Biochemical and Biophysical Research Communications 464 (2015) 94–99

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Intravenous immunoglobulin enhances the killing activity and autophagy of neutrophils isolated from immunocompromised patients against multidrug-resistant bacteria

Hidemasa Matsuo [a, b], Hiroshi Itoh [a], Naoko Kitamura [a], Yasuhiko Kamikubo [a], Takeshi Higuchi [b], Shuichi Shiga [b], Satoshi Ichiyama [c], Tadakazu Kondo [d], Akifumi Takaori-Kondo [d], Souichi Adachi [a, *]

[a] Department of Human Health Sciences, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan
[b] Department of Clinical Laboratory, Kyoto University Hospital, Kyoto 606-8507, Japan
[c] Department of Infection Control and Prevention, Kyoto University Hospital, Kyoto 606-8507, Japan
[d] Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan

ARTICLE INFO

Article history:
Received 11 May 2015
Accepted 2 June 2015
Available online 25 June 2015

Keywords:
Intravenous immunoglobulin
Neutrophil
Autophagy
Multidrug-resistant bacteria
Immunocompromised host

ABSTRACT

Intravenous immunoglobulin (IVIG) is periodically administered to immunocompromised patients together with antimicrobial agents. The evidence that supports the effectiveness of IVIG is mostly based on data from randomized clinical trials; the underlying mechanisms are poorly understood. A recent study revealed that killing of multidrug-resistant bacteria and drug-sensitive strains by neutrophils isolated from healthy donors is enhanced by an IVIG preparation. However, the effectiveness of IVIG in immunocompromised patients remains unclear. The present study found that IVIG increased both killing activity and O_2^- release by neutrophils isolated from six patients receiving immune-suppressive drugs after hematopoietic stem cell transplantation (HSCT); these neutrophils killed both multidrug-resistant extended-spectrum β-lactamase-producing *Escherichia coli* (*E. coli*) and multidrug-resistant *Pseudomonas aeruginosa* (*P. aeruginosa*). Moreover, IVIG increased the autophagy of the neutrophils, which is known to play an important role in innate immunity. These results suggest that IVIG promotes both the killing activity and autophagy of neutrophils isolated from immunocompromised patients against multidrug-resistant bacteria.

1. Introduction

Antibiotic-resistant strains of pathogenic bacteria are increasingly prevalent in hospitals where they can cause life-threating infections in vulnerable patients, particularly those that are immunocompromised. Although new antibacterial drugs have been developed, progress is slow and bacterial resistance is increasing [1]. Intravenous immunoglobulin (IVIG) preparations have been developed as an alternative approach to treating severe infectious diseases and are often used in combination with antibiotics. IVIG is a blood product prepared from the serum of between 1000 and 15 000 donors [2,3]. Although several studies report the clinical effectiveness of IVIG [4–7], most of the evidence is based on data from randomized controlled trials. Thus, the mechanisms underlying the effectiveness of IVIG against pathogenic bacteria need to be examined to develop optimum IVIG-based therapeutic strategies.

Neutrophils are the most abundant class of leukocyte and play a central role in the primary mechanisms underpinning host defense. Neutrophils bind to IgG-coated pathogenic bacteria in the presence of complement components and kill these bacteria via phagocytosis

Abbreviations: IVIG, intravenous immunoglobulin; HSCT, hematopoietic stem cell transplantation; ESBL, extended-spectrum β-lactamase; *E. coli*, *Escherichia coli*; *P. aeruginosa*, *Pseudomonas aeruginosa*; SOD, superoxide dismutase; ROS, reactive oxygen species; LC3B, microtubule-associated protein 1 light chain 3B; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FITC, fluorescein-5-isothiocyanate; DAPI, 4′,6-diamidino-2-phenylindole; Cy3, cyanine dye 3; TEM, transmission electron microscopy.

