

Intravenous Immunoglobulins (IVIG) Enhance *In vitro* Activation of Neutrophils in C57BL/6 but not in CBA Mice

Liliya Yu Basyreva¹, Ilya B Brodsky¹, Alexandr A Gusev¹, Olga N Zhapparova¹, Elena V Mikhachik¹, Sergey A Gusev^{1*}, Dmitriy G Matishov², Miri Blank³ and Yehuda Shoenfeld^{3*}

¹Scientific Research Institute of Physical-Chemical Medicine, Russia

²Institute of Arid Zones Southern Scientific Center of Russian Academy of Sciences

³Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Aviv University, Laura Schwarz-Kipp Chair for Research of Autoimmune Diseases

Abstract

Background: Intravenous immunoglobulins (IVIG) are used in the treatment of inflammatory, autoimmune conditions and cancer. However, the precise mechanism of their action and the mechanisms that underlie responsiveness and resistance of some patients to the IVIG therapy remain unknown. The anti-cancer effect of IVIG might be mediated by neutrophils, which regulate tumor development. We studied *in vitro* effects of IVIG on neutrophils from two immunologically contrasting mouse strains, CBA and C57BL/6.

Results: We examined total and differential WBC count, platelets count and the activity of normal neutrophils in intact CBA and C57BL/6 mice by method of luminol-dependent chemiluminescence (CL) of whole blood, and found significant difference in neutrophils, lymphocytes and platelets counts. Upon *in vitro* activation with opsonized zymosan (OZ), the C57BL/6 blood neutrophils showed no CL response at the absence of extracellular calcium while in the presence of calcium the CL responses of CBA and C57b blood were very similar. In the presence of IVIG, the calcium-dependent raise in OZ-activated CL response was registered only in C57BL/6 blood samples.

Conclusions: For the first time we demonstrate that IVIG cause the calcium-dependent neutrophils priming *in vitro* in whole blood of C57BL/6 but not in CBA mice.

Keywords: IVIG; Neutrophils; Chemiluminescence; Zymosan; Calcium

Abbreviations: IVIG: Intravenous Immunoglobulin; OZ: Opsonized Zymosan; PMA: Phorbol 12-Myristate 13-acetate; CL: Chemiluminescence; ROS: Reactive Oxygen Species; RBC: Red Blood Cells; WBC: White Blood Cells

Introduction

Intravenous immunoglobulins (IVIG) obtained from thousands of donors are widely used as replacement of immunoglobulins in immunodeficiency and in inflammatory, autoimmune conditions and cancer [1-4].

The mechanisms underlying the therapeutic effect of IVIG, development of IVIG-resistance or adverse effects associated with IVIG treatment are poorly understood [5-7].

Neutrophils are considered as important effectors of the innate immunity. Various proteins, including interleukins, chemokines, and growth factors, both locally at the inflammation site and systematically in the circulation, modulate their activity.

The available data concerning the effects of IVIG on circulating neutrophils indicate that these cells might mediate the therapeutic response and the adverse effects of IVIG [8-10].

The IVIG-induced alteration of neutrophil's activity can be estimated *in vitro* by measurement of reactive oxygen species produced by activated neutrophils [9,10], by the expression of reference proteins [9], and by the level of neutrophil's degranulation [8]. Direct interaction of neutrophils with IVIG can result in their activation through Fc receptors (FcR) [11]. Nagahata et al. demonstrated the possibility of cross-interaction with Fc- and complement-receptors [12]. Another direct mechanism of neutrophil's activation is induced by binding of

Fab fragments to anti-neutrophil antibodies [13]. At the same time, IgG can competitively inhibit the interaction of FcR with other ligands [14].

Generation of reactive oxygen species (ROS) results from the activation of NADPH-oxidase. In neutrophils, NADPH-oxidase is composed of multiple membrane-bound and soluble subunits. The enzyme complex can be assembled either at the plasma membrane (induced by soluble stimuli) or at the phagosome membrane after engulfment [15-17]. The rate and duration of ROS generation by neutrophils depend on stimuli and signaling pathway. Thus, soluble phorbol myristate acetate (PMA), a structural analogue of diacylglycerol, binds directly to protein kinase C and induces phosphorylation of NADPH-oxidase components. Particulate stimuli (zymosan, bacteria) can activate neutrophils through specific receptors, and in this case, signaling pathways depend on intra- and extracellular calcium [18].

