Natural antibody against neuroblastoma of TH-MYCN transgenic mice does not correlate with spontaneous regression

川久保, 尚徳

Biochemical and Biophysical Research Communications 503 (2018) 1666–1673

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Natural antibody against neuroblastoma of TH-MYCN transgenic mice does not correlate with spontaneous regression

Naonori Kawakubo [a, b], Yui Harada [a, *], Minori Ishii [a, b], Ryota Souzaki [b], Yoshiaki Kinoshita [b], Tatsuro Tajiri [c], Tomoaki Taguchi [b], Yoshikazu Yonemitsu [a]

[a] R&D Laboratory for Innovative Biotherapeutics, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan
[b] Department of Pediatric Surgery, Faculty of Medical Sciences, Kyushu University, Fukuoka, Japan
[c] Department of Pediatric Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan

ARTICLE INFO

Article history:
Received 5 July 2018
Accepted 19 July 2018
Available online 24 July 2018

Keywords:
MYCN
Neuroblastoma
Natural antibody

ABSTRACT

The mechanism underlying the spontaneous regression of neuroblastoma is unclear. Although it was hypothesized that this regression occurs via an immunological mechanism, there is no clinical evidence, and no animal models have been developed to investigate the involvement of immune systems, especially natural antibodies, against neuroblastoma. We performed an immunological analysis of homo- and heterozygous TH-MYCN transgenic mice as a model of aggressive neuroblastoma. Mice with no or small (<5 mm) tumors showed higher antibody titers in plasma than mice with large (>5 mm) tumors. A significant negative correlation was observed between the tumor diameter and the titer of antitumor antibody. This antibody had complement-dependent cytotoxicity but not antibody-dependent cellular cytotoxicity against neuroblastoma cells. Moreover, B-cell depletion had no effect on the tumor incidence *in vivo*. We revealed that TH-MYCN transgenic mice have a natural antibody against neuroblastoma that correlate with tumor size. However, this antibody does not correlate with the spontaneous regression of neuroblastoma. Thus, the function of the natural antibody is limited.

Neuroblastoma (NB) is the most common extracranial solid malignant tumor in children [1]. In some cases of infant neuroblastoma (stage 4S), this tumor undergoes spontaneous regression and has a favorable prognosis with a greater than 90% survival rate [2]. Complete regression is also seen in some stage 1 tumors [3]. However, despite the enormous amount of basic and clinical research conducted on this disease entity, neuroblastoma patients with MYCN amplification (>10 copies per cell) show particularly poor prognoses [4].

Brodeur et al. introduced four major pathways that may explain, together or in part, the spontaneous regression of NB [5]: (1) immune-mediated cell killing by antibody-dependent cellular cytotoxicity (ADCC) or by natural killer (NK) cells, (2) deprivation of neurotrophin and the activation of programmed apoptosis, (3) apoptosis caused by low levels/absence of telomerase, and (4) epigenetic changes in gene expression. However, there has been no clear evidence in support of these or any other mechanisms of spontaneous NB regression.

TH-MYCN mice, a model in which the human MYCN gene is integrated under the control of a rat tyrosine hydroxylase, develop NB tumors [6]. The histological and pathological aspects of these tumors are similar to those of human NB [7,8], and the mouse tumors show genomic aberrations with some similarity to those in human NB [9]. The mouse tumors have many of the molecular characteristics of human NB [10]. There have been very few reports on TH-MYCN mice from an immunological viewpoint. In the few studies that have been performed, infiltrations of T cells, macrophages and dendritic cells have been observed in the tumor microenvironment [11,12]. Myeloid-derived suppressor cells (MDSCs) also infiltrate to the tumor and exhibit immune suppressive activity [12]. In a xenograft model of TH-MYCN primary culture cells, treatment with anti-GD2 and NK cells achieved a good prognosis [13].

Based on these findings, we hypothesized that spontaneous regression occurs via an immunological mechanism, and we investigated this possibility by analyzing the immune response in TH-MYCN mice. Our focus is whether there is a causal relationship

* Corresponding author. R&D Laboratory for Innovative Biotherapeutics, Graduate School of Pharmaceutical Sciences, Kyushu University, 6th Floor, Collaborative Research Station I, 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812-8582, Japan.
E-mail address: rkfraile@med.kyushu-u.ac.jp (Y. Harada).

https://doi.org/10.1016/j.bbrc.2018.07.097

between spontaneous regression and the natural antibody.

1. Methods

1.1. Mice

TH-MYCN heterozygote mice [6] were kindly provided by Professor K. Kadomatsu at Nagoya University. Genotyping was carried out as described by Haraguchi et al. [14]. The tumors were detected by ultrasound using a VEVO770 Micro-Imaging System (FujiFilm VisualSonics, Toronto, ON, Canada).

Animal experiments were reviewed and approved by the Ethics Committee on Animal Experiments at the Faculty of Medical Sciences, Kyushu University. The experiments were carried out under the conditions indicated in the Regulations for Animal Experiments of Kyushu University and Law 105 and Notification 6 of the Government of Japan.

1.2. Cells and antibodies

IMR-32 was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in RPMI-1640 medium supplemented with 15% fetal bovine serum (FBS) under a humidified atmosphere containing 5% CO_2 at 37 °C.