* Corresponding author. Department of Human Health Sciences, Graduate School of Medicine, Kyoto University, 53 Kawahara-cho, Syogoin, Sakyoku, Kyoto 606-8507, Japan.
E-mail address: adachiso@kuhp.kyoto-u.ac.jp (S. Adachi).

http://dx.doi.org/10.1016/j.bbrc.2015.06.004
0006-291X/

[8]. In a recent study, we showed that IVIG enhanced both the killing activity and autophagy of neutrophils isolated from healthy donors against multidrug-resistant bacteria as well as drug-sensitive bacteria [9]. Autophagy was originally described as an essential homeostatic process that degrades damaged or unnecessary cellular components [10]; however, recent studies reveal that autophagy also functions to degrade intracellular pathogens and contributes to host defense against infectious diseases [11,12]. We found that the IVIG-mediated increase in the autophagy of neutrophils plays a role in their bactericidal function [9]; however, the effectiveness of IVIG in immunocompromised patients remains unclear.

Here, neutrophils were isolated from the peripheral blood of six immunocompromised patients receiving immunosuppressive drugs after hematopoietic stem cell transplantation (HSCT). We first examined the effect of IVIG on the killing activity of neutrophils in the presence of multidrug-resistant extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) and multidrug-resistant *Pseudomonas aeruginosa* (*P. aeruginosa*) (both of which cause severe nosocomial infections). We then examined the effect of IVIG on O_2^- release and autophagy of neutrophils. The results shed light on the mechanisms underlying the clinical effects of IVIG in immunocompromised patients.

2. Materials and methods

2.1. Reagents

Dulbecco's PBS (−), Trypticase soy broth, and Heart Infusion Agar were purchased from Nissui Pharmaceutical Co., Ltd (Tokyo, Japan). Hanks' balanced salt solution (HBSS) was purchased from Gibco® Invitrogen™ (CA, USA). Paraformaldehyde, glutaraldehyde, OsO_4, and RIPA buffer containing a protease inhibitor cocktail were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Cytochrome c was purchased from Sigma–Aldrich Corporation (St. Louis, USA). Superoxide dismutase (SOD) was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Kenketsu Venilon®-I (Teijin Pharma Ltd, Tokyo, Japan) dialysis with PBS as IVIG preparation was supplied by KAKETSUKEN (Kumamoto, Japan).

2.2. Preparation of neutrophils

Approximately 20 ml of peripheral blood was obtained from patients receiving immunosuppressive drugs after HSCT. Neutrophils were isolated by sedimentation through two-step Percoll (GE Healthcare Japan, Tokyo, Japan) gradients. The study was conducted in accordance with the principles set down in the Declaration of Helsinki and was approved by the Ethics Committee of Kyoto University. All patients provided written informed consent.

2.3. Bacteria strains

ESBL-producing *E. coli* and multidrug-resistant *P. aeruginosa* were isolated from patients at Kyoto University Hospital. Bacterial identification and antibiotic susceptibility tests were performed according to the methods recommended by the Clinical and Laboratory Standards Institute (CLSI). Each strain was cultured in Heart Infusion Broth and preserved at −80 °C in stock medium. The strains were used after overnight incubation in Trypticase soy broth. Cells were washed three times in PBS and suspended in HBSS. The concentration of bacteria was adjusted by measuring the absorbance at 600 nm.

2.4. Preparation of IgG-free serum

Human AB-type serum was used as a source of complement in the experiments designed to analyze neutrophil function. Because intact serum contains abundant IgG, this class of antibody was removed from serum by passage through protein G affinity columns so that the effect of IVIG could be assessed without interference. Removal of IgG from the serum was confirmed using a turbidimetric immunoassay (IgG levels were below the detection limit). We also confirmed that, although the levels of IgA (1.24–0.74 mg/ml), IgM (0.96–0.39 mg/ml), IgE (44.2–21.2 IU/ml), complement components C3 (0.89–0.54 mg/ml), C4 (0.24–0.15 mg/ml), and hemolytic complement activity (CH50) were reduced to approximately half that in untreated serum, the remaining levels were sufficient for the test assays.

