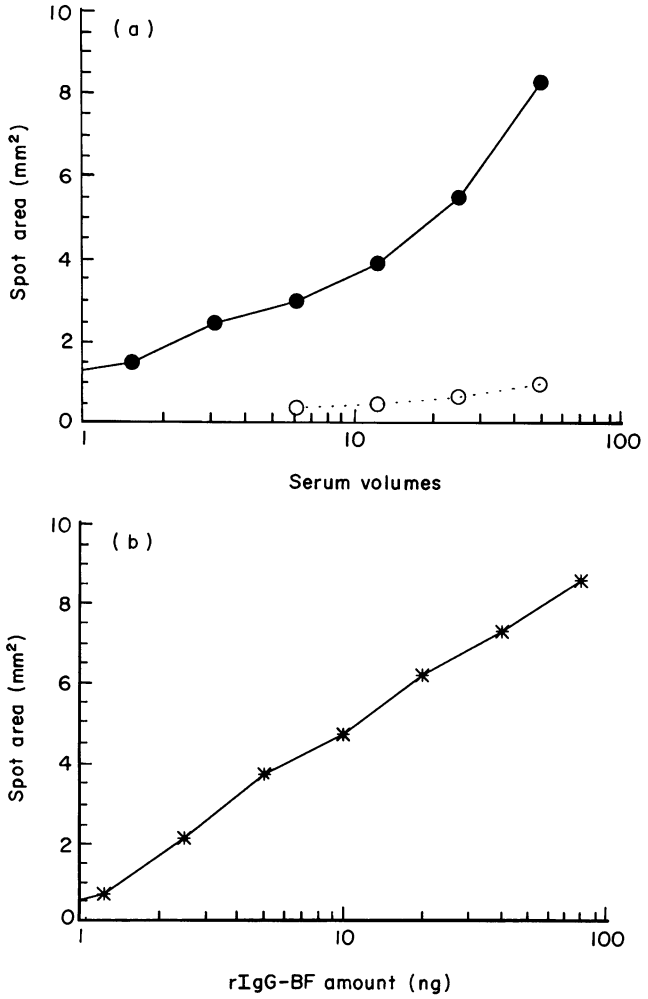

Fig. 1. Detection of IgG-BF by immunodot with [^{125}I]-labelled anti-FcγRII MoAb. (a) Various volumes of serum from (●) a BALB/c mouse bearing an IgG2a-secreting solid tumour (UN2.C3) and (○) from a control BALB/c mouse were deposited onto the nitrocellulose. (b) Various amounts of culture medium from Cu1B3 cells containing 16 μg/ml of rIgG-BF were deposited onto the same filters. Spot absorbances of autoradiographs were measured with a laser scan densitometer and peaks were integrated to obtain areas (mm^2).

