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Effects of Transfer of Hybridomas Producing Various Isotypes of Immunoglobulins on the C1q Metabolism in Mice

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In order to elucidate possible effects of immunoglobulin on C1q metabolism at the anabolic steps, serum C1q levels and C1q mRNA of peritoneal exudate cells (PEC) and spleen cells were measured in female BALB/c mice implanted intraperitoneally with complement-(C)-fixing IgG2b- or non-C-fixing class IgG3-producing hybridomas and/or with immunoglobulin-non-productive myeloma cells (p3x63-Ag.8.653) (myeloma 653) (2×10^6/0.2 ml) or without any treatment as controls. In the IgG2b-hybridoma-treated mice, the serum C1q levels and C1q mRNA in PEC increased conspicuously as compared with those in the controls, but C1q mRNA in spleen cells was almost equal to that in the control mice. On the other hand, in the IgG3-hybridoma-treated mice, the serum C1q levels decreased significantly, but the extent of such decrease and the level of C1q mRNA in their PEC were almost equivalent to those in the myeloma 653-implanted mice. The serum C1q levels and C1q mRNA in PEC fluctuated similarly in mice injected intraperitoneally with highly purified IgG2b and/or IgG3 preparations. These results suggest some anabolic interaction, as well as catabolic interaction, between the C-fixing class of immunoglobulin and C1q.

Key words: IgG2b- or IgG3-producing hybridoma, isotype dependent serum C1q-fluctuation, mouse C1q metabolism, mRNA of the C1q B-chain.

C1q is a subcomponent of the first component of complement containing unique collagenous regions in its molecule, and is the essential triggering component in the classical activation pathway. Serum C1q is considered to be mainly produced in the monocyte-macrophage lineage (*1*).

Low serum C1q levels may accompany primary humoral immunodeficiency diseases such as sex-linked (Bruton type) hypogammaglobulinemia, combined immunodeficiency, and common varied immunodeficiency (*2, 3*). Furthermore, a significant correlation was observed between C1q and IgG levels (low through high) in various strains of normal mice (*4*). Such correlations have been supposed to result from the IgG-dependent catabolism of C1q: C1q is stabilized by IgG in the circulation, probably *via* reversible interactions which reduce extravascular degradation. At higher IgG concentrations, the equilibrium may be pushed toward a higher proportion of complexed C1q with a slower catabolic rate, and at lower IgG concentrations C1q appears to be more rapidly catabolized (*3*). In fact, normalization of serum C1q in hypogammaglobulinemic patients has been achieved by means of intravenous immunoglobulin infusions (*5, 6*).

However, patients with Swiss-type lymphopenic agammaglobulinemia (*7*) and embryonically bursectomized chickens (*8*) do not show a positive correlation between their serum C1q and IgG levels. Furthermore, we have shown that serum C1q as well as IgG levels are significantly suppressed in mice treated with immunosuppressive reagents such as cyclophosphamide and anti-thymocyte-globulin (*9*).

The present investigation was therefore undertaken to study whether a C(C1q)-fixing class of immunoglobulin (IgG2b) (*10*) in the circulation may affect the serum C1q levels only at the catabolic steps or at some of the anabolic steps. We examined the possible dependence of the C1q metabolism on immunoglobulin isotypes in mice by *in vivo* intraperitoneal implantation of hybridomas producing different isotypes (C-fixing IgG2b and non-C-fixing IgG3) of immunoglobulins (*11*) and/or by intraperitoneal injection of these isotypes of immunoglobulins in highly purified forms.

MATERIALS AND METHODS

Animals—Female BALB/c mice (4 to 5 months old) were used. The mice, originally a gift from the Center for Laboratory Animals in the Research Institute for Microbial Diseases, Osaka University (Osaka), have been maintained by sister-brother mating in our laboratory.

Treatment with Hybridomas—Each mouse was intraperitoneally given 0.5 ml of pristane and 0.2 ml of IgM (μ, κ)-,

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Abbreviations: IgG, immunoglobulin G; Myeloma 653, immunoglobulin-non-productive myeloma cells (p3x63-Ag.8.653); PBS, physiological phosphate-buffered saline; PEC, peritoneal exudate cells; C, complement; PCR, polymerase chain reaction; SD, standard deviation.

