Antilymphocytic Antibodies and Marrow Transplantation. XIV. Antibody-Induced Suppression of Graft-Versus-Host Disease in C3-Decomplemented Mice Differentiates Two T-Cell–Depletion Pathways

By Stefan Thierfelder, Josef Mysliwietz, Gertrud Hoffmann-Fezer, and Udo Kummer

Remarkable differences in the suppression of graft-versus-host disease (GVHD) have been found for anti–Thy-1 antibodies to relate to (1) antigen density and antibody coating on the target cells, (2) antibody isotype, and (3) uptake of complement subcomponent C1q. Regarding (2) and (3) we now demonstrate that depletion of the third complement component C3 by cobra venom factor (CVF) differentiates two T-cell elimination pathways in mice: four rat IgG2c anti–Thy-1 monoclonal antibodies (MoAbs) with low uptake of mouse C1q lost most of their T-cell–depleting and consequently GVHD-preventing effect in C3-depleted H2 IA incompatible semiallogeneic (C57BL/6xCBA)F1 mice. In contrast, eight rat IgG2b, mouse IgG2a, and 2b anti–Thy-1 MoAbs with high affinity for C1q still remained strongly T-cell–depleting and prevented GVHD even in fully mismatched CBA mice depleted of C3. In conjunction with our observation that anti–Thy-1 MoAbs also suppress GVHD in C5-deficient AKR mice, we conclude that complete complement activation until T-cell lysis is not required for our antibodies to be effective in vivo. Activation, but only until deposition of C3b on target cells for opsonisation via C3b receptors, is necessary with the less immunosuppressive anti–Thy-1 IgG2c isotype with low affinity for C1q. Mouse C1q uptake and C3/C4 deposition on target cells were measured with labeled antibodies and localized in T-cell areas. Interestingly, not even activation until C3b is necessary with the most immunosuppressive C1q–high-affine isotypes. As far as the latter is concerned, we discuss whether elimination of antibody-coated cells via Fc receptors is enhanced by binding to C1q-receptors and/or by intercalating C1q expressed on macrophages.

IN VITRO TREATMENT of donor marrow with anti–T-cell antibodies for suppression of graft-versus-host disease (GVHD) has recently been complemented by injecting certain antibodies directly into preirradiated mice before transplantation with the advantage of reducing GVHD as well as residual host-versus-graft reactivity.[1,2] The selection of the 'right' antibody is based on studies of the mechanism(s) underlying antibody-induced T-cell depletion, which intensified once it was realized how important the selection of the isotype[3-6] and its complement-fixing activity can be. For instance, in standard GVHD mouse models it was found that IgM anti–Thy-1.2 monoclonal antibodies (MoAbs) had to be incubated with donor cells in the presence of heterologous C′ to suppress GVHD.[7] It is now clinical routine to include C′ in bone marrow transplantation (BMT) when anti–T-cell MoAbs are used. With polyclonal, specifically absorbed anti–T-cell globulin the importance of adding C′ had not been evident, although its immunosuppressive effector mechanism had been localized in the Fc part because $F(ab)_2$ fragments were ineffective.[8] The finding that there are anti–Thy-1 MoAb Ig isotypes that prevent GVHD without requiring C′ to be added in vitro furnished an explantation.[9] These isotypes share a high intrinsic affinity for C1q, a subunit of the first component of the complement cascade.[1,10] They are more immunosuppressive, depleting T cells more completely than anti–Thy-1 MoAbs with low affinity for C1q.[11] In the search to discover whether these differences might point to differing T-cell–suppressing pathways in vivo, we noticed that C1q–low-affine anti–Thy-1 MoAbs lost their immunosuppressive effect on GVHD in mice depleted of the complement component C3 by cobra venom factor (CVF). In contrast, the anti-GVHD activity of C1q–high-affine anti–Thy-1 MoAbs remained unaffected by C3 depletion, suggesting that these antibody isotypes exert their remarkably strong in vivo effect under conditions other than classical complement-mediated T-cell lysis or opsonisation, or even classical antibody-dependent cell-mediated cytotoxicity (ADCC).

MATERIALS AND METHODS

Animals. All strains of inbred mice reported in this article, originally obtained from The Jackson Laboratory (Bar Harbor, ME), were raised and maintained in our breeding facilities. Both male and female mice between 2 and 3 months of age were used in these studies.

Complement-dependent lympholysis. Lymphocytotoxicity was evaluated with the complement-dependent dye exclusion test.[12] Freshly drawn serum from male C56BL/6 mice was incubated undiluted with a suspension of mouse lymphnode cells and antibody for 1 hour.

Antibodies. Table 1 lists the MoAbs used in this study along with their characteristics. RmT6, RmT7, RmT8 rat IgG2c anti–Thy-1 was produced in our laboratory (J. Haunschild, personal communication, April 1990) as described for RmT2.[10] MoAb reacting with mouse C1 and C1q was generated according to a principle published previously,[13] where rats were immunized with antibody-coated cells intercalating mouse C1q. After native agarose gel electrophoresis it was found to bind to mouse C1q and to recognize the same band as a polyclonal POX rabbit antihuman C1q (Dakopatts, Hamburg, Germany), which crossreacts strongly with mouse C1q (E. Ego, M. Wasiliu, and E. Lederer, personal communication, April 1990). MoAbs to mouse C4 and C3 deposited on antibody-coated cells were generated in rats following the same principle of immunization with, however, a different screening system as detailed elsewhere.[14] A class switch family of anti–Thy-1.1 MoAbs producing cell clones were kindly provided by

From the Institut für Immunologie der GSF, Forschungszentrum für Umwelt und Gesundheit, GmbH, München, Germany.

Submitted December 11, 1990; accepted January 17, 1991.

Supported by Sonderforschungsbereich 217 der Ludwigs-Maximilians-Universität, München, Germany.