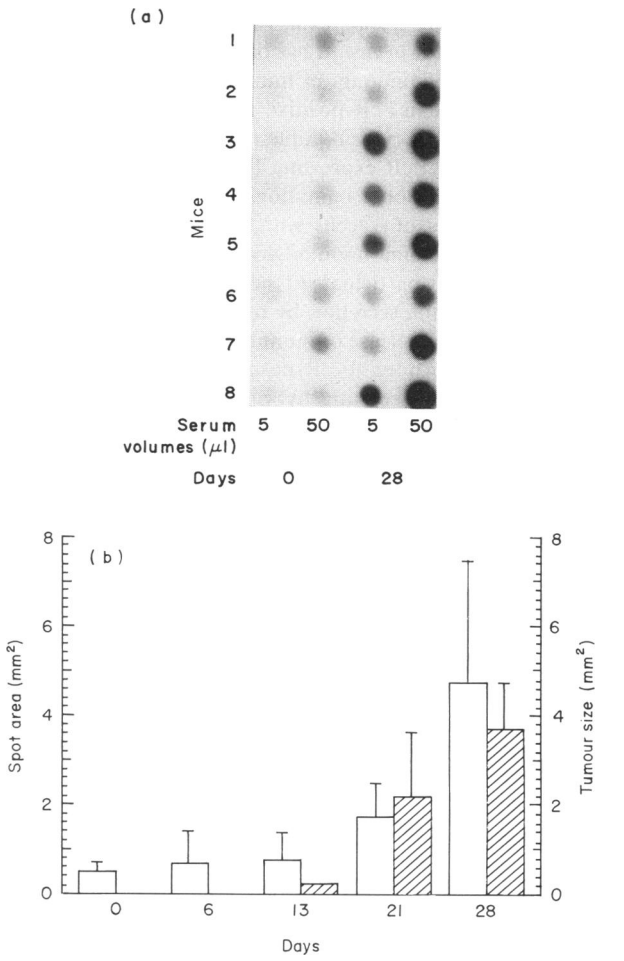

Fig. 2. Detection of IgG-BF in the sera of BALB/c mice bearing an IgG2a-secreting tumour. Eight mice were injected subcutaneously with UN2.C3 hybridoma B cells, bled at various days after inoculation and tumour sizes were measured. The amount of IgG-BF in 50 μl of serum was estimated by immunodot with [^{125}I]-2.4G2. (a) Autoradiography spots of sera obtained before (day 0) and after (day 28) injection of tumour cells. (b) IgG-BF spot areas (□) and tumour sizes (■) (mean values ± s.d.) measured at various days after inoculation.

lose) for 1 h at room temperature or overnight at 4°C. It was then washed twice in WB, once in WB/0·05% NP40, and twice more in WB. Finally, the membrane was dried and exposed to radiographic film Kodak X-Omat S (Kodak, Rochester, NY), from 2 to 3 days at −80°C. Spot absorbances of autoradiographs were measured using a laser scan densitometer (LKB, Bromma, Sweden). Results were expressed in spot area obtained for 50 μl of serum. IgG-BF augmentation factor represents the ratio of the mean value of the areas obtained with 50 μl of sera from 6–11 mice bled after tumour inoculation *versus* the mean value of the area obtained with 50 μl of sera from the same mice bled before tumour inoculation and tested in the same assay.

RESULTS

Detection of serum IgG-BF in mice bearing UN2.C3 solid tumours

In a first experiment, the amounts of IgG-BF in the serum of a mouse bearing an IgG2a-secreting solid tumour (UN2.C3, a subclone of the UN2 hybridoma cell line injected subcutaneously) and of a control mouse were tested and compared in an immunodot assay on nitrocellulose using [^{125}I]-labelled 2.4G2 anti-FcγR antibody. As shown in Fig. 1a, the spot areas increased with serum doses and reached higher values with serum from the tumour-bearing mouse (8·3 mm^2) than with serum from the control animal (0·9 mm^2). These data indicate that circulating IgG-BF is increased in the serum of the tumour-bearing mouse. Since no spot was detectable with normal serum for volumes below 50 μl, the subsequent experiments were performed routinely with 50 μl of serum. In order to quantify the concentration of IgG-BF in serum, calibrated amounts of recombinant IgG-BF were deposited on the same filter. The comparison of the spot areas obtained with serum (Fig. 1a) and the rIgG-BF standard titration curve (Fig. 1b) led to the conclusion that the serum from the mouse bearing UN2.C3 tumour contained approximately 1 μg/ml IgG-BF, whereas the normal value is around 100 ng/ml.

Table 1. Serum IgG-BF, as determined by immunodot assay during the growth of IgG-secreting tumours in BALB/c mice

Tumour cells	Characteristics and injection	FcγR expression on tumour cells*	No. of mice	Time of bleeding (days)	IgG-BF augmentation factor	Serum immunoglobulin (mg/ml)†
UN2.C3	IgG2a anti-sheep erythrocyte hybridoma (i.p.)‡	+	10	0	1	1·0±0·3
			10	15	2·9	3·8±2·5
			7	23	5·1	13·7±8·1
HOPC1	IgG2a-secreting myeloma (i.p.)	ND	10	0	1	ND
			10	20	12·2	
A20.2J	IgG2a-secreting B cell lymphoma (i.p.)	+	10	0	1	ND
			8	29	6·5	
IIA1.6	A20.2J variant (i.p.)	−	10	0	1	ND
		β gene deleted	9	28	4·4	
			7	31	7	
			6	38	10·7	
Ar13.4.9	IgG2b anti-Ars hybridoma (i.p.)	−	10	0	1	0·4±0·2
			10	7	3·6	1·2±0·4
			6	15	6·2	10·6±2·4
45TG1.7	IgG2b-secreting myeloma (i.p.)	ND	10	0	1	1·3±1
			10	13	1·1	2·6±2·3
			10	15	1·5	3·8±3·3
			8	20	4·2	11·9±10
9.3G7	IgG1 anti-Ars hybridoma (i.p.)	ND	10	0	1	0·8±0·3
			10	12	1	1·3±0·9
			8	21	1·7	6·7±9·5
			7	28	2	12·1±20

* As detected by immunofluorescence.
† Of the tumour isotype.
‡ i.p., intraperitoneal injection.
ND, not done.