IgG1 (γ1, κ)-, IgG2b (γ2b, κ)-, or IgG3 (γ3, κ)-producing hybridoma, murine plasmacytoma [MOPC-141 (γ2b, κ), J 606 (γ3, κ)] (*12, 13*) or immunoglobulin-nonproductive myeloma 653 (1×10^7 cells/ml in PBS) on day 0. These hybridomas were produced by fusing female BALB/c spleen cells forming antibodies to Staphylococcal lipoteichoic acid (Sigma Chemical, St. Louis, MO. USA) antibodies and myeloma 653 in polyethylene glycol according to a routine method of monoclonal antibody production. Almost all these hybridoma- or plasmacytoma-implanted mice fell sick and died 14-16 days after the inoculation. On day 11, blood was collected from each mouse by cutting the carotid artery and the serum was separated after 30 min incubation at 37°C for quantitation of C1q and IgG. Since a conspicuous increase and an extreme decrease of serum C1q were observed in IgG2b (γ2b, κ)-hybridoma-implanted mice and in IgG3 (γ3, κ)-hybridoma-implanted mice, respectively (Fig. 1), the mice treated with these hybridomas were killed under ether anesthesia on days 9, 10, 11, 12, and 13, in parallel with those treated with myeloma 653 and those without any treatment, and their PEC and spleen cells were used for cell counts and C1q mRNA analyses.

Purification of Mouse IgG2b and IgG3—Pooled ascitic fluid of BALB/c mice carrying IgG2b (γ2b, κ)- or IgG3 (γ3, κ)-producing hybridoma was precipitated with 50% saturated ammonium sulfate and desalted with Sephadex G-25. Each isotype of these immunoglobulins was finally purified by affinity chromatography on a Protein A column (Ampure PA column, Amersham, Tokyo). The purity of final preparations was checked by SDS-polyacrylamide gel electrophoresis (*14*) in the presence of dithiothreitol using a MULTI GEL (10/20, Daiichi Pure Chemicals, Tokyo), and each isotype of the preparations showed only two staining bands (one corresponded to H-chain and the other to L-chain of the mouse immunoglobulin) with Coomassie Brilliant Blue (Fig. 2).

Treatment with Immunoglobulin Preparations—Each mouse, which was injected with 0.5 ml of pristane intraperitoneally 10 days in advance, was intraperitoneally given 0.7 ml of a purified preparation of IgG2b (γ2b, κ) or IgG3 (γ3, κ) (10 mg/ml in PBS). Twenty-four hours later, the mice were killed in parallel with those without any treatment, and their blood and PEC were isolated and used for quantitation of C1q and IgG and/or for C1q mRNA analyses.

Immunochemical Quantitation of C1q and IgG—This was carried out by using a modification of the single radial immunodiffusion method described by Mancini *et al.* (*15*). Rabbit monospecific antiserum to mouse C1q was prepared according to the method previously described (*16*). Rabbit monospecific antiserum to mouse IgG was purchased from Miles Laboratories (Elkhard, IN, USA). The sensitivity of these methods was about 2 μg C1q/ml and 10 μg IgG/ml, respectively.

Peritoneal Exudate Cells (PEC) and Spleen Cells—PEC were harvested from each mouse by intraperitoneally injecting PBS containing 10 mM EDTA as an anticoagulant repeatedly (total volume of 10 ml). The spleen was removed surgically from each mouse and rubbed against a frosted glass plate with the inside Teflon cylinder of a syringe in PBS, and large debris in the spleen cell suspension was removed by allowing it to settle in its container for 2 min. Small aliquots of the PEC and the spleen cell suspension were used for nucleated cell counts with Turk's solution. Glass-attached and peroxidase-positive cells were measured as macrophages with an MSP kit (Japan Immunoresearch Laboratories, Gunma). The remaining cells were used for Northern blot analysis.

cDNA for B-Chain of Murine C1q—Oligonucleotide primers corresponding to Asp^{85}-Gln^{93} and Phe^{220}-Ala^{228} of the mouse C1q B-chain were synthesized by a DNA synthesizer (mode 391; Applied Biosystems, Tokyo) according to the reported cDNA sequence (*17*). Peritoneal macrophages were obtained from thioglycolate-stimulated female BALB/c mice (*18*), suspended in RNAzol™B (Biotecx Laboratories, Houston, TX, USA) and homogenized. RNA was extracted with chloroform, precipitated with isopropanol, and washed with 75% ethanol according to the

