THE EXPRESSION OF Fc GAMMA RECEPTORS ON LEUKOCYTES AND CLINICAL COURSE OF COMMON VARIABLE IMMUNODEFICIENCY (CVID)

THON V.1, VLKOVA M.1, FREIBERGER T.2, LITZMAN J.1, LOKAJ J.1

1Department of Clinical Immunology and Allergology, Masaryk University, Brno, Faculty of Medicine, St. Anne’s Faculty Hospital
2Laboratory of Molecular Genetics, Centre of Cardiovascular Surgery and Transplantation, Brno

Received after revision November 2005

Abstract

CVID is the most common symptomatic primary antibody deficiency syndrome characterised by decreased levels of serum immunoglobulin and reduced antibody production. Intravenous immunoglobulin (IVIG) replacement is a standard treatment of this disorder. The factors that influence the effect of the therapy are not elucidated and it should be supposed that various factors are involved. We have studied the correlation between the clinical course of CVID patients and the receptors for IgG (Fc gamma RI, II, III) expressed on their leukocytes.

We have investigated 41 patients (13 males, 28 females, aged 10 to 77 years, mean 43) and 26 age-related control persons. Quantitative expression of the Fc receptors on the surface of lymphocytes, monocytes, and granulocytes was determined by flow cytometry (Coulter Epics XL-MCL; monoclonal antibodies CD16, CD32 were used from Immunotech and CD64 from Dako). Gene polymorphism of Fc gamma RIIa and IIIb was determined by PCR.

The expression of CD16, CD32, and CD64 on lymphocytes and monocytes was comparable in patients and control persons, also the expression of CD16 and CD32 on granulocytes was similar in both groups. However, we have found marked differences in the expression of CD64 on granulocytes in CVID patients: the expression of CD64 (Fc gamma RI) on granulocytes was low in healthy controls (n = 26; mean = 4.7 %, SEM = 0.8). On the contrary, in the group of CVID patients there are two subgroups. The first was comparable with the control group (n = 17; mean = 4.8 %, SEM = 0.6) but the second is characterised with high expression of Fc gamma RI (n = 24; mean = 36.1 %, SEM = 4.2). No differences in genetic polymorphisms of Fc gamma RIIa and IIIb between both CVID subgroups were found. The clinical state (number of pneumonias before the diagnosis, occurrence of splenomegaly, and outcome to the standard therapy with IVIG and antibiotics) in patients with high expression of CD64 on the granulocytes was significantly worse than in persons with low expression.

Our results suggest a relationship between the clinical picture and the expression of Fc gamma RI on granulocytes in CVID patients treated.

Keywords

Immunodeficiency, Clinical course, Fc gamma receptors, Common variable immunodeficiency, Intravenous immunoglobulin
INTRODUCTION

Common variable immunodeficiency (CVID) is the most common symptomatic primary antibody deficiency syndrome characterised by decreased levels of serum immunoglobulin and reduced antibody production. Patients with CVID suffer from a variety of respiratory tract infections. The spectrum of infections is usually rather limited. The most common pathogens are non-capsulated *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and mycoplasmas. Enlarged lymphnodes and spleen may reflect a persistence of low-grade infections. The character of observed infections and inflammatory processes suggests that the deficiency of antibodies may be compounded by the failure in phagocytic functions (1–2).

Intravenous immunoglobulin replacement is a standard treatment of the CVID. The factors that influence the effect of this therapy are not elucidated and it should be supposed that the failure in phagocytic immunoglobulin receptors may be important (3).

The Fc gamma receptors (FcγR) provide a critical link between the humoral and cellular parts of the immune system (4–6). The recognition of IgG by these receptors results in activation or inhibition of various effector functions of immune cells (phagocytosis, release of toxic oxygen metabolites, production and secretion of cytokines, modulation of cell proliferation and differentiation). Three distinct classes of FcγR differing in molecular size, cellular distribution and functions have been defined on leukocytes in humans: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). The high affinity receptor CD64 is capable of binding monomeric IgG and the low affinity receptors CD32 and CD16 interact preferentially with complexed IgG. In peripheral blood, CD64 is expressed constitutively by monocytes and its expression in polymorphonuclears occurs only upon activation. CD32 is present on the plasma membrane of all phagocytes as well as on some B cells, CD16 is found on phagocytes, eosinophils, and natural killer cells (4–5).

The present study was performed to measure the expression of receptors for IgG (FcγRI, II, III) on peripheral blood leukocytes in correlation with the clinical course of CVID patients.