The variability of individual response to IVIG might be associated with the peculiarities of neutrophil's activation. Individual variations

***Corresponding authors:** Sergey A Gusev, Scientific Research Institute of Physical-Chemical Medicine, Russia, Tel: +7 (499) 246 4293, fax: +7 (499) 246 4293; E-mail: ser_gus@mail.ru

Yehuda Shoenfeld, Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Aviv University, Sackler Faculty of Medicine, Tel-Aviv University, Israel, E-mail: Yehuda.Shoenfeld@sheba.health.gov.il

Received September 03, 2013; **Accepted** October 21, 2013; **Published** October 25, 2013

Citation: Basyreva LY, Brodsky IB, Gusev AA, Zhapparova ON, Mikhachik EV, et al. (2013) Intravenous Immunoglobulins (IVIG) Enhance *In vitro* Activation of Neutrophils in C57BL/6 but not in CBA Mice. Immunome Res 9: 068. doi: 10.4172/1745-7580.1000068

Copyright: © 2013 Basyreva LY, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

in the response to IVIG treatment can be elucidated by the comparison of CBA and C57BL/6 mouse strains.

The variability of individual response to IVIG might be associated with the peculiarities of neutrophil's activation. The granulocytes can contribute to differences in the innate susceptibility of mouse strains to infection. Such a difference was shown for intracellular killing of *Salmonella typhirium* by CBA and C57BL/10 (susceptible to *Salmonella* as well as C57BL/6) granulocytes in exudates [19], for inflammation kinetics in mice CBA and C57BL/6 injected with zymosan (difference in the rate of granulocytes influx) [20], for regulation in a number of genes involved in the recruitment and function of granulocytes in mice with tuberculosis [21].

The available data indicate that these two strains can be considered as contrasting ones regarding function of their immune system [20,22,23]. And both strains are used for studying the effects of IVIG [24,25].

Here we compared the effect of IVIG on neutrophils from CBA and C57BL/6 mice *in vitro*.

We measured chemiluminescence (CL) of whole blood samples treated with opsonized zymosan (OZ), and estimated its dependence on extracellular calcium and IVIG. We revealed significant differences between mouse strains in neutrophil activation and response to IVIG.

Materials and Methods

The following reagents were used: zymosan, phorbol 12-myristate 13-acetate, luminol, Krebs-Ringer medium, EDTA (Sigma-Aldrich), IVIG (Intraglobin, Biotest, Germany).

Animals

The experiments were performed on female CBA (H-2^k) and C57BL/6 (H-2^b) mice (18-22 g) obtained from the Scientific Center for Biomedical Technologies of the Russian Academy of Medical Sciences, Andreevka branch. The animals were kept on a standard feed with free access to water. The investigation was performed after approval of the local ethics committee.

Blood sampling, preparation of whole blood smears, staining, total and differential WBC counting

The animals were subjected to cervical dislocation, then 100 μ l of blood were taken from the right ventricle after thoractomy. The blood was added to 3% EDTA (4:1) and mixed thoroughly. A 2 μ l aliquot of blood was used for smear preparation. The whole blood smears were subjected to Romanowsky-Giemsa staining and analyzed with Motic 3B microscope.

WBC differential count was calculated after analysis of 100-200 leukocytes. Total WBC number was estimated with hemocytometer after 10-fold dilution with 5% acetic acid.

Platelet counting

The numbers of individual platelets, aggregates and platelets in each aggregate were estimated in 15 microscopic fields in the central part of the blood smear at x 40 magnification.

Measurement of chemiluminescence

The chemiluminescence (CL) assay was performed using LKB Wallac luminometer in continuous measurement mode at 37°C. Final volume of the samples was 1 ml.

The cuvette was filled with Krebs-Ringer solution (Sigma) containing the following reagents (g/l): D-glucose 1.8; MgCl₂ 0.0468; KCl 0.34; NaCl 7.0; Na₂HPO₄ 0.1; NaH₂PO₄ 0.18; NaHCO₃ 1.26. CaCl₂ was added separately to final concentration of 0.144 g/l. Luminol (0.2 mM) was used as a CL-sensitizing agent. Spontaneous CL was recorded for 6 min after addition of 20 μ l of whole blood into warm medium with luminol followed by 200 μ l of IVIG (up to 10 mg/ml) or 200 μ l of 0.9% NaCl. Then after another 5 minutes, the cells were activated with opsonized zymosan (OZ) (0.2 mg/ml) and the peak value was measured. In some experiments, phorbol 12-myristate 13-acetate (PMA) (0.1 μ l / mL) was added after OZ-activated CL reached maximum. CL response to OZ and PMA was found as the difference between maximal and basement (spontaneous) CL values. The result was expressed as CL response (mV) per 10⁵ neutrophils.