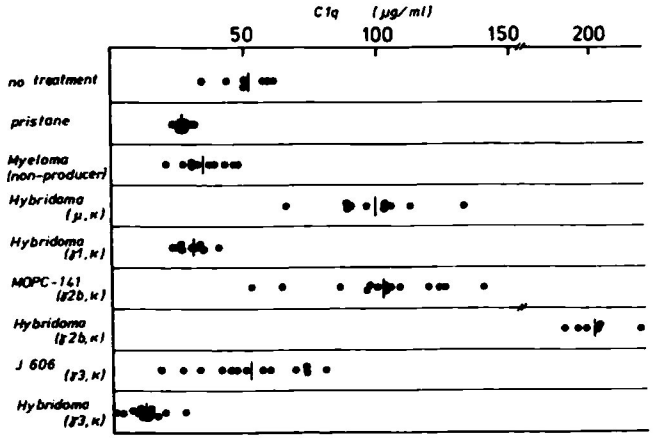

Fig. 1. **Serum C1q levels (μg/ml) in female BALB/c mice implanted intraperitoneally with various hybridoma or myeloma cells (2×10^6) on day 0.** Sera were obtained on day 11, and the C1q levels were quantified immunochemically.

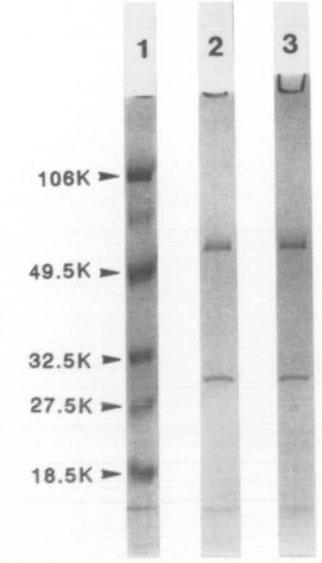

Fig. 2. **Electrophoresis of purified IgG2b (γ2b, κ) and IgG3 (γ3, κ) preparations on SDS-polyacrylamide gels.** 1, markers with known molecular weights of 106, 49.5, 32.5, 27.5, and 18.5K (Bio-Rad Lab., Hercules, CA, USA); 2, IgG2b; 3, IgG3. The anode is to the bottom, and the faint band near the anode is a non-specific band formed at the water/buffer interface.

instructions to users (*19*). Messenger RNA was purified from the extracted total RNA with oligo-dT immobilized latex beads (Nippon Roche, Tokyo), and used for synthesizing mouse macrophage cDNA together with reverse transcriptase (Amersham, Tokyo). The cDNA corresponding to Asp^{85}-Ala^{228} of the globular region of the mouse C1q B-chain was amplified with Taq DNA polymerase (Takara Biomedicals, Kyoto) by polymerase chain reactions (PCR) with the oligonucleotide primers for the mouse C1q B-chain and the macrophage cDNA. The amplified C1q cDNA was purified by agarose gel electrophoresis and extracted with SpinBind™ DNA Extraction Units (FMC BioProducts, Rockland, ME, USA). The purified DNA was identified as the cDNA of the globular region of the C1q B-chain based on its restriction fragments (*17*) obtained with *Sma*I (New England Biolabs, Beverly, MA, USA) and/or with *Pst*I (Gibco BRL Life Technologies, Gaithersburg, MD, USA).

cDNA Probes—The cDNA (430 bp) corresponding to Asp^{85}-Ala^{228} of the mouse C1q B-chain and a rat β-actin cDNA (207 bp), which has a 50% homology with the corresponding mouse β-actin, were labeled with dCTP[α-^{32}P] (NEN Research Products; DuPont, Daiichi-Kagaku, Tokyo) by using a multirandom DNA labeling kit (Ready-To-Go™; Pharmacia Biotech., Tokyo). Each probe had a specific radioactivity of approximately 6×10^8 cpm/μg DNA.