Address reprint requests to Prof S. Thierfelder, MD, Institut für Immunologie, GSF, Marchioninistr. 25, D-8000 München 70, Germany.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" *in accordance with 18 U.S.C. section 1734 solely to indicate this fact.*

0006-4971/91/7710-0002$3.00/0

Table 1. Main Features of the MoAbs Used in This Study

MoAb	Origin*	Ig Isotyp	Binding Specificity	Reference
RmC1q (7H8)	Mouse × rat	IgG1	Mouse C1q	†
RmC4 (16D2)	Mouse × rat	IgG2a	Mouse C4	14
RmC3 (5A5)	Mouse × rat	IgG1	Mouse C3	14
RmT1	Rat × rat	IgG2b	Thy-1	10
BD30-H12	Mouse × rat	IgG2b	Thy-1.2	3
RmT2	Rat × rat	IgG2c	Thy-1	10
RmT6	Rat × rat	IgG2c	Thy-1	†
RmT7	Rat × rat	IgG2c	Thy-1	†
RmT8	Rat × rat	IgG2c	Thy-1	†
MmT1	Mouse × mouse	IgG2a	Thy-1.2	15
MmT4	Mouse × mouse	IgG2a	Thy-1.2	15
5a-8	Mouse × mouse	IgG2b	Thy-1.2	‡
1.A-14	Mouse × mouse	IgG2a	Thy-1.1	16
1.A-12	Mouse × mouse	IgG2b	Thy-1.1	16
31C5.31	Mouse × mouse	IgG3	Thy-1.1	16
31.A	Mouse × mouse	IgG1	Thy-1.1	16
145.2C11	Hamster × mouse	IgG	Anti-CD3	18

*Myeloma × spleen cell donor.
†This study.
‡Cedarlane Lab, Hornby, Ontario, Canada.

I.D. Bernstein University (Seattle, WA).[16] Binding of rat anti–Thy-1 MoAb to Thy-1–positive cells was fully inhibited by mouse anti–Thy-1.1 or Thy-1.2 MoAb listed in Table 1.

X-radiation. Prospective marrow recipients received a lethal dose of total body X-radiation of 8.5 Gy at a dose rate of 1.04 Gy/min.[10]

Labeling of RmC1q MoAb with biotin. Two milligrams of biotin N-hydroxysuccinimide ester were diluted in 250 μL dimethyl sulphoxide and mixed with a 10-fold volume of purified rat antimouse C1q (RmC1q7h8) MoAb at a concentration of 1.45 mg/mL in 0.1 mol/L $NaHCO_3$ (pH 8.2). After 1.5 hours of incubation the biotin-conjugated protein was dialyzed overnight against phosphate-buffered saline (PBS) with 0.1% sodium azide, and stored at 4°C.[17]

Binding of biotinylated RmC1q to thymocytes coated with antibodies and C1q. Fifty microliters of a saturating dilution of anti–Thy-1 MoAb was incubated for 30 minutes with 5×10^5 C57BL/6 or AKR/J thymocytes in microtiter plates at 4°C for enzyme-linked immunosorbent assay (ELISA) as described elsewhere.[1] The plates were washed twice in PBS, incubated for 30 minutes with 50 μL of reciprocal dilutions of partly purified mouse C1q, and then again washed twice in PBS before 50 μL of diluted biotinylated RmC1q was added, followed by detection with POX avidin (Vector, Burlington, CA) and 1.2-phenylene-diamine (OPD) as chromogen. Partly purified mouse C1q was obtained by fractionated polyethylene glycol (PEG) precipitation as described before.[13]

Immunofluorescence of cell suspensions. Direct staining was performed with the following fluorescein or phycoerythrin (PE)-conjugated MoAbs: GK 1.5 (rat IgG2b anti-L3T4, PE); 53.6.7. (rat IgG2a anti-Lyt-2, fluorescein isothiocyanate [FITC]); 53.7.3 (rat IgG2a anti–Ly-1, FITC). All MoAbs above were received from Becton Dickinson (Heidelberg, Germany); polyclonal $F(ab)_2$ anti-mouse μ antibodies were received from Jackson Immuno Research (Hamburg, Germany); MoAbs RmT1,[10] RmC1q, RmC4, RmC3,[14] and 145-2C11 (hamster-anti-CD3),[18] a gift from Dr J. Bluestone, The Ben May Institute, Chicago, IL, were conjugated with FITC as described previously.[19] For quantitative determination of mouse C1, C4b, and C3b deposition, 0.5×10^6 of C57BL/6 lymphnode cells in fresh undiluted syngeneic serum was incubated with serial dilutions of purified RmT1 or RmT2 MoAb in fresh undiluted C57BL/6 serum for 30 minutes at 37°C. After washing the cells were double-labeled with saturating concentrations of RmC1q, RmC4, or RmC3 MoAb (FITC) and $F(ab)_2$-goat-antimouse μ antibody (PE) for 15 minutes. The samples were processed on fluorescence-activated cell analyzer (FACScan; Becton Dickinson) without final wash; propidium iodide was added to monitor the dead cells. Quantitative measurements were performed as described elsewhere.[20]

Immunohistochemistry. Five-micrometer thick, acetone-fixed (10 minutes) cryostat sections of spleens and lymph nodes of C57BL/6 mice were incubated in the first step with RmT1 together with either CVF-treated C3-depleted serum or, for positive controls, with untreated fresh serum or, for negative controls, with heat-inactivated serum of C57BL/6 mice. The second incubation step was either with biotin-labeled rat antimouse C3 (RmC3) or RmC4 MoAb. Peroxydase-labeled avidin was used in the third incubation. Each incubation lasted 60 minutes and was stopped by washing in PBS. Peroxidase activity was shown with amino-ethyl-carbazole.[21] The sections were counterstained with hematoxilin.

