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RESEARCH ARTICLE

Intravenous Immunoglobulin Suppresses Chemotherapy-Induced Peripheral Neurotoxicity Via Macrophage Modulation in Rats and Mice

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ABSTRACT

Chemotherapy-induced peripheral neurotoxicity (CIPN) is a serious adverse effect that leads to treatment discontinuation by patients receiving anticancer therapy. Treatment discontinuation is a serious and life-threatening problem for patients with cancer; hence, there is a need for drugs that suppress the induction of CIPN by anticancer drugs. Here, using rat and mouse models, we showed that intravenous immunoglobulin (IVIg) can suppress CIPN induced by not only paclitaxel but also by doxorubicin. Furthermore, the suppressive effect of IVIg is eliminated when macrophages are depleted. Here, we proposed two novel independent mechanisms underlying the alleviation of CIPN by IVIg. First, IVIg suppresses CIPN in a macrophage-dependent manner. Second, IVIg combined with anticancer drugs can avoid restrictions on the use of anticancer drugs owing to CIPN induction. However, further research is necessary for the bench-to-bedside translation of these novel applications of IVIg. Our findings lay a strong foundation for research on IVIg therapeutics.

Introduction

Patients with anticancer chemotherapy-induced peripheral neurotoxicity (CIPN) experience compromised daily life activities and lowered quality of life post treatment[1-3]. Consequently, treatment must be interrupted even if a patient is responding well to a drug. This causes undue psychological stress in patients who must deal with CIPN-related symptoms and effective drug discontinuation. Although some drugs have been approved to treat CIPN (such as duloxetine, pregabalin, and tricyclic antidepressants), there is no drug that shows a remarkable effect. Moreover, the pathogenesis of CIPN remains unclear[4].

Intravenous immunoglobulin (IVIg) was developed as a drug for patients with antibody deficiency[5]; however, IVIg is also used to treat many autoimmune disorders, such as Kawasaki disease and idiotypic immune thrombocytopenic purpura[6,7]. Most currently, it was reported that IVIg suppressed a miscarriage of Recurrent Pregnancy Loss (RPL)[8]. The proposed mechanisms underlying IVIg action in autoimmune diseases include the modulation of Fc receptors, interference with the cytokine network and complement proteins, provision of anti-idiotypic antibodies, suppression of lymphocyte effector function[6,9,10], and presentation of regulatory T cell-inducing epitope termed Tregitope[11]. Additionally, sialylated immunoglobulin G (IgG) contained in IVIg indirectly upregulates the inhibitory IgG Fc receptor FcγRIIB on effector macrophages[7]. Although IVIg has several functions and is effective against many diseases, its precise action mechanism remains unclear.

Recently, Meregalli et al. reported that IVIg exerts suppressive effects on bortezomib-induced[12] and paclitaxel-induced CIPN[13] in rat models. Additionally, they observed that IVIg reduced the infiltration of M1 macrophages into the peripheral nerves. To the best of our knowledge, their study is the first to report the effectiveness of IVIg in alleviating CIPN. However, IVIg is generally not effective for CIPN. Recently, our group focused on the multi-functional effects of IVIg and studied the mechanisms underlying the effects under various conditions such as infectious diseases[14-17], RPL[18], and neurological disease[19,20]. In the present study, we focused on whether the suppressive effects of IVIg can be extended to peripheral neurotoxicity induced by other chemotherapy drugs and whether the reduced infiltration of macrophages observed in response to IVIg administration is involved in alleviating CIPN. Therefore, we aimed to clarify the effects of IVIg on peripheral neurotoxicity induced by chemotherapeutics and studied the role of macrophages in IVIg-mediated suppression of CIPN.

Materials and Methods

Laboratory animals and code of ethics

Crl:CD (SD) male rats (7 weeks) and Crl:CD1 (ICR) male mice (6 weeks) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). The rats were maintained under specific pathogen-free conditions and a 12-h light/dark cycle; they were used in experiments after 7 weeks of age. All animal procedures were approved by the Animal Care and Use Committee of Japan Blood Products organization. The study was performed in accordance with the guidelines of the Animal Care and Use Committee of Japan Blood Products Organization, which conforms to the policies of the Japan Health Sciences Foundation {the business of the "Japan Health Sciences Foundation" was taken

over by the "Japan Pharmaceutical Information Center (https://www.japic.or.jp/calac/english.html)" on 2021 / mar / 31.}. This study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org).

