Complement-Mediated Mechanisms in Anti-GD2 Monoclonal Antibody Therapy of Murine Metastatic Cancer

Masaki Imai,1 Charles Landen,1 Rieko Ohta,1 Nai-Kong V. Cheung,2 and Stephen Tomlinson1

1Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, South Carolina and 2Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, New York

Abstract

The role of complement in antibody therapy of cancer is in general poorly understood. We used the EL4 syngeneic mouse model of metastatic lymphoma to investigate the role of complement in immunotherapy directed against GD2, a target of clinical relevance. IgG2a and IgM anti-GD2 therapy protected EL4-challenged mice from metastases and prolonged survival. Expression of CD59, an inhibitor of direct complement-mediated cytotoxicity (CMC), effectively protected EL4 cells from CMC in vitro but did not affect the outcome of monoclonal antibody therapy. Protection by IgG therapy was also unaffected in mice deficient in C3 or complement receptor 3 (CR3) but was almost completely abrogated in FcγR I/III–deficient mice. These data indicate a crucial role for antibody-dependent cell-mediated cytotoxicity (ADCC). However, at lower doses of IgG, therapeutic effect was partially abrogated in C3-deficient mice, indicating complement-mediated enhancement of ADCC at limiting IgG concentration. In contrast to IgG, the therapeutic effect of IgM was completely abrogated in C3-deficient mice. High level expression of CD59 on EL4 did not influence IgM therapy, suggesting IgM functions by complement-dependent cell-mediated cytotoxicity (CDCC), a mechanism thought to be inactive against tumor cells. Thus, IgG and IgM can operate via different primary mechanisms of action, and CDCC and complement-dependent enhancement of ADCC mechanisms are operative in vivo. The effects of complement can be supplemental to other antibody-mediated mechanisms and likely have increased significance at limiting antibody concentration or low antigen density. (Cancer Res 2005; 65(22): 10562-8)

Introduction

Passive immunization with unmodified antibodies is an increasingly used mode of therapy for the treatment of cancer, although for the most part, the in vivo mechanisms involved are not well defined (1–3). Antibody-dependent mechanisms that may be effective against cancer include induction of apoptosis, growth arrest, antibody-dependent cell-mediated cytotoxicity (ADCC), and complement-dependent activities. Complement activation on a tumor cell results in the generation and covalent attachment of C3 activation fragments that serve as opsonins for complement receptors expressed by phagocytes and natural killer (NK) cells. Engagement of complement receptors by C3-opsonized tumors (principally complement receptor 3 or CR3) can enhance ADCC (4–6) and may also promote complement-dependent cell-mediated cytotoxicity (CDCC), although the latter mechanism is not alone considered to be effective against tumor cells in the absence of adjuvant therapy (7, 8). Complement activation also results in the generation of inflammatory peptides (C3a and C5a) that may potentiate antitumor responses and, ultimately, in the formation of the cytolytic membrane attack complex (MAC). Direct MAC-mediated lysis is also referred to as complement-mediated cytotoxicity (CMC). However, tumor cells are protected from complement by membrane-bound complement inhibitory proteins. Complement inhibitory proteins expressed on human normal and tumor cells are CD59, decay-accelerating factor (DAF), and membrane cofactor protein (MCP). CD59 controls the terminal complement pathway and prevents cytolytic MAC formation by binding complement proteins C8 and C9 in the assembling MAC and preventing membrane insertion. DAF (CD55) and MCP (CD46) are inhibitors of complement activation that control complement at an earlier step in the pathway and limit the amount of C3 deposited. Several studies have shown that complement inhibitors are expressed at increased levels on tumor cells, indicating that they play a role in tumor immune evasion (9–15). Rodents express an additional membrane inhibitor of complement activation termed Crry, a functional and structural analogue of human DAF and MCP (16, 17). Crry is the preeminent inhibitor of complement activation expressed on mouse tumor cell lines.