BMT. Groups of irradiated (C57BL/6xCBA)F1 or CBA ($H\text{-}2^k$) mice were injected with a mixture of 5×10^7 spleen and 2×10^7 BM cells of C57BL/6 ($H\text{-}2^b$) mice 4 hours later as described before.[10] Anti–Thy-1 MoAb (0.25 mL, equivalent to about 1 mg of active antibody) was injected either into prospective bone marrow recipients 4 hours postirradiation or donors 3 days before BMT.[1,11]

Complement depletion with CVF. Mice were injected with 10 U of CVF (Cordis Lab, Miami, FL) from Naja haje for C3 depletion.

RESULTS

C3 depletion by CVF. Deposition of C3b and C4b on mouse T cells coated with anti–Thy-1 MoAb (RmT1) was tested following incubation with serum of mice injected with 10 U of CVF. C4b deposition was found in RmT1-coated T-cell zones, ie, periarteriolar lymphatic sheaths of spleen white pulp (Fig 1) and paracortex of lymphnodes of both CVF-treated and control mice. In contrast, C3b was present in T-cell areas only in those spleens incubated with fresh serum and lacking in spleen sections incubated with RmT1 plus CVF-treated C3-depleted serum of C57BL/6 mice. Virtually no deposition of C3b on antibody-coated cells was measured with sera collected from mice during the first 2 days following injection of CVF (Fig 2). A second injection of CVF on day 2 prolonged C3 depletion for another 2 days but did not change the isotype-related differences in immunosuppression described below.

Isotype-related consequences of CVF treatment for T-cell elimination. Table 2 summarizes T-cell counts in lymphnodes of C3-depleted mice after injection of saturating amounts (1 mg) of IgG2b (RmT1) or IgG2c (RmT2) anti–Thy-1 MoAb. Three days after injection of RmT1, a 97% reduction of cells carrying the pan T-cell marker Lyt-1 and a 90% reduction T cells in C3-depleted mice were counted. Reduction of T cells was much less with RmT2: 79% in normal and 46% in C3-depleted mice.

Isotype-related differences in immunosuppression in C3-depleted mice. Mortality from GVHD was prevented in semiallogeneic radiation chimaeras conditioned with RmT1 or RmT2 injected after irradiation and before transfusion of 2×10^7 BM together with 5×10^7 spleen cells of C57BL/6 mice. RmT2, in contrast to RmT1, failed to prevent GVHD if the mice were in addition depleted of C3 by CVF that was

