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Variations in the Levels of IgG1 and IgG2 Subclasses in the Sera of Normal, Immunized and Tumor-bearing Hamsters

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Abstract—*The concentration of IgG1 and IgG2 subclasses in the sera of hamsters bearing tumors of different origins were compared to that of normal serum and to that of sera of animals rendered resistant to tumor take by immunization with viable SV40-transformed cells. In the sera of hamsters bearing tumors induced by virus-transformed cells an augmentation of IgG2 and a diminution of IgG1 was observed during the development of the tumor compared to sera of normal hamsters. In the sera of animals bearing tumors induced by methylcholanthrene (MCH2) or by spontaneously transformed cells (EHB) the level of IgG2 was almost normal but IgG1 was barely detectable, especially in MCH2 tumors. On the other hand, the sera of animals immunized with virus-transformed cells showed a slight increase in both IgGs, but only that of IgG2 was significant. Antibody activity was tested in the sera as well as in the IgG1 and IgG2 fractions of the sera of hamsters immunized or bearing tumors induced by SV40 transformed cells. Both sera and the subclasses showed antibody activity, the activity being more pronounced in the IgG2 fraction than in the IgG1 fraction.*

INTRODUCTION

THE PARAMETERS of the immune reaction that hamsters develop during the growth of tumors induced by injection of SV40 transformed cells have been studied extensively [1–3]. We have shown that the tumor development is accompanied by important changes in the lymphoid cells that populate the spleen and thymus of tumor-bearing animals. Similar changes in different lymphoid organs were observed in the tumors induced by inoculation of cells transformed spontaneously (EHB) or by papovavirus (ZD, CTB 24). A relationship between the tumor weight and the reactions in the peritumoral area, the spleen and the thymus were seen in all cases [1, 3]. To sum up, the evolution of lymphoid populations during the simian virus 40 (SV40)-induced tumor growth can be divided into three phases. The first phase (tumors up to 5 g) was characterized by the presence of numerous plasma cells in the peritumoral area and the splenic reticulum. The second phase (tumors from 5 to 12 g) was characterized by disappearance of the peritumoral plasmatic reaction. In the third or terminal phase (tumors over 15 g) the thymus and the spleen were invaded by a new population of young lymphoid cells, giving to the spleen a pseudo-leukemic aspect. At the same time, the amount of total serum IgGs and the titer of antibodies directed against SV40-induced antigens increased in the sera of SV40 tumor bearers parallel to tumor growth.

Marked functional differences were seen between the sera of animals immunized with virus-transformed cells (immune sera) and sera of tumor-bearing animals: the *in vitro* antibody-dependent cytotoxicity mediated by spleen cells (ADCC) was enhanced when target cells were incubated in immune sera, whereas this cytotoxicity was decreased in the presence of sera of tumor-bearing animals and more so when the tumor weight was greater than 10 g (unpublished data).

Coe and Takemoto [4] reported that, in the sera of hamsters bearing polyoma virus-induced

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tumors, the IgG2 response was characteristic of tumor resistance while IgG1 antibodies were associated with tumor growth.

In order to further understand the antitumoral immune response of the hamster, the relative amounts of IgG1 and IgG2 subclasses were studied in the sera of normal, immunized and tumor-bearing animals. The technique of fractionation of hamster IgG2 and IgG1 on protein A-Sepharose allowed us to obtain IgG2 in pure form and a relatively good purification of IgG1 [5]. The antibody activity of these subclasses were also tested *in vitro*. The results presented in this communication indicate that the level of IgG1 and IgG2 fractions varied between the immunized and tumor-bearing animals.

MATERIAL AND METHODS

Hamsters

Syngeneic Syrian hamsters from the animal farm of the Institut de Recherches Scientifiques sur le cancer were employed as the source of serum. Normal serum (NS) was obtained from 4–6-month-old animals.