Induction of CIPN model

Paclitaxel (Bristol-Myers Squibb, Princeton, NJ USA) was intraperitoneally injected into rats (1 g/kg on days 0, 1, 2, 3, 4, 7, 8, 9, 10, 11, 14, and 15) or mice (3 mg/kg on days 0, 1, 2, 3, and 4) to induce PPIN. Paclitaxel was dissolved in 8.3% Cremophor EL (Nacalai Tesque, Kyoto, Japan), 8.3% ethanol (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), and 83.4% sterile saline (Otsuka Pharmaceutical Factory, Tokushima, Japan).

Doxorubicin (Nippon Kayaku, Tokyo, Japan), an anticancer drug, was intravenously injected into rats (6 mg/kg on day 0). Control group animals were administered the same amount of the solvent or saline used to dissolve the drugs.

Administration of IVIg

IVIg (Japan Blood Products Organization, Tokyo, Japan) was intravenously administered on days 0, 7, 14, and 21 at 1 g/kg to the rats treated with paclitaxel or doxorubicin and on days 0 and 5 at 1 g/kg to mice treated with paclitaxel. Control group animals were administered the same amount of saline.

Depletion of macrophage

To deplete macrophages, clodronate liposome (Hygieia Bioscience, Osaka, Japan) was intravenously injected on days 2, 5, 12, and 19 at 5.6 mg/body.

Mechanical allodynia and heat hyperalgesia

Neuropathic pain was evaluated using the dynamic test in accordance with a previously published method[21,22]. Animals were acclimated to the corresponding behavioral test environments, and baseline responses were measured before anticancer drug administration. The occurrence of neuropathic signs was monitored after 3, 4, and 5 weeks (rat) or 1 and 2 weeks (mouse) of anticancer drug injection.

Mechanical allodynia was assessed using a 26-g Semmes-Weinstein Von Frey Anesthesiometer (Tactile Test; Muromachi Kikai, Tokyo, Japan). Briefly, the animals were placed in a compartment with a wire mesh bottom, and later, a Semmes-Weinstein monofilament was applied to the plantar surface of their hind paws with a progressive increase in puncture pressure. The sensory threshold was recorded, and the mechanical sensitivity was determined by calculating the mean value of 10 repeated applications per footpad. Mechanical measurements were assessed via verification by two experimenters.

Heat hyperalgesia was measured as the latency to express pain-related behavior using a hot plate (54.5 °C). The following behaviors were observed: 1) licking of foot, 2) fluttering of foot, 3) raising of foot, 4) jumping, and the latency until one of them was expressed (latency to paw withdrawal) was measured for individual animals.

Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM). The results are representative of two or more independent experiments. Means were

compared using Student's *t*-test. First, the control group and the anticancer drug-administered group were tested. After confirming that the model was established, the anticancer drug-administered group and the IVIg-administered group were tested. Statistical analyses were performed using SAS9.3 software (SAS Institute Inc, NC, USA), and results with $P < 0.05$ (two-tailed) indicated significance.

Results

IVIg suppresses peripheral neurotoxicity induced by chemotherapeutics

We examined the effects of IVIg on peripheral neurotoxicity induced by two different anticancer drugs using not only rat but also mouse. We studied the effect of IVIg on paclitaxel-induced CIPN (p-CIPN) and doxorubicin-induced CIPN (d-CIPN) in rats. Our results showed that IVIg alleviated mechanical allodynia in p-CIPN (Fig. **1a**) and d-CIPN (Fig. 1**b**) rats. Additionally, in the mouse p-CIPN model, IVIg alleviated mechanical allodynia (Fig. 2**a**) and heat hyperalgesia (Fig. 2**b**).

Figure **1a** **b**

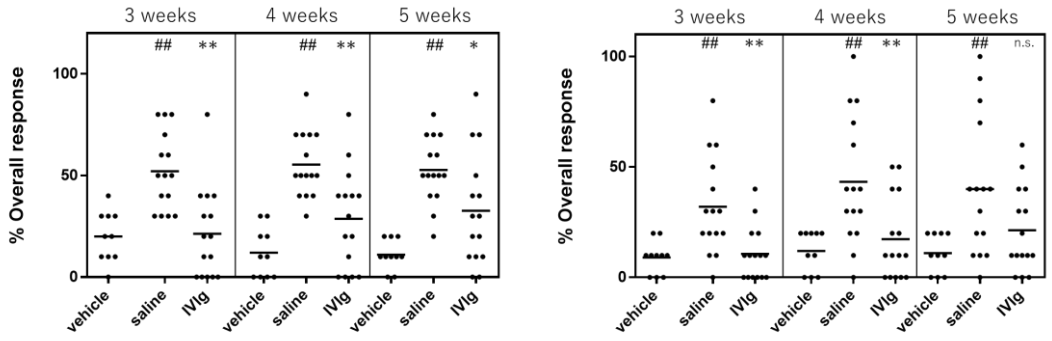


Figure 1. IVIg suppresses peripheral neurotoxicity induced by anticancer drugs in rat models

