Immunofluorescence localization of SP 10 protein on monkey spermatozoa during maturation

P. Sivashanmugam, John C. Herr* and M. Rajalakshmi

Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi, 110 029, India
*Department of Cell Biology and the Center for Recombinant Gamete Vaccinogens, University of Virginia, Virginia 22908, USA

SP 10 is a testis-specific acrosomal protein. Spermatozoa collected from the different regions of the epididymis and the ejaculate were analysed by immunofluorescence using MHS 10, the antibody to SP 10. In the rhesus monkey, majority of the spermatozoa from the initial segment, caput, corpus and cauda epididymides and the ejaculate showed immunolocalization of SP 10 only in the acrosome. During the epididymal transit of spermatozoa, the localization of SP 10 did not show any change. The presence of this antigen, in the acrosome of spermatozoa from both the rhesus monkey and the human shows that it is a conserved antigen and indicates that rhesus monkey can be used for the preclinical evaluation of SP 10-based vaccine.

SP 10 is a human testis-specific antigen arising during spermatogenesis[1]. Light and electron microscopical observations using MHS 10 monoclonal antibody derived against antigen SP 10, show that the antigen is detected first in the round spermatids at the Golgi phase of spermatogenesis and subsequently persists in the mature spermatozoa where it is associated with the acrosomal membranes[1]. Immunofluorescence studies using freshly ejaculated human spermatozoa have shown that more than 90% of the spermatozoa localized the antigen on the acrosome[2]. In the present study, the changes in the localization of SP 10 in the rhesus monkey spermatozoa during their epididymal transit were evaluated.

Three adult rhesus monkeys, *Macaca mulatta*, weighing 8–10 kg were procured from the wild and quarantined for three months. Ejaculated spermatozoa were collected by penile electrojaculation[3]. For the collection of epididymal spermatozoa, the animals were castrated under ketamine anaesthesia (10 mg/kg body weight). The epididymis was divided arbitrarily into the initial segment, caput, corpus and cauda epididymides[4]. Aliquots of spermatozoa from the different regions of the epididymis and the ejaculate were washed twice with phosphate-buffered saline (PBS; 75 mM $Na_2HPO_4 \cdot 2H_2O/KH_2PO_4$, 77 mM NaCl, pH 7.2). Spermatozoa were smeared on to glass slides, air dried and were fixed and permeabilized with methanol, The slides were rehydrated with PBS (30 min at 37°C) and then exposed to the blocking solution (1% BSA in PBS) for 1 h at room temperature, in a moist chamber. The blocking solution was removed and a uniform layer of MHS 10 antibody diluted with PBS (1:500) was added

Figure 1. Immunofluorescence localization of the SP 10 antigen on the acrosomal region of cauda epididymidal spermatozoa (× 1000).

and incubated for 1 h at 37°C. The excess antibody was removed by washing twice with PBS. The slides were exposed to FITC conjugated goat anti-mouse IgG (1:100) for 1 h, which acted as the secondary antibody, washed twice with PBS and wet mounted. The slides were observed under an epifluorescence illumination (*Laborlux S, Wild Leitz, GmbH, Germany) using* Ploemopak I 2 filter block (excitation filter BP 450–490 and suppression filter LP 515). The images were recorded on Kodak gold 100 ISO films.

Epididymal spermatozoa (88–94%) from all regions showed antigen localization only in the acrosome (Figure 1); but, a small percentage of spermatozoa (4–6%) did not show fluorescence. Further, 10% of caput and 4% each of corpus and cauda epididymal spermatozoa showed fluorescence only at the tip of the acrosome. Ejaculated spermatozoa (97%) showed antigen localization in the acrosome while 3% showed localization only at the acrosomal tip.