Zymosan opsonization: 10 mg of zymosan (Sigma) were thoroughly suspended in 1 ml of 0.9% NaCl and added to 1 ml of fresh human blood serum. The suspension was incubated for 30 min at 37°C and intermittent shaking, and then zymosan was pelleted by centrifugation and washed three times with 10-fold volume of NaCl. The obtained pellet was resuspended in 0.9% NaCl to final concentration of 4 mg/ml.

Statistics: statistical analysis was performed using Statistica® 6.0.437.0 software (StatSoft), using Mann-Whitney U-test or Wilcoxon matched-pairs test.

Results

ROS generation by neutrophils can be estimated by luminol-dependent CL of diluted zymosan-treated whole blood [26].

Experiments in calcium-free medium enable studying the processes that are independent of calcium uptake. Kinetics and rate of response were different in C57BL/6 and CBA mice. Neutrophils of CBA mice developed a weak response to OZ, while neutrophils of C57BL/6 mice remained insensitive to zymosan (Figure 1). The observed differences might depend not only on extracellular calcium, but also on its intracellular depots and on NADPH-oxidase functioning. Since CL induced by phorbol myristate acetate (PMA) depends only on intracellular calcium, we added PMA to zymosan-treated samples. PMA induced similar increase of CL in neutrophils of CBA and C57BL/6 mice (Figure 1). The average PMA-activated CL response was 5.4 ± 1.1 and 7.4 ± 1.9 mV/10⁵ neutrophils for CBA and C57BL/6, respectively.

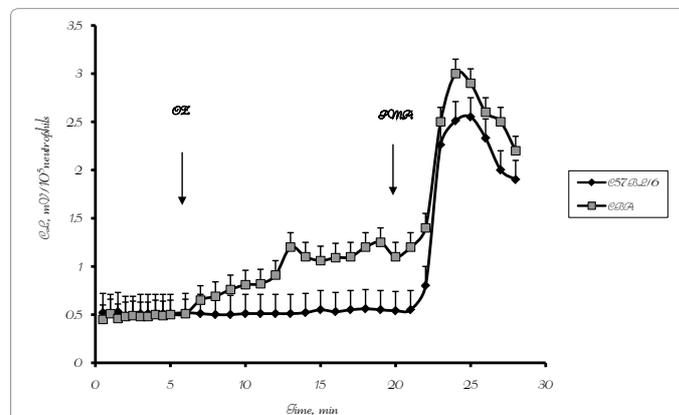


Figure 1: Kinetics of luminol-dependent CL of whole blood from CBA and C57BL/6 mice after addition of OZ and PMA. Final dilution of blood was 1:50. The data represent mean \pm standard error of mean. Each group contained 5 animals.

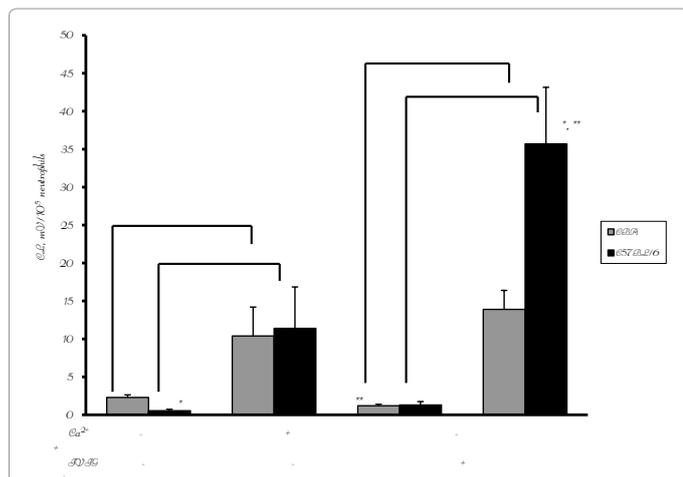
Therefore, the differences in neutrophil activation do not depend on calcium uptake, intracellular calcium depots or NADP-oxidase functioning, but require signaling reactions upstream of diacylglycerol.

In the presence of exogenous calcium (which resembled natural conditions), the level of neutrophil's activation in response to zymosan was increased in both mouse strains and became almost equal (Figure 2).