To confirm that IgG-BF is increased in mice bearing UN2.C3 tumours, eight mice were injected subcutaneously with UN2.C3 hybridoma cells. Tumour sizes were measured and the amount of circulating IgG-BF was assessed by the immunodot assay before and at various times after inoculation. As shown in Figs. 2a and b, the mean values of spot areas, reflecting the serum IgG-BF contents, increased progressively and concurrently with tumour size up to 11-fold between day 0 (0·40 ± 0·30 mm²) and day 28 (4·6 ± 3·0 mm²).

Levels of circulating IgG-BF in mice bearing IgG-secreting tumours

In order to extend this finding, UN2.C3 and various IgG-secreting tumours were injected intraperitoneally in BALB/c mice. Circulating IgG-BF and serum IgG of the isotype produced by the tumour cells were titrated before injection and at several times during tumour growth. As shown in Table 1, the i.p. injection of four IgG2a-secreting tumour B cells (the UN2.C3 hybridoma, the HOPC1 myeloma and the A20.2J and IIA1.6 lymphomas) as well as the i.p. injection of two IgG2b-secreting hybridoma cells (Ar13.4.9 and 45TG1.7) led to a 4·2–12·2-fold increase in serum IgG-BF levels, accompanied by a circulating concentration of IgG of the tumour isotype greater than 10 mg/ml. The injection of 9.3G7 hybridoma cells secreting IgG1 antibodies also increased the amount of circulating IgG-BF, but to a lesser extent, as the level on day 28 was twice that observed before injection of the tumour cells. Also on day 28, the tumour had grown *in vivo* and the concentration of IgG1 in serum was around 12 mg/ml.

Since the rise in serum IgG-BF could be due to the shedding of FcγR from the tumour cells, the presence of FcγR on the tumour cells was analysed by indirect immunofluorescence with 2.4G2. As shown in Table 1, some cell lines expressed membrane FcγR. However, Ar.13.4.9 hybridoma cells, which induced a 6·2-fold increase in serum IgG-BF levels, did not express detectable levels of membrane FcγR as tested by immunofluorescence (Table 1) and did not contain detectable mRNA coding for FcγR as tested by Northern blots (data not shown). Moreover, IIA1.6 lymphoma cells, which have a deletion in the FcγRβ gene and do not react with 2.4G2, induced a 10·7-fold increase in serum IgG-BF.

Levels of circulating IgG-BF in mice bearing various non-IgG-secreting tumours

Five non-IgG-secreting tumours of lymphoid or non-lymphoid origin were transplanted into syngeneic mice. Groups of 10–11

Table 2. Serum IgG-BF, as determined by immunodot assay, during the growth of non-IgG-secreting tumours in normal mice

Tumour cells	Characteristics and injection	FcγRII expression on tumour cells*	No. of mice	Time of bleeding (days)	Spot area (mm^2)	Augmentation factor
S.107.3	IgA anti-PC myeloma (i.p.)†	ND	10	0	0·137±0·08	1
			9	13	0·150±0·33	1·1
			9	20	0·150±0·18	1·1
SP2/O	Non-Ig-secreting hybridoma†	ND	10	0	0·19±0·18	—
			10	20	0·59±0·16	3
BW5147	Thymoma‡	—	10	0	0·20±0·18	—
			10	20	0·59±0·17	2·9
B16F10	Melanoma§	—	11	0	0·77±0·43	—
			11	7	2·40±1·26	3·1
			11	11	0·52±0·12	<1
			8	18	0·62±0·57	<1

* As detected by immunofluorescence.
† BALB/c mice injected intraperitoneally.
‡ AKR mice injected intraperitoneally.
§ C57BL/6 mice injected subcutaneously.
ND, not done.