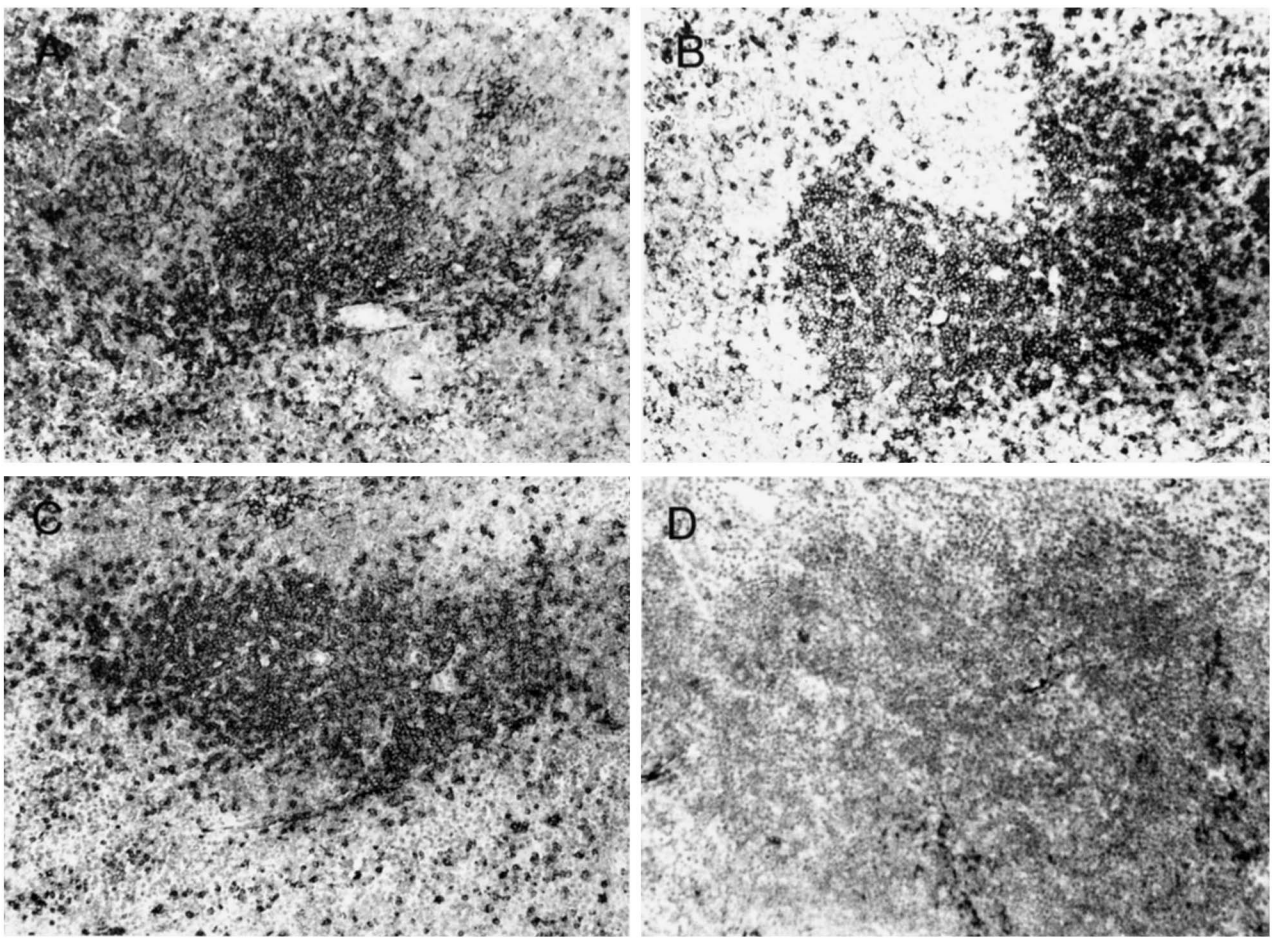

Fig 1. Immunohistochemical staining of spleen sections for the presence of labeled MoAb to C4b and C3b deposited on T cells during C′ activation (from fresh or CVF-treated mouse serum) by anti–Thy-1 MoAb (RmT1). Spleen periarteriolar lymphatic zone shows labeled anti-C4b (A) or anti-C3b (B) on RmT1-coated cells incubated with fresh serum and anti-C4b (C) or absence of anti-C3b (D) after incubation with CVF-treated C3-depleted serum of C57BL/6 mice.

injected after irradiation (Fig 3). A comparable reversal of prevention of GVHD with 100% mortality between days 20 to 68 was noted when further rat IgG2c (RmT6, RmT7, RmT8) anti–Thy-1 MoAbs were injected together with CVF. Prevention of GVHD following injection of RmT1 or further anti–Thy-1 MoAb of rat IgG2b (BD 30-H12), mouse IgG2a (MmT1, MmT4), or IgG2b (5a-8) isotype remained unaffected by C3 depletion even in homozygous fully H-2, IA mismatched CBA mice. When tested approximately 100 days later these mice were full (95% and better) chimaeras tolerating marrow donor skin grafts. In further experiments, RmT1 or RmT2 antibodies were injected in prospective C57BL/6 donors depleted of C3 by CVF. Three days later their spleen and BM cells were transferred to irradiated (C57BL/6xCBA)F1 mice. Again, failure to survive from GVHD was noted for recipients of cells from donors treated with RmT2 but not RmT1.

Uptake of C1q by T cells coated with various anti–Thy-1 MoAb isotypes. In contrast to four rat IgG2c anti–Thy-1 MoAbs, a plateauing retention of C1q on thymocytes saturated with rat IgG2b or mouse IgG2a and IgG2b anti–Thy-1 MoAbs was found after incubation with dilutions of partly purified mouse C1q (Fig 4). Similar differences were obtained for C57BL/6 lymphocytes. Comparison of uptake of C1q by thymocytes coated with mouse IgG3 anti–Thy-1.1 MoAb or its IgG2a, IgG2b, and IgG1 switch variants showed low uptake of C1q for IgG3 similar to the rat IgG2c isotype and marginal C1q binding of IgG1 (Fig 5).

Deposition of C1, C4, and C3 on antibody-coated T cells. C1, C4b, and C3b were bound on C57BL/6 lymph node cells during incubation with rat IgG2b (RmT1) or IgG2c (RmT2) in the presence of undiluted fresh serum from C57BL/6 mice. Their amounts were measured with FITC-labeled anti-C1, anti-C4b, and anti-C3b MoAbs in FACS. No difference between RmT1 and RmT2 was found for C3b. C1 was about four times more on cells incubated with RmT1 as compared with RmT2; C4b was about one third (Fig 6).

DISCUSSION

Although suppression of GVHD is usually approached by incubation of antibodies with donor cells and comple-

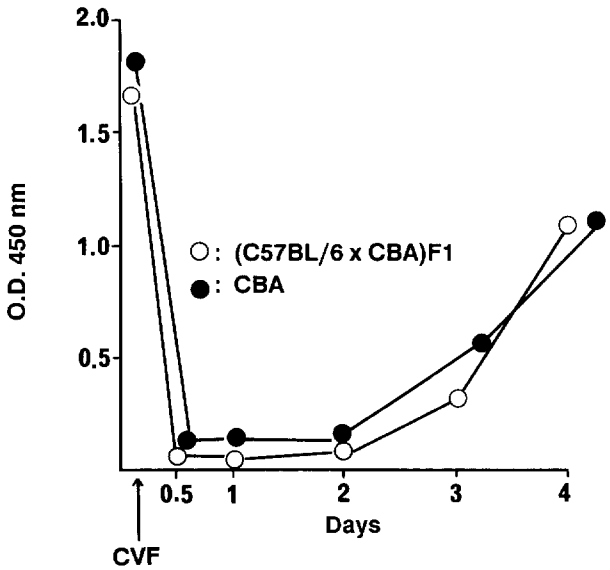

Fig 2. Depletion of C3 in (C57BL/6xCBA)F1 (○) or CBA (●) mice injected with 10 U of CVF. C3 levels were assayed in ELISA using labeled anti-C3 MoAb in the presence of thymocytes that had been preincubated with RmT1 and serum of mice collected after injection of CVF.

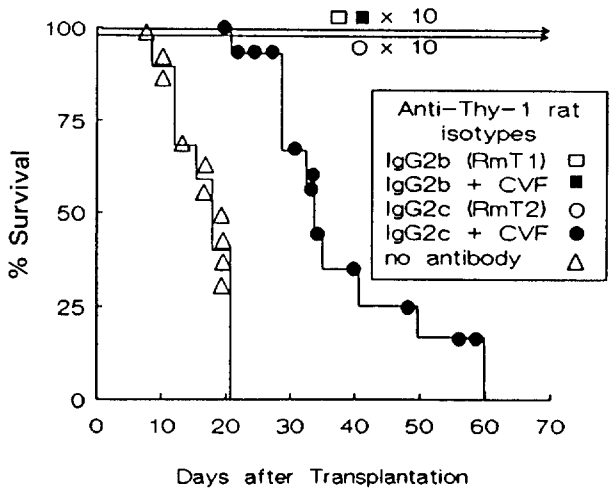

Fig 3. Survival of (C57BL/6xCBA)F1 mice depleted of C3 by injection of CVF after irradiation followed by 1 mg of anti-Thy-1 MoAb 4 hours before transplantation of BM and spleen cells of C57BL/6 mice.