Addition of IVIG into the probes showed different effects on CL of neutrophils from CBA and C57BL/6 mice. In case of CBA mice, IVIG induced a significant decrease in neutrophil's response in calcium-free medium, while in C57BL/6 the neutrophil response was increased.

In the presence of calcium, the neutrophil response was more prominent in C57BL/6 mice, whereas in CBA IVIG treatment did not affect the CL (Figure 2).

Analysis of the results indicate that zymosan-induced response of neutrophils from CBA mice was less dependent on exogenous calcium compared to neutrophils from C57BL/6. Neutrophils of C57BL/6 mice generated trace amounts of ROS in calcium-free medium, and



* Significant difference from CBA ($p < 0.05$)
 ** Significant difference from control samples (without IVIG) ($p < 0.05$)

Figure 2: The level of luminol-dependent CL of neutrophils from whole blood of intact mice treated with OZ regarding the presence of calcium (0.144 g/l) and IVIG (10 mg/ml). Final dilution of blood was 1:50. The data represent mean \pm standard error of mean. Each group contained 5 animals. Dashed lines indicate reliable effect of intracellular calcium ($p < 0.05$).

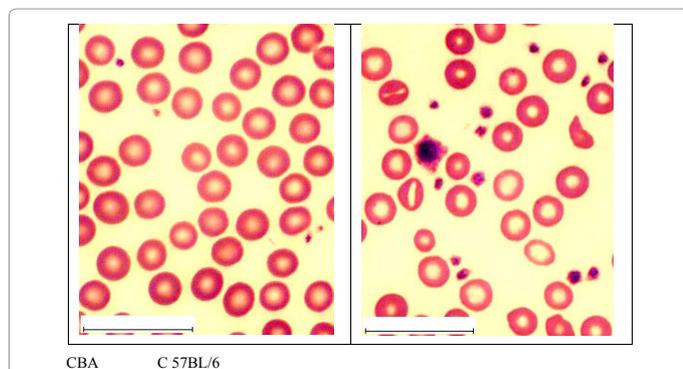


Figure 3: RBC and platelets from CBA and C57BL/6 mice. Romanowsky-Giemsa staining. Multiple deformed RBCs are visible in C57BL/6 mice. Scale bar 20 μ m.

increased ROS synthesis by 20 times in the presence of calcium (the neutrophils of CBA mice generated 5.5-fold more ROS in the presence of calcium).

Further increase of C57BL/6 neutrophil response was observed after addition of IVIG in the presence of calcium.

Thus, we have shown for the first time that in C57BL/6 mice IVIG did not induce ROS generation by circulating neutrophils, but enhanced neutrophil's response to opsonized zymosan *in vitro*. The effect was more pronounced in the presence of calcium.

Other differences between strains were found after microscopic examination: analysis of blood smears revealed a significant amount of hypochromic RBC in C57BL/6 mice. In CBA mice, the RBCs were normal (Figure 3).

C57BL/6 mice contained an increased number of stomatocytes and small deformed RBC (resembling deformed RBC in thalassemia). Such abnormal erythrocytes were rarely detected in CBA mice.

C57BL/6 mice contained multiple intensively stained platelets. Some large and giant (equal to RBC in size) platelets were also detected. Often platelets were aggregated. CBA mice contained less platelets, which were small, weakly stained and less aggregated (Figure 3) (Table 1).

The number of WBC in the examined mouse strains was almost similar. The leukocytes contained 1-2% eosinophils, 40-60% lymphocytes and about 25% monocytes (Table 1). CBA mice had more neutrophils and less lymphocytes compared to C57BL/6 mice (Table 1).

Thus, C57BL/6 mice had an increased number of hypochromic RBC, 40% more platelets and twice less neutrophils than CBA mice.

Such significant differences in hematological characteristics of mouse strains should be taken into account for analysis of whole blood chemiluminescence.

Discussion

The CBA and C57BL/6 mouse strains develop different response to infection and radiation [20,22]. Some authors suggested that the observed phenomena might be explained by the differences in T- and B-lymphocytes [23], and peculiarities of T-cell differentiation [27]. Our results indicate that CBA and C57BL/6 mice vary in hemoglobin content in RBC, in number and size of platelets and in number of neutrophils.

These observations suggest that hemopoietic system functions differently in CBA and C57BL/6 mice.

