Institute of Experimental Morphology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 13
Sofia • 2008

Immunocytochemical Localization of Antigen Involved in Sperm-Zona Pellucida Interaction

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Previously we have produced Mab 4B12 which recognized a surface membrane-associated protein located in the acrosome portion of the boar capacitated spermatozoa with role in a primary sperm-zona pellucida binding. The objective of the present study is to determine the tissue specificity of Mab 4B12 cognate antigen. The reaction of Mab 4B12 against saline extracts of boar reproductive and somatic organs was examined by indirect ELISA and immunoperoxidase technique. The ELISA results showed a dose-depended reaction of the antibody with testis as well as with caput, corpus and cauda epididymis, but not with somatic extracts used. Immunocytochemical analysis of paraffin-embedded somatic and reproductive boar tissue showed positive reaction on Leydig cell cytoplasm as well as on elongated spermatids. In the epididymis positive reaction was observed on the apical epithelium cells and on the spermatozoa in the lumen. Somatic tissue sections studied as well as HEP-2 cells were negative in immunoperoxidase test. The results showed tissue specificity of the 4B12 protein with possible doublet secretion in the testis and epididymis, proposing its significance in the fertilization.

Key words: sperm-egg interaction, monoclonal antibody, sperm antigens.

Introduction

Fertilization in mammals involves a series of specific interactions between ligand and receptor molecules on complementary gametes [6, 7]. In the majority of species these molecules are located on the limiting surfaces of spermatozoa and egg; in the case of spermatozoa, specifically on the plasma membrane overlying the acrosomal domain and in the case of eggs, on the zona pellucida (ZP) [5]. The identification of sperm receptors and the complementary molecules to which they bind in the zona pellucida is of a great importance for studying mechanisms of gamete recognition and initial binding processes during the first steps of fertilization.

Previously we produced a series of monoclonal antibodies (Mabs) against boar capacitated sperm [2] and we found Mab 4B12 to recognize a surface membrane-associated protein localized on the acrosomal portion of capacitated sperm, shared by

different animal species. In addition we showed protein 4B12 is not connected with sperm motility and secondary sperm-ZP binding as well as with acrosome reaction of spermatozoa, either spontaneous or induced [4]. The results from biological experiments demonstrated that protein 4B12 participates in the primary binding of sperm to ZP [3]. The present study was aimed on tissue specificity of Mab 4B12 cognate antigen.

Material and Methods

Tissue specificity of the 4B12 protein was studied by means of enzyme linked immunosorbent assay (ELISA) and immunocytochemistry. The reactivity of Mab 4B12 against boar reproductive and somatic organs as well as against somatic cell line HEP-2 was examined. Boar capacitated spermatozoa ($1\text{-}5 \times 10^6$) as positive control, boar seminal plasma as negative control (diluted 1:1 in bicarbonate coating buffer pH 9.6) or saline extracts (1mg/ml protein) of boar reproductive (testis and epididymis-caput, corpus and cauda) and somatic (spleen, stomach, kidney, muscle, lung, intestines, colon, liver, lymph node, heart) organs in PBS were coated onto PVC 96-well U-bottomed microtitre plates (Costar Ltd., USA) and the procedure for ELISA reported earlier [1] was applied. The optical density was read at Titertek ELISA reader at 492 nm. Pieces of the same tissues were fixed and embedded in paraffin. Tissue sections (5 μm) after deparaffinization and dehydratation as well as smears of HEP-2 somatic cell line were stained by immunoperoxidase technique. Endogenous peroxidase activity was blocked by incubation in 1.2% H_2O_2 in medium for 10 min. The non-specific binding was blocked by 5% normal goat serum. The tissues and smears were incubated with Mab 4B12 (culture supernatant) overnight at 4°C and with HRP- conjugated goat anti- mouse IgG at dilution 1:80 for 60 min. The reaction was developed with 0.1% DAB in the presence of 0.02% H_2O_2 The sections were counterstained with Harris's hematoxilyn and observed on Zetopan microscope (Reichert, Vienna, Austria). Control probes were examined using supernatant of nonspecific monoclonal antibody — Mab 3G2 against melanoma associated antigens.