ment, we had previously found that, except for the IgM isotype, which was not included in this study, 1 mg of anti-Thy-1 MoAb, whether used in vitro or injected in recipients before transplantation or in donors, produced comparable results.[1,11] In vivo T-cell depletion by these antibodies could thus be related to its immunosuppressive consequences by probing the cells of the antibody-treated donor in the GVHD model. We used MoAbs that reacted with Thy-1.2 cells. Their isotypes include the most immunosuppressive examples found so far. Pan T Lyt-1 (CD5), Lyt-2 (CD8), or L3T4 (CD4) antigens are less densely expressed on T cells. Injections of corresponding MoAbs do not prevent GVHD in fully mismatched mice. Even the concentration of the Thy-1.1 alloantigen on T lymphocytes is too low for optimal antibody coating, complement activation, and suppression of GVHD.[20] Regarding the depletory effect of CVF, we found an almost complete lack of C3b deposition, as a consequence of C3 depletion, on antibody-coated cells in the presence of serum samples collected from mice until 48 hours after injection of CVF (Figs 1 and 2). This finding is concordant with the markedly diminished C3 levels (>1%) shown in immune adherance activity that were reported for sera of guinea pigs and mice treated with CVF.[22]

The main focus of our investigation was to differentiate between the C′-fixing rat IgG2c anti-Thy-1 isotype and C′-fixing rat IgG2b, mouse IgG2a and IgG2b isotypes regarding the suppression of GVHD. Only the former lost most of its T-cell–reducing and immunosuppressive effect in C3-depleted mice, whereas the latter isotypes showing high uptake of C1q remained little affected by CVF. The high intrinsic affinity for human C1q of the rat IgG2b

Table 2. T-Cell Elimination in Lymph Nodes of C3-Depleted C57BL/6 Mice Three Days After Intravenous Injection of RmT1 (IgG2b) or RmT2 (IgG2c) Rat anti-Thy-1 MoAb

	Specificity of Diagnostic Antibody Combination				
Treatment	$CD3^+/L3T4^+$	$CD3^+/Lyt\text{-}2^+$	$Ly\text{-}1^+/\mu^-$	μ^+	Rat Ig^+
Untreated	34.5*	24.0	59.0	32.0	ND
RmT1	1.3	0.3	2.0	96.2	0
RmT1 + CVF†	4.3	2.1	6.0	89.6	5.4
RmT2	12.6	2.4	12.7	80.0	11.6
RmT2 + CVF	24.0	8.3	32.0	62.0	32.0

Abbreviation: ND, not done.

*Percentage of positive lymphoid cells from pooled lymph nodes of four normal or treated mice was determined with FITC- or PE-labeled MoAbs in FACS.

†Ten units of CVF per mouse was injected 6 hours before RmT1.

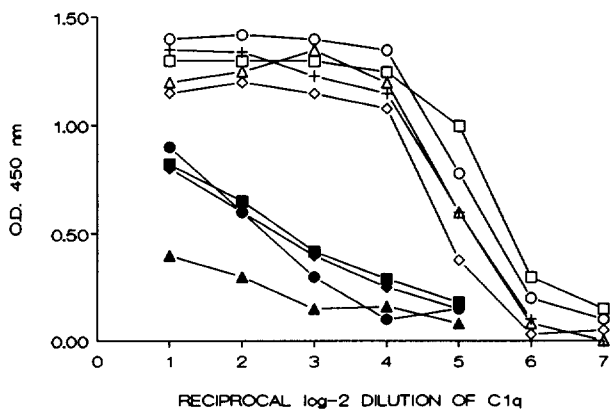

Fig 4. Uptake of C1q by C57BL/6 (Thy-1.2) thymocytes precoated with rat anti-Thy-1 MoAbs of various isotypes. Cells were saturated with MoAb and incubated with dilutions of C1q partly purified from mouse serum. C1q uptake was measured in ELISA with biotinylated rat antimouse C1q MoAb. Rat IgG2b: (—○—) RmT1, (—△—) BD30-H12. Rat IgG2c: (—●—) RmT2, (—◆—) RmT6, (—■—) RmT7, (—▲—) RmT8. Mouse IgG2a IgG2b: (—□—) MmT1, (—+—) MmT4, (—◇—) 5a-8.

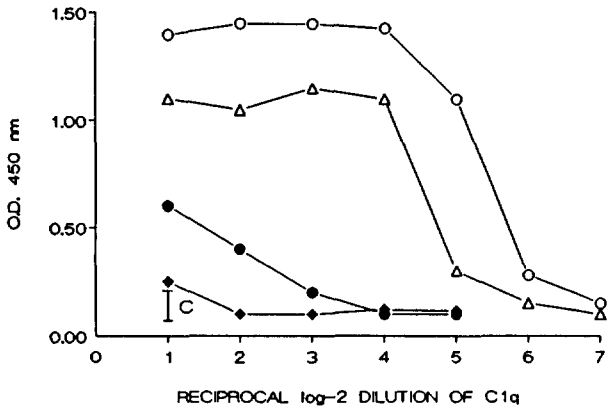

Fig 5. Uptake of C1q by AKR/J (Thy-1.1) thymocytes precoated with mouse anti–Thy-1 MoAbs of switch variants. C: range of controls with heat-inactivated partially purified C1q. Conditions as mentioned in legend of Fig 4. (—○—) 1. A-14 IgG2a. (—△—) 1.A-12 IgG2b. (—●—) 31C5.31 IgG3. (—◆—) 21.A IgG1.

