CD8-like Molecules on Human Spermatozoa

(CD8-like molecules / CD4-like molecules / human ejaculated spermatozoa / leucocytospermia / globozoospermia / human subfertility / human infertility)

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Abstract. The object of the present study was to investigate whether there were differences in the presence of CD8-like molecules on human ejaculated spermatozoa from fertile donors and subfertile patients (with leucocytospermia). In our previous report we defined CD4-like molecule heterogeneity on normozoospermic and globozoospermic human spermatozoa. In this investigation the results from indirect and absorption ELISA, as well as the indirect IEM and IIF findings, demonstrated the presence of CD8 immunopositive spermatozoa in all samples studied. The ELISA data showed that anti-human MoAb CD8 recognized an epitope common to the human spermatozoa with normal morphology and foetal thymocytes. During absorption experiments MoAb CD8 was preincubated with spermatozoa and allowed to react with thymocytes. A significant decrease of the reactivity was obtained for MoAb CD8 by ELISA. Localization of the antigenic determinants, identified by MoAb CD8, in the acrosomal region, in the neck and on the sperm-tail plasma membrane was defined in normozoospermic samples. Similar in localization but different in intensity, CD8-like sperm immunoreactivity was found in leucocytospermic samples in comparison to normozoospermic samples. The obtained results proved the heterogeneity in the presence, localization and expression of CD-like antigen determinants on human spermatozoa and enlarged the information about CD8-like antigen characteristics of the spermatozoa from fertile donors and subfertile patients.

In the literature, of special interest are the data about the presence of cross-reacting antigenic determinants of human spermatozoa and leucocytes (Mathur et al., 1981a, b; Witkin and Sonnabend, 1983; Anderson et al., 1986; Gobert et al., 1990) because of the existence of different hypotheses for their yet unclear biological role (Mathur et al., 1981a, b; Witkin and Sonnabend, 1983; Mavligit et al., 1984; Witkin and Yu, 1985; Gobert et al., 1990; Root-Bernstein and Hobbs, 1993; Trubner et al., 1997). The expression of adhesion CD-like molecules on human spermatozoa of healthy probands (D'Cruz and Lambert, 1997; Trubner et al., 1997) and possible association of infertility with sperm-specific abnormality of CD-like molecules (Kitamura et al., 1997) were analysed.

Numerous studies suggested the presence of CD4-like molecules (Gobert et al., 1990) or of molecules similar in structure to the receptor for HIV, described on the CD4-neural and colonic epithelial cell lines (Brogi et al., 1995; 1996), on the membrane of human spermatozoa as a receptor structure. Anti-CD4 antibodies cross-react with sperm (Gobert et al., 1990) and sperm is thought to bind to class II major histocompatibility complex (MHC) receptor proteins on lymphocytes through these CD4-like homologues (Root-Bernstein and Hobbs, 1993). On the other hand, there are few data in the literature concerning the detection of CD7 and CD19-like molecules on human spermatozoa and the distribution of CD8-like molecules on human spermatozoa as receptor structures. Different hypotheses for their biological role have been suggested.

In our previous report we defined CD4-like molecule heterogeneity on normozoospermic and globozoospermic human spermatozoa (Dimitrova-Dikanarova et al., 1998). The object of the present study was to investigate the differences in the presence of some other CD-like molecules (CD8-like molecules) on human ejaculated spermatozoa from fertile donors and subfertile patients (with leucocytospermia).

Material and Methods

Human semen samples were collected from 100 normal healthy donors of proven fertility (according to the criteria of WHO, 1992) and after the "swim-up" method (Koyama et al., 1988), motile spermatozoa were obtained. Spermatozoa were collected from subfertile patients with leucocytospermia (>10^6 WBC/ml semen, according to the criteria of WHO, 1992) attending the Seminological Laboratory of Medical Faculty,

Received October 10, 2000. Accepted May 2, 2001.

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Abbreviations: FITC – fluorescein isothiocyanate, ELISA – enzyme-linked immunosorbent assay, IEM – immunogold electron microscopy, IIF – indirect immunofluorescence, MHC – major histocompatibility complex, MoAb – monoclonal antibody, WBC – white blood cells.

Sofia. Thymic lymphocytes derived from 8–9 lunar month old human foetuses (Haynes, 1990) were examined, too. The following methods were applied:

a) Indirect enzyme-linked immunosorbent assay (ELISA). The microassay plates were coated alternatively with sperm suspension after the "swim-up" method (from fertile donors and subfertile patients) and with foetal thymocytes (1×10^7 cells/ml). After methanol fixation, ELISA was carried out as described in detail elsewhere (Dimitrova et al., 1993). Anti-human monoclonal CD8 antibody (MoAb), mouse IgG2a isotype, clone UCHT-4 (Cat. No. C 7423; Sigma, Co., St. Louis, MO), and MoAb CD8, clone MEM-31, IgG2a isotype (Cat. No. 11-207; EXBIO Praha, Czech Republic) at a starting dilution 1 : 40 were applied in serial dilutions as a primary antibody.

