European Journal of Internal Medicine 20 (2009) e62 – e65

EUROPEAN JOURNAL OF INTERNAL MEDICINE

www.elsevier.com/locate/ejim

Original article

Selective defect of anti-pneumococcal IgG in a patient with persistent polyclonal B cell lymphocytosis

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Received 8 June 2008; accepted 19 August 2008
Available online 1 October 2008

Abstract

Background: Persistent polyclonal B cell lymphocytosis (PPBL) is a rare condition characterized by increased IgM and large excess of B cells with an IgD^+ $CD27^+$ phenotype. In normal individuals, these cells play a central role in the defense against pneumococcal infection. So far, few studies have characterized humoral immune responses in PPBL patients. We therefore measured IgG directed against *S. pneumoniae* antigens in a 51 yr-old woman with PPBL before and after vaccination with a pneumococcal 23-valent polysaccharide vaccine.
Methods: Antibodies against pneumococcal antigens were measured first with an overall immunoassay using microplates coated with the 23-valent pneumococcal vaccine. A serotype-specific test was also performed according to the WHO consensus protocol.
Results: Despite a large number of IgD^+ $CD27^+$ cells, our patient had low baseline titers of IgG directed against pneumococcal antigens and did not significantly respond to a 23-valent polysaccharide vaccine against *S. pneumoniae*. On the contrary, she had good titers of IgG directed against tetanus toxoid.
Conclusion: IgM^+ IgD^+ $CD27^+$ cells which accumulate in this patient with typical PPBL patient failed to perform IgG isotype switch after a polysaccharide vaccine. The potential mechanisms and relationships with the main features of PPBL are discussed. Further studies on a larger number of similar patients are needed.

Keywords: Immunoglobulin class switching; B lymphocyte; Lymphocytosis; Pneumococcal vaccines

1. Introduction

Persistent polyclonal B cell lymphocytosis (PPBL) is a rare condition characterized by increased numbers of B cells with abnormal binucleated forms and high levels of polyclonal IgM [1,2]. This condition mainly affects smoking women with the HLA-DR7 haplotype [3]. The expansion of B cells is associated with an abnormal resistance to apoptosis and with multiple bcl-2/Ig gene rearrangements [4,5]. Most of the accumulating B cells have an IgM^+ IgD^+CD27^+ phenotype [6,7]. In normal conditions, B cells expressing IgD and CD27 are usually considered as IgM memory B cells [8]. An important function is to secrete high affinity IgM directed against polysaccharide antigens during the early stages of infection [9]. Accordingly, IgM^+ IgD^+CD27^+ play a major role in the control of pneumococcal infections [10,11] and their decrease in aging patients [12] or in pathologic conditions such as common variable immunodeficiency [13] or HIV infection [14] is correlated with a higher risk of severe pneumococcal infections. The role of IgM^+

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doi:10.1016/j.ejim.2008.08.005

IgD^+CD27^+ B cells in the response to polysaccharide antigens is not limited to the early secretion of high affinity IgM. It has been shown that these cells also have a high IgG switch capacity after T independent stimulation [15]. Therefore they are the main B cell subset responsible for the production of IgM and IgG directed against pneumococcal antigens after vaccination.

Little is known about the function of IgM^+ IgD^+CD27^+ in PPBL patients. These cells clearly have initiated Ig V gene somatic mutations [7] but there is no evidence of positive antigenic selection following somatic hypermutation. This suggests a failure to eliminate low affinity IgM^+ IgD^+CD27^+ B cells [16]. In the only functional study published so far, Himmelman et al. showed that B cells from PPBL patients produce high levels of IgM after *in vitro* stimulation with IL-2 and *Staphylococcus aureus* Cowan strain I [6].

In the present report we describe a typical case of polyclonal B cell lymphocytosis in a 52 yr-old women with a strong increase of IgM^+ IgD^+CD27^+ B cells. In view of the central role of IgM^+ IgD^+CD27^+ B cells in the defense against pneumococcal infection, we investigated IgG responses to several pneumococcal serotypes before and after vaccination with a 23-valent non conjugated vaccine. Interestingly, the patient presented with very low titers of IgG directed against all pneumococcal serotypes and did not respond against most serotypes of the vaccine despite good titers of antibodies against protein antigens such as tetanus toxoid.