Cell lines

*ZDC*125 (ZD) arose from an undifferentiated tumor appearing in the inbred Syrian hamster injected at birth with 10^5 PFU of SV40 virus, the cells then being maintained in culture after cloning.

*CTB*24 was obtained by *in vitro* transformation of syngeneic hamster (AIB/Mey) kidney cells with polyoma virus (a generous gift from Dr Meyer Marseille).

*MCH*2 arose from a fibrosarcoma induced in an adult syngeneic Syrian hamster after one injection of methylcholanthrene dissolved in olive oil. The tumor cells were maintained in culture since 1980.

EHB spontaneously transformed hamster fibroblasts.

*CV*1 were SV40 productively infected monkey cells.

Tumor induction

Tumors were induced by injecting 2×10^4 transformed cells (ZDC125, CTB24, MCH2 or EHB) in the right flank of 5-month-old hamsters. The tumors were palpable after 3 weeks. The hamsters were bled at different times after the injection of neoplastic cells and the sera were pooled according to the weight of the tumors.

Immunization procedure

Hamsters were immunized against ZDC125 tumor cells by serial inoculations of small doses of *in vitro* cultured ZDC125 cells. It has been demonstrated that s.c. injections of 2×10^2 or 1×10^3 cells on days 0, 7 and 12 protected the animals against the tumor take. The hamsters were considered as immunized when they did not develop tumors after injection of the tumoral dose of 2×10^4 cells.

Preparation and evaluation of IgG2 and IgG1

Hamster IgG2 and IgG1 were fractionated according to the technique described by Escribano *et al.* [5], using protein A-Sepharose and buffers of decreasing pH. Pure IgG2 was eluted from protein A-Sepharose at pH 6. IgG1 contaminated with a small amount of IgG2 was eluted at pH 5. The amount of each subclass was evaluated (a) by optical density at 280 nm, taking as the coefficient $E^{1\,cm}_{1\%} = 14$; and (b) by the Laurell technique [6] using 1% C agarose (Pharmacia, France).

Antisera to IgG subclasses

Rabbit antisera specific for hamster immunoglobulins were obtained by injecting rabbits with IgG2 and IgG1 fractions obtained after fractionation of normal hamster serum using protein A-Sepharose. The rabbits received three subcutaneous injections in the footpad at 10-day intervals of 1.3 mg protein in 0.1 ml saline emulsified in 0.1 ml complete Freund's adjuvant.

Rabbit serum anti-IgG1 (aIgG1) was rendered monospecific by absorbing anti-IgG2 antibodies on gluteraldehyde-polymerized IgG2. (The rabbit anti-total-IgG and anti-IgG1 were diluted 1:200 and the anti-IgG2 1:100 for use.)

Antibody activity

The antibody activity of whole sera, IgG2 and IgG1 fractions were tested *in vitro* using the immunofluorescent technique on acetone-fixed CV1 cells previously infected for 16–18 hr with SV40 virus. The presence of antibodies directed against SV40-induced membrane antigens was shown using the radioactive protein A technique as described by Nayak *et al.* [7] and modified by Duthu *et al.* [8]. The precipitation characteristics of each subclass was tested by SDS-PAGE. The SV40-induced antigens were labelled with [^{35}S]-methionine (100 μCi/ml, 750–930 Ci/mmol; Radio Chemical Centre, Amersham, U.K.) and immunoprecipitated with the whole sera and their subclasses. The immunoprecipitates were isolated by immunoaffinity chromatography on formalin-fixed protein A-producing *Staphylococcus aureus* [9] denatured and analyzed on SDS-polyacrylamide gels (12.5%). The gels were stained with Coomassie blue, destained, vacuum-dried and exposed to Kodirex X-ray films for 2–16 days at -70°C. The following molecular weight standards were used to calibrate the gels: myosin (200K) phosphorylase b (100 and 92K), bovine

serum albumin (69K), ovalbumin (46K), carbonic anhydrase (30K) and lysozyme (14.3K).