b) Absorption ELISA. Absorption ELISA was applied according to the method that we described previously (Dimitrova et al., 1993). MoAb CD8 (at a starting dilution 1 : 40) was preabsorbed with an equal volume of "swim-up" normozoospermic sperm suspension (1×10^7 spermatozoa/ml). The supernatant obtained after centrifugation (at $300 \times g$ for 10 min) was added in serial dilutions (as a primary antibody) to plates (× 50 µl/well) coated with foetal thymocytes (× 50 µl/well; 1×10^7cells/ml). ELISA was carried out as described previously. The values of optical densities were used to plot the absorption curve, which was compared to the one obtained for MoAb CD8 without absorption. Points from different curves corresponded to the same dilution of MoAb (with or without absorption). The correlation of the respective average OD_{492nm} values showed the blocking effect of human normozoospermic spermatozoa on the binding of CD8 MoAb to its corresponding antigen on foetal thymocytes. Statistical tests (Graph Pad Instate Software V2.04a, Boston, MA) were used for evaluation of the immunological methods applied.

c) Indirect immunofluorescence (IIF) was performed according to the protocols as described by Jassim et al. (1993), Markova and Marinova (1999). The same MoAbs as these used in ELISA were applied 1 : 100 diluted as primary antibodies. Anti-mouse IgG (whole molecule) – FITC conjugated (Cat. no. F-3008; Sigma, Co., St. Louis, MO), diluted 1 : 100 – was applied as a secondary Ab.

d) Indirect immunogold electron microscopy (IEM) and indirect immunogold silver staining method (IGSS) were performed according to the standard protocols as described by Manara et al. (1990), Jassim et al. (1993), Marinova and Fichorova (1993), Markova and Marinova (1999), respectively. MoAb CD8 diluted 1 : 100 and anti-mouse IgG (whole molecule), gold conjugated (10 nm and 5 nm) (Cat. No. G-7652; G-7527, Sigma, Co., St. Louis, MO), diluted 1 : 20, and silver enhancer kit (Cat. No. SE-100, Sigma, Co., St. Louis, MO) were applied as a primary and secondary antibody, respectively.

In the positive controls of both immunocytochemical reactions the primary antibody was replaced by polyclonal mouse serum against human sperm antigens, and in the negative controls by RPMI 1640 containing 10% foetal calf serum (Cat. No. F-2442, Sigma Co., St. Louis, MO).

Results and Discussion

The ELISA results demonstrated the presence of $CD8^+$ immunopositive normozoospermic spermatozoa and thymocytes. In the course of the absorption experiments, MoAb CD8 was preincubated with normozoospermic spermatozoa and allowed to react with thymocytes. A significant blocking effect (62.30%) was obtained for MoAb CD8 by ELISA (Fig. 1). Our ELISA and IIF data suggested that human spermatozoa and T lymphocytes share membrane CD8 antigenic determinants. Localization of the antigenic determinants, identified by MoAb CD8 in the acrosomal region, including the equatorial segment, was determined in normozoospermic samples. Weak CD8 immunopositivity was observed additionally on the neck and on the sperm-tail plasma membrane (Fig. 2). The localization of sperm surface CD8-like molecules was estimated by IEM and IGSS, too. The antigen-antibody-colloidal gold complexes were observed equally distributed or patched along the plasmalemma of the acrosomal region, including the equatorial segment (Fig. 3a), and on the sperm tail of the human spermatozoa from donors with normozoospermia. The middle piece was immunonegative (Fig. 3b). These data were confirmed by use of the IGSS method, too (data not shown).

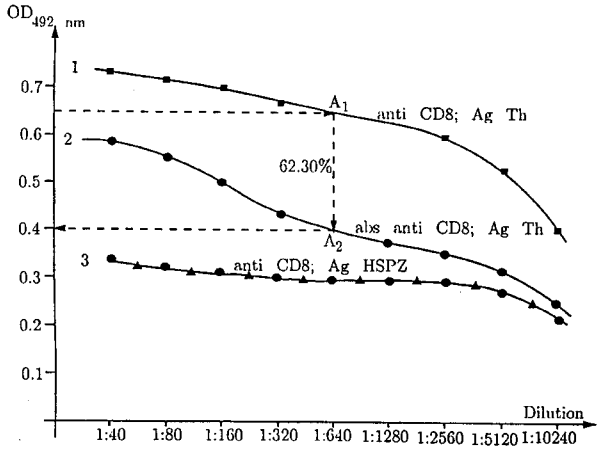

Fig. 1. Reactions of monoclonal anti-human CD8 Ab in ELISA:
(1) ■ ■ ■ – Ag: human thymic lymphocytes (Th) 9 LM; 1st Ab: anti-CD8 without absorption
(2) ● ● ● – Ag: human thymic lymphocytes (Th) 9 LM; 1st Ab: anti-CD8 after absorption with human normozoospermic spermatozoa
(3) ● ▲ ● – Ag: human normozoospermic spermatozoa; 1st Ab: anti-CD8 without absorption