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Influence of FcγRIIIb polymorphism on its ability to cooperate with FcγRIIa and CR3 in mediating the oxidative burst of human neutrophils

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ABSTRACT

Considering that human neutrophil FcγRIIa and FcγRIIIb receptors interact synergistically with CR3 in triggering neutrophil functional responses, allelic polymorphisms in these receptors might influence such interactions. We assessed whether FcγRIIIb polymorphisms affect FcγR/CR cooperation in mediating the neutrophil oxidative burst (OB), in particular the FcγRIIIb/CR3 cooperation that occurs via lectin-saccharide-like interactions. The OB of human neutrophil antigen (HNA)-1a-, HNA-1b-, and HNA-1a/-1b-neutrophils stimulated with immune complexes, opsonized or not with serum complement, was measured by the luminol-enhanced chemiluminescence assay. Compared with HNA-1a-neutrophils, HNA-1b-neutrophils exhibited reduced FcγR-stimulated OB, but increased FcγR/CR-stimulated OB. It suggests that (i) FcγR and CR cooperate more effectively in HNA-1b-neutrophils, and (ii) the HNA-1b allotype influences the FcγRIIIb cooperation with FcγRIIa, but not with CR3. HNA-1a- and HNA-1b-neutrophils exhibited similar OB responses elicited via CR3 alone or via FcγR/CR-independent pathways. In addition, the level of FcγRIIIb, FcγRIIa, and CR3 expression did not differ significantly among the neutrophil groups studied. Together, these results demonstrate that the HNA-1b allotype influences the functional cooperation between FcγRIIIb and FcγRIIa, and suggest that the difference in the glycosylation pattern between HNA-1a and HNA-1b does not affect the FcγRIIIb cooperation with CR3.

1. Introduction

Stimulating neutrophils with soluble and particulate agents triggers an oxidative burst (OB) characterized by increased oxygen consumption and reactive oxygen species (ROS) generation [1]. Both IgG (FcγR) and complement (CR) receptors mediate this process, which enables neutrophils to effectively eradicate invading microorganisms [1–3]. In the last thirty years, many researchers have reported that the cooperative response of FcγR and CR is important to the host defense, and markedly enhances the efficiency of IgG-mediated effector functions of neutrophils [2,3]. Thus, it is essential to understand how FcγR and CR interact to

Abbreviations: AUC, area under the time-course CL curve; BSA, bovine serum albumin; CL, chemiluminescence; cpm, photon counts per minute; CR, complement receptor; EDTA-Na_2, disodium ethylenediaminetetraacetate; FcγR, Fc gamma receptor; FITC, fluorescein isothiocyanate; HBSS, Hank's balanced saline solution; HNA, human neutrophil antigen; IC, immune complex; INHS, inactivated normal human serum; MCFI, median channel fluorescence intensity; NHS, normal human serum; OB, oxidative burst; PE, phycoerythrin; PMA, phorbol 12-myristate-13-acetate; ROS, reactive oxygen species; SLE, systemic lupus erythematosus.

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activate the functional responses of human neutrophils [1,2,4]. The description of molecular features of FcγR [5] and CR [6,7] has improved comprehension of the effector functions of human neutrophils triggered via these receptors, but the specific role of polymorphic variants remains unclear.

The binding of IgG to FcγR induces a wide range of biological responses [3], which provides an important link between cellular and humoral immunity. Polymorphisms in FcγR may influence susceptibility to diseases [8,9]. There are three families of FcγR in humans: FcγRI (CD64, located at chromosome 1q21.1–2), FcγRII and FcγRIII (CD16 and CD32, respectively, both located at 1q23.3) [10]. The FcγRIIIb isoform (CD16) is a low-affinity receptor constitutively expressed in neutrophils, which is codified by the *FCGR3B* gene [11]. *FCGR3B* has three allelic variants – FCGR3B*01, FCGR3B*02, and FCGR3B*03 – that codify three different allotypes: human neutrophil antigen (HNA) -1a, -1b and -1c, respectively [12]. The allelic variants FCGR3B*01 and FCGR3B*02 differ in four amino acids; such differences result from substituting five nucleotides on the genomic DNA level [13]. HNA-1b has two more N-linked glycosylation sites than HNA-1a, and these allotypes – HNA-1a and -1b – exhibit different IgG-binding capacities [10]. FCGR3B*02 and FCGR3B*03 differ in the nucleotide at position 266: they contain adenine and cytosine, respectively. It leads to substitution of alanine for aspartic acid at residue 78 of FcγRIIIb [14], resulting in the less frequent HNA-1c variant [15]. HNA-1a is more efficient than HNA-1b in binding and phagocytosis of IgG1- and IgG3-containing immune complexes (IC) [11,16].

Neutrophils express the FcγRIIa isoform that is codified by the *FCGR2A* gene. A single nucleotide change of arginine (R) for histidine (H) at amino acid position 131 determines an increased affinity of this receptor for IgG2 [17]. Neutrophils from individuals with homozygosis for both FcγRIIa-R131 and FcγRIIIb-HNA-1b alleles exhibit a lower phagocytic activity than neutrophils from homozygous FcγRIIa-H131 and FcγRIIIb-HNA-1a individuals [9,18]. FcγR and CR polymorphisms are associated with immune abnormalities and increased risk to develop autoimmune diseases [19,20]; however, the influence of these polymorphisms on the neutrophil OB has not been fully elucidated.