2. Materials and methods

The patient gave an informed consent before the vaccination procedure. Flow cytometry was performed with the BD FACSCalibur system and BD monoclonal antibodies. Anti-pneumococcal IgG titers were determined by two different enzyme-linked immunosorbent assay (ELISA): an overall assay measuring IgG against the 23 serotypes present in the 23-valent polysaccharide pneumococcal vaccine (PPV-23) and a serotype-specific assay. These assays were described elsewhere [17]. Briefly, diluted sera (1/10 and 1/100) were first pre-incubated with free pneumococcus cell wall polysaccharide (25 μg/ml) and serotype 22F (10 μg/ml) (ATCC) for the absorption of non-specific antibodies. The overall assay was performed with microtiter plates coated overnight with 10 μg/ml of the 23-valent pneumococcal vaccine. After washing and blocking the plates, peroxidase-labeled anti-human IgG was added at 1/5000 followed by the addition of tetramethyl-benzidine. The total anti-pneumococcal IgG titers were calculated by measuring the absorbance at 450 nm and by converting these in arbitrary units (AU) by reference to a calibration curve that was made by serial dilutions of a reference pooled serum. A level less than 11 AU/ml was considered as a low antibody concentration. Serotype-specific (serotypes 1, 7F, 14, 19F and 23F) pneumococcal IgG were measured by ELISA according to the WHO consensus protocol which use an international reference serum (89-S, FDA). The cut-off values for significant antibody titers were based on the results obtained with the serum of non-immunized children and were 100 ng/ml for serotype 1, 200 ng/ml serotype 7F and 23F, 500 ng/ml for serotype 19F and 700 ng/ml for serotype 14. For the determination of anti-tetanus toxoid antibody titers, diluted sera (1/200 and 1/2000) were incubated on Tetanus Toxoid coated plates (1/5000) (TT, Staten Serum Institute, Copenhagen). After incubation and washing, fixed IgG were revealed by the addition of peroxidase-labeled anti-human IgG and the absorbance was measured at 450 nm. The anti-TT IgG levels were calculated by reference to a standard curve established by serial dilutions of a preparation of human tetanus immunoglobulins with a know concentration of anti-TT IgG (Tetabulin 250, Baxter Bioscience). An anti-TT level above 0.01 UI/ml was considered as protective.

3. Case description

This is a 51 yr-old woman who was referred to our hospital for a suspicion of lymphoproliferative disease. She had a marked increase of B cells (57% or 2462 cells/μl) and of IgM (5.9 g/l). Her medical history revealed a hereditary angioedema with a low level of C1 esterase inhibitor (no genetic analysis was performed) and several episodes of lymphocytic infiltration of the skin (Jessner Kanof disease). She is a heavy smoker since she is 17 (more than 50 pack-years) and does not drink alcohol. The patient complaints of asthenia and diffuse pain evoking

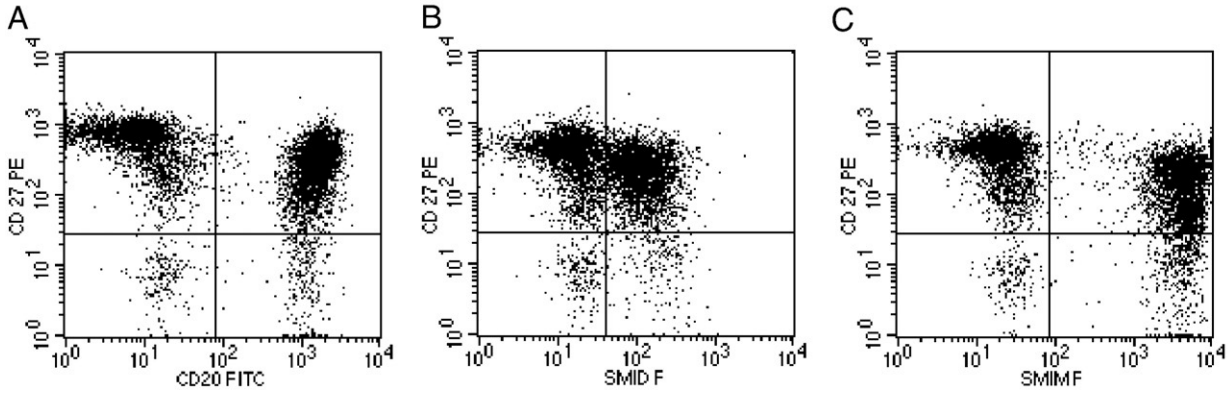

Fig. 1. Dot plots illustrating the accumulation of B cells in our PPBL patient. The three dot plots correspond to the same total lymphocyte gate. (A) illustrates the increased proportion of $CD20^+$ (B cells) most of which have a $CD27^+$ phenotype. The other dot plots show that more than 70% of these B cells have a IgD^+ (B) and IgM^+ phenotype.