RESULTS

Obtention of IgG2 and IgG1 from the sera of normal, immunized and tumor-bearing animals

Three types of tumor cells differing in their origin and antigenicity were chosen and used: (1) SV40- or polyoma-transformed cells (ZD or CTB24 respectively). These cells were highly antigenic, possessing all the virally induced neoantigens. They elicited antibody titers in the sera that increased as a function of tumor weight [1]. Specific antibodies against SV40 or Py antigens could be detected; (2) spontaneously transformed EHB cells induced tumors that appeared more rapidly than ZD tumors. Specific antibodies directed against EHB cells could not be detected; (3) methylcholanthrene-transformed cells, MCH2, induced tumors similar to ZD tumors to grow. The study of the characteristics of MCH2 cells is in progress.

Protein A-Sepharose and buffers of decreasing pH were used to obtain IgG1 and IgG2 subclasses from normal hamster serum (NHS), from the sera of hamsters immunized with viable SV40 transformed cells (Im.S) and from the sera of animals bearing tumors (TBS) induced by s.c. injection of transformed cells. The elution pattern of immunoglobulins from 1 ml of NHS, Im.S and TBS (ZD) absorbed at pH 8 on protein A-Sepharose is given in Fig. 1 (a, b, c). The nature of each fraction was assessed by immuno-electrophoresis as described for normal serum [5].

The amount of IgG2 and IgG1 obtained by this technique was evaluated and the ratio of IgG2/IgG1 calculated, and is given in Table 1. With this technique the amount of IgG2 was more or less of the same magnitude in the three sera. In contrast, important variations in the levels of IgG1 could be seen in the sera of immunized or tumor-bearing animals. The IgG1 level increased in the serum of immunized animals and this augmentation depends on the number of immunizing injections. The IgG1 level in the sera

Table 1. Amount (in mg) of IgG_2 and IgG_1 obtained on protein A-Sepharose from 1 ml of serum of normal, tumor-immunized and ZD tumor-bearing hamsters

Source of serum	Experiment No.	IgG_2	IgG_1	$\frac{IgG_2}{IgG_1}$
Normal	1	4.25	1.24	3.4
	2	4.10	1.14	3.6
	3	4.20	1.20	3.5
Tumor-immunized	1*	3.60	1.30	2.77
	2	3.80	1.28	2.96
	3	3.80	2.15	1.77
	4	3.60	2.00	1.80
Tumor-bearing Tumor weight:				
<1 g	1	4.30	1.30	3.23
	2	4.13	1.28	3.22
1-3 g	1	4.05	0.98	4.10
	2	4.27	1.28	3.89
>5 g	1	4.20	0.82	5.2
	2	4.15	0.71	5.8

*The serum employed in experiments 1 and 2 came from one series of immunized animals whereas those of experiments 3 and 4 came from another series. The latter animals showed a better protection against the tumor take than those of the first series.