The CD11b subunit of CR3 (CD11b/CD18) is encoded by the *ITGAM* gene. A single nucleotide polymorphism in this receptor changes the arginine at amino acid position 77 to histidine (R77H). The 77H CD11b variant has been associated with systemic lupus erythematosus (SLE) [21,22], but its functional significance remains unclear.

In neutrophils, the FcγRIIIb isoform has no signal sequence in the cytoplasm; thus, it is not able to trigger the neutrophil effector functions alone, but cooperates with the FcγRIIa and CR3 to enhance the functional responses of these leukocytes [23]. Considering that FcγRIIIb/CR3 cooperation occurs via lectin-saccharide-like interactions [24], and that the HNA-1a and HNA-1b allotypes differ in the number of glycosylation sites, we assessed whether the FcγRIIIb polymorphism affect FcγR/CR cooperation in mediating the neutrophil OB. To exclude the influence of FcγRIIa and CR3 polymorphisms, only individuals with the HR131 (FcγRIIa) and 77RR (CD11b subunit of CR3) variants were included in this study. This is the first study that investigates the role of FcγRIIIb polymorphism in the neutrophil OB without the interference of FcγRIIa and CR3 polymorphisms.

2. Material and methods

2.1. Volunteers

Blood samples were collected from healthy blood donors at Hemonúcleo Regional de Araraquara, Faculdade de Ciências Farmacêuticas da Universidade Estadual Paulista (UNESP, Araraquara – SP, Brazil). The study included twenty seven healthy adult volunteers –10 males and 17 females – aged 33.0 ± 9.7 years, who were not taking any drugs. All the participants signed a written informed consent to participate in the study, approved by the local Research Ethics Committee.

2.2. Neutrophil isolation

Whole blood was collected into vacutainer tubes containing disodium ethylenediaminetetraacetate (EDTA-Na_2) as anticoagulant. Neutrophils were purified by the gelatin method [25]. Briefly, the anticoagulated blood samples were centrifuged (755×*g*, 4 °C, 10 min). Gelatin solution (2.5% in 0.15 M NaCl) was added to the pellet, and the mixture was incubated for 30 min, at 37 °C. The neutrophil-rich supernatant was centrifuged (755×*g*, 25 °C, 10 min), and the resulting pellet was washed with PBS pH 7.4 (480×*g*, 4 °C, 10 min). To lyse remaining erythrocytes, the pellet was suspended in 0.83% NH_4Cl pH 7.2, incubated for 5 min, at 37 °C, and centrifuged (480×*g*, 4 °C, 10 min). Neutrophils were washed with PBS pH 7.4 (480×*g*, 4 °C, 10 min), and finally suspended in Hank's balanced saline solution (HBSS) pH 7.2 supplemented with 0.1% gelatin for use. Cell suspensions contained 92% of neutrophils, and less than 8% of non-viable cells, as established by exclusion with Trypan blue. All reagents and glassware were lypopolysaccharide-free when tested with E-TOXATE® (Sigma, St. Louis, MO, USA; product no. 210).

2.3. Immune complex preparation

Immune complexes (IC) were prepared at equivalence with bovine serum albumin (BSA; Sigma–Aldrich, St. Louis, MO, USA) as antigen, and rabbit anti-BSA IgG antibody or its F(ab')2 fragments – optimal antigen-antibody proportion has been previously established on the basis of a quantitative precipitin curve. The antigen-antibody mixtures were incubated for 60 min, at 37 °C, and thereafter overnight at 4 °C. The mixtures were centrifuged, and the precipitated IC was washed with PBS pH 7.4. Finally, IC was suspended in PBS pH 7.4 for use [26]. Total protein concentration in the precipitate was determined by absorbance readings at 280 nm and expressed as μg mL^{-1}.

IC was opsonized with complement from normal human serum (NHS) or not (IC-IgG). Heat-inactivated NHS (INHS) at 56 °C for 30 min was used as negative control for complement activity. Briefly, aliquots of IC-IgG or IC-F(ab')2 (1 μg) were incubated with 1 mL of NHS or INHS – previously diluted 1:2 in veronal-buffered saline pH 7.2, containing 0.25 mM $CaCl_2$ and 0.83 mM $MgCl_2$ – for 30 min, at 37 °C. Reaction was stopped by adding cold PBS pH 7.4 to the mixture, and centrifuging it (12,000×*g*, 4 °C, 10 min). The opsonized IC were washed with PBS pH 7.4, and suspended in HBSS pH 7.2 for use.

2.4. Oxidative burst (OB) assay

Neutrophils (5×10^5 mL^{-1} of reaction) were mixed with 10^{-4} M luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma–Aldrich, St. Louis, MO, USA) and stimulated with: 10^{-7} M phorbol 12-myristate-13-acetate (PMA; Sigma–Aldrich, St. Louis, MO, USA), or 30 μg of IC-IgG, IC-IgG/NHS, IC-IgG/INHS, IC-F(ab')2/NHS, or IC-F(ab')2/INHS. Neutrophils incubated with luminol but not stimulated with IC were used as control. The chemiluminescence (CL) reaction was monitored in a luminometer (AutoLumat Plus LB 953 luminometer, EG&G Berthold, Bad Wildbad, Germany) for 20 min, at 37 °C, and data were recorded as photon counts per minute (cpm). Results are reported as area under the time-course CL curve (AUC) [27].