Representative results of the mechanical allodynia monitoring experiments to evaluate neuropathic signs after 3, 4, and 5 weeks of anticancer drug injection are shown. (**a**) Rats were intraperitoneally injected with paclitaxel (1 g/kg) to develop the p-CIPN model. (**b**) Rats were intravenously injected with doxorubicin (6 mg/kg) to develop the d-CIPN model; IVIg (1 g/kg) was intravenously administered on days 0, 7, 14, and 21 [data are presented as mean ± SEM {n = 10 (control group) or 15 (d-CIPN and p-CIPN groups), $^{\#\#}P < 0.01$ (vs. same time vehicle group by Student's *t*-test), $^{*}P < 0.05$ and $^{**}P < 0.01$ (vs. same time saline group by Student's *t*-test), n.s. = not significant]

Figure **2a** **b**

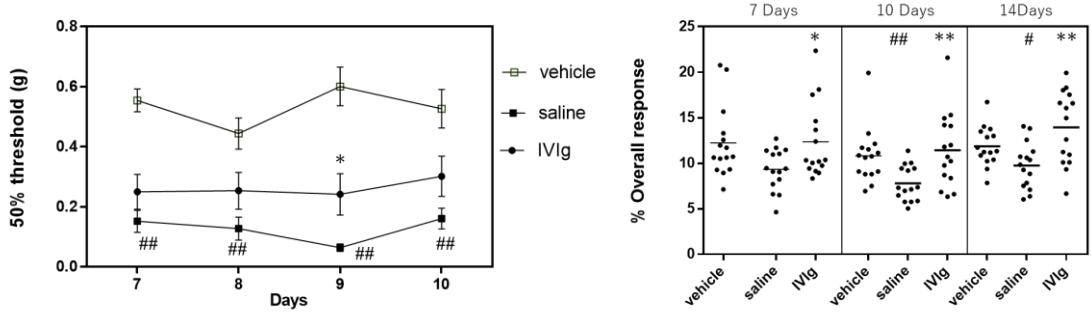


Figure 2. IVIg suppresses p-CIPN in mice

Representative results of the experiments to monitor the occurrence of neuropathic signs—mechanical allodynia (**a**) and heat hyperalgesia (**b**)—after 7, 10, and 14 d of anticancer drug injection are shown; IVIg (1 g/kg) was intravenously administered on days 0 and 5. Mice were intraperitoneally administered paclitaxel (3 mg/kg) to induce p-CIPN [data are presented as mean ± SEM (n = 15), $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ (vs. same time vehicle group by Student's *t*-test), *P < 0.05 and **P < 0.01 (vs. same time saline group by Student's *t*-test), n.s. = not significant. Results are representative of two or more independent experiments]

Role of macrophages in IVIg action to alleviate CIPN

To clarify the role of macrophages in the action of IVIg, we investigated the influence of macrophage depletion on the suppressive effects of IVIg in the rat p-CIPN model. Interestingly, IVIg failed to suppress p-CIPN in the rat model when macrophages were depleted (Fig. 3).