It is well documented that complement inhibitory proteins afford tumor cells with protection from antibody and complement in vivo, but the contributions of complement in antitumor monoclonal antibody (mAb) therapy in vivo are in most cases not well understood. In the current study, we used the well-characterized EL4 syngeneic mouse model of metastatic lymphoma to investigate the role of complement in antibody immunotherapy. The EL4 lymphoma cell line expresses ganglioside GD2, and antibodies to GD2 administered subsequent to tumor challenge have been shown to eliminate EL4 micrometastases and provide protective immunity (18). GD2 is also a human tumor-associated antigen, and anti-GD2 antibodies have shown antitumor effects in clinical trials (19–23). EL4 lymphoma cells stably expressing human CD20 have also been used in mouse models to investigate the mechanism of action of anti-CD20 mAbs, including Rituximab, a chimeric anti-CD20 mAb approved for the treatment of non-Hodgkin’s lymphoma (24, 25). Studies indicate an important role for complement in Rituximab therapeutic activity, but there is not yet consensus on the in vivo mechanisms involved (26).

The most success with mAb therapy in the clinic has been realized using mAbs against hematologic malignancies, and it has been proposed that effective mAb therapy may be efficacious for solid tumors in the setting of circulating micrometastases (18).
In this regard, the anti-GD2/EL4 model of metastatic lymphoma is a suitable model for investigating mechanisms of antibody immunotherapy and the role of complement. A better understanding of the role of complement in antibody therapies may facilitate the development of strategies to enhance complement-dependent effector mechanisms that may be additive to all other mAb effector mechanisms.

**Materials and Methods**

**Cell lines and cDNA.** The mouse lymphoma cell line EL4 was grown at 37°C in 5% CO2 in RPMI 1640 with 10% heat-inactivated FCS (Gemini Bio-Product, Woodland, CA), 100 units/mL penicillin, and 100 μg/mL streptomycin. cDNA encoding mouse CD59a was provided by Dr. B.P. Morgan (ref. 27; University of Wales, Cardiff, United Kingdom). A stably transfected EL4 cell population expressing mouse CD59 was selected by fluorescence-activated cell sorting as described (28).

**Antibodies and serum.** Anti-mouse CD59a mAb 3B3, anti-mouse Cry mAb 5D5, and anti-mouse DAF mAb RIKO-3 were provided by Drs. B.P. Morgan and V.M. Holers (University of Colorado Health Sciences Center, Denver, CO) and Dr. H. Okada (Nagoya City University, Nagoya, Japan), respectively. Anti-GD2 mAb 14G2a (mouse IgG2a) was the gift of Dr. R.A. Reisfeld (Scripps Research Institute, La Jolla, CA), and anti-GD2 mAb 3G6 (mouse IgM) mAb was described previously (29). Goat anti-mouse C3 IgG was obtained from ICN Pharmaceuticals (Aurora, OH), and all other secondary antibodies were purchased from Sigma (St. Louis, MO). Normal mouse serum was prepared from C57BL/6 mice and stored in aliquots at −80°C until use.

**Flow cytometry.** Analysis of cell surface GD2, complement inhibitor expression, and C3 deposition was done as described (28). For analysis of mouse C3 deposition on EL4 cells in vitro, cells (5 × 10⁶) were resuspended in 20 μL PBS or anti-GD2 mAb (either 14G2a or 3G6) at 50 μg/mL and incubated for 30 minutes at 4°C. After washing, cells were resuspended in 20 μL PBS or complement inhibitor blocking mAB (5D5 and/or 3B3; final concentration, 50 μg/mL) and incubated at 4°C for 30 minutes. Cells were then washed and incubated in 30 μL of 30% mouse serum diluted in gelatin veronal-buffered saline (GVB; Sigma) at 37°C for 30 minutes. Cells were then washed with EDTA-GVB, incubated with FITC-conjugated goat anti-mouse C3 (30 minutes/4°C), and washed twice. Finally, cells were suspended in PBS containing 2 μg/mL propidium iodide and analyzed by flow cytometry. Propidium iodide–positive (dead) cells were excluded when fluorescence intensity was calculated.

**Complement lysis assay.** CMC was determined by ⁵¹Cr release as described (30).

**Cell proliferation assay.** EL4 cells (4 × 10⁵ per well) were plated in 96-well tissue culture plates in serum-containing medium and then treated with either 14G2a or 3G6 anti-GD2 mAB at indicated concentrations for 24 hours at 37°C. After incubation, cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt tetrazolium assay kit according to manufacturers instructions (CellTiter 96, Promega, Madison, WI) or by trypan blue staining. Both methods gave similar results.