Specifically, the following monoclonal antibodies (mAbs) were used in this study: Alexa488-conjugated anti-mouse IgM (Abcam, Cambridge, UK), Dylight488-conjugated anti-mouse IgG (Abcam), FITC-conjugated anti-mouse CD3 (BioLegend, San Diego, CA), FITC-conjugated anti-mouse IgG1 (BioLegend), FITC-conjugated anti-mouse IgG2a (BioLegend), FITC-conjugated anti-mouse IgG2b (BioLegend), FITC-conjugated anti-mouse B220 (BioLegend), PE-conjugated anti-mouse CD8a (BioLegend), FITC-conjugated anti-mouse IgG3 (Santa Cruz Biotechnology, Dallas, TX), PerCP/Cy5.5-conjugated anti-mouse CD4, PerCP/Cy5.5-conjugated anti-mouse CD49b, PerCP/Cy5.5-conjugated anti-mouse CD19, FITC-conjugated anti-human IgM (BioLegend), and Alexa-488-conjugated anti-human IgG Fc (BioLegend).

1.3. Flow cytometric analysis

The cells reacted with appropriate mAbs were analyzed using a FACSCalibur™ cell analyzer with CellQuest software (Becton Dickinson, Tokyo). Dead cells were excluded by staining with propidium iodide. The data analyses were performed using FlowJo 7.6 software (Tree Star, Ashland, OR).

1.4. In vivo depletion

The administration schedule was as follows: For NK cell depletion, anti-asialo GM1 (Wako, Osaka, Japan) was given intraperitoneally (i.p.) (100 μg/dose) to mice 3 × /week beginning when the mice were 3 weeks old until they were 22 weeks old. $CD4^+$ or $CD8^+$ cells in the mice were eliminated by an i.p. injection of mAbs (Clone GK1.5 for $CD4^+$ cells depletion, Clone 53–6.72 for $CD8^+$ cell depletion; Bio X Cell, West Lebanon, NH) 2 × /week beginning when the mice were 3 weeks old until they were 22 weeks old.

For the depletion of peritoneal B cells, 100 μl of 2% thioglycolic acid was i.p.-administered to newborn mice, and the next day, 250 μg of anti-CD20 mAb (Clone 5D2, Genentech, San Francisco, CA) was i.p.-injected. The schedule of administration was days 0, 7, 14, 42, and 70. For the depletion of the mothers' peritoneal B cells, 100 μl of 2% thioglycolic acid and anti-CD20 mAb was i.p.-administered to the mother before pregnancy 1 × /week for 3weeks.

1.5. Detection of anti-neuroblastoma antibody

The tumors obtained from TH-MYCN mice were dissociated using a Tumor Dissociation Kit (Miltenyi Biotec, Cologne, Germany). The tumor cells were incubated with mouse plasma obtained from TH-MYCN mice at 4° for 30 min, then washed three times with D-PBS and stained with secondary antibody (anti-mouse IgG/IgG1/IgG2a/IgG2b/IgG3/IgM).

For the detection of human anti-NB antibody, IMR-32 was incubated with human plasma obtained from healthy adult volunteers at 4° for 30 min, washed three times with D-PBS, and stained with secondary antibody (anti-human IgG/IgM). All participants gave informed consent for the use of their data, and ethical approval was obtained from the Ethics Committee of the Institute of Health Science at Kyushu University.

1.6. Purification of human IgG

Purification of IgG from human plasma was done using Dynabeads™ Protein G (Veritas, Tokyo). A 10-μl aliquot of human plasma was diluted in 200 μl of D-PBS with Tween-20, then incubated with Dynabeads™ Protein G for 10 min at room temperature. The beads-IgG complex was eluted by elution buffer, and the purified IgG was obtained.

1.7. Complement-dependent cytotoxic assay

First, 4×10^4 TH-MYCN primary cells suspended in 15 μl of RPMI-1640 containing 1% FBS were mixed with 5 μl of mouse plasma. Then, 20 μl of human complement and 60 μl of RPMI-1640 containing 1% FBS were added and the mixture was incubated at 37 °C for 5 min. The viable cells were then immediately counted using a hemocytometer and trypan blue staining.

1.8. CDC assay

First, 4×10^4 IMR-32 cells suspended in 90 μl RPMI-1640 medium containing 15% FBS were cultured with 10 μl of human plasma for 1 h at 4 °C. Then, 10 μl of human complement (Sigma-Aldrich Japan, Tokyo) was added and the mixture was incubated at 37 °C for 60 min. The viable cells were then immediately counted using a hemocytometer and trypan blue staining.

1.9. ADCC assay

For the evaluation of ADCC, 1×10^5 TH-MYCN primary cells were labeled with DiOC18 (Sigma-Aldrich Japan) according to the manufacturer's protocol. Labeled target cells were washed twice, suspended in 100 μl RPMI-1640 medium containing 10% FBS, and mixed with 20 μl of mouse plasma for 30 min at 4°. NK cells were obtained from spleens of wild-type mice using EasySep™ Mouse NK Cell Isolation Kit (Stemcell Technologies, Vancouver, Canada). NK cells were added at 1:1 and 5:1 effector:target cell ratios. After 4 h of coincubation, all cells were harvested and stained with propidium iodide according to the manufacturer's protocol. Duplicate samples were assessed by flow cytometry, and the numbers of dead cells (DiOC18-positive and PI-positive) were determined. The percentage of specific killing was calculated using the following formula: (percentage of total dead target cells^{target} − percentage of total dead target cells^{control})/(100 − percentage of total dead target cells^{control}) × 100.

1.10. Statistical analysis

All data are expressed as the mean ± SEM. The statistical

significance of differences was determined using the Mann-Whitney *U* test, and p-values <0.05 were considered significant.