Figure 3

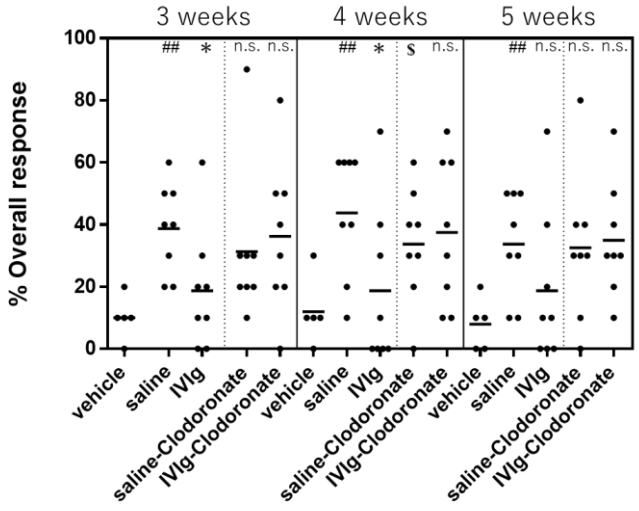


Figure 3. Suppressive effect of IVIg disappeared following macrophage depletion

Representative results of the experiments to monitor the occurrence of neuropathic signs of mechanical allodynia after 3, 4, and 5 weeks of anticancer drug injection are shown; IVIg (1 g/kg) was intraperitoneally administered on days 0, 7, 14, and 21; clodronate liposomes (5.6 mg/body) were intraperitoneally administered 2 d before the administration of IVIg. Rats were intraperitoneally injected with paclitaxel (1 g/kg) to induce CIPN [data are presented as mean ± SEM {n = 5 (control group) or 8 (non-control groups)}, $^{\#\#}P < 0.01$ (vs. same time vehicle group by Student's *t*-test), *P < 0.05 (vs. same time saline group by Student's *t*-test), $^{\$}P < 0.05$ (vs. same time saline vehicle by Student's *t*-test), n.s. = not significant. Results are representative of two or more independent experiments]

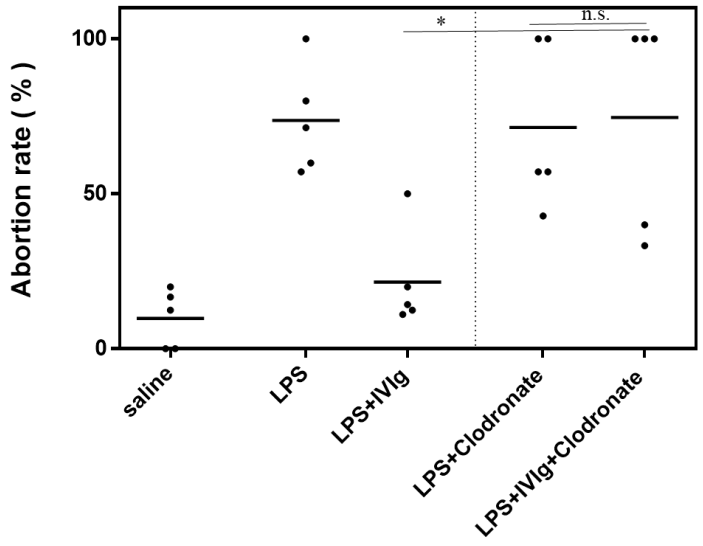
Discussion

CIPN is a serious adverse effect that leads to treatment discontinuation by patients receiving anticancer therapy. Currently there is no effective drug for CIPN. Here, we focused on whether IVIg exerts suppressive effects on CIPN. To the best of our knowledge, this study is the first to establish that IVIg exerts suppressive effects in a macrophage-dependent manner. We demonstrated that IVIg suppresses p-CIPN and d-CIPN via macrophage modulation in rats and mice. Although the mechanism of CIPN induction varies with the anticancer drug, our results indicate that IVIg could have a suppressive effect on peripheral neurotoxicity caused by these drugs regardless of drug type, such as platinum compounds, anti-tubulins, thalidomide, and bortezomib[1,2]. In other words, IVIg does not directly interfere with anticancer drugs and may act on a factor, which is common in the CIPN pathway.

Notably, our results showed that in the absence of macrophages, IVIg loses its CIPN-suppressive effect. Furthermore, IVIg can normalize immune system-related abnormalities. Since macrophages are key coordinators of the immune system, we speculate that the immunomodulatory effects of IVIg are mediated by macrophages. Although the mechanism underlying the development of p-CIPN after macrophage depletion remains unclear, it is possible that here, clodronate did not completely deplete the macrophages in the peripheral neurons. Moreover, the subset of macrophages essential for IVIg action may differ from the subset required for the onset of p-CIPN. However, further exploratory investigations are required in this regard. Nevertheless, based on our results, we can conclude that macrophages are essential for IVIg suppressive action.

Our study emphasizes a strong relationship between IVIg mechanism of action and macrophages. Interestingly, we have confirmed the same phenomenon in a RPL animal model, where IVIg is shown to suppress miscarriage[18]. Correspondingly, in the model, macrophage depletion eliminated the IVIg-suppressive effect (Supplementary Fig. 1). Moreover, in our previous mouse model study of chronic inflammatory demyelinating polyneuropathy, we found that IVIg can modulate macrophage levels[19]. IVIg exhibits immunostimulatory effects in infectious diseases and immunosuppressive effects in autoimmune diseases. These contradictory effects can be explained by understanding how IVIg modulates macrophages that are key coordinators of the immune system. IVIg may act by normalizing the immune system via macrophage modulation. Thus, our study provides new insights in elucidating the mechanisms underlying the contrasting roles of IVIg.

