Brief Definitive Reports

QUANTITATIVE STUDIES ON TUMOR ENHANCEMENT IN MICE

I. Enhancement of Sarcoma I Induced by IgM, IgG1, and IgG2*

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The mechanism whereby passively-transferred antibodies lead to enhancement of tumor allografts in mice is still unclear. Different hypotheses have been derived from conflicting results on similarly constructed experiments, and controversy extends to the immunoglobulin class of the responsible antibodies (1–8). However, the absolute quantity of antibody used has not been determined, which prevents resolution of these discrepancies. We have determined immunochemically the amounts of specific alloantibody in three purified mouse immunoglobulin preparations and have shown that IgM, IgG1, and IgG2 antibodies are all capable of inducing tumor enhancement when used in adequate concentrations.

Materials and Methods

Mice.—All animals used were purchased from the Jackson Laboratories, Bar Harbor, Maine.

Tumor.—Sarcoma I (SaI), a strain A fibrosarcoma, was kindly provided by Dr. N. Kaliss, Jackson Laboratories. It was maintained in the ascites form by serial passage in A/J mice. Allografts consisted of 10^6 SaI ascites cells, harvested 8 days after inoculation, suspended in 0.1 ml of sterile Hank's balanced salt solution and injected subcutaneously (s.c.) in the backs of the recipient mice.

Preparation of Immune Ascites.—30 female C57BL/6 (B6) mice were hyperimmunized with A/J spleen cells. Ascites was then induced by four intraperitoneal (i.p.) injections of complete Freund's adjuvant (Difco Laboratories, Inc., Detroit, Mich.).

Fractionation of Ascitic Fluid.—The hemagglutinating activity of the material was followed with the polyvinylpyrrolidone (PVP) method (9).

IgM: IgM was obtained from the ascending limb of the excluded fraction of Sephadex G-200 gel filtration (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). A contaminating α-globulin was removed by Pevikon block electrophoresis (10).

IgG: IgG was separated by anion exchange chromatography through DEAE-Sephadex, (pH 7, 0.001 M, Tris) (Pharmacia Fine Chemicals). By elution with starting buffer IgG2 only was found in the first fractions while most of the IgG1 appeared in the last fractions, contaminated with IgG2. The beginning and final fractions were separately pooled and each was freed of transferrin by Sephadex G-100 gel filtration (Pharmacia Fine Chemicals). Protein

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content was determined by absorbance at 280 nm[1] and by the method of Lowry (12), with bovine serum albumin (BSA) (Schwartz-Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.), as standard. Immunoglobulin concentration was found by radial immunodiffusion (13). Purity of the fractions was ascertained by immunoelectrophoresis and immunoglobulin classes were identified by double-diffusion in agar (14).

Determination of Specific Antibody Content.—The single-dilution radioimmunoassay method of Paul and Esposito (15) was used with minor modifications. Briefly, an aliquot of each antibody preparation was trace-labeled (15) with ^{125}I (New England Nuclear, Boston, Mass.) achieving approximately one atom of I and 0.002 of ^{125}I per molecule of immunoglobulin. Antibody uptake was determined with 10^7 nucleated spleen cells pretreated with 0.25% glutaraldehyde, (Sigma Chemical Co., St. Louis, Mo.) 1-ml vol of different dilutions of labeled immunoglobulins were incubated with glutaraldehyde-treated A/J or B6 spleen cells for 2 h at 22°C with continuous mixing. After incubation, the cellular contents were washed eight times in chilled BSS, and bound radioactivity was determined in a gamma-counter (Superscaler II, Nuclear Chicago Corp., Des Plains, Ill.), using a 20–80 kev energy range.

RESULTS

Antibody Concentration in Immunoglobulin Fractions.—Three immunoglobulin fractions, isolated from two separate pools of immune ascites, were studied as shown in Table I. The recovery of immunoglobulins (not shown) varied from 15% to 30% from the concentrations in the starting fluid.

Specific Antibody Content of the Three Immunoglobulin Preparations.—Table II depicts the procedure for the estimation of the total antibody concentration in the labeled fractions.