**Mice and tumor model.** Normal male C57BL/6 mice were obtained from the National Cancer Institute (Frederick, MD). The C3<sup>−/−</sup> and CR3<sup>−/−</sup> knockout mice on C57BL/6 background and wild-type littermates were purchased from the Jackson Laboratory (Bar Harbor, ME). FcγR receptor γ chain–deficient mice were purchased from Taconic (Germantown, NY). Mice were housed in a clean room, and food and water was sterilized. All other experiments, mice were used at 6 to 8 weeks of age, with between 6 and 11 animals in each group. EL4 cells (3 × 10⁶) suspended in 0.1 mL of PBS were injected into the tail vein. Groups of mice received either EL4 mAb or EL4/mCD59 transfectants with mouse CD59 or empty plasmid. In some experiments, mice were injected i.v. with 100 μg of anti-GD2 mAb 14G2a or PBS 2 days after tumor challenge. In other experiments, mice were injected i.v. with 200 μg of anti-GD2 mAb 3G6 at days 1 and 2 after tumor challenge. In an alternative protocol, all animals were sacrificed on the 23rd day after the injection. In both types of experiment, necropsies were done to examine the number of liver tumors, liver weight, and liver appearance.

**Statistical analyses.** Unpaired Welch’s t tests were used to determine statistical differences. The log-rank test was used to compare differences on survival curves. Significance was accepted at the P < 0.05 level.

**Results**

**Complement inhibitor and GD2 expression by EL4 cells.** Flow cytometry revealed that EL4 cells, unlike most human tumor cells, were CD59 and DAF negative (MCP expression was not analyzed, because in rodents expression is restricted to the testes). EL4 cells did, however, express the rodent inhibitor of complement activation, Crry, a structural and functional analogue of DAF and MCP (Fig. 1). EL4 cells were previously reported to express GD2 (8, 31), and recognition of EL4 by anti-GD2 mAbs14G2a (IgG) and 3G6 (IgM) was also confirmed (Fig. 1). Nearly all human tumor cells and cell lines express CD59 and are resistant to MAC-mediated lysis (CMC) by human serum. To investigate the role of CD59 and the terminal complement pathway in immune resistance in a mouse model, EL4 cells were stably transfected with mouse CD59 for subsequent in vitro and in vivo analyses. A population of EL4 cells stably expressing mouse CD59 was isolated by cell sorting. CD59 expression did not affect the expression of GD2 or the other complement inhibitors on EL4 (Fig. 1).

**Effect of complement inhibitors on complement deposition and complement-mediated lysis in vitro.** CD59 inhibits the terminal pathway of complement and, as anticipated, the expression of CD59 on EL4 cells did not significantly alter the level of C3 deposition on EL4 cells sensitized with either 14G2a or 3G6 anti-GD2 mAb (Fig. 2A). The mouse CD59 blocking mAb 3B3 also had no effect on C3 deposition. Crry inhibits at the earlier C3 activation
step and, also as anticipated, the blocking of endogenous Crry activity with mAb 5D5 resulted in increased levels of deposited C3 on anti-GD2-sensitized EL4 cells (Fig. 2A).

We next examined the effect of CD59 expression on CMC of EL4 cells in vitro. Wild-type and control-transfected EL4 cells lack CD59 and were susceptible to homologous (mouse) complement-mediated lysis in vitro following sensitization with either 14G2a or 3G6 mAb (Fig. 2B). However, EL4 cells stably transfected with mouse CD59 were almost completely resistant to lysis by mouse complement, a phenotype resembling that of most human cancer cell lines. CD59 function was confirmed by the restoration of complement susceptibility by CD59-blocking mAb 3B3 (Fig. 2B). Blocking both CD59 and Crry activity with mAb 3B3 and 5D5 F(ab’)_2, respectively, had an additive effect on enhancing complement sensitivity. Lysis of control-transfected EL4 cells was also enhanced by blocking Crry activity, but as expected, CD59-blocking antibody had no effect on the lysis of CD59-negative cells. The blocking mAbs alone (in the absence of anti-GD2 mAb) had no significant effect on complement deposition or lysis. Note that it is well documented that isolated mouse complement is generally poorly lytic, and the levels of complement-mediated lysis shown are low relative to levels of lysis often obtained using complement from other species. We also determined that neither 14G2a nor 3G6 anti-GD2 mAb had any effect on cell viability or proliferation in the absence of complement (Fig. 2C).