2.5. Bactericidal assay

A mixture (volume, 0.5 ml) containing 1% IgG-free serum, bacteria (2.5×10^6 cfu/ml), and 1 mg/ml of IVIG preparation was incubated with or without human neutrophils (2.5×10^6 cells/ml) for 60 or 120 min at 37 °C with gentle shaking. The reaction mixture was then diluted in an excess amount of sterile distilled water. Appropriate amounts of diluted sample were poured onto Heart Infusion Agar plates and cultured for 24–48 h. The number of colonies was then counted.

2.6. O_2^- release assay

A reaction mixture (volume, 0.5 ml) containing cytochrome c (80 μM), neutrophils (2.5×10^6 cells/ml), bacteria (2.5×10^7 cfu/ml), and 1% IgG-free serum was incubated with or without 1 mg/ml of IVIG for 30 min at 37 °C. The supernatant was collected after centrifugation at 1000× *g* for 10 min at 4 °C. The amount of O_2^- released by neutrophils was calculated by subtracting the absorbance at 550 nm in the presence of SOD (150 units/ml) from that in the absence of SOD. The results are expressed as nanomoles of superoxide/1.25×10^6 cells (calculated using an absorbance coefficient of $2.1 \times 10^4\ M^{-1}\ cm^{-1}$). The data were obtained from average value of more than duplicate per one assay.

2.7. Immunoblot analysis

Approximately 5×10^6 treated neutrophils were washed with PBS and lysed with RIPA buffer containing a protease inhibitor cocktail. After centrifugation, the protein content of the supernatants was measured in a DC Protein Assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Equal amounts of whole cell lysate were separated on 15% SDS polyacrylamide gels and transferred to PVDF membranes (GE Healthcare Japan, Tokyo, Japan). The membranes were blocked for 1 h in 0.3% skim milk/TBS-T, followed by an overnight incubation with an anti-LC3B pAb (1/500; L7543 Sigma–Aldrich, St. Louis, MO, USA) or an anti-GAPDH mAb (1/200; SC47724: Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) at 4 °C. After a thorough wash in TBS-T, the membranes were incubated with a HRP-conjugated anti-rabbit Ab (NIF824; GE Healthcare Japan, Tokyo, Japan) or an anti-mouse Ab (NIF825; 1/5000; GE Healthcare Japan, Tokyo, Japan) for 1 h at room temperature. Immunoreactive proteins were detected using the Novex ECL HRP Chemiluminescent Substrate Regent Kit (Invitrogen, CA, USA) and the signals were captured. The intensity of the bands was quantified using the Chemi Doc XRS System (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Table 1
Patient characteristics.

Patient no.	Age (y)	Sex	Diagnosis	Stem cell source	Days after transplantation	Immunosuppressive agents	CRP (mg/dl)	WBC (/μl)	Differential WBC count	IgG (mg/dl)	Administration of IVIG
1	43	M	Ph1-ALL	UCB	336	Tacrolimus 0.8 mg Prednisolone 5 mg	0.1	6100	Neutrophil 46% Lymphocyte 32% Monocyte 8% Eosinophil 14%	463	+
2	32	M	PTCL-NOS	UBM	137	Tacrolimus 0.4 mg Prednisolone 5 mg	0.1	4400	Neutrophil 63% Lymphocyte 23% Monocyte 9% Eosinophil 5%	623	+
3	20	M	AML	UCB	148	Tacrolimus 3.5 mg Prednisolone 15 mg	0	7500	Neutrophil 93% Lymphocyte 6% Monocyte 1%	472	+
4	63	M	Follicular Lymphoma	UBM	442	Tacrolimus 0.5 mg Prednisolone 5 mg	0	5300	Neutrophil 75% Lymphocyte 12% Monocyte 9% Eosinophil 3% Basophil 1%	586	+
5	28	F	Therapy-related MDS	UBM	160	Tacrolimus 3 mg Prednisolone 15 mg	0.4	5900	Neutrophil 69% Lymphocyte 18% Monocyte 9% Eosinophil 3% Myelocyte 1%	507	–
6	64	M	MDS	UCB	149	Tacrolimus 1.4 mg Prednisolone 20 mg	0.1	5400	Neutrophil 81% Lymphocyte 14% Monocyte 5%	392	–