Supplementary Fig. 1



Supplementary Figure 1. Effect of macrophage depletion on IVIg-mediated suppression of recurrent abortion in pregnant mice

Pregnant CBA/J mice of each group were injected with LPS (0.8 μg/mouse) and IVIg (1000 mg/kg) on gestational day (gd) 7.5. Evaluation of abortion was performed on gd 13.5 by macroscopic examination. Clodronate was injected on gd 5.5 at 5.6 mg/body via intravenous injection [data are given as mean ± SEM (n = 4-5). Results are representative of two or more independent experiments. *$P < 0.05$, n.s. > 0.05 (two-tailed) vs. same time in the control group (Student's *t*-test)]

In view of its dual role, IVIg may have two potential effects on cancer therapy via anticancer drugs, i.e., suppression of CIPN and activation of an immunosuppression state. Thus, IVIg could be used in combination with anticancer drugs if the latter induce CIPN. IVIg administration allows for the continued use of anticancer drugs and is also expected to enhance immune function. As IVIg does not cause serious adverse effects and is a multifunctional drug, combination therapy is a rational strategy. Therefore, IVIg may prove beneficial owing to its dual role of suppressing CIPN and alleviating immune deficiency caused by anticancer drugs.

Here, we demonstrated two distinct and important possibilities of therapeutic application through IVIg suppression in CIPN. First, we found that IVIg acts on macrophages normalized from a weakened immune system due to chemotherapy. Thus, our study further validates that macrophages could be a new target for the development of novel multifunctional drugs[23]. Second, our results indicate that it is advantageous to utilize combination therapy with IVIg to continue treatment with anticancer drugs in order to reduce adverse effects. To date, all studies in this field have focused on the direct effects of drugs on cancer. The range of cancer therapy is expected to expand in the future if IVIg is indeed found useful in combination therapy with drugs that are effective in combating cancer.

We expect to examine this possibility (e.g., evaluation using a mouse cancer model) in the future to expedite therapeutic strategies for cancer patients.

Conclusion

Our findings will help to elucidate the action mechanism of IVIg and its future therapeutic applications. Moreover, to our knowledge, the current therapeutic guidelines for CIPN do not include IVIg therapy. In this study, we conducted experiments only on rodent models; hence, further studies on other animal models are necessary to verify the effectiveness of IVIg therapy in alleviating CIPN. We conclude that our study emphasizes this urgent need.

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Author contributions

MK conceived and designed the study and collated and analyzed the data. JT interpreted the data, directed the research, and wrote the manuscript draft.

References

1 Brown, M. R., Ramirez, J. D. & Farquhar-Smith, P. Pain in cancer survivors. *British journal of pain*. 2014; 8(4): 139-53. doi:10.1177/2049463714542605.

2 Flatters, S. J. L., Dougherty, P. M. & Colvin, L. A. Clinical and preclinical perspectives on Chemotherapy-Induced Peripheral Neuropathy (CIPN): a narrative review. *British journal of anaesthesia*. 2017; 119(4): 737-749. doi:10.1093/bja/aex229.

3 Shimozuma, K. *et al*. Taxane-induced peripheral neuropathy and health-related quality of life in postoperative breast cancer patients undergoing adjuvant chemotherapy: N-SAS BC 02, a randomized clinical trial. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2012; 20(12): 3355-64. doi:10.1007/s00520-012-1492-x.

4 Hershman, D. L. *et al*. Prevention and management of chemotherapy-induced peripheral neuropathy in survivors of adult cancers: American Society of Clinical Oncology clinical practice guideline. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2014; 32(18): 1941-67. doi:10.1200/jco.2013.54.0914.

5 Bruton, O. C. Agammaglobulinemia. *Pediatrics*. 1952; 9(6): 722-8.

6 Ballow, M. The IgG molecule as a biological immune response modifier: mechanisms of action of intravenous immune serum globulin in autoimmune and inflammatory disorders. *The Journal of allergy and clinical immunology*. 2011; 127(2): 315-23; quiz 324-5. doi:10.1016/j.jaci.2010.10.030.