Effect of CD59 on anti-GD2 immunotherapy. Direct complement-dependent cytolysis is a proposed contributory mechanism of action for anti-GD2 mAbs and for antibodies against hematologic malignancies, such as rituximab. Complement-dependent cytolysis has also been proposed to play a role in eradication of EL4 micrometastases in syngeneic mice by anti-GD2 antibodies (18), and we used the EL4 model to investigate the role of CMC in mAb therapy. Following i.v. challenge with EL4, mice develop hepatic metastases and a 2- to 3-fold increase in liver mass at death, which occurs 25 to 35 days after challenge. Mice receiving a single injection of 100 μg 14G2a 2 days after tumor challenge survived >70 days (at which time, mice were sacrificed) and remained tumor free. Similar protection was obtained with a different anti-GD2 mAb, 3F8 (IgG3), and is consistent with previous reports using 3F8 (18). To test the role of CMC during anti-GD2 therapy, mice were challenged with CD59-positive EL4 (cells that are almost completely resistant to lysis by mouse complement). Irrespective of the presence of CD59, mice were completely protected by therapy with 14G2a (Fig. 3) or 3F8 (data not shown), indicating that neither CMC nor CD59 play a mechanistic role in antibody therapy in this model. There was no difference in survival (Fig. 3), liver weight, or number of tumors in the liver (data not shown) between CD59-positive and CD59-negative EL4-challenged mice. Tumor cells isolated from mice on day 25 following challenge with CD59-positive EL4 continued to express CD59, indicating that there was no in vivo selection (analyzed by flow cytometry; data not shown).

Effect of complement, complement receptor, and Fc receptor deficiency on anti-GD2 immunotherapy. Complement activation and C3 deposition on a target cell may enhance antibody-dependent cell-mediated cytotoxicity and may promote complement-dependent cell-mediated cytotoxicity (CDCC) via engagement of CR3 on leukocytes. To investigate whether these mechanisms play a role in anti-GD2 therapy of EL4 lymphoma, immunotherapy experiments were done using C3- and CR3-deficient mice. Neither C3 nor CR3 deficiency had any significant effect on 14G2a mAb efficacy, indicating that complement is not involved in the antibody-mediated protection from EL4 (Fig. 4). There was also no difference in survival of wild-type and C3- or CR3-deficient mice following challenge with CD59-positive EL4 continued to express CD59, indicating that there was no in vivo selection (analyzed by flow cytometry; data not shown).

3 M. Imai. mAb 3B3 does not activate complement, unpublished observation.
antibody therapy (Fig. 4). In contrast, 14G2a therapy of EL4 lymphoma in FcγR I/III–deficient mice provided almost no protection (P = 0.004), indicating that ADCC is the fundamental mechanism of action for anti-GD2 therapeutic activity in this model.

Anti-GD2 IgM therapy of EL4 lymphoma. Together, the above data indicate that ADCC was necessary and sufficient for anti-GD2 immunotherapy using the EL4 model. However, we found that immunotherapy with 3G6, an anti-GD2 IgM mAb, was also protective, albeit less so than 14G2a IgG. Mouse immune effector cells are not known to express Fc receptors for this class of antibody, appearing to rule out a role for ADCC in the mechanism of action of 3G6. Compared with PBS treatment, immunotherapy of EL4 lymphoma with 3G6 (≥ 200 µg) resulted in significantly prolonged survival, reduced number of liver metastases, and lower liver mass (P < 0.01; Fig. 5). The antibody 3G6 alone had no effect on EL4 viability in vitro (Fig. 2C). These data suggest a role for complement in 3G6 therapy, although 3G6 was no more effective than 14G2a at depositing complement on EL4 cells in vitro (Fig. 2A).

To investigate whether the terminal complement pathway and CMC is involved 3G6-mediated antitumor activity, we did 3G6 immunotherapy experiments in mice challenged with CD59-negative EL4. There was a significant difference between PBS control and 3G6-treated mice when challenged with CD59-positive EL4 in terms of survival (P < 0.05), liver metastases (P < 0.01), and liver mass (P < 0.05), but there was no difference in survival following 3G6 treatment between CD59-positive and CD59-negative EL4-challenged mice (compare Figs. 5 and 6). Thus, CD59 expression did not interfere with 3G6 efficacy, indicating that CMC does not have a mechanistic role in anti-GD2 IgM therapy and suggesting the involvement of complement at an earlier stage in the pathway. We therefore repeated the IgM immunotherapy experiments in C3-deficient mice. Immunotherapy with 3G6, in contrast to therapy with 14G2a, was not effective in C3-deficient mice. Whereas C3-deficient mice challenged with EL4 were completely protected by 14G2a therapy, 3G6 therapy had no protective effect in terms of survival, liver metastases, or liver mass (Fig. 7). These data indicate anti-GD2 IgM therapy is complement dependent and operates via CMC.