Results and Discussion

ELISA of Mab 4B12 with saline extracts of boar somatic organs showed negative reaction. Strong concentration-dependent reaction of Mab 4B12 with positive control (boar capacitated spermatozoa) and no reaction with negative control (boar seminal plasma) was observed. A weak positive reaction with an antigen from stomach only was registered (Fig. 1). The ELISA results with saline extracts of reproductive organs showed a dose-depended reaction of the antibody with testis as well as with caput, corpus and cauda epididymis, the reaction being weakly exposed in comparison with control capacitated spermatozoa (Fig. 2). The tissue specificity of 4B12 protein was further confirmed by immunocytochemical analysis of paraffin-embedded sections of the boar somatic and reproductive tissues. In the testis weak staining of the Leydig cell cytoplasm as well as of elongated spermatids was detected (Fig. 3). In the epididymis weak positive reaction was observed on the apical epithelium cells as well as on the spermatozoa in the lumen (Fig. 4). Somatic tissue sections used were negative. The data that Mab 4B12 did not stain HEP-2 cells in immunoperoxidase test confirmed the lack of appreciable cross reactivity of antibody with antigenic determinants on somatic tissues.

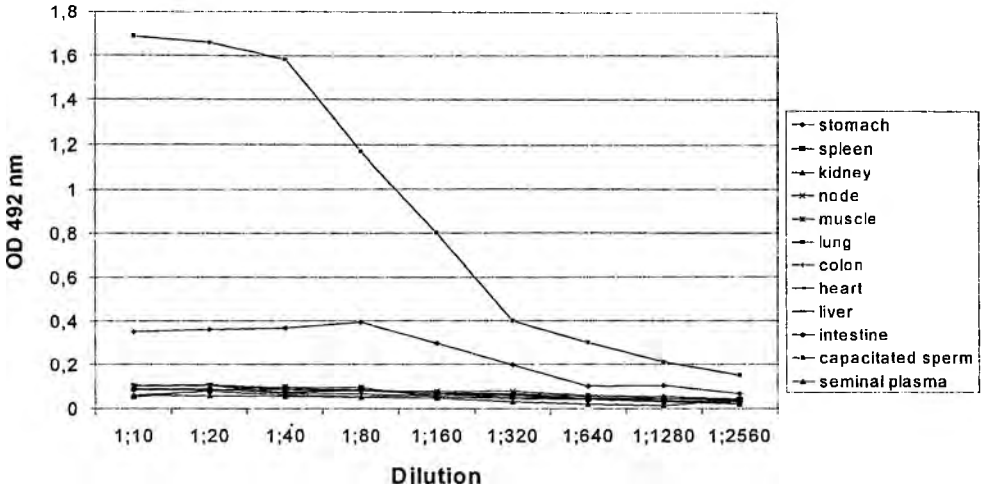

Fig.1. Negative reaction of Mab 4B12 with saline extracts of somatic tissues in ELISA is registered. Strong dose-depended reaction with positive control (capacitated spermatozoa) and weak positive reaction with an antigen from stomach only was observed

