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Case report

Functional T-cell anergy in a case of persistent polyclonal B-cell lymphocytosis

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Abstract

The T-cell population of a patient with persistent polyclonal B-cell lymphocytosis (PPBL) presenting with an intermittent Epstein-Barr virus (EBV)-associated disease was studied. Unstimulated T-cells did not express CD40 ligand (CD40L), whereas activation with IL-2 led to expression of this costimulatory molecule. CD40L expression was inhibited upon incubation with the supernatant of an EBV-positive B-cell line (SM) which had been grown spontaneously from the patient's peripheral blood cells. The supernatant of SM cells effectively inhibited cytotoxic T-cells. Elevated levels of IL-10, TNF-alpha and soluble CD40 were found in the supernatant of SM cells. Additionally, enhanced levels of LMP-1 protein were detected.

Keywords: Persistent polyclonal B-cell lymphocytosis; CD40; CD40 ligand; LMP1; EBV

1. Introduction

Persistent polyclonal B-cell lymphocytosis (PPBL) is a rare B-cell disorder characterized by a mild leukocytosis with an absolute and persisting B-cell lymphocytosis [1–5]. Morphologically, some of the B-cells are binucleated or bilobated and the B lymphocytes produce polyclonal immunoglobulins of the IgM type. The majority of PPBL patients express an HLA DR 7 type, are cigarette smokers and most patients are female, whereas symptomatic male PPBL patients seem to be rather rare [1–3]. Cytogenetic abnormalities, such as trisomy 3 and an additional chromosome long arm i(3)(q10) were repeatedly found in PPBL patients suggesting that this lymphoproliferative syndrome is associated with a higher frequency of chromosome 3 instability [4]. Furthermore, PPBL patients have been shown to present multiple Bcl2/JH gene rearrangements [5], whereas no monoclonal rearrangement of immunoglobulins could be detected using a sensitive PCR technology [6].

Several different causative agents have been suggested to be involved in the pathogenesis of PPBL. First, the simultaneous occurrence of PPBL in HLA identical twins, the presence of binucleated lymphocytes in related family members and the HLA DR 7 expression in the majority of PPBL patients all strongly point towards a genetic predisposition [6–8]. Second, nearly all PPBL patients with HLA DR7 expression are heavy smokers, and therefore a relationship between cigarette smoking and PPBL has been discussed since the first description [9–11]. Indeed, cessation of smoking sometimes leads to a decrease in lymphocytosis and IgM production [12]. Finally, Epstein-Barr virus might contribute to some of the cases because serological, PCR and in situ hybridization studies [10,13–16] all demonstrated the presence of this herpes

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virus in the B-cells or in the serum of PPBL patients. In addition, there is evidence for a higher replicative activity of certain EBV phenotypes at least in some virus isolates [17]. In a more recent study, the EBV-encoded oncoprotein LMP1 has been detected in all PPBL patients tested and was discussed as possibly being involved in the observed dysfunction of PPBL derived B-lymphocytes. Additionally, defects in the CD40 signalling pathway have been observed in this study although neither a defect in CD40 structure nor abnormalities in the early signalling cascade have been found [18].

Of interest, X-linked hyper-IgM syndrome (X-HIM) type 1 is caused by mutations of the gene encoding CD40 ligand (CD40L) and has been described as being associated with immunodeficiency and T-cell anergy [19]. Patients with X-linked lymphoproliferative disease are characterized by symptoms of immunosuppression with susceptibility to opportunistic infections [20,21]. Moreover, an extreme vulnerability to EBV has been shown in X-HIM type 1 [22].

In contrast to the X-HIM type 1, PPBL patients are mostly females and there is no clear evidence so far that they regularly suffer from recurrent infections, although a recent publication suggested that also PPBL may be associated with varying minor clinical features of immunodeficiency [12]. The fact that our case presented with intermittent symptoms of a chronic EBV syndrome [15] also suggests that some PPBL cases may be associated with different grades of severity of immunosuppression.