2. Results

2.1. Spontaneous regression of neuroblastoma in TH-MYCN heterozygote mice

TH-MYCN mice generally developed retroperitoneal neuroblastoma (Suppl. Fig. S1). We first investigated changes in the tumors in heterozygote mice, on a weekly basis. The microscopic findings of the superior mesenteric ganglion (SMG) in 2- to 3-week-old heterozygote mice revealed that the SMG of all of these mice contained solitary or multiple tumor foci (Fig. 1A). The frequency of tumorigenesis decreased at 4 weeks of age in these mice (Fig. 1B). All of the homozygote mice developed a neuroblastoma and died within 9 weeks of age (Fig. 1C). Then, we checked the effects of effector cell ($CD4^+$, $CD8^+$, and NK cell) (Fig. 1D). These cells were depleted, and we determined the survival rate by Kaplan-Meier analysis. The depletion of each type of effector cells was confirmed weekly(Suppl. Fig. S2). The tumor incidence tended to increase in the asialo-GM1-administered group, however, due to the limitation to the removal efficiency of NK cells, it is difficult to conclude whether the cells affected tumor regression.

2.2. Anti-neuroblastoma antibody in the TH-MYCN mice

Next, we focused on an antitumor antibody in the mice. The plasma was cultured with tumor cells that were dissected from other heterozygote mice, followed by incubation with anti-mouse IgG or anti-mouse IgM as a secondary antibody. As shown in Fig. 2A, there was a significant negative correlation ($p < 0.05$) between the tumor diameter and the mean fluorescence intensity (MFI) of antitumor IgM/IgG. These antibodies were detectable in the heterozygote mice in which the diameter was <5 mm (Fig. 2B and C). We monitored the antitumor antibody during the tumor progression (Fig. 2D). Surprisingly, the wild-type mice had levels of antitumor IgG/IgM similar to those of the tumor-free heterozygote mice. These data indicated that the antitumor antibody level might be correlated with the spontaneous regression and progression of neuroblastoma in this model, and that the antibody is a natural antibody because it was detected in the wild-type mice.

The subclass of antitumor IgG was examined by flow cytometry. The tumor cells were stained by anti-mouse IgG1/IgG2a/IgG2b/IgG3 after being incubated with plasma obtained from wild-type mice. Anti-mouse IgG3 and anti-mouse total IgG showed the same titer, whereas the titers of the other classes of antibody did not (Fig. 2E). We then focused on the peritoneal B-cell subset B1 cells, known as the main source of natural IgM and IgG3 [15,16]. The B-cell ELISpot analysis revealed that peritoneal B cells were the main source of anti-NB antibody in this mouse model (Suppl. Fig. S3A,B). Moreover, the tumor-bearing mice had lower numbers of peritoneal B cells than the tumor-free mice (Suppl. Fig. S4).

Next, we examined whether human plasma also contains anti-NB antibodies. IMR-32 was then incubated with the healthy adult plasma and then with anti-human IgM/IgG. All of the plasma samples contained anti-NB IgM, and a low level of anti-NB IgG was detected (Fig. 2F and G). The purified IgG from healthy adult plasma showed significantly higher MFI (Fig. 2H), suggesting that, in humans, the natural IgM and IgG recognize the same epitope of NB cells.

2.3. The complement-dependent cytotoxicity of the natural anti-NB antibody

To confirm the *in vitro* role of this antibody, we investigated the complement-dependent cytotoxicity (CDC) The tumor cells were