CRP, C-reactive protein; WBC, white blood cells; Ph1-ALL, Philadelphia chromosome-positive acute lymphoblastic leukemia; PTCL-NOS, peripheral T-cell lymphoma not otherwise specified; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; UCB, unrelated cord blood; UBM, unrelated bone marrow.

2.8. Confocal laser microscopy

To observe the formation of LC3B aggregates, treated neutrophils were attached to glass slides using Cytofuge2 (StatSpin Technologies, Norwood, Massachusetts, USA) and then stained for LC3B. Briefly, cells were washed and fixed with 4% paraformaldehyde, blocked in 10% serum, incubated with an anti-LC3B mAb (1/50; SAB4200361; Sigma–Aldrich), and detected by a Cy3-conjugated sheep anti-mouse LC3B Ab (1/20; C2181; Sigma–Aldrich) secondary antibody. *E. coli* were detected using a FITC-conjugated anti-*E. coli* pAb (1/20; PA1-73029; Thermo Fisher Scientific K.K., Yokohama, Kanagawa, Japan). The slides were then washed and mounted with Prolong-Gold antifade reagent (P36935; Life Technologies) and analyzed under a confocal laser scanning microscope (Fluoview FV10i, Olympus, Tokyo, Japan). Images were processed using FV10-ASW viewer (Olympus, Version 03.00.03.00).

2.9. Transmission electron microscopy

Briefly, 2.5×10^6 treated neutrophils were pelleted by centrifugation, fixed in 4% paraformaldehyde/2% glutaraldehyde/0.1 M phosphate buffer at 4 °C, washed in isotonic phosphate-buffered sucrose, re-fixed in phosphate-buffered 1% OsO4, dehydrated through a graded series of ethanol solutions, and embedded in Luveak 812 (Nacalai Tesque). Thin sections (70–90 nm thick) were cut with a diamond knife on an EM UC6 ultramicrotome (Leica, Heidelberg, Germany), stained with uranyl acetate and lead citrate, and observed using a Hitachi H-7650 electron microscope (Hitachi, Tokyo, Japan). The number of autophagosomes with bacteria within each cell was counted (50 per each sample).

2.10. Statistical analysis

Data were analyzed using the Student's paired t-test and expressed as the mean ± SE. All results were representative of at least three independent experiments. Statistical significance was defined as follows: $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***).

3. Results and discussion

3.1. Patient characteristics

The clinical features of the six patients are summarized in Table 1. The mean age was 42 years (range, 20–64 years). All

Table 2
Antibiotic susceptibility of the bacterial strains.

	Source	Drug sensitivity															
E. coli	Urine	ABPC	PIPC	TAZ/PIPC	CPDX	CEZ	CMZ	FMOX	CAZ	CTX	CPZ	CFPM	AZT	IPM	LVFX	AMK	GM
		R	R	S	R	R	S	S	R	R	R	R	R	S	S	S	S
P. aeruginosa	Blood	PIPC	TAZ/PIPC	CTX	CPZ	CAZ	CZOP	CFPM	AZT	IPM	MEPM	MINO	AMK	GM	TOB	CPFX	ST
		S	S	R	S	S	S	S	I	R	I	R	R	I	R	R	R

S, Susceptible; I, Intermediate; R, Resistant; ABPC, ampicillin; PIPC, piperacillin; TAZ/PIPC, tazobactam/piperacillin; CPDX, cefpodoxime proxetil; CEZ, cefazolin; CMZ, cefmetazole; FMOX, flomoxef; CAZ, ceftazidime; CTX, cefotaxime; CPZ, cefoperazone; CFPM, cefepime; AZT, aztreonam; IPM, imipenem; LVFX, levofloxacin; AMK, amikacin; GM, gentamicin; CZOP, cefozopran; MEPM, meropenem; MINO, minocycline; TOB, tobramycin; CPFX, ciprofloxacin; ST, sulfamethoxazole-trimethoprim.