Enhancement of SaI.—In experiment 1, groups of three male B6 mice each were injected s.c. with tumor and simultaneously i.p. with different quantities of immunoglobulin preparations, as shown in Table III. The highest doses of IgM used, 30–15 μg, suppressed tumor growth, while lower doses of the same preparation enhanced it. Treatment of the IgM preparation with 0.1 M 2-mercaptoethanol (not shown) resulted in loss of both enhancing and suppressive effects; IgG1 and IgG2 were not affected by this treatment. Thus, the activities found in the IgM preparation were not due to undetected contamination with antibodies of either IgG class. Enhancement resulted from the injection of 0.06 μg of IgG1 in three of three recipients; the same dose of IgG2 induced enhancement in only one of three mice. Thus, the IgG2 contaminant in IgG1 is unlikely to be responsible for its enhancing properties.

In experiment 2, larger quantities of purified immunoglobulins were available. As shown in Table III, again high (25–50 μg) doses of IgM were suppressive of tumor growth while lower ones led to enhancement. IgG2 in molar concentrations ~100-fold higher (600 μg) also suppressed tumor growth. Doses of 200 μg of either IgG2 or IgG1 resulted in enhancement. Unfortunately, IgGl was not available in quantities that permitted the use of the 600 μg dose. At the lower end of the scale, again IgG1 and IgM were slightly more efficient than IgG2 in inducing enhancement (in molar terms).

[1] Average extinction coefficient = 0.0100 OD/μgN/ml (11).

TABLE I

Protein and Immunoglobulin Content of Ascitic Fluid Fractions

Preparation	Protein content		Immunoglobulin content (Mancini assay)		
	Absorbance (280 nm)	Lowry	Specific Ig	Other Ig	Total
	mg/ml	*mg/ml*	*mg/ml*	*mg/ml*	*mg/ml*
Exp. 1					
IgM	1.5	1.9	1.1	0	1.1
IgG2	2.7	3.0	2.0	0	2.0
IgG1	3.2	3.5	1.9	0.4*	2.3
Exp. 2					
IgM	1.9	2.7	1.45	0	1.45
IgG2	2.8	3.0	2.25	0	2.25
IgG1	2.9	3.4	1.6	0.3*	1.90

* IgG2.

TABLE II

Specific Antibody Content of Immunoglobulin Preparations

Prepara-tion	Spec act	Ig content*	Δ Counts from aliquots containing:			Specific antibody‡ (μg/ml unlabeled preparations)
			10 μg	50 μg	100 μg	
	cpm/μg	*mg/ml*				
Exp. 1						
IgM	1.91×10^3	1.0	439	2,465	5,122	27.5
IgG2	15.00×10^3	1.9	2,908	13,951	31,833	40
IgG1	13.4×10^3	2.1	2,151	10,822	22,193	37 (IgG1 30, IgG2 7)
Exp. 2						
IgM	2.27×10^3	1.3	357	2,039	4,209	22
IgG2	9.48×10^3	2.1	2,213	10,870	20,981	45.5
IgG1	8.54×10^3	1.8	1,811	8,875	16,021	36.5 (IgG1 31, IgG2 5.8)

* Estimated by radial immunodiffusion.

‡ Calculations: (*a*) Spec act = cpm/immunoglobulin concentration (μg); (*b*) Δcount = (cpm on A/J) − (cpm on B6); (*c*) Specifically bound antibody (SBA) = (Δcount/spec act) (μg); (*d*) Concentration of bindable antibody = (SBA at given dilution) × (dilution) × (protein concentration in unlabeled preparation/protein concentration in labeled preparation).

Table IV shows the effect of IgM given together with either IgG1 or IgG2. Suppressive doses of IgM were neutralized by IgG1 and led to enhancement while IgM plus IgG2 was still suppressive. In subenhancing doses both IgG2 and IgG1 had additive effects to IgM so that the mixtures led to enhancement.

DISCUSSION

Quantitative methodology developed for the estimation of Rh antibody in immunoglobulin preparation (15) has allowed comparison of the effect on tumor growth of antibodies of different immunoglobulin classes in similar molar concentrations. IgM suppressed the initial growth of the tumor when used in high concentrations, presumably because it is cytolytic for the neoplastic cells (7) and so did IgG2 in much higher doses, as could perhaps be expected from its relatively lower complement-fixing efficiency. In lower dosage, both led to enhancement, as did comparable molar amounts of IgG1. In our preparations,