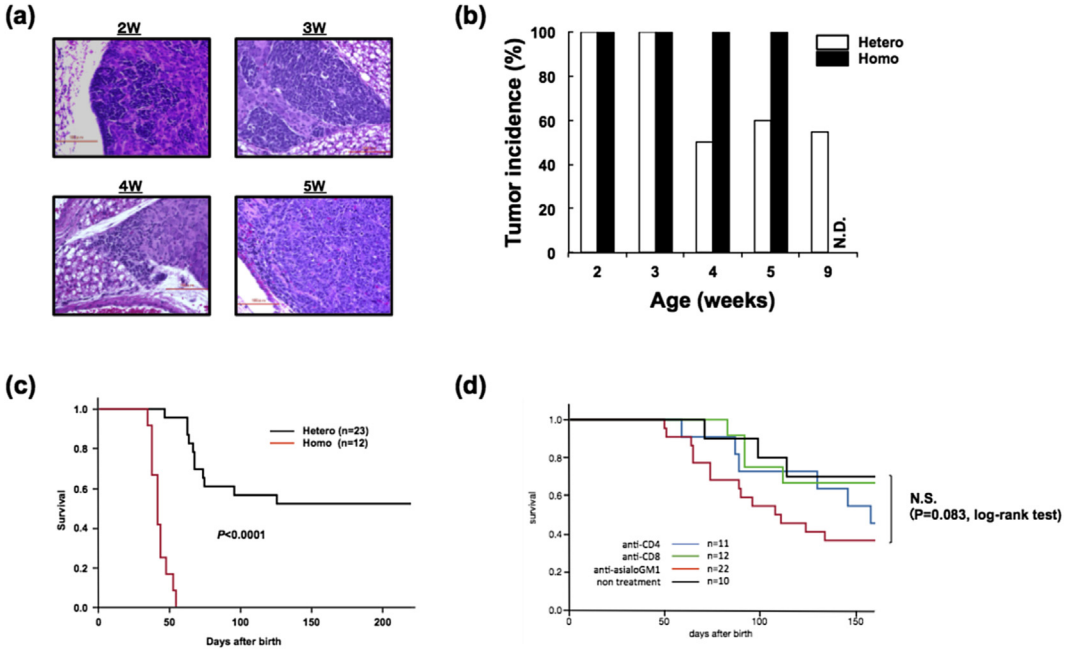

Fig. 1. Natural course of the TH-MYCN mice and spontaneous regression. **A:** H&E staining of the SMG of heterozygote mice at the end of each wk (week). The images reveal that the ganglia of mice contained tumor foci in each wk. **B:** Tumor incidence for each wk. The ganglia of all mice at 2 and 3 wks of age contained tumor foci. Hetero: n = 7 each (2 and 3 wks old), n = 10 each (4 and 5 wks old), and n = 11 (9 wks old). Homo: n = 5 each. The data of 9-wk-old homozygote mice are labeled "not detected" because all of the homozygote mice died within 9 wks of age. **C:** Kaplan-Meier analysis of heterozygote and homozygote mice. Approximately half of the heterozygote mice developed retroperitoneum tumors and died within 20 wks of age. All of the homozygote mice developed tumors and died. **D:** Effect of effector cell depletion. A significant difference was not observed.