isotype was discovered by Hughes-Jones et al using synergistic MoAbs specific for the rat class I major histocompatibility antigen RT1A[a], which is also expressed on rat red cells.[23] Synergistic, ie, noncompetitive, binding of these antibodies to distinct epitopes on the same cell membrane antigen[24] produced pairs of closely positioned MoAbs that intercalated C1q on the red cells. We had noted that Thy-1.2 is more densely expressed on T cells, allowing, for example, an average concentration of 59,000 RmT1 molecules per lymphnode cell,[25] enough for normal, nonsynergistic rat IgG2b MoAb to intercalate C1q with high affinity. Furthermore, we demonstrated that a correlation exists between uptake of human C1q by cell-bound anti–Thy-1 MoAb or polyclonal rabbit or rat ATG and their immunosuppressive effect on GVHD and/or skin graft rejection.[1,10] C′-dependent cell lysis is a classic but not a reliable indicator for in vivo antibody activity. Our anti–Thy-1 rat IgG2b and 2c MoAb isotypes are fully lytic, in the presence of mouse complement, for their target T cells despite their different uptake of C1q and immunosuppressive potency. This finding underlines the fact that intrinsic affinity for C1q and, consequently, uptake of C1q, rather than cell lysis, is a better indicator of in vivo potency of the tested anti–Thy-1 MoAb regarding cell depletion and immunosuppression. Using polyclonal rabbit antihuman C1q crossreacting strongly with mouse C1q or a monoclonal antimouse C1q antibody that we generated in rats, we have shown in the present study that the isotype-related differences found for uptake of human C1q also hold true in principle for mouse C1q, although we find an even lower uptake of human compared with mouse C1q for the rat IgG2c isotype (data not shown).

Studying the clearance of anti-RT1A[a] antibody-coated rat red cells, Yousaf et al measured a prolongation in rats treated with CVF.[26,27] It was explained by the CVF-induced lack of C3 that could no longer be deposited on the antibody-coated cells and contribute to their binding to corresponding receptors on phagocytes. We interpret our results with CVF and rat IgG2c anti–Thy-1 MoAb as due to a similar weakening of the classical[28] Fc and C3-receptor–dependent effector mechanism.

It remains to be explained why the C1q-affine anti–Thy-1 MoAbs were so little affected by C3 depletion that they eliminated T cells and prevented GVHD not only in C3-depleted semiallogeneic F1 mice but also in homozygous H2 IA region two haplotype incompatible chimaeras. In the latter, considerably more difficult combination the C1q–low-affine rat IgG2c anti–Thy-1 MoAb failed to delay GVHD even when C3 depletion by CVF had been omitted.

We did not find clear differences in the amount of deposition of C3b on target T cells and only small differences for C4b. The question, therefore, remains whether the isotype-related difference in uptake of C1q is the sole explanation for in vivo differences in antibody effector mechanisms. Opsonization by macrophages, complement-mediated lysis, and ADCC have been discussed as important effector mechanisms in cell suppression of certain antibody isotypes.[16,29-31] As such, ADCC does not require C′-activation and would not explain the correlation between C1q uptake and immunosuppression. Classical ADCC with K cells as effectors is not ruled out by the present findings. However, there is recent evidence for a mechanis-

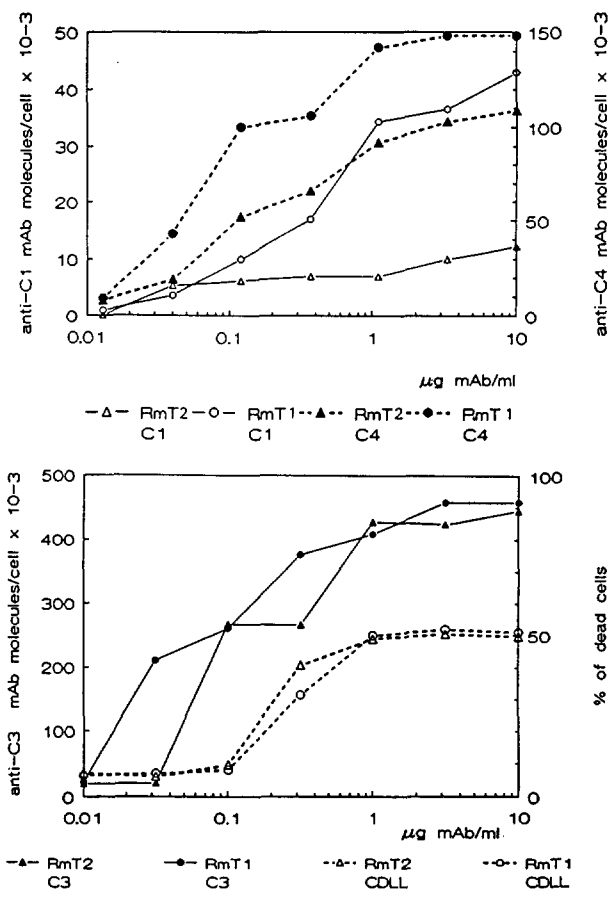

Fig 6. Binding of FITC-labeled MoAb to C1, C4b, and C3b deposited on lymph node T cells from C57BL/6 mice during activation of C′ by RmT1 (IgG2b) or RmT2 (IgG2c) rat anti–Thy-1 MoAb in the presence of fresh syngeneic serum. For comparison, complement-dependent lymphocyte lysis (CDLL) is also shown in FACS.

tic role of C1q in ADCC of IgG-coated cells, too. Murine resident peritoneal macrophages with low levels of endogenous C1q synthesis were reconstituted in their ADCC of antibody-coated red cells by exogenous C1q.[32] In contrast, reconstitution with serum C1q did not increase ADCC above the level that activated, 'inflammatory' macrophages induce all on their own. Macrophages synthesize and secrete C1q by themselves,[33,34] at least when they are activated. Whether they use it directly as a link with intercalation of the globular heads of C1q in preformed immune complexes and the fibrillar stems to be bound to C1q-specific receptors on macrophages is still open.[32,35,36] Leu et al, when discussing their findings, emphasized a pivotal role of C1q in the regulation of receptor-mediated phagocytosis and ADCC activation.[32]