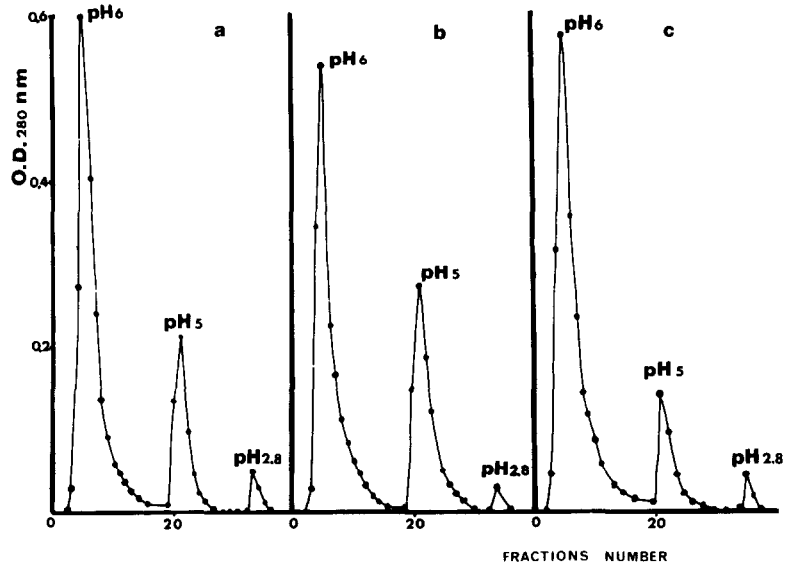

Fig. 1. Elution patterns of IgG2 (pH6) and IgG1 (pH5) obtained after fractionation on protein A-Sepharose. (a) 1 ml of normal hamster serum; (b) 1 ml of ZD-immunized hamster serum; (c) 1 ml of ZD tumor-bearing hamster serum.

of tumor bearers varied; the bigger the tumor, the lower the level of IgG1. The IgG2/IgG1 ratio (Table 1) was approximately 3.5 in normal sera, but was 2.5 in immune sera, reflecting the increasing amount of IgG1. In animals bearing large tumors (over 15 g) the ratio is 5.5, and this indicates an important diminution in the amount of IgG1. It should be emphasized that no differences in the IgG ratios were seen in the sera of hamsters with small tumors.

Evaluation by the Laurell-technique of the level of IgG2 and IgG1

The sera of tumor-bearing animals were routinely tested at different periods of tumor growth by a different method using the Laurell technique. Individual normal hamster age-matched sera were tested at the same time, and as the individual variations were small (Fig. 2), pooled normal sera were used thereafter.

A pool of 10 normal sera and pure fractions of IgG2 and IgG1 were used as reference with monospecific rabbit antisera against IgG2 and IgG1 in the agarose C gels. Figure 2(A) shows the relative amounts of IgG2 in the sera of ZD-immunized animals and in the sera of tumor bearers taken at different tumor weights, and Fig. 2(B) shows that of IgG1.

(a) In the sera of animals immunized against viable ZD cells, IgG2 level was slightly increased ($P < 0.05$) compared to the control, while IgG1 level was not statistically significantly higher.

(b) In the sera of hamsters bearing viral-induced tumors, the amount of IgG2 increased as a function of tumor growth and decreased during the terminal phase, when the animals were dying ($P < 0.001$ for all tumor weights less than 30 g).

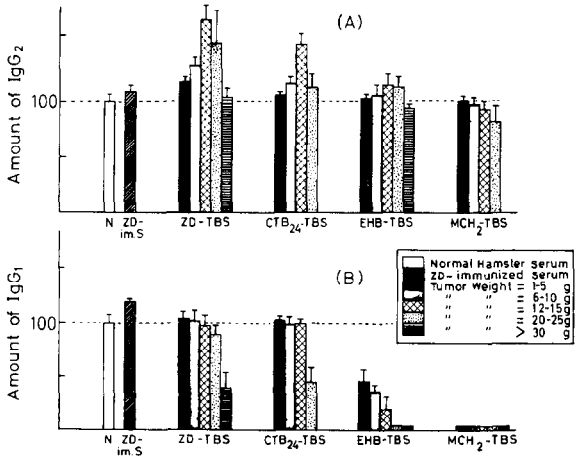

Fig. 2. Relative amounts of IgG2 (A) and IgG1 (B) evaluated by the rocket immunoelectrophoresis technique in the sera of normal and immunized hamsters and hamsters bearing different tumors taken during the tumor growth. (Bars = standard deviations).

This increase was more important in the sera of animals bearing SV40-induced tumors than in those of Py-induced tumors. The level of IgG1 as compared to normal serum showed no significative difference for tumor weights less than 15 g and decreased as tumors grew bigger.

(c) In the sera of EHB tumor bearers a weak and unsteady augmentation of IgG2 was noted, whereas IgG1 was difficult to detect for all tumor weights ($P = 0.01$ for 12–15-g tumors).

(d) In the sera of MCH2 tumor bearers the IgG2 level was lower than in controls, and for the same dilutions IgG1 was undetectable.