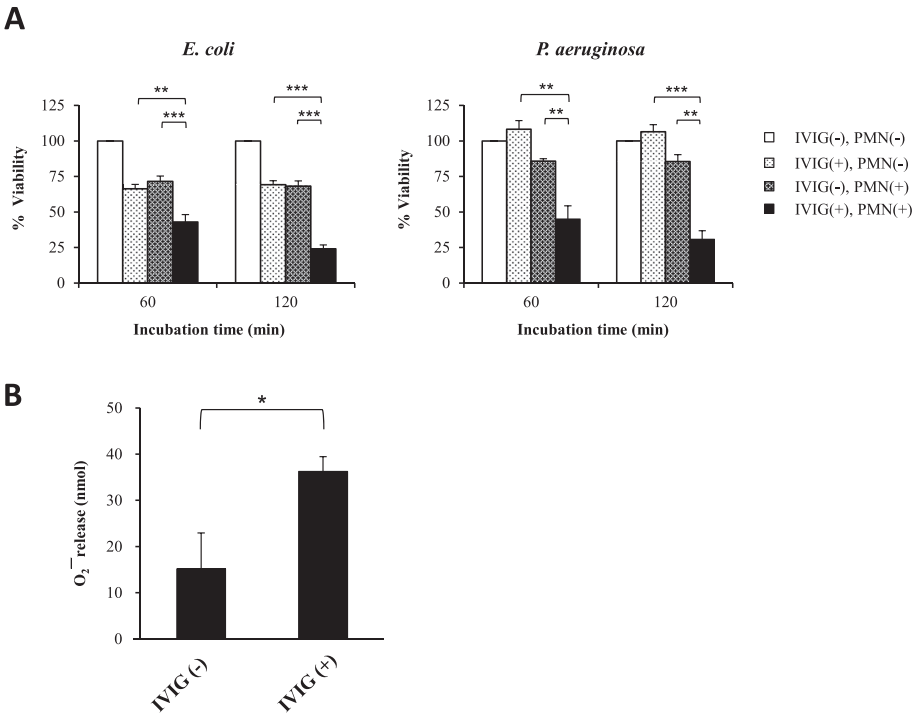

Fig. 1. Bactericidal activity and O_2^- release by neutrophils exposed to multidrug-resistant bacteria. (A) The effect of IVIG on neutrophil-mediated bactericidal activity against multidrug-resistant *E. coli* and *P. aeruginosa* (n = 6). The number of viable bacteria remaining after exposure to 1% IgG-free serum (in the presence or absence of human neutrophils) plus 1 mg/ml of IVIG for the indicated times. Data are expressed as a percentage of the control value (no neutrophils or no IVIG). (B) The effect of IVIG on O_2^- release by human neutrophils exposed to multidrug-resistant *E. coli* in the presence of 1% IgG-free serum in the presence or absence of 1 mg/ml of IVIG (n = 4). Data represent the mean ± SE. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

patients received an unrelated cord blood or unrelated bone marrow transplant to treat a hematological disorder (Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph1-ALL); peripheral T-cell lymphoma not otherwise specified (PTCL-NOS); *de novo* acute myeloid leukemia (AML); therapy-related myelodysplastic syndrome (MDS); and *de novo* MDS). The median period from HSCT was 229 days. All patients received immunosuppressive drugs (tacrolimus (FK506) and prednisolone (PSL)). The C-reactive protein (CRP) levels and white blood cell (WBC) counts were normal; however, the serum IgG concentration was low (mean: 507 mg/dl, range: 392–623 mg/dl). Four patients had received IVIG when the specimens were collected for this study.