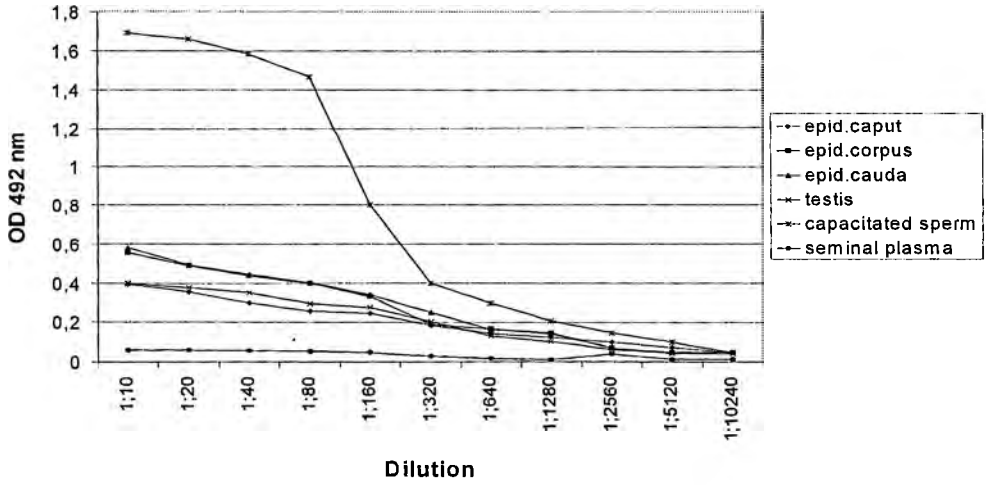

Fig.2. Dose-depended positive reaction of Mab 4B12 with saline extracts of boar reproductive tissues (testis and caput, corpus and cauda epididymis) is seen, the reaction being weakly exposed in comparison with positive control (capacitated spermatozoa)

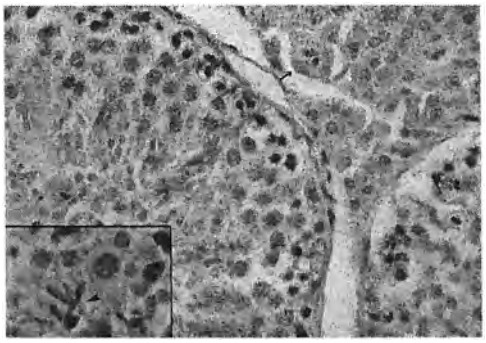

Fig. 3. Immunocytochemical analysis of boar reproductive tissues (testis) with Mab 4B12: labelling of the Leydig cell cytoplasm (arrow) as well as of the elongated spermatids was detected (arrowhead). × 480

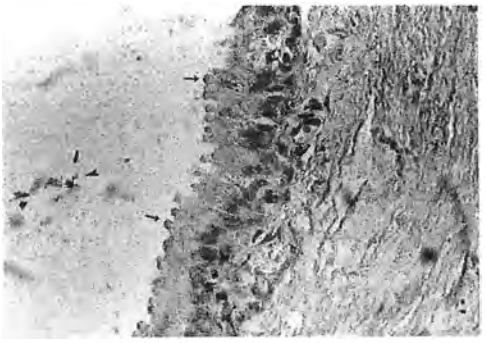

Fig. 4. Immunocytochemical analysis of boar reproductive tissues (cauda epididymis) with Mab 4B12: labelling of the apical epithelium cells (arrows) as well as of the caudal spermatozoa in the lumen was observed (arrowhead). × 320

The results on the positive, although weak reaction of Mab 4B12 with the testis and the epididymis but not with somatic tissues, obtained after the application of two different methods, suggest that the corresponding antigen seemed to be an intrinsic cell membrane protein secreted by Leydig and epididymal cells and connected with sperm maturation. Positive reaction of Mab 4B12 with both reproductive tissues and capacitated sperm may result from an unmasking and/or transformation of ligand (s) that is already present in the sperm and becomes accessible to antibody after plasma membrane modifications accompanying sperm capacitation. It is possible that 4B12 corresponding antigen is secreted by Leydig cells, transported to the Sertoli cell cytoplasm and bound to the sperm cell membrane during their terminal differentiation stage. The immunocytochemical staining of Leydig cells as well as of epididymal epithelial cells together with positive reaction of Mab 4B12 with an antigen from extracts of the reproductive organs tested in ELISA gives ground to assume doublet secretion of the antigen in the testis and epididymis, proposing its significance in the fertilization process.

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