Parameter	Units	CBA	C57BL/6
Number of WBC	Cells/ml	3761 \pm 573	3892 \pm 719
monocytes	%	25 \pm 3	27 \pm 4
lymphocytes	%	43 \pm 2	53 \pm 9 *
neutrophils	%	29 \pm 1	18 \pm 3**
eosinophils	%	1.1 \pm 0,5	1.5 \pm 0,4
Single platelets*	N in 15 microscopic fields	167 \pm 11	233 \pm 13*
Platelets in aggregates	N in 15 microscopic fields	64 \pm 4	142 \pm 10**
Number of platelets aggregates		19 \pm 2	31 \pm 4*

*- significant difference between strains ($p < 0.05$)
 **- significant difference between strains ($p < 0.01$)
 Significance was estimated with Mann-Whitney criterion.

Table 1: WBC differential count and the number of platelets from blood of CBA and C57BL/6 mice. Mean \pm standard error of mean is shown.

While there are published data concerning adaptive immunity of CBA and C57BL/6 mice, the peculiarities of innate immunity (neutrophils functioning) remain poorly studied.

The functioning of neutrophils (and their activation in particular) can be estimated by the ROS generation [28]. Generation of ROS is regulated by membrane-bound NADPH-oxidase. The major NADPH-oxidase subunits are present in neutrophil's plasma membrane, and some soluble subunits are stored in intracellular depots [17]. Neutrophil's activation is associated with phosphorylation of NADPH-oxidase subunits, their translocation to plasma membrane and assembly of NADPH-oxidase complex [28]. Phosphorylation of NADPH-oxidase subunits require the elevation of intracellular calcium [17].

Neutrophil's activation can be induced by receptor-mediated signaling (following interaction with zymosan, bacteria or immune complexes) or without transmembrane receptors (PMA).

Zymosan, a preparation of *Saccharomyces cerevisiae* cell walls, can induce respiratory burst in neutrophils [26]. Most prominent activation of neutrophils is observed after preliminary opsonization of zymosan by complement proteins and immunoglobulins, which bind polysaccharides exposed on zymosan and interact with CR and FcR on neutrophils [12]. Zymosan polysaccharides can interact directly with lectin- and Toll-like receptors on neutrophils [29]. That is why zymosan activates NADPH-oxidase even in the absence of opsonizing proteins [30].

The first slow stage of neutrophil's activation by opsonized zymosan is accompanied by calcium release from intracellular depots, then subsequent uptake of extracellular calcium enhances the response [18] and leads to activation of protein kinase C, assembly of NADPH-oxidase and generation of ROS. Therefore, extracellular calcium is required for neutrophil's activation with opsonized zymosan.

PMA, unlike zymosan, interacts directly with protein kinase C [31], which phosphorylates p47phox subunit of NADPH-oxidase [30]. That is why neutrophil's response to PMA is regulated only by intracellular calcium depots and is independent of extracellular calcium [15]. This mechanism functions similarly in both mouse strains.

The differences between CBA and C57BL/6 mice in ROS generation by whole blood neutrophils treated with opsonized zymosan in calcium-free medium might arise from the differences in the surface receptors and/or in signaling cascades that induce phosphorylation of NADPH-oxidase subunits. In CBA mice, neutrophil's activation was independent of extracellular calcium, while in C57BL/6 such a pathway played a negligible role.

Under the same conditions (in calcium-free medium), PMA activated neutrophils of both mouse strains. Since we did not detect differences in neutrophil's response to PMA in CBA and C57BL/6 mice, we suggested that the absence of zymosan-induced neutrophil's activation in C57BL/6 might arise from the peculiarities of signaling cascades upstream of protein kinase C.

Addition of calcium eliminated the differences in neutrophil's activation, and enhanced the overall response. Thus, neutrophils of both mouse strains can develop respiratory burst associated with calcium uptake from extracellular medium.

It is well known that some cytokines, lipopolysaccharides and other compounds influence neutrophils and make them primed. Priming is associated with structural alteration of NADPH-oxidase, phosphorylation of its subunits and/or their translocation from cytosol

to plasma membrane, although the assembly of the enzyme complex is completed only under activation stimuli [28].

We showed that in the presence of calcium IVIG added to medium prior to zymosan markedly enhanced neutrophil's activation in C57BL/6 strain (Figure 2). In case of CBA mice, neutrophil's response was not enhanced under these conditions.

