Short Communication

Monoclonal Antibody to Human Sperm Acrosomal Protein

(monoclonal antibody / sperm acrosomal proteins)

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Abstract. A new monoclonal antibody designated Hs-14 was generated after immunization of BALB/c mice with the acid extract of human sperm. In indirect immunofluorescence Hs-14 mAb binds to the acrosome of permeabilized sperm cells and consequently recognizes some intra-acrosomal protein. Western blotting analysis revealed that under non-reducing conditions the Hs-14 mAb detects a protein with a molecular mass of 220 kDa. Under reducing conditions the Hs-14 recognizes several peptide bands within the range from 55 kDa to 110 kDa. Beside human sperm the antibody positively reacts also with sperm of some other mammalian species. Using Hs-14 mAb it is possible to evaluate the acrosomal integrity of spermatozoa and to reveal sperm pathology.

Several monoclonal antibodies to mammalian sperm acrosomal proteins have been generated and described. Some monoclonal antibodies recognize individual domains of the acrosomal region, i.e. the anterior acrosome, principal segment or equatorial segment of living cells. Other monoclonal antibodies bind to sperm after the permeabilization of their plasma membrane with aceton or methanol and recognize internal antigens, intra-acrosomal proteins. These antibodies react either with the acrosomal content or with the acrosomal membrane. Such monoclonal antibodies were obtained to serin proteases acrosin and proacrosin (Elce et al., 1986; Pěknicová and Moos, 1990; Gallo et al., 1991), but predominantly antibodies that bind to fixed acrosome-intact spermatozoa were generated (Jassim et al., 1993; Brucker et al., 1994; Jimenez et al., 1994; Yoshiki et al., 1995, 1996; Dorjee et al., 1997; Geussová et al., 1997; Yoshiki et al., 1997). In immunoblotting the corresponding antigens to these monoclonal antibodies showed bands at different molecular masses within the range from 16 kDa to 100 kDa.

The new monoclonal antibody (mAb) designated Hs-14 detects an intra-acrosomal protein and belongs to this group. The Hs-14 monoclonal antibody was obtained according to the protocol that we succesfully used for production of antibodies to boar sperm acrosin (Pěknicová et al., 1986; Pěknicová and Moss, 1990). BALB/c mice were immunized with lyophilized acid extract of human sperm dissolved in PBS. After the third immunization, fusion of the splenocytes with Sp2/0 myeloma cells followed. Positive clones were selected by ELISA with human sperm acid extracts and by indirect immunofluorescence (IIF) test with human sperm.

The specificity of Hs-14 mAb was studied by immunofluorescence and by the Western blotting technique. Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis was carried out on 12% slab gels according to the method of Laemmli (1970), and Western blotting was performed as described by Towbin et al. (1979).

Indirect immunofluorescence of human spermatozoa dried onto slides after their permeabilization with aceton showed the reaction of Hs-14 mAb with acrosomal cap region of the sperm head. The Hs-14 mAb strongly labelled the acrosome of about 60–90% of aceton-fixed ejaculated human spermatozoa (Fig. 1). Non-permeabilized intact sperm cells were not stained. Immunoblotting analysis revealed that under non-reducing conditions the Hs-14 mAb specifically detected a high molecular 220 kDa protein. Under reducing conditions the Hs-14 mAb recognized several distinct peptide bands within the range from 55 kDa to 110 kDa. A prominent signal was given by 55 kDa, 98 kDa and 110 kDa peptides (Fig. 2). As concerns the 220 kDa protein, this band is usually weaker or is completely missing in the reduced

Table 1. Cross-reactivity of Hs-14 mAb with sperm of various mammals

Species	Indirect immunofluorescence	Western blotting
human	+	+
boar	+	+
bull	+	-
mouse	+	ND[1]

[1]not done

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Abbreviations: IIF – indirect immunofluorescence, mAb – monoclonal antibody, SDS – sodium dodecyl sulphate.