Northern Blot Analysis—Total RNA was extracted from PEC and spleen cells of each mouse as described above (see "*cDNA for B-Chain of Murine C1q*"). The RNA samples (6 μg each) were denatured at 65°C for 15 min and electrophoresed in a 1% agarose gel containing 0.66 M formaldehyde. The gel was stained with ethidium bromide to confirm the 18S and 28S bands of the ribosomal RNA. The RNA was blotted for 30 min onto a nylon membrane (Hybond-N+; Amersham). Hybridization was performed with random-primed ^{32}P-labeled cDNA probes of the mouse C1q B-chain and of the rat β-actin (5×10^4 cpm) in a hybridization solution (QuikHyb™ solution; Stratagene, La Jolla, CA, USA) at 68°C for 2 h. The membrane was washed twice with 2×SSC/0.1% SDS at room temperature for 15 min and finally twice with 0.1×SSC/0.1% SDS at 60°C for 15 min. The washed filter was exposed to an Imaging Plate (IP; Fuji-Films, Kanagawa) for 2 h. Radioactivity was measured by using a Bioimaging Analyzer (BAS1000MAC; Fuji Film), and results for the RNA samples on day 11 were printed on autoradiographic films by using a Pictrography 3000 (Fuji Film).

Statistical Analysis of Data—This was carried out by the methods of Snedecor and Cochran (*20*). The statistical significance of differences among the means of the experimental groups was calculated by use of the Fisher-Behrens z test (*20*). In experiments using small numbers of mice, statistical comparison between experimental groups was performed by use of the Wilcoxon rank sum test.

RESULTS

Does Inoculation of Immunoglobulin-Producing Hybridomas/Myelomas Affect Serum C1q Levels (Preliminary Experiment)?—Protein concentrations of serum C1q were quantified on day 11 in 75 mice implanted intraperitoneally with 2×10^6 cells of various hybridomas or myelomas [10 with myeloma 653; 10 with IgM-producing hybridoma; 9 with IgG1-producing hybridoma; 14 with IgG2b-producing myeloma (MOPC-141); 6 with IgG2b-producing hybridoma; 13 with IgG3-producing myeloma (J606); 13 with IgG3-producing hybridoma], as well as in 8 mice with pristane treatment and in 7 mice without any treatment. The mean value in each experimental group was calculated and is presented in Fig. 1 in parallel with the C1q level. The mean C1q level in mice with IgM-hybridoma or MOPC-141 increased significantly ($0.001<p<0.01$), and the mean in mice with IgG2b-hybridoma increased conspicuously in comparison with that in normal mice ($p<0.01$). On the other hand, the levels in the IgG3-hybridoma-implanted mice were lower than those in normal non-treated mice ($0.001<p<0.01$). The C1q levels in mice treated with pristane, or implanted with IgG-non-productive myeloma 653 or non-C-fixing IgG1-hybridoma or J606 were not altered significantly, and were almost equivalent to those in the normal mice.

Serum C1q and IgG Levels in Hybridoma-Treated Mice—Protein concentrations of serum C1q and IgG were quantified in 108 mice (22 implanted with IgG2b-hybridoma; 18 with IgG3-hybridoma; 37 with myeloma 653; 31 without any treatment) on day 11. The mean value in each experimental group was calculated and is presented in Table I. The C1q levels in the IgG2b-hybridoma-implanted mice were conspicuously high, and corresponded to approximately 470% of the levels in the normal non-treated mice

TABLE I. **Serum levels of C1q and IgG in female BALB/c mice (Mean±SD).**

Treatment	Number	C1q(μg/ml)	IgG(mg/ml)
Hybridoma (γ 2b, κ)	22	160.36 ± 69.90	27.23 ± 10.29
Hybridoma (γ 3, κ)	18	14.00 ± 5.07	5.65 ± 1.75
Myeloma 653	37	17.16 ± 6.80	2.01 ± 0.73
No treatment	31	34.29 ± 5.92	1.90 ± 0.64

NS, statistically not significant ($p>0.05$). *Statistically significantly different with $p<0.001$.

($p<0.001$). On the other hand, in the IgG3-hybridoma- and myeloma 653-treated mice, the C1q levels were markedly low (40% for the former and 50% for the latter) in comparison with those in the non-treated mice ($p<0.001$). The IgG levels in these hybridoma-implanted mice were much higher than those in the control mice, apparently owing to the increase in isotypes of IgG produced by the implanted hybridomas, on the basis of the electrophoretic pattern on zone electrophoresis (data not shown).