3.2. IVIG promotes the neutrophil-mediated killing of multidrug-resistant bacteria

First, to examine the effect of IVIG on the neutrophil-mediated killing of multidrug-resistant bacteria, neutrophils were incubated with multidrug-resistant *E. coli* and *P. aeruginosa* in the presence of IgG-free serum (as a source of complement). The results of the antibiotic susceptibility tests are summarized in Table 2. In the presence of neutrophils, IVIG significantly reduced the viability of bacteria in a time-dependent manner (Fig. 1A). Neutrophils from each patient yielded similar data (Supplementary Fig. 1). Thus, due to the limited number of neutrophils isolated from each patient, all other experiments were performed using multidrug-resistant *E. coli*. Next, we measured O_2^- release to examine the mechanism underlying efficient neutrophil-mediated killing in the presence of IVIG. O_2^- is a reactive oxygen species (ROS) essential for the bactericidal activity of neutrophils [13]. We found that IVIG treatment led to a significant increase in O_2^- release (Fig. 1B). Data from each individual patient are shown in Supplementary Fig. 2. Taken together, the results suggest that IVIG promotes the neutrophil-mediated killing of multidrug-resistant bacteria by inducing the release of O_2^-.

3.3. IVIG promotes autophagy by neutrophils

Next, we examined whether IVIG affected the autophagy of neutrophils. Some reports suggest that ROS are required to activate antibacterial autophagic mechanisms [14,15], and others suggest that the autophagy of neutrophils is induced by phagocytosis followed by ROS production [16]. We previously showed that autophagy plays a role in the bactericidal activity of neutrophils derived from healthy volunteers [9]. Thus, we next examined the effect of IVIG on the autophagy of neutrophils isolated from immunocompromised patients.

The conversion of LC3B-I to LC3B-II, a well-established marker of autophagy [17], was analyzed by immunoblotting. When neutrophils were stimulated with multidrug-resistant *E. coli* in the presence of IgG-free serum, increased conversion of LC3B-I to LC3B-II was observed in IVIG-treated neutrophils, suggesting that IVIG promotes autophagy (Fig. 2A). Next, we used confocal microscopy to examine LC3B aggregate formation within neutrophils. Consistent with the immunoblotting results, IVIG treatment led to an increase in LC3B aggregation (Fig. 2B). Colocalization of aggregated LC3B with FITC-conjugated *E. coli* was frequently observed, suggesting that autophagy may play a role in the antibacterial function of neutrophils. Autophagy is best characterized by autophagosome (double membrane) formation within the cell cytoplasm [18]. Transmission electron microscopy (TEM) analysis showed that the number of autophagosomes with bacteria in the cytoplasm of IVIG-treated neutrophils was significantly greater than that in non-treated cells (Fig. 2C). This suggests that the autophagy of neutrophils (even those from immunocompromised patients) against multidrug-resistant *E. coli* is enhanced by IVIG treatment.