(e) The mixture of normal serum and of serum of MCH2 tumor bearers (1:1) did not lead to artifactually low values of either IgG1 or IgG2 from normal serum.

Antibody activity of IgG2 and IgG1 fractions

One milliliter of immune serum and of ZD tumor serum (TBS) (tumor weight = 10–15 g) were fractionated, IgG1 and IgG2 fractions were obtained, concentrated to the original volume of the serum and tested for their antibody function on SV40-infected CV1 cells and on ZD cells. In the two sera IgG1 and IgG2 subclasses showed antibody activity against T antigen, as revealed by nuclear immunofluorescence in CV1 cells infected for 16–18 hr by SV40 virus. Both subclasses of immunoglobulin G gave a specific fixation of radioactive protein A on the membranes of SV40-transformed cells. However, the activity was stronger with IgG2 than with IgG1 in the sera of both immunized and tumor-bearing animals.

The whole immune sera, SV40 tumor sera and their IgG1 and IgG2 fractions were tested by immunoprecipitation to find out the antigens recognized.

By SDS-PAGE, the whole immune serum (Fig. 3.2) precipitated 94K (large T), 56K and, weakly, 17K (small t) antigens. IgG1 from this serum (Fig. 3.3) only precipitated very weakly 94K and IgG2 (Fig. 3.4) precipitated 94K, 56K and not 17K.

Whole ZD-TBS (Fig. 3.5) precipitated 120K (super T), 94K, 56K, 17K and 32K. IgG1 (Fig. 3.6) precipitated 94K, 17K and weakly 120K and 32K, and IgG2 (Fig. 3.7) precipitated 120K, 94K, 56K, 17K and 32K.

DISCUSSION

A great deal of research has been done in recent years in attempting to develop immunodiagnostic procedures for the detection of cancer. Much of this effort has been centered on a comparison of immune responses in normal and tumor-bearing hosts. Although most investigators have reported a general suppression of host immune responses with progressive tumor

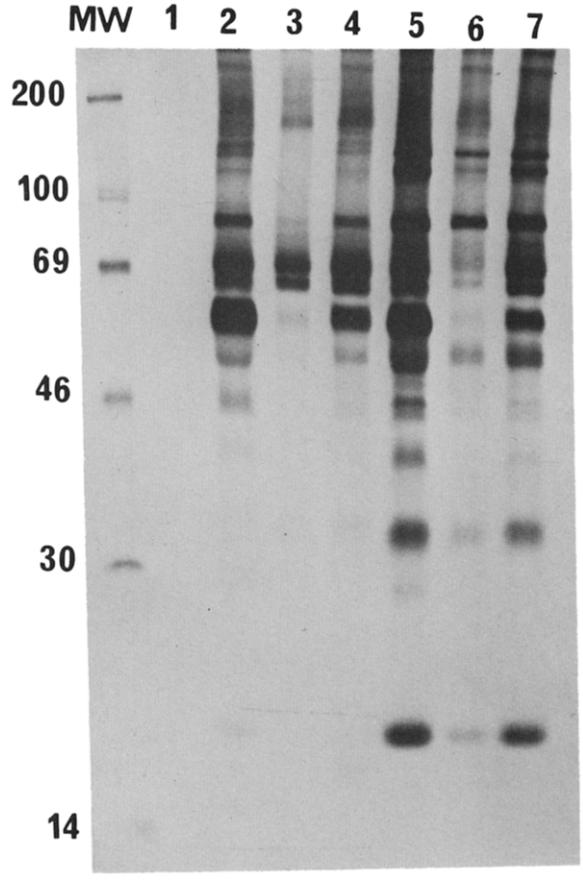

Fig. 3. SDS polyacrylamide gel analysis: extracts were obtained from ZD cells and immunoprecipitated by normal hamster serum (1), *whole immune serum* (2), *its IgG1* (3) *and IgG2* (4) *fractions, whole tumor-bearing hamster serum* (5) *and its IgG1* (6) *and IgG2* (7) *fractions. Molecular weight values are expressed in* K_d.

growth [10, 11], conflicting reports also exist. Some investigators explain the tumor escape by the presence of suppressor cells [2], while others expect the existence of enhancing antibodies [12] that block the activity of cytotoxic effector cells.