7 Anthony, R. M., Wermeling, F. & Ravetch, J. V. Novel roles for the IgG Fc glycan. *Annals of the New York Academy of Sciences*. 2012; 1253: 170-80. doi:10.1111/j.1749-6632.2011.06305.x.

8 Yamada, H. *et al*. Intravenous immunoglobulin treatment in women with four or more recurrent pregnancy losses: A double-blind, randomised, placebo-controlled trial. *EClinicalMedicine*. 2022; 50: 101527. doi:10.1016/j.eclinm.2022.101527.

9 Ibanez, C. & Montoro-Ronsano, J. B. Intravenous immunoglobulin preparations and autoimmune disorders: mechanisms of action. *Current pharmaceutical biotechnology*. 2003; 4(4): 239-47.

10 Tanaka, J. *et al*. Intravenous immunoglobulin suppresses IL-10 production by activated B cells in vitro. *Open Journal of Immunology*. 2012; Vol.02No.04: 12. doi:10.4236/oji.2012.24019.

11 De Groot, A. S. *et al*. Activation of natural regulatory T cells by IgG Fc-derived peptide "Tregitopes". *Blood*. 2008; 112(8): 3303-11. doi:10.1182/blood-2008-02-138073.

12 Meregalli, C. *et al*. High-dose intravenous immunoglobulins reduce nerve macrophage infiltration and the severity of bortezomib-induced peripheral neurotoxicity in rats. *Journal of neuroinflammation*. 2018; 15(1): 232. doi:10.1186/s12974-018-1270-x.

13 Meregalli, C. *et al*. Human Intravenous

Immunoglobulin Alleviates Neuropathic Symptoms in a Rat Model of Paclitaxel-Induced Peripheral Neurotoxicity. *International journal of molecular sciences*. 2021; 22(3. doi:10.3390/ijms22031058.

14 Nakae, T., Hirayama, F. & Hashimoto, M. [Neutralizing activity of human immunoglobulin preparation against toxic shock syndrome toxin-1]. *Kansenshogaku zasshi. The Journal of the Japanese Association for Infectious Diseases*. 2002; 76(3): 195-202.

15 Nakae, T., Tanaka, J., Nakano, A. & Ono, Y. Complement-mediated bactericidal effect of antibodies in human intravenous preparation against multi-drug resistant pseudomonas aeruginosa. *The Japanese journal of antibiotics*. 2008; 61(6): 379-87.

16 Tanaka, J. *et al*. Effective concentration of intravenous immunoglobulin for neutralizing Panton-Valentine leukocidin in human blood. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy*. 2018. doi:10.1016/j.jiac.2017.12.023.

17 Tanaka, J. *et al*. Complement-mediated bacteriolysis after binding of specific antibodies to drug-resistant Pseudomonas aeruginosa: morphological changes observed by using a field emission scanning electron microscope. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy*. 2010; 16(6): 383-7. doi:10.1007/s10156-010-0074-4.

18 Tanaka, J. *et al*. Intravenous Immunoglobulin Suppresses Abortion Relates to an Increase in the CD44bright NK Subset in Recurrent Pregnancy Loss Model Mice. *Biology of reproduction*. 2016; 95(2): 37. doi:10.1095/biolreprod.116.138438.

19 Kajii, M. *et al*. Intravenous immunoglobulin preparation attenuates neurological signs in rat experimental autoimmune neuritis with the suppression of macrophage inflammatory protein -1alpha expression. *Journal of neuroimmunology*. 2014; 266(1-2): 43-8. doi:10.1016/j.jneuroim.2013.10.011.

20 Kajii, M. *et al*. Prevention of excessive collagen accumulation by human intravenous immunoglobulin treatment in a murine model of bleomycin-induced scleroderma. *Clinical and experimental immunology*. 2011; 163(2): 235-41. doi:10.1111/j.1365-2249.2010.04295.x.

21 Flatters, S. J. & Bennett, G. J. Ethosuximide reverses paclitaxel- and vincristine-induced painful peripheral neuropathy. *Pain*. 2004; 109(1-2): 150-61. doi:10.1016/j.pain.2004.01.029.

22 Nieto, F. R. *et al*. Tetrodotoxin inhibits the development and expression of neuropathic pain induced by paclitaxel in mice. *Pain*. 2008; 137(3): 520-31. doi:10.1016/j.pain.2007.10.012.

23 Cassetta, L. & Pollard, J. W. Targeting macrophages: therapeutic approaches in cancer. *Nature reviews. Drug discovery*. 2018; 17(12): 887-904. doi:10.1038/nrd.2018.169.