MATERIALS AND METHODS

Patients

We investigated 42 patients (14 females, 28 males, aged 10–77 years, mean 43) and 44 age- and sex-related control healthy persons after approval by the Ethical Committee at the Faculty Hospital of Brno. The severity of the disease, the clinical course and the effectivity of standard intravenous immunoglobulin (IVIG) therapy (Endobulin, Baxter Czech, Praha, Czech Republic) were assessed by the same physician.

Flow cytometric measurement of surface antigen expression: Peripheral blood was collected into vials coated with EDTA. The expression of CD64, CD32, and CD16 on polymorphonuclear cells and monocytes was measured by flow cytometry in whole blood. All the antibodies used in this study were mouse-derived monoclonal antibodies labelled with fluorescein isothiocyanate (FITC), phycoerythrin.
(PE), and with PE-tandem dyes ECD (PE-Texas Red) and PC5 (PE-Cy5). The following reagents were purchased: specific to CD3, CD4, and CD8 (Coulter, Fullerton, CA), CD64 (DakoCytomation, Praha, Czech Republic), CD32 and CD16 (Immunotech, Marseille, France). Flow cytometric analysis was performed using a Coulter Epics MCL-XL analyser. The percentages of the selected cell populations stained were determined on an average of ca. \(10^4\) cells.

Gene polymorphism of FcgRIIa and FcgRIIIb was determined by polymerase chain reaction (PCR). The clinical course was evaluated as a score with the occurrence of pneumonias, number of infections during a year, response to the IVIg and antibiotic treatment, and the development of splenomegaly.

For statistical evaluation of the difference between patients and controls the T-test and Mann-Whitney test were employed. P-values of less than 0.05 were considered to be significant.

RESULTS

The expression of CD16, CD32, and CD64 on lymphocytes and monocytes in patients with CVID was comparable to healthy control persons, as the expression of CD16 and CD32 on granulocytes in both groups (Fig. 1). The expression of CD64 on granulocytes (FcgRI) was low in the healthy volunteers (n = 44, mean = 4.5 %, s.e.m. = 0.6 %) (Fig. 1).

As concerns CD64, the group of CVID patients could be divided into two subgroups. The first was comparable to the control group (n = 17, mean = 4.8 %, s.e.m. = 0.6 %), but in the second the expression of CD64 was highly elevated (n = 25, mean = 36.8 %, s.e.m. = 4 %, p < 0.0001) (Figs. 2 and 3). No differences in genetic polymorphisms of FcgRIIa and FcgRIIIb between both CVID subgroups were found.

The clinical state of CVID patients was objectively evaluated with the number of pneumonias before the diagnosis, the occurrence of splenomegaly, and the response to the standard treatment with intravenous immunoglobulin (Fig. 2). In patients with high expression of CD64 on the granulocytes the clinical state was significantly worse than in persons with low expression of this receptor (Fig. 3).

DISCUSSION

Primary immunodeficiency diseases including those with impaired antibody production, e.g. common variable immunodeficiency (CVID), X-linked agammaglobulinemia (XLA), are relatively rare but require difficult and expensive treatment. As no causative therapy is available in those diseases, a lifelong immunoglobulin replacement is necessary. Almost exclusively intravenous immunoglobulins (IVIG) are used at present (1–2).

A long-term follow-up of those patients in our department and others (7–10) showed that the real clinical effect of the treatment with IVIG (in the same scheme) may be markedly different in patients with the same diagnosis. Although the majority of the patients do well on standard (400 mg/kg/4 weeks) IVIG doses, other patients have frequent/complicated infection even on relatively high replacement of IVIG doses. Immunoglobulins interact through the Fc fragment with the membrane
Fig. 1
The expression of FcgR (CD16, CD32, CD64) on granulocytes, monocytes and lymphocytes. Flow cytometric analysis was performed with monoclonal antibodies as described in Materials and Methods.

Fig. 2
Evaluation of clinical state. Number of pneumonias before the diagnosis, occurrence of splenomegaly (score: 1 – with splenomegaly, 0 – without splenomegaly) and response to the standard treatment with intravenous immunoglobulin (score: 1 – very good, 2 – good, 3 – poor) were evaluated.
receptor (FcR) and have important influence on cells of the immune system. The Fc fragment of the immunoglobulin molecule triggers a broad spectrum of biological effects (4). It is evident that FcR can modulate the response of the cells of the immune system to exogenously administered intravenous immunoglobulins (11–12). Moreover, the regulatory function of FcR has an influence on the phenotype of infectious and autoimmune diseases and FcR stands out as a potential therapeutic target (4, 5). Activation of phagocytic cells through Fc receptors triggers a functional cascade with a consequent respiratory burst, release of intracellular granules, cellular cytotoxicity, and activates various genes with successive protein synthesis, including cytokines (4). The functional activity of FcR may be influenced also by other membranous structures (e.g. complement receptors) and humoral factors (cytokines) (5).