TABLE II. **Macrophage counts in peritoneal exudate cells (PEC) and in spleen cells of each mouse (Mean±SD).**

Source	Number	Macrophage Count($\times 10^6$)
PEC		
Hybridoma (γ2b, κ)	11	7.30 ± 2.78
Hybridoma (γ3, κ)	6	16.25 ± 5.53
Myeloma 653	11	13.35 ± 3.69
No treatment	13	2.10 ± 0.65
Spleen cells		
Hybridoma (γ2b, κ)	8	16.40 ± 4.92
Hybridoma (γ3, κ)	6	29.71 ± 9.53
Myeloma 653	7	30.57 ± 11.62
No treatment	5	21.06 ± 10.93

NS, statistically not significant ($p>0.05$). Statistically significantly different with $^*p<0.001$ or with $^\dagger 0.001<p<0.05$.

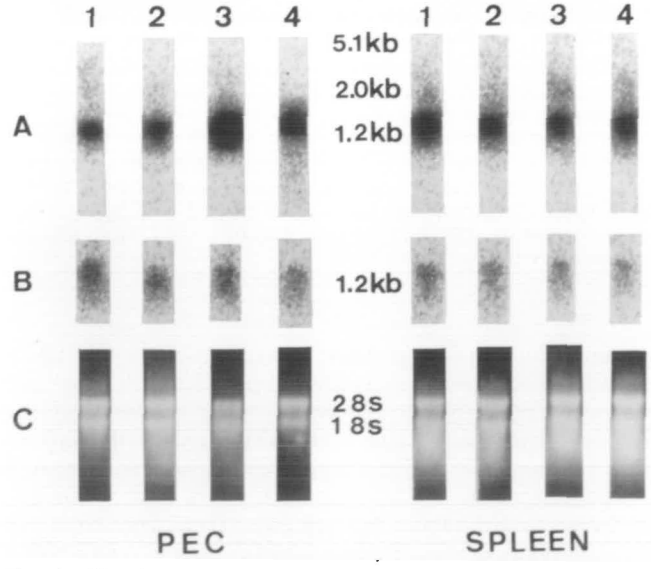

Fig. 3. **Northern blot analysis of total RNA from PEC and spleen cells.** These cells were harvested on day 11. Filters were hybridized with ^{32}P-labeled cDNA probes for the mouse C1q B-chain (A), and for the rat β-actin (B). 28S- and 18S-ribosomal RNAs are indicated on agarose gels with ethidium bromide before blotting (C). Experimental groups: 1, non-treated control; 2, myeloma 653-implanted; 3, IgG2b-hybridoma-implanted; 4, IgG3-hybridoma-implanted.

Macrophages in PEC and in Spleen Cells of Hybridoma-Treated Mice—Mean values of peritoneal and splenic macrophage counts of each mouse killed on day 11 were calculated in groups implanted with hybridoma or myeloma cells and in a normal control group, and are presented in Table II. Peritoneal macrophages increased greatly in the mice implanted with IgG2b- or IgG3-hybridoma or myeloma 653, and the counts were equivalent to 347-774% of those in the normal control mice ($p<0.001$). The extent of such increase was relatively low in the IgG2b-hybridoma-treated mice, and the mean deviation from the values in the mice of the other 2 treated groups was statistically significant ($p<0.001$).

The splenic macrophage counts in the mice of these hybridoma- and myeloma 653-treated groups did not deviate significantly from the mean in the normal non-treated group ($p>0.05$). The mean in the IgG2b-hybridoma-implanted group was significantly lower than that in the IgG3-hybridoma- or myeloma 653-treated group ($0.01<p<0.05$).