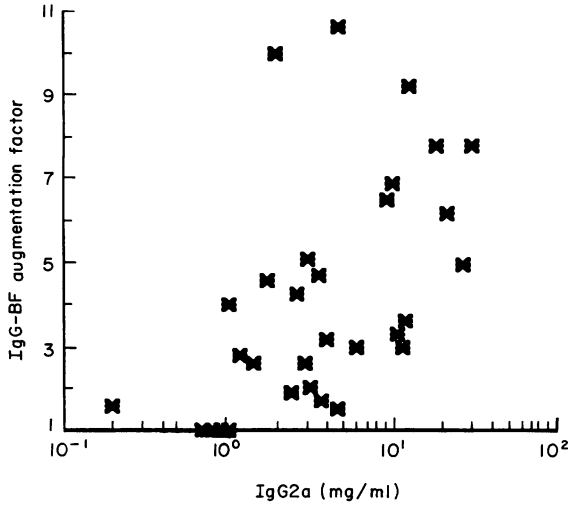

Fig. 3. Comparison of IgG-BF augmentation factor and IgG2a concentration in 32 serum samples from BALB/c mice bearing UN2.C3 tumours (correlation coefficient = 0·45; $P = 0·02$).

mice were injected intraperitoneally with S.107.3, a myeloma line producing anti-phosphonyl choline (PC) IgA antibodies, SP2/O, a non-immunoglobulin-secreting hybridoma, BW5147, a thymoma or B16F10, a melanoma.

As shown in Table 2, all these tumours induced a modest (less than three-fold) increase in serum IgG-BF, although they grew *in vivo* as demonstrated by: (i) the presence of ascites when they were injected intraperitoneally; (ii) a 8·3±5·1 mg/ml serum IgA concentration in the case of S.107.3 tumours; or (iii) solid tumours measuring 300 mm^2±100 mm^2 18 days after injection of B16F10 melanoma cells. It is noteworthy that the BW5147 thymoma cells and B16F10 melanoma cells, which do not express detectable levels of membrane FcγR (Table 2), increased the serum IgG-BF levels three-fold.

Role of tumour-secreted IgG

Since, with the exception of 9.3G7, the IgG-secreting tumours induced levels of circulating IgG-BF higher than those observed with non-IgG-secreting tumours, the role of IgG was then investigated. In a first set of experiments, the levels of serum IgG-BF and monoclonal IgG2a were compared in 32 serum samples of BALB/c mice injected with UN2.C3 hybridoma cells. As shown in Fig. 3, whereas no significant modifications of serum IgG-BF levels were found in mice with less than 1 mg/ml serum IgG2a, for sera containing more than 1 mg/ml IgG2a, the levels of serum IgG-BF levels increased progressively with IgG2a concentration. These observations suggest that IgG produced by the hybridoma cells may play a role in this model.

In order to establish whether IgG2a by itself is capable of increasing the levels of serum IgG-BF, 6 mg of purified monoclonal IgG2a were injected, over a period of 3 days, into the peritoneal cavity of three BALB/c mice. The serum IgG-BF levels as well as the circulating concentration of IgG2a were measured before and several days after the injection of IgG2a. These injections, which increased the serum IgG2a concentration from 1·6±1·8 to 4·4±0·9 mg/ml, increased the serum IgG-BF levels two-fold. Similar conclusions were drawn from the two mice injected with 9 mg of IgG2a in which the IgG-BF augmentation factor was around 3.

DISCUSSION

This study investigated the levels of serum IgG-BF in tumour-bearing mice and in mice injected with high doses of IgG. The results show that in mice bearing IgG2a- or IgG2b-secreting B cell tumours, levels of circulating IgG-BF increased 4–12-fold.

The increase was less than three-fold in sera from mice bearing IgG1-, IgA- or non-immunoglobulin-secreting tumours, as well as in sera of mice injected with 6–9 mg of monoclonal IgG2a.

Soluble FcγR in mouse sera have been previously identified by other groups [3,5]. When interpolated from dilutions of a standard curve containing dilutions of recombinant IgG-BF, sera from mice bearing immunoglobulin-secreting tumours were found to contain IgG-BF at a concentration of around 1 μg/ml ($2{\cdot}5 \times 10^{-8}$ M), whereas normal serum contained around 100 ng/ml ($2{\cdot}5 \times 10^{-9}$ M) IgG-BF. A circulating concentration of 10^{-9}–10^{-8} M was reported by Puré *et al.* [3].