Since some clinical and laboratory characteristics of PPBL, namely the clinical features of immunodeficiency and the overproduction of polyclonal IgM, are similar to the X-linked hyper-IgM syndrome type 1, caused by a defective CD40L/CD40 interaction, the aim of this study was to examine a possible CD40L/CD40-related pathological mechanism in PPBL. As an experimental model we used purified T-cells from a PPBL patient and the B-cell line SM which had been grown spontaneously from the same PPBL patient's peripheral blood mononuclear cells. SM cells not only show similar characteristics as the atypical PPBL B-cells [16] but also have previously been shown to harbour an EBV with a 69bp deletion of the LMP1 gene, which has been suspected to play a role in lymphoproliferative disorders (for review see [23]). Furthermore, we investigated the role of different cytokines for the pathogenesis of our case of PPBL.

2. Materials and methods

2.1. Cells

Peripheral blood mononuclear cells (PBMC) from a 30-year-old woman, who presented with a typical PPBL with intermitting symptoms compatible with a chronic fatigue syndrome, recurrent erythema nodosum and multiforme [15] were isolated by density gradient centrifugation [16]. Cells were cultured in RPMI-1640 medium (BioWhittaker, Walkersville, MD, USA) supplemented with 10% fetal calf serum, L-glutamin and antibiotics without suppression of T-cell response. Cells were kept in culture until spontaneous outgrowth of a permanent EBV-positive B-cell line after 2 month. The resulting cell line was referred to as SM cell line [16].

As controls lymphocytic cell lines (LCL) were used, which had been produced by spontaneously outgrowth of PBL from healthy donors infected with wild-type EBV in vitro.

2.2. Immunophenotyping of PPBL patient's peripheral blood mononuclear cells and SM cells

Immunophenotypic analysis of the patient's peripheral blood mononuclear cells (PBMC) which were isolated from heparinized blood samples by density gradient centrifugation (LeucoSep, Arnika, Milan, Italy) and SM cells grown in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamin and antibiotics (Life Technologies, Paisley, Scotland, UK) was performed as described in [15] with the following monoclonal antibodies (mAb) conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE), respectively: CD3, CD4, CD5, CD8, CD10, CD16, CD19, CD20, CD21, CD22, CD23, CD25, CD54, CD56, CD80, HLA DR, FMC7 all from Becton Dickinson (Mountain View, CA, USA), anti-CD138 (BB4) from IQP (Coulter/Immunotech, Milan, Italy), unconjugated anti-CD40L (clone MK13A4) from Bender Medsystems (Vienna, Austria), anti-CD40L (clone TRAP1) and anti-CD40 from Pharmingen (San Diego, CA, USA). 20,000 cells were analysed using a FACScan (Becton Dickinson) equipped with an argon laser.

2.3. Cytokine measurements in PPBL patient's serum and supernatant of SM cells

Cytokine measurements in PPBL patient's serum and supernatant of SM cells were performed using commercial IL-4 (BioSource, Camarillo, CA, USA), IL-10 (CLB, Amsterdam, The Netherlands), IL-12 (Biomedica Vienna, Austria), IL-15 (BioSource), TNF-alpha (BioSource), and Interferon gamma (BioSource) enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Human sCD40L was measured using an ELISA system of Bender Diagnostics (Vienna, Austria) and human sCD40 using an ELISA method described earlier [24]. For control, serum samples of 10 healthy blood donors and supernatants of EBV-positive Burkitt's lymphoma cell line Raji and EBV-negative B-cell lymphoma cell line BJAB grown in RPMI 1640 medium with 10% FCS, L-glutamin and antibiotics as well as the cell culture medium alone were tested.

2.4. Purification of T-cells from PPBL patient

PBMC of the PPBL patient were obtained by LeucoSep density gradient centrifugation. Briefly, B-cells were removed by negative selection using a cocktail of anti-CD19