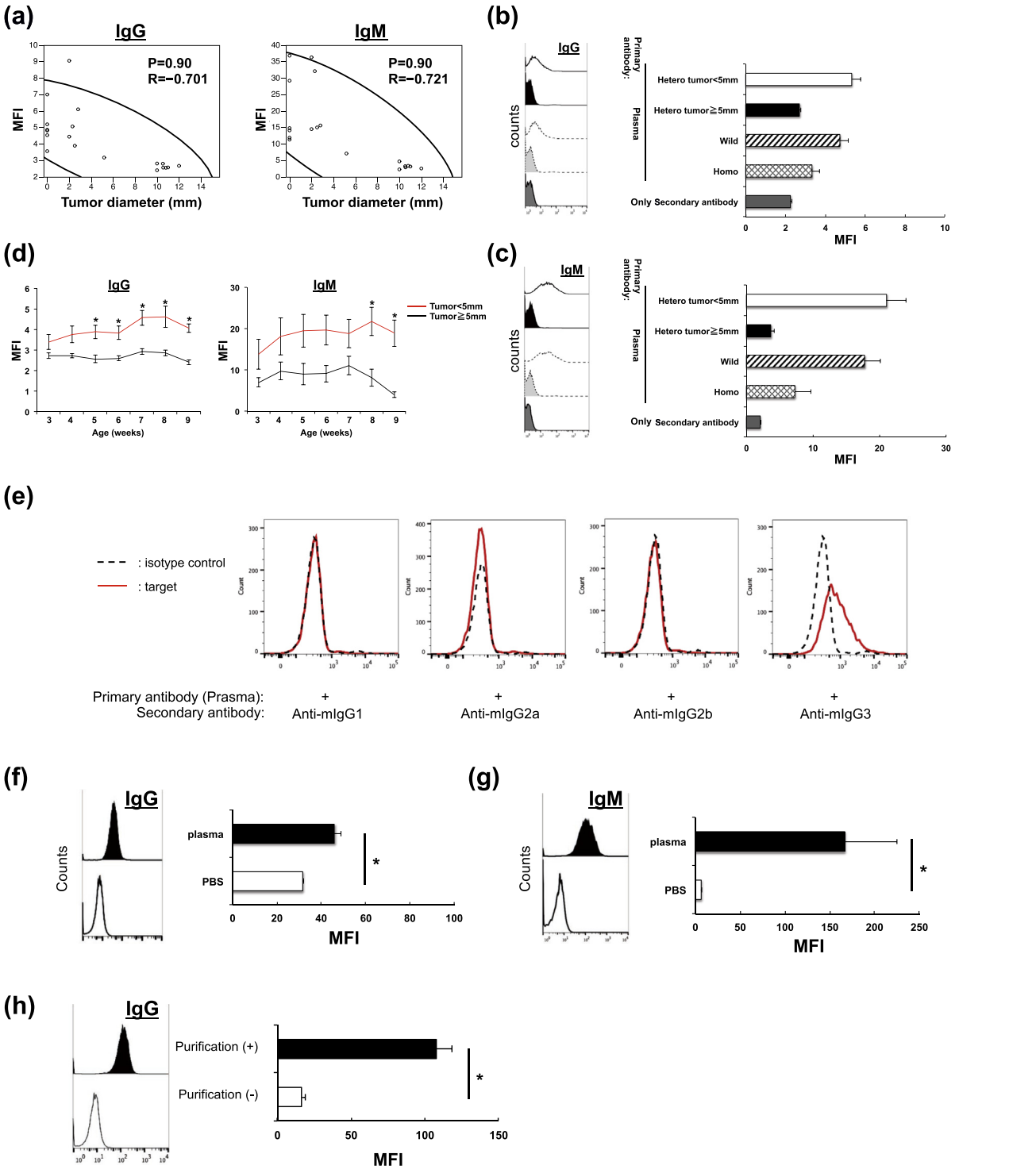

Fig. 2. Natural antitumor antibody in TH-MYCN mice. To detect the antitumor antibody in these mice, we obtained the plasma from 9-wk-old heterozygote mice, 6-wk-old homozygote mice, and 9-wk-old wild-type mice. The plasma was incubated with the tumor cells dissected from heterozygote mice and then with anti-mouse IgG or anti-mouse IgM as a secondary antibody. **A:** The scatter plot showed a significant negative correlation ($p < 0.05$) between the tumor diameter of heterozygote mice and the MFI of antitumor IgG/IgM. The tumor diameter was estimated by sonogram at 9 wks of age. **B,C:** Histograms and graphs showing that the mice that developed small tumors (<5 mm) had higher MFI of antitumor IgG/IgM than did those developing large tumors (>5 mm) or homozygote mice. Wild-type mice had antitumor IgG/IgM similar to those of the hetero tumor<5 mm mice. $p < 0.01$ vs. the hetero tumor>5 mm group. **$p < 0.05$ vs. the homo mice. Error bars: mean + S.E. Hetero tumor<5 mm: n = 11; Hetero tumor>5 mm: n = 8; Wild: n = 12; Homo: n = 4. **D:** Trends in the MFI of antitumor IgG/IgM in the heterozygote mice at each wk. In the hetero tumor<5 mm group, a higher amount of antitumor IgG/IgM was observed from 5 or 7 wks of age. Tumor<5 mm: n = 8 (IgG)/n = 7 (IgM); Tumor>5 mm: n = 4 (IgG), n = 3 (IgM). **E:** Tumor cells were stained by anti-mouse IgG1/IgG2a/IgG2b/IgG3 as the secondary antibody after being incubated with plasma obtained from wild-type mice. Anti-mouse IgG3 and anti-mouse IgG showed the same titer of antitumor antibody, whereas the titers of the other classes of antibody did not.

Flow cytometric analysis of the binding of natural IgG (**F**) or IgM (**G**) to IMR-32 from normal human plasma. PBS served as a control (white). The graphs show the MFI of the anti-NB IgM and IgG of normal human plasma (n = 5). All of the plasma samples contained anti-NB IgM as well as a low level of anti-NB IgG. **H:** Purified IgG showed significantly higher MFI. *$p < 0.01$.

incubated with the plasma or culture medium alone as a control. The microscopic images in Fig. 3 show the tumor cells incubated with plasma and complement, and the inset shows trypan blue staining. Because the cytotoxicity was cancelled when the cells were incubated with inactivated complement, this cytotoxicity depended on the complement. The human NB cell line was also killed by the addition of human plasma containing anti-NB antibody, and the cytotoxic activity was shown to depend on the complement (Fig. 3B). We then examined the ADCC using primary NB cells obtained from TH-MYCN mice. As shown in Fig. 3C, the cytotoxicity did not change between antibody-rich and -poor plasma. We also confirmed the cytotoxicity toward YAC-1 cells (Suppl. Fig. S5).