One might wonder about the advantage of exploiting the earliest subcomponent of the complement cascade as a powerful cell-depleting effector mechanism. In the race between cell depletion and cell renewal, antibodies that deplete as promptly as possible will be most successful. Integrating C1q may be safer than using only later complement components, which may, eg, be deficient. For instance GVHD was also completely prevented by injection of C1q–high-affine anti–Thy-1 MoAbs in prospective AKR/J recipient mice challenged with C57BL/6 spleen and BM cells. AKR mice are genetically fully deficient of C5. Even depletion of C3 and the later components of the C′-cascade by CVF from Naja naja did not reverse the anti-GVHD effect of C1q–high-affine RmT1. Teleologically speaking, depletion of antibody-coated particles may be safer with isotypes that more stably intercalate C1q as the earliest complement subcomponent from serum and/or as expressed on phagocytes.

The question remains as to whether mouse IgG3 anti–Thy-1 MoAbs in mice correspond to the rat IgG2c isotype. Their heavy chain constant region genes have a high (87%) degree of homology.[37] As for rat IgG2c, we found low uptake of mouse C1q on IgG3 MoAb-coated Thy-1.1 thymocytes. Unfortunately Thy-1.1 density on postthymic T cells is too low to cause strong T-cell depletion and prevention of GVHD comparable with what is possible with anti–Thy-1.2 MoAb.[20] Testing whether CVF reduces the C3b-dependent effector mechanism of IgG3 anti–Thy-1 MoAb in the same way as we found for rat IgG2c anti–Thy-1 MoAb must, therefore, be adjourned until generation of an anti–Thy-1.2 mouse IgG3 isotype. However, it is of interest that in another, lymphoma cell-depleting mouse model, Denkers et al[16] found this C′-fixing antibody less effective in vivo than its C′-fixing IgG2a and 2b switch variants, which would be concordant with the differential uptake of C1q that we found for the same antibodies.

In summary, differentiation between a C3-9–independent effector mechanism of antibody isotypes with high intrinsic affinity for C1q and a less potent C3-dependent effector mechanism of antibody isotype(s) with low affinity for C1q led us to focus our attention on the earliest subcomponent of the complement cascade regarding antibody-induced cell depletion and immunosuppression.

ACKNOWLEDGMENT

The authors express their gratitude to Dr I.D. Bernstein (Seattle, WA) for making available the anti–Thy-1.1 MoAb producing hybridoma switch variants and to Dr R. Burger (Berlin, Germany) for a critical reading of the manuscript. The authors thank Hannelore Jennen, Julika Jasny, Ursula Hartl, and Barbara Glöckner for excellent technical assistance.

REFERENCES

1. Thierfelder S, Kummer U, Schuh R, Mysliwietz J: Antilymphocytic antibodies and marrow transplantation. VIII. Recipient conditioning with C1q-affine monoclonal anti-Pan T antibodies prevents GVHD in homozygous fully mismatched mice. Blood 68:818, 1986

2. Cobbold SP, Martin G, Qin S, Waldmann H: Monoclonal antibodies to promote marrow engraftment and tissue graft tolerance. Nature 232:164, 1986

3. Ledbetter LA, Herzenberg LA: Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol Rev 47:63, 1979

4. Bernstein ID, Nowinski RC, Tam MR, Mcmaster B, Houston LI, Clark EA: Monoclonal antibody therapy of mouse leukemia, in Kennet RH, McKearn TJ, Bechtel KB (eds): Hybridomas: A New Dimension in Biological Analysis. New York, NY, Plenum, 1988, p 275

5. Opitz HG, Opitz U, Hewlett G, Schlumberger HD: A new model for investigations of T-cell functions in mice: Differential immunosuppressive effects of two monoclonal anti-Thy-1.2 antibodies. Immunobiology 160:438, 1982

6. Cobbold SP, Thierfelder S, Waldmann H: Immunsuppression with monoclonal antibodies. A model to determine the rules for effective serotherapy. Mol Biol Med 1:285, 1983

7. Vallera DA, Soderling CCB, Carlson GJ, Kersey JH: Bone marrow transplantation across major histocompatibility barriers in mice. Effect of elimination of T cells from donor grafts by treatment with monoclonal Thy-1.2 plus complement or antibody alone. Transplantation 31:218, 1981

8. Rodt H, Thierfelder S, Eulitz M: Antilymphocytic antibodies and marrow transplantation. IV. Comparison of the effects of antibody fragments directed against immunoglobulin or lymphocyte antigens on acute secondary disease. Exp Hematol 2:195, 1974

9. Thierfelder S, Cobbold SP, Kummer U, Waldmann H, Schuh R: Antilymphocytic antibodies and marrow transplantation. VII. Two out of nine monoclonal anti-Thy-1 antibodies used for pretreatment of donor marrow suppressed graft-versus-host reactions without added complement. Exp Hematol 13:948, 1985

10. Kummer U, Thierfelder S, Hoffmann-Fezer G, Schuh R: In vivo immunosuppression by pan-T cell antibodies relates to their isotype and to their C1q uptake. J Immunol 138:4069, 1987

11. Antica M, Hofmann-Fezer G, Thierfelder S: Antilymphocytic antibodies and marrow transplantation. IX. T-cell depletion in marrow donors with C1q high and low affinity antibodies for suppression of GVHD in fully mismatched mice. Exp Hematol 17:942, 1989

12. Mittal KK, Mickey MR, Singal DP, Terasaki PI: Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity. Transplantation 6:913, 1968