Anti-GD2 IgG therapy of EL4 lymphoma at limiting antibody concentration. The above data show that the primary mechanism of action is different for anti-GD2 IgG and IgM-mediated therapy in the EL4 model. Effective IgG therapy was dependent on ADCC, whereas IgM therapy was complement dependent. However, because anti-GD2 IgM was no better than anti-GD2 IgG at activating and depositing complement on EL4 cells, the data suggest that complement-dependent antitumor activity is operational during IgG therapy but is redundant to ADCC at high IgG concentration. The effect of complement opsonization on enhancement of ADCC is well characterized in vitro and to investigate this phenomenon in vivo, we did low-dose anti-GD2 IgG immunotherapy and compared the effect of treatment in wild-type and C3-deficient mice. A single dose of 100 µg 14G2a resulted in complete protection of both wild-type and C3-deficient mice inoculated with EL4 (see Fig. 4). A single dose of 50 or 25 µg, however, resulted in reduced survival rates in C3-deficient mice compared with wild-type mice, which was significant at 50 µg (P < 0.05; Fig. 8). These data suggest a cooperative role for complement in ADCC that becomes significant at low antibody concentration.

Discussion

The clinical use of mAbs as a treatment modality for cancer is gaining acceptance. Many antitumor mAbs have been shown to activate complement, but strong evidence for an important role for complement in cancer regression exists only for Rituximab, a humanized anti-CD20 mAb for treating non-Hodgkin’s lymphoma (26, 32–35). For most mAbs, the role of complement, if any, in their mechanism of action is not known. Indeed, complement is generally considered to play little or no role in mAb efficacy against tumors, due in large part, to the expression of membrane-bound complement inhibitory proteins. On the other hand, ADCC is recognized as an important effector function of mAbs. Although it is well known that complement deposition on target cells can enhance ADCC via CR3 costimulation in vitro, there is no direct evidence indicating that such activity occurs against tumor
cells in vivo in the absence of adjuvant therapy. In this study, immunotherapy experiments were conducted with IgG2a and IgM mAbs specific for the ganglioside GD2, an antigen of relevance to tumor therapy (19–23, 36, 37). Mouse IgG2a can mediate ADCC and can activate complement and has a similar activity profile to human IgG1, the most common isotype of chimeric or humanized antitumor mAb (mouse IgG1 does not activate complement). IgM is a strong activator of complement but has no known role in ADCC or CDC of tumor cells, although a Fcγ/μR is expressed on human macrophages (ref. 38; expression has not been reported in mice).

We show that anti-GD2 immunotherapy with IgG at high concentration (as generally used in therapeutic protocols in rodent models) is dependent on ADCC for efficacy, with no apparent role for complement. Similar conclusions were reached previously with other mAbs and tumor models in FcγR-deficient mice (39, 40). However, a dose response study done in C3-deficient mice showed that complement enhances antibody therapy at lower mAb concentration. Because our data seem to rule out a role for direct MAC-mediated lysis, the effect is presumably mediated through ligation of CR3 by iC3b/C3dg-opsonized tumor cells resulting in enhancement of ADCC (4–6). This finding is of clinical relevance, because in many circumstances, there will be limited antibody bioavailability, particularly in solid tumors, and maintaining nontoxic concentrations of mAb may be problematic. In the same way, it is also likely that complement will lower the threshold for antibody-mediated destruction of tumor cells with low target antigen density. By validating a role for complement in enhancing ADCC in vivo, the data support previous suggestions that C3 opsonization of B cells enhances Rituximab therapy of leukemia and lymphoma (24, 35).

A previous study showed that CR3 was essential for effective ADCC of melanoma in a mouse model, but the effect was complement independent (41).