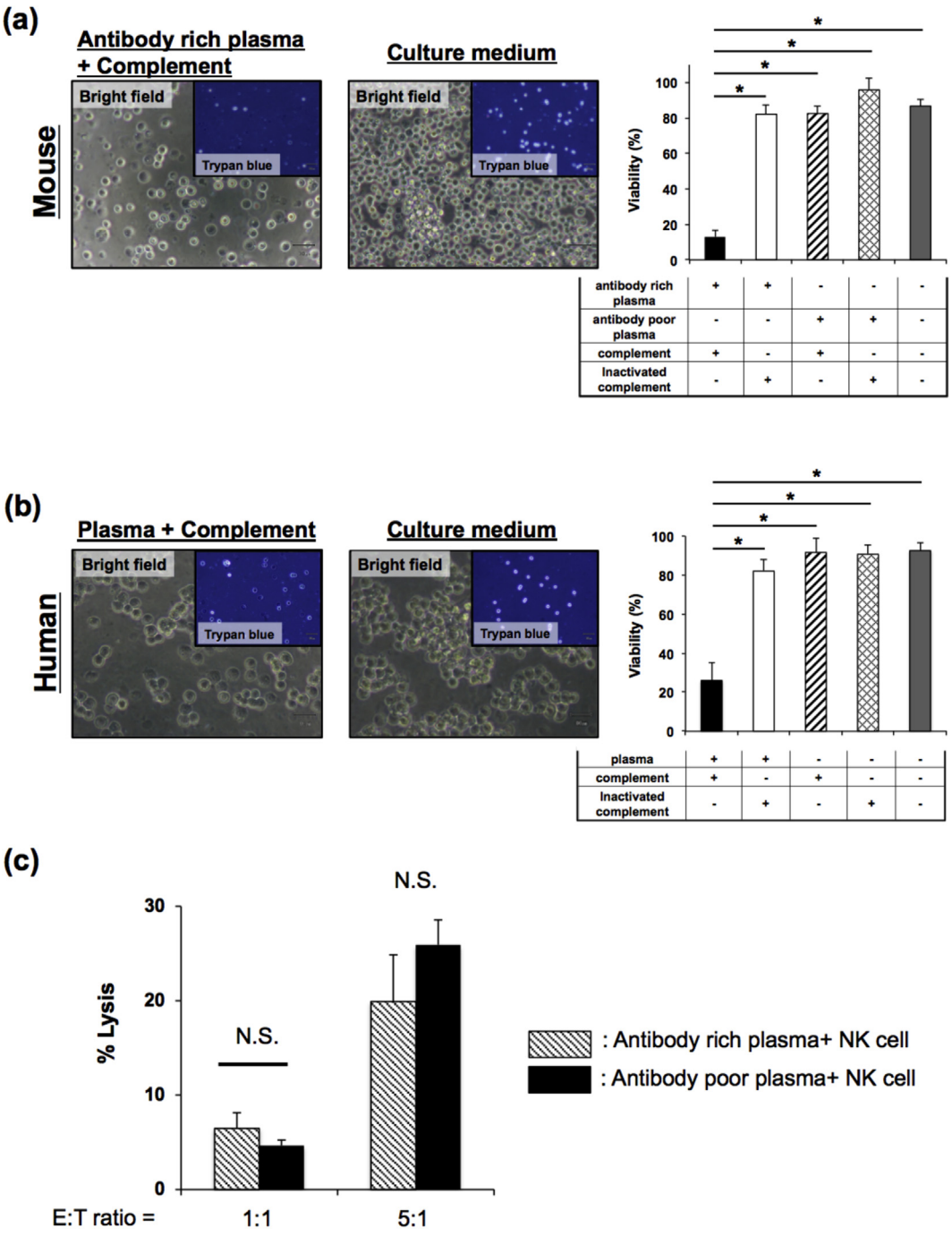

Fig. 3. CDC and ADCC of the natural antitumor antibody. A: Microscopic images of TH-MYCN primary tumor cells after incubation with antitumor antibody-rich plasma and complement (*left images*) and culture medium and complement (*right images*). The dead cells were stained by trypan blue. Graphs show the viability of TH-MYCN primary tumor cells after incubation under each condition. The combination of antibody-rich plasma and complement induced the cell death of TH-MYCN tumor cells. *$p < 0.05$ vs the other groups. Each examination is $n = 4$. **B:** Microscopic images of IMR-32 after incubation with normal human plasma and complement (*left images*), and culture medium and complement (*right images*). The dead cells were stained by trypan blue. Graphs: The viability of IMR-32 cells after incubation under each condition. The combination of normal human plasma and complement induced the cell death of IMR-32 cells. *$p < 0.05$ vs. the other groups. The plasma was obtained from five healthy volunteers, and viability was estimated by duplicate analysis. **C:** Cytotoxicity of NK cells toward the TH-MYCN primary tumor cells after incubation with antibody-rich plasma or antibody-poor plasma. The cytotoxicity did not change between the antibody-rich plasma and -poor plasma. Each examination is $n = 3$.