It is interesting to determine whether in tumour-bearing mice, serum IgG-BF comes predominantly from the host or from the tumour. In fact, circulating IgG-BF could represent soluble FcγR shed by tumour cells since some tumours either constitutionally express FcγR or may acquire such receptors *in vivo* [39]. It is noteworthy that the development of IIA1.6 tumours which have a deletion in the FcγRβ gene and hence do not express FcγRII is accompanied by a 10·7-fold increase in serum IgG-BF level. It is highly improbable that such an increase reflects the shedding or secretion of soluble FcγRIII by tumour cells, since (i) IIA1.6 cells do not express detectable amounts of FcγRIII in *in vitro* cell cultures or after passage *in vivo*, as demonstrated by indirect immunofluorescence and by Northern blots (data not shown); and (ii) these cells do not shed detectable amounts of soluble FcγR as tested by the immunodot assay (data not shown). Such observations are in accordance with the fact that FcγRIII are expressed by macrophages and natural killer cells but not by B lymphocytes [25] and show that IIA1.6 cells do not acquire such receptors *in vivo*. Thus, serum IgG-BF secreted in response to IIA1.6 tumours comes from the host rather than from the tumour. Moreover, Ar13.4.9 and BW5147 cells which induce a 6·2- and 2·9-fold increase in serum IgG-BF respectively, do not express detectable amounts of membrane FcγR and FcγRIIβ mRNA. Hence, it seems likely that the majority of IgG-BF produced during tumour growth comes from the host rather than from the tumour.

The cellular origin of serum IgG-BF is unknown. It reacts with 2.4G2, a MoAb directed against FcγRII and III expressed by lymphocytes and macrophages. IgG-BF produced by lymphocytes *in vitro* are 38–40 kD glycoproteins with 19–23 kD degradation products [21]. Recent studies using cells expressing a recombinant FcγRII showed that 38–40-kD IgG-BF can be generated by enzymatic cleavage of FcγRII [23]. The cellular origin and the structure of serum IgG-BF and its mechanisms of production are under investigation.

The present work shows that five tumours secreting IgG2a or IgG2b induced 4–12-fold increases in serum IgG-BF. Five tumours which do not secrete IgG, or secrete IgA, trigger the production of lower amounts of IgG-BF *in vivo* (less than three-fold) than most of the immunoglobulin-secreting tumours. These findings require confirmation using other tumours. However, if the majority of serum IgG-BF produced in response to tumours comes from the host, these results raise several comments concerning the mechanism(s) of production of these molecules. We have previously shown that *in vivo*, alloactivation enhances the serum IgG-BF levels [35]. Thus, it is possible that the three-fold increases observed during growth of non-IgG-secreting tumours reflects activation of the immune system which occurs during tumour growth. The production of IgG-BF may be increased by the interaction of FcγR-positive cells with cytokines secreted by tumour cells or by host cells. On the other hand, the tumour-secreted IgG may trigger the production of IgG-BF as suggested by the present data. The interaction of the IgG-BF-producing FcγR-positive cells with IgG may increase IgG-BF production, as previously shown *in vitro* [28] and/or trigger the production of cytokines [40] which may in turn enhance IgG-BF production. Therefore the stronger enhancing effect of IgG-secreting tumours on serum IgG-BF levels may be due to the fact that they both activate the immune system and secrete IgG.

If this is the case, it seems surprising that the serum IgG-BF levels increased only two-fold in mice bearing the IgG-secreting tumour 9.3.G7, since low affinity FcγR binds murine IgG2a, IgG2b and IgG1 [20]. This finding was confirmed in mice bearing another IgG1-secreting tumour (U182.5, an IgG1-anti-sheep erythrocyte hybridoma cell line) (data not shown). Such an observation is reminiscent of previous findings showing that some IgG isotypes, although interacting with FcγR, are unable to trigger certain FcγR-mediated signals. For instance in man, some IgG3 are unable to mediate ADCC although they interact with FcγR [41]. Mast cell degranulation is known to occur with certain but not all IgG subclasses which bind to FcγR [42]. Consequently, the monoclonal IgG1 used in our study may be unable to activate immune cells. The other possibility is that 9.3G7 cells do not produce or stimulate the production of other signals—e.g. cytokines—which may be required, in addition to IgG, to trigger the production of large amounts of IgG-BF.

The biological role of elevated levels of serum IgG-BF in tumour-bearing mice may be of importance. In the case of IgG-secreting tumours, IgG-BF may interfere directly with tumour cells, probably through its binding to membrane immunoglobulin, and may exert a negative control on myeloma immunoglobulin secretion and tumour growth as suggested by *in vitro* experiments [32–34]. Conversely, IgG-BF may participate in immune suppression of the host and may facilitate escape from immune surveillance by interfering with IgG-mediated functions and decreasing antibody production. The recent availability of biologically active recombinant IgG-BF may help to clarify the physiological role of IgG-BF in normal and pathological situations.

ACKNOWLEDGMENTS

We wish to thank F. Guivel and A. Galinha for technical assistance and I. Lefranc for typing assistance. This work was performed with funds from INSERM, Institut Curie, and Roussel Uclaf (grant No. 1221).

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