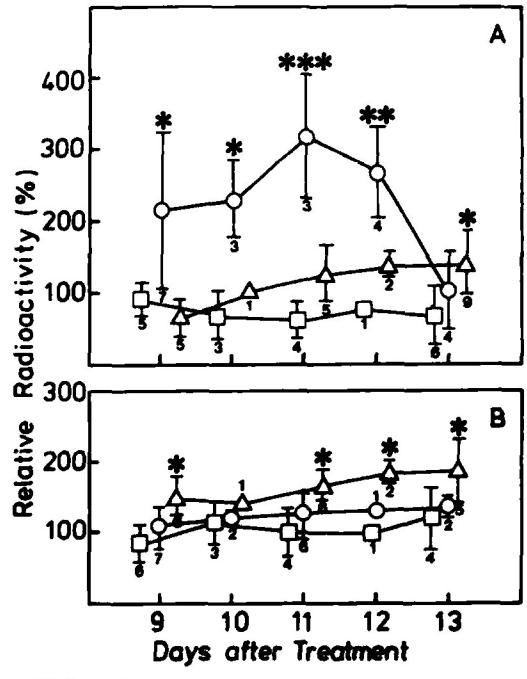

Fig. 4. **Bioimaging analysis of Northern blots of total RNA from mouse PEC (A) and spleen cells (B).** Filters were hybridized with cDNA probes for the mouse C1q B-chain and for the rat β-actin. Radioactivity was quantified on a special IP plate (Fuji Film) by using a Bioimaging Analyzer (Fuji Film) and the values were corrected on the basis of those obtained for the rat β-actin probe. Values were then calculated as the relative intensity to the mean of radioactivities in the non-treated control mice on each corresponding day, and are represented as the mean±SD. Arabic numerals attached to solid bars are the numbers of mice used. Experimental groups: mice implanted with IgG2b-hybridoma (○), with IgG3-hybridoma (△), and with myeloma 653 (□). Statistically significant at levels of $^*0.01<p<0.05$, $^{**}0.001<p<0.01$, and $^{***}p<0.001$.

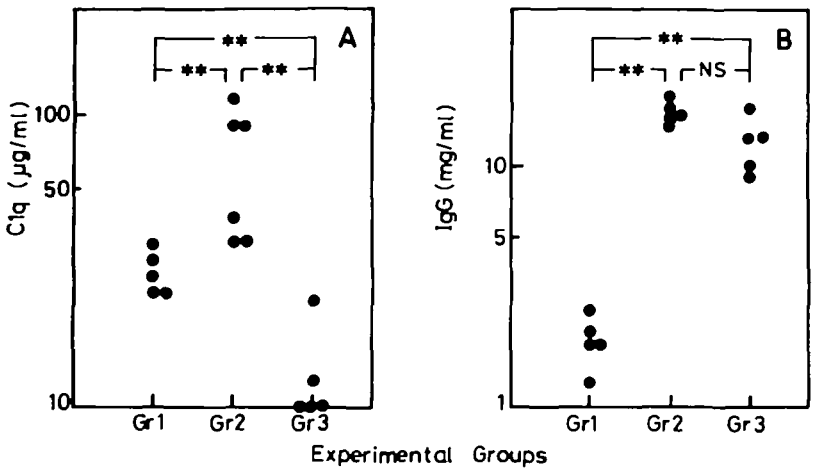

Fig. 5. **Serum C1q (A) and IgG (B) levels in female BALB/c mice.** Gr 1, mice not injected with any immunoglobulin preparation; Gr 2, mice treated with IgG2b (7 mg/mouse); Gr 3, mice treated with IgG3 (7 mg/mouse). Statistically significant at the level of $^{**}p<0.01$. NS, not significant ($0.05<p$).

Expression of mRNA of the C1q B-Chain in Hybridoma-Treated Mice—Heat-denatured total RNA from mouse PEC and spleen cells obtained on day 11 was electrophoresed and stained with ethidium bromide. The 28S and 18S bands owing to ribosomal RNA were clearly seen (Fig. 3C). The RNA was transferred to a nylon membrane and Northern blot analysis was carried out by hybridizing with ^{32}P-labeled cDNA probes for Asp^{85}-Ala^{228} of the mouse C1q B-chain (Fig. 3A) or for the rat β-actin (Fig. 3B). The analysis revealed only one band of approximately 1.2 kb with both probes. The signals obtained with the rat β-actin probe were comparable in these PEC and in spleen cells among the 4 groups of mice (1: control non-treated; 2: implanted with myeloma 653; 3: with IgG2b-hybridoma; 4: with IgG3-hybridoma). With the C1q B-chain probe, the highest signal in PEC was found in the group implanted with IgG2b-hybridoma, and the signals in spleen cells were almost equal among these 4 experimental groups, except for slightly higher signals in the IgG3- and IgG2b-hybridoma-treated groups.