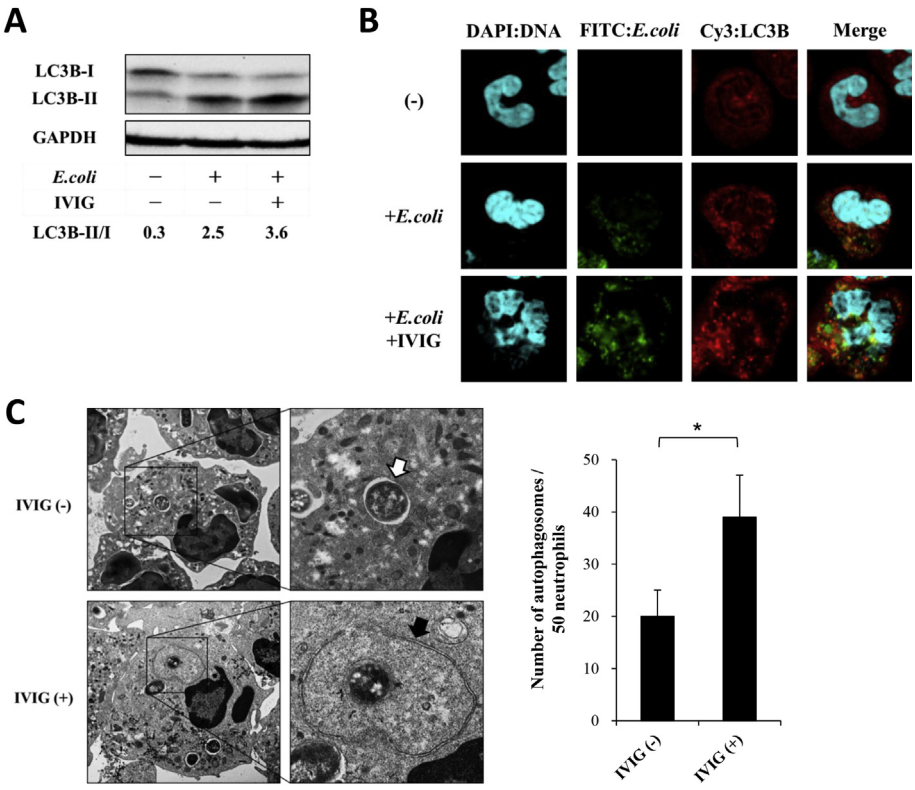

Fig. 2. Effect of IVIG on the autophagy of neutrophils. (A) Immunoblot analysis. Neutrophils were incubated for 90 min in 1% IgG-free serum in the presence or absence of multidrug-resistant *E. coli* and IVIG. The samples were then subjected to immunoblot analysis to examine the expression of LC3B (18 kDa and 16 kDa bands), which is a marker of autophagy. GAPDH (37 kDa) was used as the control. (B) Immunohistochemical analysis. The formation of LC3B aggregates was examined under a confocal laser scanning microscope. Neutrophils were incubated for 90 min with (+*E. coli*) or without (none) bacteria and IVIG in the presence of 1% IgG-free serum. The cells were then examined after indirect immunostaining with fluorescently-labeled antibodies. The cell nuclei are blue (DAPI), *E. coli* are green (FITC), and LC3B is red (Cy3). Data are representative of three independent experiments (magnification, ×600). (C) Transmission electron microscopy (TEM) analysis. Neutrophils were incubated for 90 min with multidrug-resistant *E. coli* either without or with IVIG in the presence of 1% IgG-free serum. The samples were then examined by TEM. Representative magnified images are shown on the right. The white arrow in the image of the IVIG (−) sample indicates bacteria contained within a phagosome (single membrane). The black arrow in the image of the IVIG (+) sample indicates bacteria contained within an autophagosome (double membrane). The number of autophagosomes with bacteria was counted in 50 neutrophils (n = 3). Data are expressed as the mean ± SE. $^{*}P < 0.05$.

In the presence of IVIG, neutrophils from immunocompromised hosts showed increased bacterial killing, increased O_2^- release, and increased autophagy. These results provide evidence that IVIG is effective against multidrug-resistant bacteria in immunocompromised patients. Together with the results of our recent study [9], which examined neutrophils isolated from healthy volunteers, our findings suggest that phagocytosis-mediated autophagy (dependent on the generation of O_2^-) is involved in the IVIG-mediated enhancement of neutrophil-mediated bacterial killing. Since more and more pathogens are acquiring resistance to antibiotics, outbreaks of nosocomial infection are extremely hazardous, particularly to immunocompromised patients. Thus, IVIG is an alternative therapeutic option for the treatment of nosocomial infections in such patients. The *in vivo* efficacy of IVIG should be examined in detail in future studies.

