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Production and characterization of a novel monoclonal antibody against progesterone-induced blocking factor (PIBF)

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Abstract

Progesterone-induced blocking factor (PIBF) has been described as an active factor intimately involved in regulation of the immune response in pregnancy. It has been shown that PIBF biased the cytokine balance to Th2-type in pregnancy and inhibited the activity of NK cells. The biological roles of PIBF would be better defined if methods for its detection and measurement in biological fluids are available. However, so far, reliable antibodies have not been developed to be used as specific probes. A monoclonal antibody designated as MAB 3A6 was produced and characterized. MAB 3A6 reacts specifically with PIBF. It can detect this protein in biological fluids when tested by immunoblot and recognizes PIBF expressed on the surface of lymphocytes of pregnant women stimulated *in vitro* with progesterone. The characteristics of MAB 3A6 makes it the possible basis for development of a clinically applicable assay to assess the presence and concentration of PIBF in biological samples.

Keywords: Progesterone-induced blocking factor; Monoclonal antibody; Quantitative assay

1. Introduction

Progesterone-induced blocking factor (PIBF) was described initially by Szekeres-Bartho et al. (1985, 1989a) as an active factor intimately involved in regulation of the immune response in pregnancy. Subsequently, it has been shown that binding of antibodies to the γδ T-cell receptor expressed by γδ CD8+ T cells down-regulated the production of PIBF and increased NK cell activity (Polgar et al., 1999). This would suggest that γδ T cells are either the source of this immunomodulatory factor or in some way control its secretion. The hypothesis suggested by the authors is that activated γ/δ CD8+ lymphocytes express *de novo* functional progesterone receptors (PR) on their surface. Detailed investigations have shown that PIBF enhanced the secretion of IL-3, IL-4 and IL-10 by spleen cells of mice when cultured *in vitro*, while the production of INF-γ was not modified in comparison to controls (Szekeres-Bartho and Wegmann, 1996). Kozma et al. (2006) reported that the effect of PIBF is mediated via a novel IL-4 receptor which consists most probably of IL-4R α-chain and the PIBF receptor which is a GPI-anchored protein. The activation signal induced phosphorylation and nuclear

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translocation of STAT-6 and inhibition of the STAT-4 pathway.

Since it has been proven that PIBF induces dominance of the Th2 immune response, it is tempting to hypothesize that the protein may have a role in anti-tumor immunity and this has attracted the attention of a number of researchers. Srivastava et al. (2004) detected the presence of PIBF mRNA in tumor cell lines, thus suggesting that PIBF might be a novel target for immunotherapy.

It is necessary to use specific antibodies as probes to detect the expression of PIBF by normal and/or malignant cells, or to detect its presence in biological fluids. To this end, using polyclonal antibody against lymphocyte-secreted PIBF to test the product of an identified cDNA clone Polgar et al. (2003) have shown that PIBF can be detected in at least two forms—a full-length protein (∼90 kDa) and located in the nucleus, and a short-length form of 34 kDa detected in the cytoplasm. The biological activity seems to be mediated by the N-terminal part of the protein molecule, as shown by experiments of the same research group. In these studies, the authors have used polyclonal antibodies against secreted 34 kDa protein, or fusion 89-recombinant PIBF-GST protein, and monoclonal antibodies produced against 48 kDa recombinant polypeptide. However, it is not clearly stated whether these monoclonal antibodies reacted against the secreted PIBF detected in human biological fluids. Despite numerous attempts, there is no antibody produced so far which can be applied in development of diagnostic tests for detection of PIBF in biological fluids.

The aim of our experiments was to produce a monoclonal antibody which would react specifically with PIBF in tissue lysates and biological fluids. The results presented here demonstrate that the monoclonal antibody designated as MAB 3A6 reacts with PIBF in different tests, such as immunoenzyme assay, immunochemistry, Western blot and flow cytometry, which makes it suitable as a basis for the development of clinically applicable tests for assessment of PIBF in patients.

2. Experimental procedures

2.1. Subjects

Peripheral blood (7 ml) was collected by vein puncture from women with normal pregnancy at 4–6 l.m. ($n = 11$) and non-pregnant women ($n = 24$) after signed informed consent was obtained in accordance with the recommendations of the Ethical Commission of Ob/Gyn Hospital 'Dr. Shterev', Sofia, Bulgaria.

2.2. Biological samples

Urine samples were collected from women with normal pregnancy (4–6 l.m.) ($n = 10$) and terminal prostate carcinoma patients ($n = 7$), and immediately tested by Western blot for the presence of PIBF. Paraffin-embedded tissue sections (five samples) were prepared for routine histochemistry from term placenta collected immediately after delivery. Similarly, tissue sections (four samples) were prepared from early placenta (8–10 gestational weeks) after abortion for social reasons.

2.3. GST-PIBF fusion protein

Glutathione *S* transferase-PIBF fusion protein was isolated from *E.coli* strain cells transformed with a GST-PIBF vector kindly donated by Prof. J. Szekeres-Bartho (Pecs University Medical School, Pecs, Hungary).

2.4. Antibodies

A sample of polyclonal anti-PIBF antibody was kindly donated by Prof. Szekeres-Bartho and used as control antibody in all experiments to characterize the selected monoclonal antibody. Additionally, a rabbit polyclonal antibody was produced after injection of rabbits with GST-PIBF following the procedure used in the laboratory and used as a positive control antibody in the experiments.

Monoclonal antibodies were produced after immunization of mice with GST-PIBF. Each animal received 5 s.c. injections of 10 μg GST-PIBF each; the first was in complete Freund's adjuvant and the next injections were with incomplete Freund's adjuvant (Sigma–Aldrich, St Louis, MO, USA). Animals were bled through puncture of the retro-orbital plexus and the sera obtained were tested for reactivity against GST-PIBF. Splenocytes from positively reacting animals were fused with P3U1 cells as described by Kyurkchiev et al. (1988). Supernatants from wells with growing hybridoma cells were simultaneously tested against PIBF-GST or GST by ELISA. Selected 3A6 hybridoma was found to be reacting against GST-PIBF, but was negative against GST. This hybridoma was further cloned by limiting dilution, grown in mass culture and aliquots frozen for storage in liquid nitrogen. The antibody secreted by hybridoma 3A6 was shown to be of the IgG isotype.

2.5. Peripheral blood mononuclear cells (PBMC)

PBMC were isolated from blood collected by vein puncture with 7 ml vacutainer with LH 119 I.U. (Bec-