Addition of IVIG to calcium-free medium caused moderate increase of neutrophil's response to zymosan in C57BL/6 and decrease in CBA strain.

Thus, in the presence of calcium, IVIG primed neutrophils from C57BL/6 mice and had no effect on neutrophils from CBA mice.

We suggest that the interaction of IVIG with plasma membrane of C57BL/6 neutrophils causes their priming either via FcR-coupled mechanisms [11,32], or via other surface antigens, which might include transporter proteins [33]. IVIG can compete with opsonized zymosan for binding to FcR on neutrophils.

For the interpretation of data obtained with neutrophils from whole blood preparations, the effect of platelets and other cells should be taken into account. Platelets from CBA and C57BL/6 mice vary morphologically, which might affect their interaction with neutrophils even in diluted blood samples (1:50). Platelets might vary in their functional activity and in their response to extracellular calcium in particular [34]. Platelets were shown to enhance neutrophil's motility [35], regulate neutrophil's recruitment to the lesion site [36] and secrete various neutrophil's activators including proinflammatory cytokines [37]. In addition, IVIG were shown to induce aggregation and activation of platelets [38].

One can suppose the participation of platelets in neutrophil reactions in a contact-dependent manner [34] through P-selectin [39] and CD11/CD18 integrins which in their turn are sensitive to FcR stimulation by IgG [40].

Thus, immunoglobulins could interact with neutrophils or platelets and facilitate or interfere with their contact-dependent reactions.

We do not exclude that the observed difference in activation of IVIG-treated neutrophils might result from different interaction of neutrophils and platelets in CBA and C57BL/6 strains.

Overall, comparison of data obtained with IVIG-treated and untreated samples reveal an IVIG-sensitive calcium-dependent pathway of neutrophil's activation in C57BL/6 mice. This pathway regulates ROS generation by neutrophils pretreated with opsonized zymosan and primes neutrophils *in vitro*.

At the same time, IVIG inhibited ROS generation by neutrophils of CBA mice in calcium-free medium and had no effect on their activation in the presence of calcium.

There are at least two possible mechanisms here: the presence of antibodies to the mouse neutrophils in IVIG (for example, ANCA-like antibodies [14,41]) and the effects through neutrophil Fcγ-receptors. The second mechanism could implicate Ca-dependent FcγR clustering [42]. The difference in IVIG effects could result from specific interaction of immunoglobulins via Fab with antigens of neutrophils in CBA or C57BL/6 or via Fc with Fc-receptors.

IVIG is used for treatment of immunodeficiencies and autoimmune diseases, and exhibits anti-cancer potency. However, the details of its therapeutic effect remain poorly understood, and the mechanisms

underlying variable sensitivity to IVIG treatment are unknown. The interaction of IVIG with immune cells and their subsequent activation can be considered as potent mechanisms of the IVIG effect. Here we analyzed the ability of IVIG to activate neutrophils obtained from immunologically opposite mouse strains. The observed differences depict that CBA and C57BL/6 mouse strains can be used as a model system for studying systemic effects of IVIG.