Northern blots of total RNA in PEC and spleen cells obtained on days 9, 10, 11, 12, and 13 were quantified by using a special analyzer (Bioimaging Analyzer) before being exposed to autoradiographic films. Radioactivities with the C1q B-chain probe were corrected on the basis of those obtained with the rat β-actin probe, following which the values in the hybridoma- and/or myeloma-implanted groups were computed as the relative intensity with respect to the mean value in the control non-treated group on each corresponding day (Fig. 4). In the mice implanted with IgG2b-hybridomas, the mean value in PEC increased gradually from day 9 through 11 to approximately 300% ($p<0.001$) and returned to the level in the normal control mice on day 13, but the values in spleen cells remained relatively constant at this time. In the mice with IgG3-hybridomas, the value in PEC was low on day 9 and then increased gradually to approximately 150% on day 13. In spleen cells of these IgG3-hybridoma-implanted mice, the mean values were increased slightly (approximately 160%; $0.01<p<0.05$). In the myeloma 653-implanted mice, these values both in PEC and in spleen cells were almost equal to those in the normal non-treated group.

Serum C1q and IgG Levels in Mice Injected with IgG2b and/or IgG3 Preparations—Protein concentrations of serum C1q and IgG in 11 mice injected with two isotypes of immunoglobulin (7 mg/mouse; 6 with IgG2b; 5 with IgG3) 24 h in advance were quantified in parallel with those in 5 normal control mice. The levels were plotted and are presented in Fig. 5. C1q levels (Fig. 5A) in the IgG2b-injected mice (Gr 2) were conspicuously high as compared with those in the control mice (Gr 1) ($p<0.01$). On the other hand, in the IgG3-injected mice (Gr 3), the C1q levels were lower than those in the control mice (Gr 1) ($p<0.01$). The IgG levels (Fig. 5B) in the mice injected with IgG2b (Gr 2) or with IgG3 (Gr 3) were higher than those in the control mice (Gr 1) ($p<0.01$).

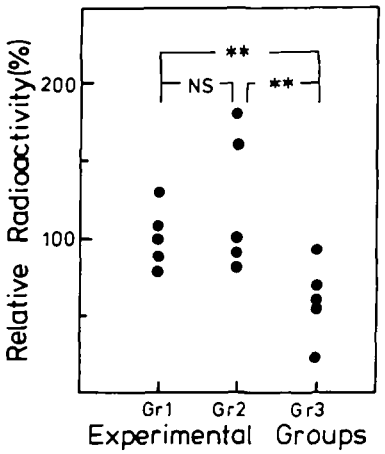

Fig. 6. **Bioimaging analysis of Northern blots of total RNA of PEC of mice injected intraperitoneally with highly purified IgG2b (γ2b, κ) or IgG3 (γ3, κ) preparations.** Filters were hybridized with cDNA probes for mouse C1q B-chain and for rat β-actin. Radioactivity was quantified on a special IP plate (Fuji Film) by using a Bioimaging Analyzer (Fuji Film) and the values were corrected on the basis of those obtained with rat β-actin probe. Values were then calculated as the relative intensity with respect to the mean of radioactivities in the non-treated control mice. Gr 1, mice not injected with any immunoglobulin preparation; Gr 2, mice treated with IgG2b (7 mg/mouse); Gr 3, mice treated with IgG3 (7 mg/mouse). Statistically significant at levels of $^{**}p<0.01$. NS, not significant ($0.05<p$).

Expression of mRNA of the C1q B-Chain in Mice Injected with IgG2b and/or IgG3 Preparations—Northern blots of total RNA in PEC of mice injected with IgG2b or IgG3 (7 mg/mouse) 24 h in advance were quantified in

parallel with those in the control non-treated mice by using the Bioimaging Analyzer. Radioactivities with the C1q B-chain probe and with the rat β-actin probe were counted, and relative radioactivities in mice injected with IgG2b or with IgG3 to those in the control mice were computed as described above (see "*Expression of mRNA of the C1q B-Chain in Hybridoma-Treated Mice*") and are presented in Fig. 6. In the mice injected with IgG2b (Gr 2), relative radioactivities of 2 mice were clearly higher and those of the others (3 mice) were almost equal to those of the control non-treated mice (Gr 1). In mice injected with IgG3 (Gr 3), the relative radioactivities were significantly lower than those of the control mice ($p<0.01$).