In this study we compared the relative amounts of IgG1 and IgG2 found in the sera of normal, immunized or tumor-bearing hamsters. Three types of tumor cells were chosen according to their immunogenic status: (a) the highly antigenic cells transformed by DNA viruses (SV40 and Py); (b) a weakly antigenic methylcholanthrene-transformed cell line (MCH2); and (c) a non-antigenic spontaneously transformed cell line (EHB).

In view of our preceding paper reporting a close relationship between the antibody titer in the sera and the weight of the tumor [1], we were interested in utilizing our three different tumor systems to compare the levels of antibody response and IgG synthesis taken as the expression of the humoral response of the host against tumor-associated antigens.

Two main conclusions can be drawn from our results: in the sera of hamsters bearing tumors induced by virally transformed cells, an important increase of the level of IgG2 is noted which can reach 150% of the normal. It can be postulated that this increase is directly correlated with the continual presence of virally induced antigens associated with the transformed cells. By contrast, the IgG2 level remains more or less normal in the two other tumoral conditions. The second fact that seems fairly important is the dramatic decrease of the IgG1 subclass, which is almost undetectable, for example, in the sera of animals bearing MCH2-induced tumors. The experiment of mixing an equal volume of normal serum and of the serum of tumor-bearing hamster showed that no factors such as the rheumatoid factor were leading to artifactually low values of IgG1 or IgG2 in the sera of tumor-bearing hamsters. Furthermore, in a previous paper [13] it was demonstrated that antibodies eluted from SV40 tumors were mostly of the IgG2 subclass. Antibodies eluted from these tumors, radiolabeled and injected into SV40 tumor-bearing animals showed no preferential fixation onto tumoral tissue [Vaux St Cyr, personal communication]. It appears that suppressive cells on factors progressively inhibit the expression of IgG1 in the sera of tumor-bearing animals. However, the presence of antigens associated with the vitally transformed cells seems able to maintain or increase the level of IgG2. The IgG1 subclass appears to be more sensitive to the suppressive activity (work in progress).

By contrast, in the sera of animals immunized against SV40-transformed cells and able to reject tumor grafts, a slight increase of IgG1 and IgG2 can be seen. Only IgG2 and IgG1 subclasses from a pool of sera from ZD-immunized hamsters and hamsters bearing tumors (8–12 g) induced by SV40-transformed cells were tested for their antibody activity: both subclasses showed approximately similar activity against SV40-induced antigens when tested by immunofluorescence techniques, as well as by precipitation and by fixation on the cell membranes. Only IgG2 fixed the complement *in vitro* [5] and the antibody-dependent cell cytotoxic activity was not related to one or the other subclass.

How could these facts be related with the findings of other investigators about the role of IgG1 and IgG2 antibodies in regulation of the immune response? Reports, mostly on mice, from different laboratories have given conflicting results. For some, IgG2 antibodies were able to exert a rejection activity on grafts and IgG1 could have the opposite activity [14]. For others both subclasses were found to be enhancing [15], and Fabre and Batchelor [16], working on passive enhancement of renal allografts, found no link whatsoever between enhancing and cytotoxic antibodies. In hamsters with polyoma tumors, Coe and Takemoto [4] reported that in the sera, the IgG2 response was characteristic of tumor resistance, while IgG1 antibodies were associated with tumor growth. In our study, when tumor cells were mixed with IgG1 or IgG2 from immune sera and injected into the hamsters no significant protection nor enhancement of tumor take and growth was observed with both subclasses (unpublished data). The probable role played by IgG1 and IgG2 in the antitumoral response still remains uncertain.

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