References

1. Sewell WAC, Jolles S (2002) Immunomodulatory action of intravenous immunoglobulin. *Immunology* 107: 387-393.
2. Sapir T, Shoenfeld Y (2005) Uncovering the hidden potential of intravenous immunoglobulin as an anticancer therapy. *Clin Rev Allergy Immunol* 29: 307-310.
3. Imbach P, Lazarus AH, Kühne ET (2009) Intravenous immunoglobulins induce potentially synergistic immunomodulations in autoimmune disorders. *Vox Sanguinis* 2009 98: 385-394.
4. Shoenfeld Y, Krause I (2004) IVIG for autoimmune, fibrosis, and malignant conditions: our experience with 200 patients. *J Clin Immunol* 24: 107-114.
5. De Vlieghere FC, Peetermans WE, Vermeylen J (1994) Aseptic granulocytic meningitis following treatment with intravenous immunoglobulin. *Clin Infect Dis* 18: 1008-1010.
6. Blank M, Gisondi P, Mimouni D, Peserico A, Piaserico S, et al. (2006) New insights into the autoantibody-mediated mechanisms of autoimmune bullous diseases and urticaria. *Clin Exp Rheumatol* 24: S20-S25.
7. Nussinovitch U, Shoenfeld Y (2008) Intravenous immunoglobulin – indications and mechanisms in cardiovascular diseases. *Autoimmun Rev* 7: 445 - 452.
8. Teeling JL, De Groot ER, Eerenberg AJM, Bleeker WK, Van Mierlo G, et al. (1998) Human intravenous immunoglobulin (IVIG) preparations degranulate human neutrophils *in vitro*. *Clin Exp Immunol* 111: 264-270.
9. Casulli S, Topçu S, Fattoum L, von Gunten S, Simon H-U, et al. (2011) A differential concentration-dependent effect of IVIG on neutrophil functions: relevance for anti-microbial and anti-inflammatory mechanisms. *PLoS ONE* 6: e26469.
10. Hiragushi S, Machino Y, Suzuki E, Suzuki M, Kohoroki J, et al. (2012) Both the Fab and Fc domains of IgG are essential for ROS emission from TNF- α -primed neutrophils by IVIG. *Biochem Biophys Res Comm* 417: 794-799.
11. Jakus Z, Németh T, Verbeek JS, Mócsai A (2008) Critical but overlapping role of Fc γ RIII and Fc γ RIV in activation of murine neutrophils by immobilized immune complexes. *J Immunol* 180: 618-629.
12. Nagahata H, Higuichi H, Inanami O, Kuwabara M (2007) Costimulatory effects of complement receptor type 3 and Fc receptor for IgG (Fc γ R) on superoxide production and signal transduction in bovine neutrophils. *J Vet Med Sci* 69: 993-997.
13. Falk RJ, Terrell RS, Charles LA, Jennette JC (1990) Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals *in vitro*. *Proc Natl Acad Sci USA* 87: 4115-4119.
14. Bodman-Smith KB, Melendez AJ, Campbell I, Harrison PT, Allen JM, et al. (2002) C-reactive protein-mediated phagocytosis and phospholipase D signaling through the high-affinity receptor for immunoglobulin G (FcRI). *Immunology* 107: 252-260.
15. DeChatelet LR, Shirley PS (1982) Chemiluminescence of human neutrophils induced by soluble stimuli: effect of divalent cations. *Infect Immun* 35: 206-212.
16. Lee WL, Harrison RE, Grinstein S (2003) Phagocytosis by neutrophils. *Microbes Infect* 5: 1299-1306.
17. Brécharad S, Tschirhart E (2008) Regulation of superoxide production in neutrophils: role of calcium influx. *J Leukoc Biol* 84: 1223-1237.
18. Leino L, Tuominen H, Lehtola K, Akerman KEO, Punnonen K (1995) Biphasic formation of inositol phosphates in opsonized zymosan-stimulated human neutrophils. *Cell Signal* 7: 397-402.
19. Van Dissel JT, Stikkelbroeck JJ, Leijh PC, van Furth R (1986) Difference in initial rate of intracellular killing of *Salmonella typhimurium* by granulocytes of *Salmonella*-susceptible C57BL/10 mice and *Salmonella* – resistant CBA mice. *J Immunol* 136: 1074-1080.
20. Mazur-Bialy AI, Majka A, Wojtas L, Kolaczowska E, Plytycz B (2011) Strain-specific effects of riboflavin supplementation on zymosan-induced peritonitis in C57BL/6L/6J, BALB/c and CBA mice. *Life Sci* 88: 265–271.
21. Keller C, Hoffman R, Lang R, Brandau S, Hermann C, et al. (2006) Genetically determined susceptibility to tuberculosis in mice causally involves accelerated and enhanced recruitment of granulocytes. *Infect Immun* 74: 4295-4309.
22. Sharplin J, Franko AJ (1989) A quantitative histological study of strain-dependent differences in the effects of irradiation on mouse lung during the early phase. *Radiat Res* 119: 1-14.