In vitro studies using tumor cell lines have frequently shown a critical role for CD59 in providing protection from MAC-mediated lysis (42–46). Furthermore, CD59 is frequently overexpressed on tumor cells (10, 14, 47, 48), and expression has been reported to correlate with resistance to anti-CD20 immunotherapy (32). However, although stable transfection of EL4 cells with CD59 made them highly resistant to complement lysis in vitro, it...
provided no protection from mAb therapy in a metastatic setting. Thus, caution should be applied to the extrapolation of in vitro data regarding the role of CD59 and MAC-mediated lysis in tumor growth and immunity. In vitro studies reported here using Crry-blocking mAb also showed that endogenous Crry on EL4 cells provided a degree of protection from complement deposition and lysis. However, it was not possible to do anti-Crry-blocking experiments in vivo due to the widespread expression of Crry on normal tissue and the immunogenicity of the anti-Crry mAb (only rat mAbs to mouse Crry are available). A previous proof-of-principle study showed that the endogenous expression of Crry on a rat tumor cell line provided protection from mAb-mediated therapy. In this previous study, anti-rat Crry mAb was administered to immune-deficient mice challenged with rat tumor cells, thus allowing for tumor-specific targeting of rat Crry (49).

Clinical studies have indicated a role for anti-ganglioside IgM antibodies (including anti-GD2) in passive and active immunity against some cancers (21, 50–54). Their mechanism(s) of action is not clear, but a recent study in which mice transgenic for anti-GD2 IgM antibody were protected from EL4 metastasis and death indicated a role for IgM, complement and NK cells (55). In the current study, we show that in contrast to IgG therapy, the therapeutic effect of IgG was complement dependent, because the protective effect of anti-GD2 IgM was completely abolished in C3-deficient mice. IgM therapy required a higher dose compared with IgG and is probably a reflection of different mechanisms of action and/or the relative instability and short circulatory half-life of administered IgM antibodies. Because mouse leukocytes are not known to express an FcμR (or FcεR/μR), and because the expression of high levels of CD59 did not effect therapeutic outcome, the operative mechanism of action is likely CDCC. This result is interesting and potentially controversial, because although CDCC is the major complement-mediated effector mechanism against microbial pathogens, it is considered to be inactive against tumor cells. An exception is when CR3 is coligated at its lectin binding site with soluble forms of polysaccharides, such as β-glucan, which are expressed on microbial surfaces but not cancer cells (8, 56, 57). Our data, together with the above reported data using anti-GD2 IgM transgenic mice indicate a CDCC mechanism mediated by NK cells. It was recently reported that (high dose) anti-CD20 therapy in the EL4 metastatic lymphoma model was complement dependent, because the protective effect was abolished in C1q-deficient mice (24). In these studies, an IgG2a mAb was also used, and these findings seem at odds with the current data using C3-deficient mice (binding of C1q to cell-bound antibodies initiates the classic pathway of complement and subsequent C3 deposition). One possible explanation for the different results is the involvement of C1q receptor-mediated CDCC. Nevertheless, such a mechanism has not previously been described for tumor cell lysis, and the depletion of NK and neutrophils did not affect therapeutic activity of anti-CD20 therapy in the reported studies (24).

In summary, this study shows that antibody-mediated complement-dependent mechanisms are effective against tumor cells in vivo, even when the tumor cell expresses high levels of complement inhibitors. Our data show that IgG and IgM can operate via different primary mechanisms of action, introducing the possibility that combination therapy may be synergistic. In our model, inhibition of MAC-mediated lysis by expression of CD59 did not hinder the efficacy of mAb therapy, and evidence is presented indicating CDCC and complement-dependent enhancement of ADCD mechanisms operate in vivo. The effects of complement can be supplemental to other antibody-mediated mechanisms of action, and these effects may have increased significance at limiting antibody concentration (which may depend on setting) or low density of the target antigen on tumor cells.

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Figure 8. Survival curves of wild-type mice and C3-deficient mice treated with low dose of anti-GD2 IgG after challenge with EL4 cells. Mice were injected i.v. with 3 × 10^6 EL4 cells and treated with 100 μg of 14G2a at indicated dose on day 2 (n = 8-10 per group).
References

4. Irie RF, Morton DL. Regression of cutaneous melanoma by the chimeric anti-GD2 monoclonal antibody 181.151–9.
7. Irie RF, Morton DL. Regression of cutaneous melanoma by the chimeric anti-GD2 monoclonal antibody 181.151–9.
13. Irie RF, Morton DL. Regression of cutaneous melanoma by the chimeric anti-GD2 monoclonal antibody 181.151–9.
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