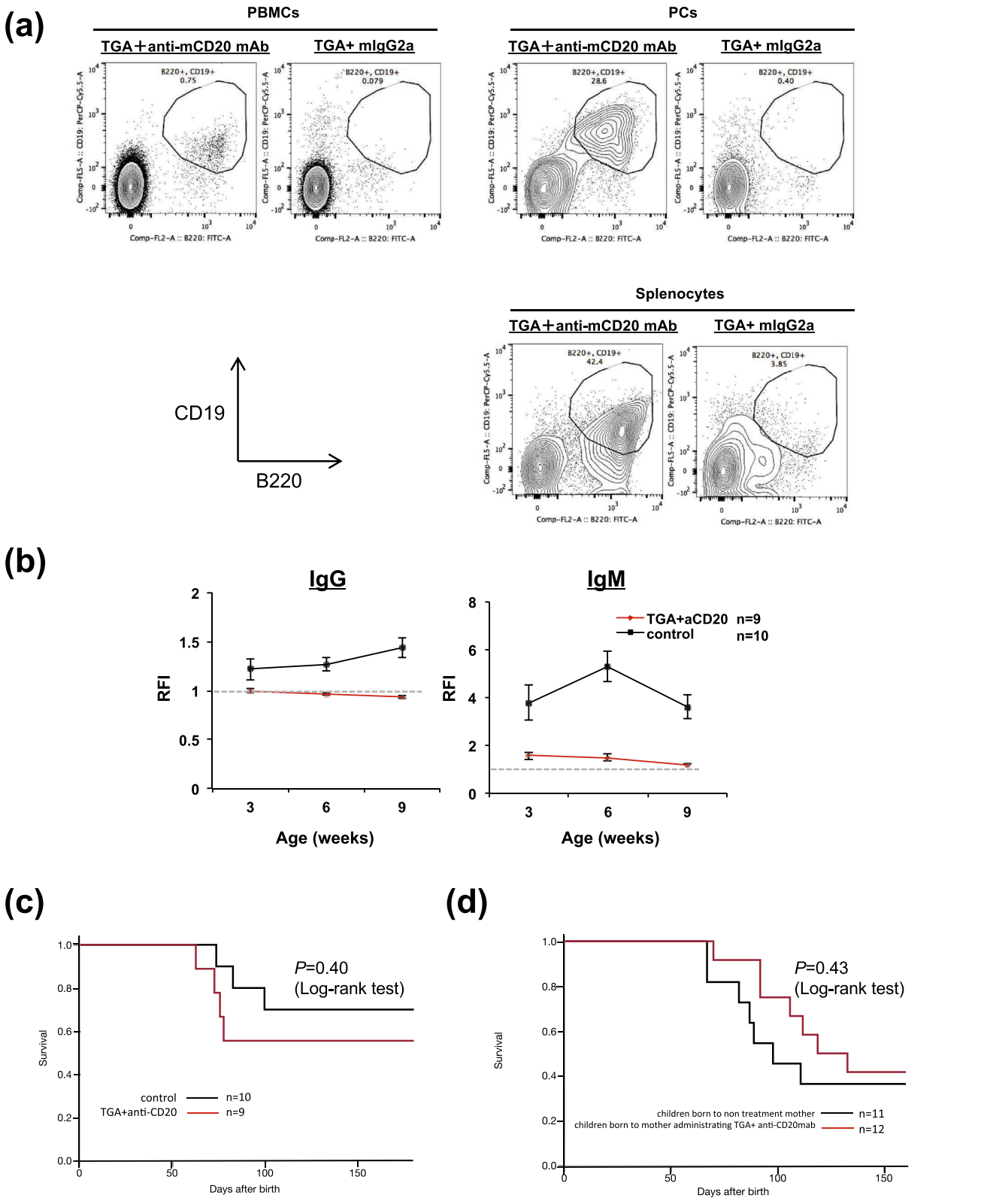

Fig. 4. Effect of the peritoneal B-cell depletion. A: For the deletion of peritoneal B cells, 100 μl of 2% thioglycolic acid (TGA) was i.p.-administered to newborn mice, and the next day, 250 μg of anti-CD20 mAb (5D2, Genentech) was i.p.-injected. The flow cytometric analysis revealed that the administration of TGA+anti-CD20 mAb diminished the peritoneal B cells compared to control (administration of thioglycolic acid+mouse IgG2a). B-cell: $B220^{+}CD19^{+}$. **B:** Trends in the MFI of antitumor IgG/IgM in the administration of TGA+anti-CD20 mAb. RFI = MFI/MFI of the negative control. **C:** Kaplan-Meier analysis of the mice injected with TGA+anti-CD20 and control mice. There was no significant difference between the two groups: $p = 0.40$ **D:** Kaplan-Meier analysis of offspring born to a mother injected with TGA+anti-CD20 and the control mice. There was no significant difference between the two groups: $p = 0.43$.

2.4. Effect of peritoneal B-cell depletion

For B-cell depletion to confirm the *in vivo* role of this antibody, we administered anti-CD20 mAb intraperitoneally to mice once a week. We combined anti-CD20 mAb and TGA for the depletion of peritoneal B cells [17], and after 3 weeks of administration, peritoneal B cells were completely removed and the production of antitumor IgG/IgM was inhibited (Fig. 4A).

The depletion of antitumor IgG/IgM from 3 to 9 weeks old was confirmed by a flow cytometric analysis in all mice (Fig. 4B). Unexpectedly, the tumor incidence did not increase in the B-cell depletion group compared to the control group (Fig. 4C). Lastly, we depleted the maternal antibody by the administration of anti-CD20 + TGA to mother mice in order to evaluate the influence of maternal antibody, and the depletion of the antitumor antibody in the mothers' plasma was confirmed by flow cytometric analysis (data not shown). In this experiment too, the tumor incidence of mice from the mothers injected with anti-CD20 and TGA did not increase (Fig. 4D), suggesting that these antibodies do not contribute to the spontaneous regression of the tumors in this mouse model.

3. Discussion

The prognosis of high-risk or recurrent neuroblastoma remains very poor, despite the advances in the treatment of these tumors. Preclinical models are needed to better understand the features of neuroblastoma and to evaluate novel therapies. Some researchers have used TH-MYCN mice as a model of aggressive neuroblastoma. The tumor foci in the ganglia of heterozygote mice have been described as “hyperplastic lesions,” and researchers have speculated that these lesions might be the precancerous [17]. A high expression of Midkine has been also reported in the ganglia containing the precancerous lesion [18], and another report indicated that FACT (a histone chaperone) is highly expressed in the lesion, and that a FACT inhibitor delays the tumor growth in TH-MYCN homozygote mice [19]. However, there have been no reports indicating a correlation between the precancerous lesion and immunological mechanisms.

In our study, the titers of natural IgM and IgG were depleted only when the peritoneal B cells were diminished with the combination of thioglycolic acid and anti-CD20 mAb. We therefore suspect that the peritoneal B1 cells are the main production source of these natural antibodies. Previous research examined only anti-NB IgM, because the detection level of IgG is much lower than that of IgM [20]. However, purified IgG in human plasma showed a high MFI level, indicating that both anti-NB IgM and IgG recognize the same epitope of IMR-32.

In a clinical study, Fukuda et al. reported that stage 4 NB patients had lower levels of anti-NB IgM than patients with nonmalignant surgical disease, and the MFI of anti-NB IgM and CDC activity increased after primary tumor resection and chemotherapy [21]. Those authors thus suggested that the low level of natural antibody in patients with stage 4 NB was caused by adsorption of the antibody by tumor cells.

Although in our *in vitro* study the cytotoxic activity of this antibody was confirmed, and several researchers speculated that this natural antibody was the main effector of the spontaneous regression [21,22], we concluded that this natural antibody is not correlated with the spontaneous regression and that the function of this antibody is very limited. In a future study, we need to identify the epitope of this antibody and confirm the reason that the titer of antitumor antibody differs among individuals.

From our depletion study of NK cells (Fig. 1D) and the *in vitro* ADCC assay (Fig. 3C), it appears that NK cells may be involved in the spontaneous regression, but no significant findings were obtained in the study. As shown in Fig. 1C, the depletion of NK cells was inadequate despite the use of anti-asialo GM1 antibody. This might because anti-antibody was produced in these mice, and we thought that this is a limitation of our depletion study.

One of the major reasons for the dearth of investigations into the cause of spontaneous regression is that, until now, there has been no mouse model of spontaneous regression. We propose that the present mouse model of neuroblastoma will improve this situation as a useful tool that mimics the spontaneous regression occurring in human NB. The immunological aspect of this mouse model also mimics that of human NB patients in terms of the natural antibodies produced against this tumor. We propose that these mice are a key model to elucidate this phenomenon.

