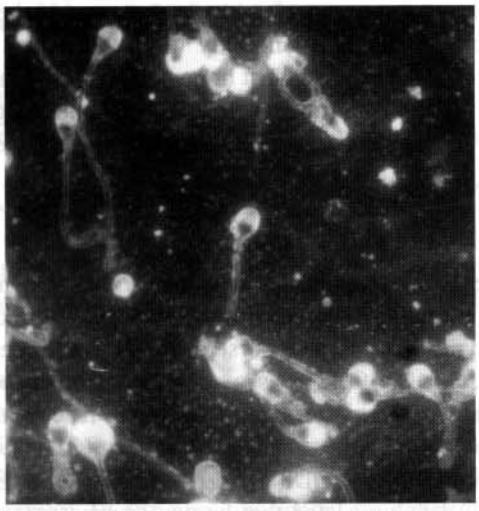

Fig. 2. IIF on methanol-fixed human spermatozoa (normozoospermia) with monoclonal anti-human CD8 Ab showing its reaction in the acrosomal region, including the equatorial segment. Weak CD8 immunopositivity was observed additionally on the neck and on the sperm-tail plasma membrane. Magnification 400x.

The immunocytochemical reaction on the spermatozoa from leucocytospermic samples was similar in localization but different in intensity (and their reaction usually more intensive) as compared to that of the normozoospermic spermatozoa (data not shown).

Numerous data in the literature concern the detection and localization of a large number of CD-like molecules on human spermatozoa as receptor structures or adhesion molecules (Trubner et al., 1997). Our previous results (Dimitrova-Dikanarova et al., 1998; Dimitrova-Dikanarova and Marinova, 2000) are in accordance with the results of Gobert and colleagues (1990) indicating that spermatozoa possess a CD4-like molecule (similar to the receptor for HIV described on mononuclear cells). To our knowledge, in the literature, a limited amount of data exists concerning the detection of CD7 and CD19-like molecules on human spermatozoa and on the distribution of CD8-like molecules on human spermatozoa as receptor structures, as well as on the existence of different hypotheses for their yet unclear biological role.

T-suppressor cells ($CD8^+$) predominate in the subepithelial tissue of the epididymis and vas deferens (El-Demiry et al., 1985) and play a role in the defence against autoimmunization to sperm antigens in the male (Marshburn and Kutteh, 1994). Activation of these T-suppressor cells will dull the antigen recognition by mature B cells and diminish humoral responses to sperm antigens (Marshburn and Kutteh, 1994). According to the data summarized by Witkin (1988), the development of autoimmune response against sperm antigens and antisperm Ab production, due to a breakdown of sperm immune tolerance, may occur as a result of the decrease in number or activity of T-suppressor cells.

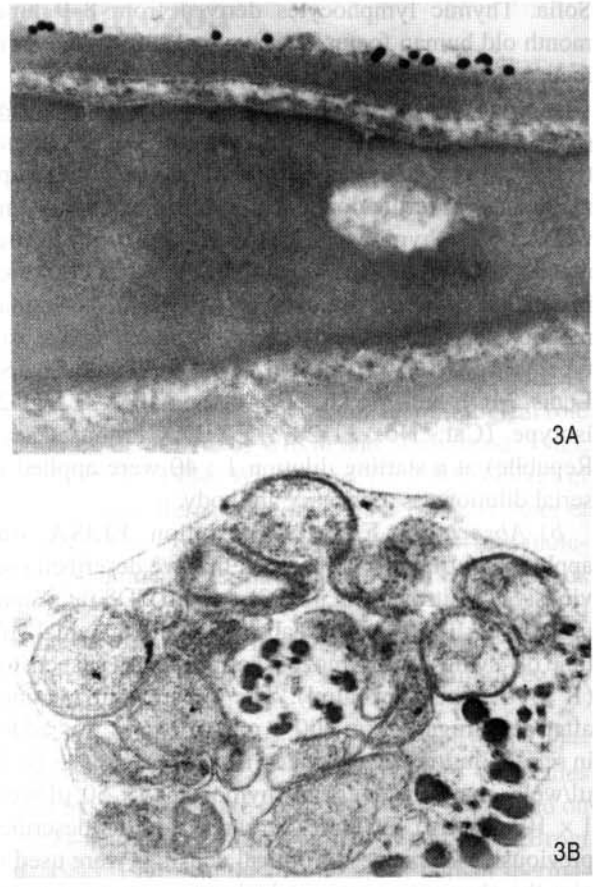

Fig. 3. A) A human spermatozoon (normozoospermic sample) labelled with monoclonal anti-human CD8 Ab and IEM method. The antigen-antibody-colloidal gold complexes were observed equally distributed or patched along the plasmalemma of the acrosomal region, including the equatorial segment of human spermatozoa. Magnification 42000x. B) The middle piece was immunonegative. Magnification 42000x.

Antigenic cross-reactivity between lymphocytes and spermatozoa has been reported (Sugi et al., 1993). Antisperm antibodies, cross-reacted with T cells, have lymphocytotoxic activity (Mathur et al., 1981a; Witkin and Sonnabend, 1983; James and Hargreave, 1984; Malvigit et al., 1984). The ELISA and IIF results discussed by Witkin and Yu (1985) demonstrated that human spermatozoa, rat spermatozoa and human T lymphocytes possess cross-reacting CD3 and CD8 antigenic determinants. Few IIF investigations illustrated the difference in the presence of CD3, CD4 and CD8 corresponding antigens on epididymal and ejaculated mammalian spermatozoa (Witkin and Yu, 1985).

We have detected CD8-like molecules on the human spermatozoa, although their entry mechanisms remain unknown. We suggested that sperm in seminal fluid might passively acquire these molecules. Lymphocytes, macrophages and granulocytes are detectable in most human semen samples from fertile or infertile men, but white blood cell (WBC) subpopulations were significant-

ly higher in the infertile patient groups (Wolff and Anderson, 1988; Gil et al., 1998). Moreover, some studies investigated a possible relationship between the semen leucocyte subpopulation (i.e. $CD8^+$ T lymphocyte prevalence in the semen of the infertile group) and antisperm antibodies, sperm motility and failure of sperm penetration in IVF attempts (Wolff and Anderson, 1988; Barratt et al., 1990). The data obtained in the experiments enlarged the information about CD-like molecules shared by human spermatozoa from fertile donors and T lymphocytes. Our results proved the heterogeneity in the presence, localization and intensity of CD8-like molecules on spermatozoa from fertile donors and subfertile patients.

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