13. Wasiliu M, Kremmer E, Thierfelder S, Felber E, Hoffmann-Fezer G, Kummer U: Monoclonal antibodies to complement

without the need of their prior purification. I. Antibodies to mouse C1q. Hybridoma 8:615, 1989

14. Kremmer E, Thierfelder S, Felber E, Hoffmann-Fezer G, Wasiliu M: Monoclonal antibodies to complement components without the need of their prior purification. II. Antibodies to mouse C3 and C4. Hybridoma 9:309, 1990

15. Kremmer E, Thierfelder S, Kummer U, Lederer R, Mysliwietz J: Neutralization of immunosuppression by antibodies against variable as well as constant regions of monoclonal anti-Thy-1 xenoantibodies and their ability to be suppressed by initial T cell depletion. Transplantation 47:641, 1989

16. Denkers EY, Badger CC, Ledbetter JA, Bernstein ID: Influence of antibody isotype on passive serotherapy of lymphoma. J Immunol 135:2183, 1985

17. Goding JW: Monoclonal antibodies: Principles and practice: Production and application of monoclonal antibodies in cell biology, biochemistry and immunology. San Diego, CA, Academic, 1983

18. Leo O, Foo M, Sachs DH, Samelson LE, Bluestone JA: Identification of a monoclonal antibody specific for a murine T3 polypeptide. Proc Natl Acad Sci USA 84:1374, 1987

19. Hudson L, Hay F: Practical Immunology (vol 11). New York, NY, Blackwell, 1976

20. Mysliwietz J, Thierfelder S, Hoffmann-Fezer G, Kummer U: Antilymphocytic antibodies and marrow transplantation. XI. Evidence that reduced Thy-1 expression in Thy-1.1 mice prevents suppression of graft-versus-host disease with anti-Thy-1 monoclonal antibodies. Transplantation 49:749, 1990

21. Schaefer HE, Fischer R: Der Peroxidasenachweis an Ausstrichpräparaten sowie an Gewebeschnitten nach Entkalkung und Paraffineinbettung. Klin Wochenschr 46:1228, 1968

22. Cochrane CG, Müller-Eberhard HJ, Aikin BS: Depletion of plasma complement in vivo by a protein of cobra venom: Its effect on various immunologic reactions. J Immunol 105:55, 1970

23. Hughes-Jones NC, Gorick BD, Howard JC: The mechanism of synergistic complement-mediated lysis of rat red cells by monoclonal IgG antibodies. Eur J Immunol 13:635, 1983

24. Howard JC, Butcher GW, Galfre G, Milstein C, Milstein CP: Monoclonal antibodies as tools to analyze the serological and genetic complexities of major transplantation antigens. Immunol Rev 47:139, 1979

25. Kummer U, Thierfelder S, Mysliwitez J: Antigen density on target cells determines the immunosuppressive potential of rat IgG2b monoclonal antibodies. Eur J Immunol 20:107, 1990

26. Yousaf N, Howard JC, Williams BD: Studies in the rat of antibody-coated and N-ethylmaleimide-treated erythrocyte clearance by the spleen. II. Effect of immune complex infusion. Immunology 59:81, 1986

27. Yousaf N, Howard JC, Williams BD: Studies in the rat of antibody-sensitized and N-ethylmaleimide-treated erythrocyte clearance by the liver: Effects of immune complex infusion and complement activation. Immunology 64:193, 1988

28. Ehlenberger AG, Nussenzweig V: The role of membrane receptors for C3b and C3d in phagocytosis. J Exp Med 145:357, 1977

29. Herlyn D, Koprowski H: IgG2a monoclonal antibodies inhibit human tumor growth through interaction with effector cells. Proc Natl Acad Sci USA 79:4761, 1982

30. Cobbold SP, Jayasuriya A, Nash A, Prospero TD, Waldman H: Therapy with monoclonal antibodies by elimination of T-cell subsets in vivo. Nature 312:548, 1984

31. Hale G, Clark M, Waldmann H: Therapeutic potential of rat monoclonal antibodies: Isotype specificity of antibody-dependent cell-mediated cytotoxicity with human lymphocytes. J Immunol 134:3056, 1985

32. Leu RW, Zhou A, Rummage JA, Kennedy MJ, Shannon BJ: Exogenous C1q reconstititutes resident but not inflammatory mouse peritoneal macrophages for Fc receptor-dependent cellular cytotoxicity and phagocytosis. Relationship to endogenous C1q availability. J Immunol 143:3250, 1989

33. Loos M: Biosynthesis of the collagen-like C1q molecule and its receptor functions for Fc and polyanionic molecules on macrophages. Curr Top Microbiol Immunol 102:1, 1983

34. Martin H, Heinz HP, Reske K, Loos M: Macrophage C1q: Characterization of a membrane form of C1q and of multimers of C1q subunits. J Immunol 138:3863, 1987

35. Tenner AJ, Cooper NR: Analysis of receptor-mediated C1q bind to human peripheral blood mononuclear cells. J Immunol 125:1658, 1980

36. Bobak DA, Gaither TA, Frank MM, Tenner AJ: Modulation of FcR function by complement: Subcomponent C1q enhances the phagocytosis of IgG-opsonized targets by human monocytes and culture-derived macrophages. J Immunol 138:1150, 1987

37. Brüggemann M, Delmastro-Galfre P, Waldmann H, Calabi F: Sequence of a rat immunoglobulin γ 2c heavy chain constant region cDNA: Extensive homology to mouse γ 3. Eur J Immunol 18:317, 1988

1991 77: 2285-2291

Antilymphocytic antibodies and marrow transplantation. XIV. Antibody- induced suppression of graft-versus-host disease in C3-decomplemented mice differentiates two T-cell-depletion pathways

S Thierfelder, J Mysliwietz, G Hoffmann-Fezer and U Kummer

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.