The other studies showed that FcgammaR genotype and its expression belong to predisposing factors which influence the clinical course of various diseases. They can also be important prognostic markers which can modify the treatment approach in some patients, but there is no analysis evaluating the relation of clinical manifestation of humoral immunodeficiency, membranous structures of the immune cells inclusive of FcR, and their importance for the effect of a replacement immunoglobulin treatment (12–14).

Our results suggest that the gravity of the clinical course of CVID patients and efficacy of intravenous immunoglobulin therapy may relate to the upregulation of the FcgRI (CD64) on their granulocytes. We found that the clinical state in patients with high expression of CD64 on granulocytes was significantly worse than in persons with low expression of this receptor and that the expressions of other

Fig. 3
The expression of FcgRI (CD64) on granulocytes and the clinical course of CVID patients.
Comparison between CVID subgroups and healthy controls was performed. P-values of less than 0.05 were considered to be significant.
Fc gamma receptors were comparable to healthy control persons. Pathogenic importance of this phenomenon is not obvious. The function of the FcγRs depends not only on their cellular expression patterns but also on whether they transduce activating or inhibitory signals. An aberrant regulation of the FcγR system might be involved in the progression of immunodeficiencies and might impact the efficacy of immunoglobulin therapy of these diseases.

Acknowledgement

Supported by the grant No. NI/7138–3 of the Ministry of Health, Czech Republic.

Thon V., Vlková M., Freiberger T., Litzman J., Lokaj J.

EXPRESE FC GAMA RECEPTORŮ PRO IGG NA LEUKOCYTECH A KLINICKÝ STAV U PACIENTŮ S BĚŽNOU VARIABILNÍ IMUNODEFICIENCÍ (CVID)


Receptory pro Fc-fragment IgG (FcγR I - CD64, FcγR II - CD32, FcγR III - CD16) propojují protilátkové a buněčné struktury imunitního systému. FcγR I, IIa, IIIa jsou aktivační, FcγR IIb má funkci inhibiční, což se projevuje především na lymfocytech a profesionálních fagocytech. Signály, které jsou těmito receptory přenášeny, mají vliv na imunologickou reaktivitu, ale mohou také ovlivňovat účinnost gamaglobulinové terapie. Studovali jsme proto vztah mezi klinickým stavem pacientů s CVID a receptory pro IgG (FcγR) na jejich leukocytech.

Vyšetřili jsme 41 pacientů (28 žen a 13 mužů ve věku 10–77 let) s diagnózou běžné variabilní imunodeficience (CVID) a 26 kontrolních zdravých osob. Expresi CD64, CD32 a CD16 na lymfocytech, granulocytech a monocytech byla sledována průtokovou cytometrií. Genový polymorfismus FcγR byl vyšetřen metodou PCR.

Expresi všech tří FcγR na lymfocytech a monocytech byla u pacientů a kontrolních osob stejná. Naproti tomu, zřetelné rozdíly jsme zaznamenali v expresi FcγR I (CD64) na granulocytech: u kontrolních zdravých osob byl počet granulocytů CD64+ nízký (4,7 %, SEM = 0,8), ve skupině pacientů s CVID bylo možno vymezit podskupinu srovnatelnou v tomto parametru se skupinou kontrolní (n = 17, 4,8 %, SEM = 0,6) a podskupinu s vysokým zastoupením granulocytů CD64+ (n = 24, 36,1 %, SEM = 4,2). Závažnost klinického stavu (počet pneumonií před stanovením diagnózy, splenomegalie) byla větší a odpovídavost na standardní intravenózní gamaglobulinovou terapii byla horší u pacientů s vysokým počtem granulocytů exprimujúcích CD64.

Naše výsledky ukazují, že závažnost klinického stavu a nižší odpovídavost na gamaglobulinovou terapii u pacientů s CVID pozitivně koreluje s expresi CD64 na granulocytech, což nepřímo svědčí o jejich aktivaci.

REFERENCES