Authors' contributions

N.K. contributed to all experiments. M.I. provided assistance with the genotyping and prepared the supplementary figures. R.S., Y.K., and T.T. advised from the clinical point of view. Y.H., T.T. and Y.Y. supervised the experiments. N.K. and Y.H. wrote the manuscript. All authors read and approved the manuscript.

Additional information

Conflict of interest statement: Y.H. is a member of the Scientific Advisory Board of GAIA BioMedicine, Inc. Y.Y. is a director of GAIA BioMedicine, Inc.

Acknowledgments

We thank Ryoko Nakamura for her help with the animal experiments, and Drs. Kenji Kadomatsu and Satoshi Kishida, Department of Biochemistry, Nagoya University Graduate School of Medicine for their advice about the breeding of the TH-MYCN mice. This work was supported in part by a Grant-in-Aid (to Y.H, Y.Y.) from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (16K20346).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2018.07.097.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2018.07.097.

References

[1] M.C. Henry, D.B. Tashjian, C.K. Breuer, Neuroblastoma update, Curr. Opin. Oncol. 17 (2005) 19–23.
[2] T. Monclair, et al., The International Neuroblastoma Risk group (INRG) staging system: an INRG task Force report, J. Clin. Oncol. 27 (2009) 298–303.
[3] B. Hero, et al., Localised infant neuroblastomas often show spontaneous regression: results of the prospective trials NB95-S and NB97, J. Clin. Oncol. 26 (2007) 1504–1510.
[4] G.M. Brodeur, Neuroblastoma: biological insights into a clinical enigma, Nat. Rev. Canc. 3 (2003) 203–216.
[5] G.M. Brodeur, R. Bagatell, Mechanisms of neuroblastoma regression, Nat. Rev. Clin. Oncol. 11 (2014) 704–713.
[6] W.A. Weiss, K. Aldape, G. Mohapatra, B.G. Feuerstein, J.M. Bishop, Targeted expression of MYCN causes neuroblastoma in transgenic mice, EMBO J. 16 (1997) 2985–2995.
[7] H.C. Moore, et al., Histological profile of tumors from MYCN transgenic mice, J. Clin. Pathol. 61 (2008) 1098–1103.
[8] L.M. Hansford, et al., Mechanisms of embryonal tumor initiation: distinct roles for MycN expression and MYCN amplification, Proc. Natl. Acad. Sci. U.S.A. 101

(2004) 12664–12669.
[9] C.S. Hackett, et al., Genome-wide array CGH analysis of murine neuroblastoma reveals distinct genomic aberrations which parallel those in human tumors, Canc. Res. 63 (2003) 5266–5273.
[10] A.J. Cheng, et al., Cell lines from MYCN transgenic murine tumors reflect the molecular and biological characteristics of human neuroblastoma, Eur. J. Canc. 43 (2007) 1467–1475.
[11] L.M. Carlson, et al., Low-dose aspirin delays an inflammatory tumor progression in vivo in a transgenic mouse model of neuroblastoma, Carcinogenesis 34 (2013) 1081–1088.
[12] G. Santilli, et al., Polyphenon E enhances the antitumor immune response in neuroblastoma by inactivating myeloid suppressor cells, Clin. Canc. Res. 19 (2013) 1116–1125.
[13] M. Kroesen, et al., A transplantable TH-MYCN transgenic tumor model in C57Bl/6 mice for preclinical immunological studies in neuroblastoma, Int. J. Canc. 134 (2014) 1335–1345.
[14] S. Haraguchi, A. Nakagawara, A simple PCR method for rapid genotype analysis of the TH-MYCN transgenic mouse, PLoS One 4 (2009), 10.1371/journal.pone.0006902.
[15] K. Hayakawa, et al., Ly-1 B cells: functionally distinct lymphocytes that secrete IgM autoantibodies, Proc. Natl. Acad. Sci. U.S.A. 81 (1984) 2494–2498.
[16] S. Panda, J. Zhang, N.S. Tan, B. Ho, J.L. Ding, Natural IgG antibodies provide innate protection against ficolin-opsonized bacteria, EMBO J. 32 (2013) 2905–2919.
[17] Y. Hamaguchi, et al., The peritoneal cavity provides a protective niche for B1 and conventional B lymphocytes during anti-CD20 immunotherapy in mice, J. Immunol. 174 (2005) 4389–4399.
[18] S. Kishida, et al., Midkine promotes neuroblastoma through Notch2 signaling, Canc. Res. 73 (2013) 1318–1327.
[19] D.R. Carter, et al., Therapeutic targeting of the MYC signal by inhibition of histone chaperone FACT in neuroblastoma, Sci. Transl. Med. 7 (2015), 10.1126/scitranslmed.aab1803.
[20] M.W. Ollert, et al., Normal human serum contains a natural IgM antibody cytotoxic for human neuroblastoma cells, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 4498–4503.
[21] M. Fukuda, C. Nozaki, Y. Ishiguro, K. Horibe, Natural Antibody against Neuroblastoma among Japanese children with or without neuroblastoma, Cancer 86 (1999) 2166–2170.
[22] K. David, et al., Growth arrest of solid human neuroblastoma xenografts in nude rats by natural IgM from healthy humans, Nat. Med. 2 (1996) 686–689.