23. Shkurupiy VA, Tkachev VO, Potapova OV, Luzgina NG, Bugrimova JS, et al. (2011) Morphofunctional characteristics of the immune system in CBA and C57bl/6g mice. *Bull Exp Biol Med* 150: 725-729.
24. Roy E, Stavropoulos E, Brennan J, Coade S, Grigorieva E, et al. (2005) Therapeutic efficacy of high-dose intravenous immunoglobulin in *Mycobacterium tuberculosis* infection in mice. *Infect Immun* 73: 6101–6109.
25. Tha-In T, Bayry J, Metselaar HJ, Kaveri SV, Kwekkeboom J (2008) Modulation of the cellular immune system by intravenous immunoglobulin. *Trends Immun* 29: 608-615.
26. Lindena J, Burkhardt H, Dwenger A (1987) Mechanisms of non-opsonised zymosan-induced and luminol-enhanced chemiluminescence in whole blood and isolated phagocytes. *J Clin Chem Clin Biochem* 25: 765-778.
27. Watanabe T, Miyatake T, Kumamoto H, Mafune N, Kubota S, et al. (2004) Adenovirus-mediated CTLA4 immunoglobulin G gene therapy in cardiac xenotransplantation. *Transplant Proc*. 2004 36: 2478-2479.
28. Sheppard FR, Kelher MR, Moore EE, McLaughlin N, Banerjee A, et al. (2005) Structural organization of the neutrophil NADPH oxidase: phosphorylation and translocation during priming and activation. *J Leukoc Biol* 78: 1025-1042.
29. Gantner BN, Simmons RM, Canavera SJ, Akira S, Underhill DM (2003) Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J Exp Med* 197: 1107-1117.
30. Makni-Maalej K, Chiandotto M, Hurtado-Nedelec M, Bedouhene S, Gougerot-Pocidal MA, et al. (2013) Zymosan induces NADH oxidase activation in human neutrophils by inducing the phosphorylation of p47phox and the activation of Rac2: involvement of protein tyrosine kinases, PI3kinase, PKC, ERK1/2 and p38MAPkinase. *Biochem Pharmacol* 85: 92-100.
31. Regier DS, Greene DG, Sergeant S, Jesaitis AJ, McPhail LC (2000) Phosphorylation of p22phox is mediated by phospholipase D-dependent and -independent mechanisms. Correlation of NADPH oxidase activity and p22phox phosphorylation. *J Biol Chem* 275:28406-28412.
32. Mirre E, Teeling JL, Meer JWM, Meeker WK, Hack CE (2004) Monomeric IgG in intravenous Ig preparations is a functional antagonist of Fc gamma RII and Fc gamma RIIB. *J Immunol* 173: 332-339.
33. Lcal PM, Barasoain I, Sánchez A, García-Sancho J, Flores I, et al. (1992) A monoclonal antibody that detects a specific human neutrophil antigen involved in phorbol myristate acetate- and formyl-methionyl-leucyl-phenylalanine-triggered respiratory burst. *J Immunol* 148:161-168.
34. Ruf A, Schlenk RF, Maras A, Morgenstern E, Patscheke H (1992) Contact-induced neutrophil activation by platelets in human cell suspensions and whole blood. *Blood* 80: 1238-1246.
35. Bengtsson T, Frydén A, Zalavary S, Whiss PA, Orselius K, et al. (1999) Platelets enhance neutrophil locomotion: evidence for a role of P-selectin. *Scand J Clin Lab Invest* 59:439-449.
36. Hara T, Shimizu K, Ogawa F, Yanaba K, Iwata Y, et al. (2010) Platelets control leukocyte recruitment in a murine model of cutaneous arthus reaction. *Am J Pathol*. 176: 259-269.
37. Holmsen H (1994) Significance of testing platelet functions *in vitro*. *Eur J Clin Invest* 24: 3-8.
38. Pollreis A, Assinger A, Hacker S, Hoetzenecker K, Schmid W, et al. (2008) Intravenous immunoglobulins induce CD32-mediated platelet aggregation *in vitro*. *Br J Dermatol* 159: 578-584.
39. Piccardoni P, Sideri R, Manarini S, Piccoli A, Martelli N, et al. (2001) Platelet/polymorphonuclear leukocyte adhesion: a new role for SRC kinases in Mac-1 adhesive function triggered by P-selectin. *Blood* 98: 108-116.

-
40. Kusunoki T, Tsuruta S, Higashi H, Hosoi S, Hata D, et al. (1994) Involvement of CD11b/CD18 in enhanced neutrophil adhesion by Fc gamma receptor stimulation. *J Leukoc Biol* 55: 735-742.
41. Jarius S, Eichhorn P, Albert MH, Wagenpfeil S, Wick M, et al. (2007) Intravenous immunoglobulins contain naturally occurring antibodies that mimic antineutrophil cytoplasmic antibodies and activate neutrophils in a TNFalpha-dependent and Fc-receptor-independent way. *Blood* 109: 4376-4382.
42. Vossebeld PJM, Kessler J, von dem Borne AE, Roos D, Verhoeven AJ (1995) Heterotypic FcγR clusters evoke a synergistic Ca²⁺ response in human neutrophils. *J Biol Chem* 270: 10671-10679.