Acknowledgments

This work was supported by Grants-in-Aid for Challenging Exploratory Research to S. A. (Research Project Number: 26670277) and H. I. (Research Project Number: 26670484) from the JSPS. The authors thank Keiko Furuta and Haruyasu Kohda (Division of Electron Microscopic Study, Center for Anatomical Studies, Graduate School of Medicine, Kyoto University) for technical assistance. We also thank Asami Shindome-Matsumura and Sachio Okuda (KAKETSUKEN) for preparing and analyzing the IgG-free serum.

Conflicts of interest

The authors have no conflicts of interest to declare.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.004.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.06.004.

References

[1] M.N. Alekshun, S.B. Levy, Molecular mechanisms of antibacterial multidrug resistance, Cell 128 (2007) 1037–1050.
[2] S. Jolles, W.A. Sewell, S.A. Misbah, Clinical uses of intravenous immunoglobulin, Clin. Exp. Immunol. 142 (2005) 1–11.
[3] G. Ferrara, A. Zumla, M. Maeurer, Intravenous immunoglobulin (IVIg) for refractory and difficult-to-treat infections, Am. J. Med. 125 (2012) e1–8.
[4] I. Schedel, U. Dreikhausen, B. Nentwig, et al., Treatment of gram-negative septic shock with an immunoglobulin preparation: a prospective, randomized clinical trial, Crit. Care Med. 19 (1991) 1104–1113.
[5] K. Werdan, G. Pilz, Supplemental immune globulins in sepsis: a critical appraisal, Clin. Exp. Immunol. 104 (1996) 83–90.

[6] T. Masaoka, H. Hasegawa, F. Takaku, et al., The efficacy of intravenous immunoglobulin in combination therapy with antibiotics for severe infections, Jpn. J. Chemother 48 (2000) 199–217.
[7] K.B. Laupland, A.W. Kirkpatrick, A. Delaney, Polyclonal intravenous immunoglobulin for the treatment of severe sepsis and septic shock in critically ill adults: a systematic review and meta-analysis, Crit. Care Med. 35 (2007) 2686–2692.
[8] D.J. Scribner, D. Fahrney, Neutrophil receptors for IgG and complement: their roles in the attachment and ingestion phases of phagocytosis, J. Immunol. 116 (1976) 892–897.
[9] H. Itoh, H. Matsuo, N. Kitamura, et al., Enhancement of neutrophil autophagy by an IVIG preparation against multidrug-resistant bacteria as well as drug-sensitive strains, J. Leukoc. Biol. (2015) (Epub ahead of print).
[10] D.J. Klionsky, S.D. Emr, Autophagy as a regulated pathway of cellular degradation, Science 290 (2000) 1717–1721.
[11] B. Levine, N. Mizushima, H.W. Virgin, Autophagy in immunity and inflammation, Nature 469 (2011) 323–335.
[12] J. Huang, J.H. Brumell, Bacteria-autophagy interplay: a battle for survival, Nat. Rev. Microbiol. 12 (2014) 101–114.
[13] J. El-Benna, P.M. Dang, M.A. Gougerot-Pocidalo, Priming of the neutrophil NADPH oxidase activation: role of p47phox phosphorylation and NOX2 mobilization to the plasma membrane, Semin. Immunopathol. 30 (2008) 279–289.
[14] J. Huang, V. Canadien, G.Y. Lam, et al., Activation of antibacterial autophagy by NADPH oxidases, Proc. Natl. Acad. Sci. U S A 106 (2009) 6226–6231.
[15] R. Scherz-Shouval, Z. Elazar, Regulation of autophagy by ROS: physiology and pathology, Trends Biochem. Sci. 36 (2011) 30–38.
[16] I. Mitroulis, I. Kourtzelis, K. Kambas, et al., Regulation of the autophagic machinery in human neutrophils, Eur. J. Immunol. 40 (2010) 1461–1472.
[17] N. Mizushima, T. Yoshimori, How to interpret LC3 immunoblotting, Autophagy 3 (2007) 542–545.
[18] S.R. Carlsson, A. Simonsen, Membrane dynamics in autophagosome biogenesis, J. Cell. Sci. 128 (2015) 193–205.