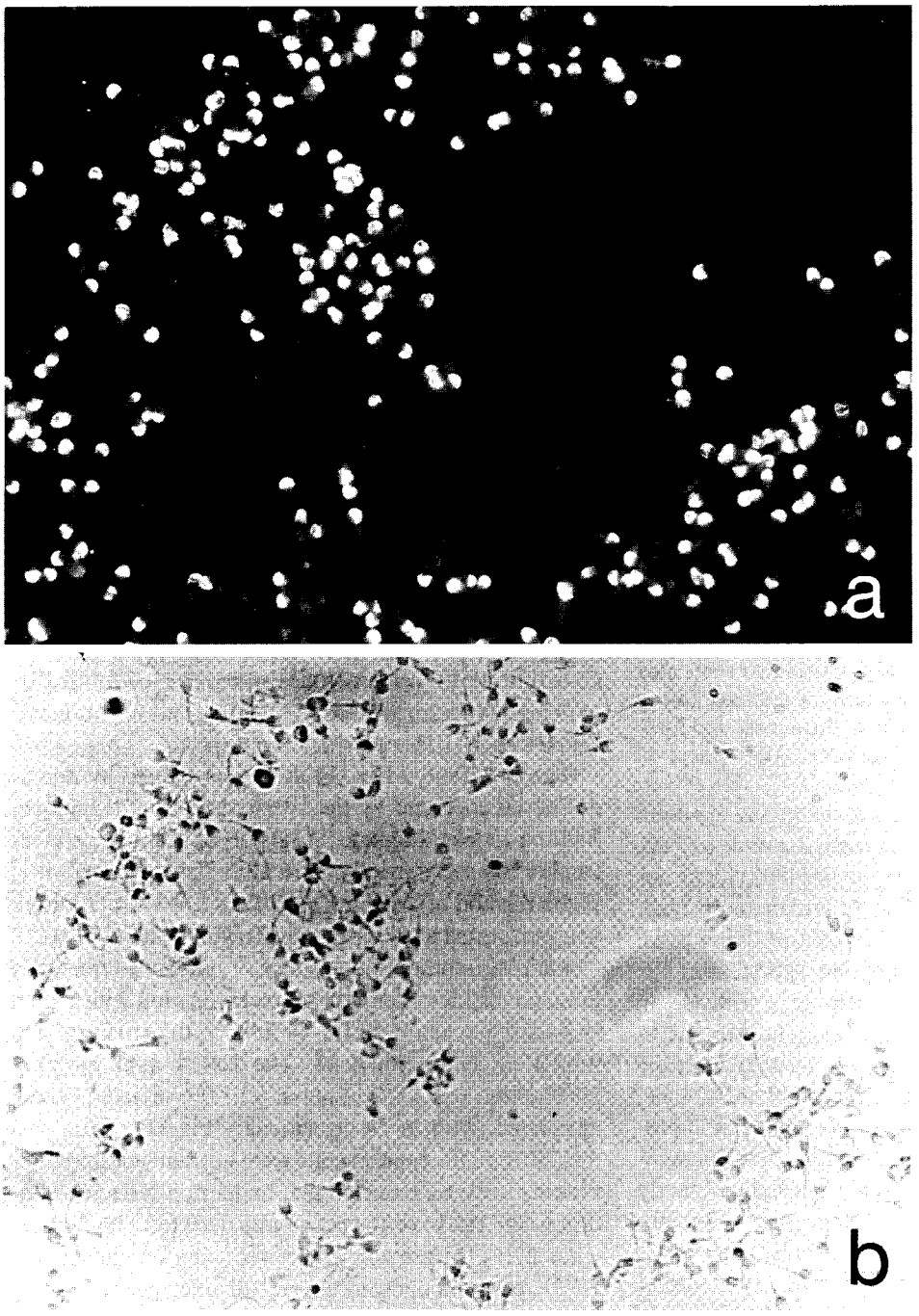

Fig. 1. Immunostaining of aceton-fixed human ejaculated spermatozoa with Hs-14 monoclonal antibody. (a) mAb intensively stained the acrosomes of sperm heads, (b) phase-contrast micrographs corresponding to the fluorescent image. Magnification 400x.

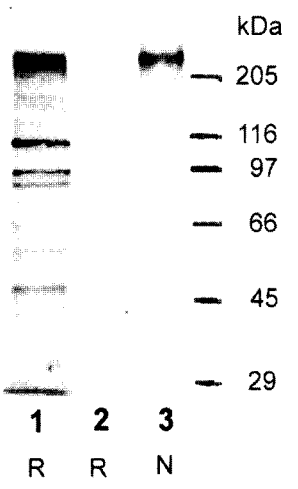

Fig. 2. Immunolabelling of proteins extracted by SDS from human sperm, run on 9% acrylamide gel and blotted to nitrocellulose. Lanes 1 and 3 were labelled with Hs-14 mAb and lane 2 (control) with supernatant from Sp2/0 myeloma cell line. Reactions were visualized with an enhanced chemiluminiscence kit (ECL) on X-OMAT LS film (KODAK). Molecular weight markers are shown on the right side. N – non-reduced sample, R – reduced sample.

state. Such reaction pattern of a reduced protein with an antibody on Western blot is not exceptional and there are other monoclonal antibodies against sperm which react similarly. Interspecies distribution of sperm protein(s) identified by Hs-14 mAb was tested on immunoblots of boar and bull sperm extracts and also in indirect immunofluorescence with bull, boar and mouse sperm cells (Tab. 1). We have found that in enhanced luminescence Western blotting the Hs-14 mAb reacts positively also with boar spermatozoa, and in IIF Hs-14 labelled not only human sperm, but also sperm of all other tested mammalian species, i.e. boar, bull and mouse. It suggests that the relevant antigen (protein(s)) is common and occurs in other mammalian species.

The Hs-14 monoclonal antibody was used to investigate the acrosomal integrity of human ejaculated spermatozoa before the acrosome reaction. We have found a significantly reduced number of spermatozoa labelled by Hs-14 mAb in human ejaculates with pathological spermiograms (Chládek et al., 2000). In assisted reproduction such spermatozoa were not able to fertilize the egg provided that intrauterine insemination and/or standard *in vitro* fertilization were used, but fertilization was achieved using the intracytoplasmic sperm injection method (Teplá et al., 1999). The

Hs-14 monoclonal antibody appears as a useful immunological reagent, which can serve not only for evaluation of sperm quality, but even for selection of suitable fertilization method in assisted reproduction.

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