DISCUSSION

As reported for patients with certain immunodeficiency diseases such as hypogammaglobulinemia, myeloma, and systemic lupus erythematosus (*2, 3, 7*), and in normal mice (*4*), IgG in the circulation may affect serum C1q levels at catabolic steps due to reversible C1q-IgG complex formation that reduces C1q degradation (*3*); in accordance with this, significantly positive correlations have been found between C1q and IgG levels (*2-4*) and further, C1q levels were normalized upon intravenous immunoglobulin infusion in hypogammaglobulinemic patients (*5, 6*).

In this study, we have shown that implantation of IgG-producing hybridomas affects the extent of expression of mRNA of the C1q B-chain as well as the serum C1q levels in mice (Figs. 3 and 4). The effects depend on the isotypes of immunoglobulins produced in implanted hybridomas: C-fixing classes of immunoglobulin (IgM, IgG2b) enhanced the C1q metabolism but non-C-fixing classes of immunoglobulin (IgG1, IgG3) had almost no effect (Fig. 1). Serum C1q levels increased markedly in the mice with IgG2b-hybridomas (Table I). The conspicuously intensified signals of mRNA of the C1q B-chain in PEC of mice with IgG2b-hybridomas (Figs. 3A and 4A) appear to be caused by real enhancement of C1q anabolism per cell and not by increase of the number of C1q-synthesizing cells per mouse, because the macrophage counts both in PEC and in spleen cells in each IgG2b-hybridoma-implanted mouse were rather low as compared with those in the other treated groups (Table II). In the mice treated with IgG3-hybridomas or with immunoglobulin-non-producing myeloma 653, serum C1q levels were significantly decreased (Table I). This suggests that pristane administered simultaneously has some inhibitory effect on C1q metabolism. Indeed, pristane had some suppressive effects on serum C1q levels (Fig. 1).

In the mice treated with IgG3-hybridoma, almost normal intensity of the signals of the C1q B-chain mRNA in PEC, slightly intensified signals in the spleen cells (Fig. 4), and reduced serum C1q (Fig. 1, Table I) levels were found. In the IgG2b-hybridoma treated mice, greatly intensified signals of C1q B-chain mRNA in PEC and almost normal signals in the spleen cells were observed (Fig. 4). Overall, it seems unlikely that serum C1q is synthesized in spleen macrophages; this is in agreement with previous reports that serum C1q is synthesized mainly by the macrophage/monocyte lineage (*1, 21*) and also confirms the report that the strongest signal of the C1q B-chain mRNA was found in peritoneal macrophages (*17*).

Serum C1q levels and expression of C1q-B-chain mRNA in PEC were influenced by injecting chromatographically purified IgG3 (Figs. 5A and 6) in the same way as by IgG3-hybridoma implantation (Table I, Fig. 4). The lower C1q levels in mice treated with IgG3 were probably caused by the administration of pristane. Serum C1q levels and mRNA of the C1q B-chain of PEC in the mice injected with a purified IgG2b preparation increased similarly to those in IgG2b-hybridoma-implanted mice (Table I, Figs. 4, 5A, and 6), though the extent of the increase in some mice in the former group seemed somewhat less. More detailed kinetic analyses of the time course of the expression of mRNA are required, since the quantitation may not have been carried out at the optimum time in the mice treated with IgG2b. Another possibility is that the injected IgG2b preparations might influence C1q metabolism only at catabolic steps. The serum IgG levels remained high 24 h after the injection of these immunoglobulins (Fig. 5B), which might be a consequence of using mice of the same inbred strain BALB/c as those used as the immunoglobulin source.

The data obtained from the studies by using purified IgG2b and/or IgG3 preparations may support the idea that such effects on C1q metabolism result from the immunoglobulin itself instead of some cytokine(s) unique to the isotype of immunoglobulin produced in the hybridoma cells. The molecular mechanism by which expression of the C1q B-chain mRNA was enhanced with the C-fixing IgG2b remains to be established, and an *in vitro* experiment is in progress.

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