In the present study, the immunolocalization of SP 10 in the acrosome of majority of monkey ejaculated spermatozoa is similar to that in the human[1]. During epididymal transit, the localization of SP 10 antigen in rhesus monkey spermatozoa did not show any change, indicating that this is essentially a testis-specific antigen[1] which does not undergo changes during epididymal maturation. Similar results were observed by Western blot analysis of extracts of human caput and cauda epididymal spermatozoa, using MHS 10 antibody[5]. Further, Western blot analysis of the extract of spermatozoa from baboon (*Papio papio*), cynomolgus monkey (*Macaca fasicularis*) and the human, using MHS 10 antibody, showed fourteen distinct immunoreactive bands ranging in molecular weight from 18 to 34 kD, indicating the heterogeneity in the nature of antigens in the human and the non-human primate species[6]. This antigen shows minimal or absence of cross-reactivity with somatic cells. On the basis of this observation, Anderson *et al.*[7] designated SP 10 as a primary vaccine candidate for immunocontraception. The presence of this antigen, common to spermatozoa of the human and the rhesus monkey, indicates that this is a conserved antigen. This study also shows that the rhesus monkey can be used as non-human primate model to screen the vaccine developed against SP 10 antigen, since this antigen is conserved in the rhesus monkey.

1. Herr, J. C., Flickinger, C. J., Homyk, M., Klotz, K. and John, E., *Biol. Reprod.*, 1990, **42**, 181–193.
2. Herr, J. C., Wright, R. M., John, E., Foster, J. and Flickinger, C. J., *Biol. Reprod.*, 1990, **42**, 377–382.
3. Mastroianni, L. and Manson, W. A., *Proc. Soc. Exp. Biol. Med.*, 1963, **112**, 1025–1027.
4. Kaur, J., Ramakrishnan, P. R. and Rajalakshmi, M., *Anat. Rec.*, 1992, **234**, 62–72.
5. Foster, J. A., Klotz, K. L., Flickinger, C. J., Thomas, T. S., Wright, R. M., Castillo, J. R. and Herr, J. C., *Biol. Reprod.*, 1994, **51**, 1222–1231.
6. Herr, J. C., Wright, R. M. and Flickinger, C. J., in *Reproductive Immunology* (eds Mettler, L. and Billington, W. D.), Elsevier, Amsterdam, 1989, pp. 319–322.
7. Anderson, D. J., Johnson, P. M., Alexander, N. J., Jones, W. R. and Griffin, P. D., *J. Reprod. Immunol.*, 1987, **10**, 231–257.

ACKNOWLEDGEMENTS. This work was supported by grants from the Indian Council of Medical Research and the Council of Scientific and Industrial Research, Government of India. One of us (P.S.) is grateful to All India Institute of Medical Sciences for the award of Fellowship. MHS 10 was donated by Dr J. C. Herr.

Received 19 March 1996; accepted 1 May 1996.

Radium-228 in the Kaveri river ecosystem

P. Shahul Hameed, K. Shaheed and M. A. R. Iyengar*

Post-Graduate Department of Zoology, Jamal Mohamed College, Tiruchirappalli 620 020, India
*Health Physics Division, Bhabha Atomic Research Centre, Kalpakkam 603 102, India

In this article we present the results of a study aimed at investigating the radium-228 level in water, sediment and biota (plankton, weed, bivalve, prawn and fish) of the Kaveri river ecosystem extending to a stretch of 95 km. The results show a dissolved Ra-228 concentration in river water ranging from 4.43 to 7.67 mBq/l (mean: 6.1 mBq/l). The sediment samples recorded a Ra-228 activity of 15.1 Bq/kg. The aquatic organisms demonstrated differential accumulation of the radionuclide with enhanced bioaccumulation in shells and bones. The bivalve mollusc, *Lamellidens marginalis*, was identified to accumulate higher concentrations of Ra-228 in their soft tissues (0.92 Bq/kg) and shell (3.86 Bq/kg), suggesting that they could serve as a biomonitor of Ra-228 radionuclide in a riverine system. The concentration factors (CFs) calculated for the aquatic organisms ranged from ~10 to ~10^2. However, CFs observed in shells and bones were higher than in soft tissues and muscle. Gamma spectrometry of the primordial radionuclides indicated an elevated Th-232 activity (45 Bq/kg) than U-238 activity (15 Bq/kg) in Kaveri river sediment. The significance of the results of Ra-228 radionuclide in the environment of the Kaveri river is discussed.

AQUATIC organisms display considerable ability to accumulate toxic elements and radionuclides from water, although the concentration levels of the individual element or radionuclide in the water may be exceedingly small. Reviews on the bioaccumulation by organisms of