TABLE III

Modification of the Growth of SaI in B6 Hosts by Specific Antibodies

Preparation	Quantity injected	Molecules $\times 10^{12}$‡	Tumor at day 8	Death with growing tumor at days
	μg			
	Total immunoglobulins			
Nonimmune	300		0/3	—*
Ascitic fluid	30		0/3	—*
30 mg. prot./ml	3		0/3	—*
Immune	300		3/3	14—19—20
Ascitic fluid	30		3/3	18—18—19
28 mg prot./ml	3		3/3	17—17—24
Exp. 1	Specific antibody			
IgG2	3	11.3	3/3	13—15—19
	0.3	1.13	3/3	14—16—23
	0.06	0.22	1/3	22 (two without tumors)
	0.03	0.11	0/3	—*
IgG1 (+IgG2)	3	11.3	3/3	11—15—15
	0.3	1.13	3/3	14—17—19
	0.06	0.22	3/3	13—20—22
	0.03	0.11	1/3	19 (two without tumors)
IgM	30	20.1	0/3	—*
	15	10	1/3	12 (two without tumors)
	1.5	1	3/3	15—18—21
	0.15	0.1	2/3	16—20 (one without tumor)
Exp. 2				
IgG2	600	2,300	0/2	—*
	200	750	2/2	14—17
	60	230	3/3	15—17—17
	20	80	3/3	15—19—19
	6	23	3/3	17—19—23
	0.6	2.3	3/3	19—21—33
	0.06	0.23	3/3	21—21—26
	0.03	0.12	0/3	— (1 died at day 39 w/o tumor)
	0.15	0.06	0/3	—*
IgG1	200	750	2/2	17—21
	20	75	2/2	17—17
	2	7.5	3/3	19—19—21
	0.2	0.8	3/3	17—21—23—
	0.1	0.4	3/3	17—21—21
	0.05	0.2	2/3	19—21 (one without tumor)
	0.02	0.1	1/3	21— (two without tumor)
	0.01	0.05	0/3	—*
IgM	50	38	0/2	—*
	25	19	0/2	—*
	12.5	10	3/3	19—21—23
	6	5	3/3	19—21—21
	3	2.5	3/3	17—19—21
	1.5	1.26	3/3	8—19—21
	0.8	0.6	3/3	21—21—23
	0.4	0.3	3/3	17—17—21
	0.2	0.15	3/3	19—19—21
	0.1	0.07	2/3	17—21 (one without tumor)
	0.05	0.03	0/3	—*

* No deaths within 30 days.

‡ Mol wt assumed to be: IgG1 and IgG2 = 160.000, IgM = 900.000

TABLE IV

Growth of SaI Allografts in Hosts Given Passive IgM Plus Either IgG1 or IgG2

	Tumor growth	Death with growing tumor
25 μg IgM + 60 μg IgG2	0/2	0/2
0.05 μg IgM + 0.03 μg IgG2	5/5	3/5
25 μg IgM + 10 μg IgG1	2/2	2/2
50 μg IgM + 10 μg IgG1	2/2	2/2
0.05 μg IgM + 0.02 μg IgG1	5/5	4/5

however, quantities of about 2×10^5 antibody molecules per injected tumor cell caused enhancement with the three immunoglobulins; 1×10^5 did so for IgG1 and IgM but not for IgG2. The wide range of survival times and the small number of animals per group preclude further elaboration of the data. Mixtures of suppressive doses of IgM and enhancing amounts of IgG2 were suppressive of tumor growth, while similar mixtures of IgM and IgG1 led to enhancement. This experiment suggests differences in the mechanisms of action of IgG2 and IgG1 as proposed by Voisin (2), although differences in avidity may (also) be at work. In low concentrations, both IgG2 and IgG1 exerted effects additive to those of IgM.

It is probable that avidity, in addition to quantity, is important in determining the enhancing efficiency of antibody, and we are currently trying to relate K-values for antibodies of the different immunoglobulin classes to enhancing efficiency. Studies are also under way to determine whether the mechanism whereby the different immunoglobulins lead to tumor enhancement is the same. In conclusion, these results indicate that enhancement is a property of antibodies of most or all immunoglobulin classes and that the contradictory results of other studies may result from quantitative differences.

SUMMARY

The concentration of specific alloantibody in purified mouse immunoglobulin preparations was determined. When passively transferred in adequate doses, IgM, IgG1, and IgG2 antibodies all induced tumor enhancement in allogeneic hosts. IgM and IgG2 antibodies in high concentration led to inhibition of tumor growth. IgM and either IgG1 or IgG2 had additive effects on tumor enhancement. IgG1, but not IgG2, suppressed the inhibitory effect of IgM in high concentration.

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