Volume 261, number 2, 339–342 FEBS 08163 February 1990

CD4-like molecules in human sperm

Bernard Gobert, Corinne Amiel, Jianqing Tang, Patricia Barbarino, Marie Christine Béné and Gilbert Faure

Laboratoire d'Immunologie, Faculté de Médecine, Université de Nancy I, BP 184, Vandoeuvre-les Nancy 54500, France

Received 20 December 1989

The expression of a molecule recognized by CD4 monoclonal antibodies was investigated on human sperm using immunolabelling, biochemical and immunochemical methods. Flow cytometry detected a significant fluorescence signal. SDS-PAGE analysis and Western blotting identified a molecule of 60 kDa, consistent with a CD4-like structure as confirmed after selective immunoseparation. Additional bands reacting with anti-CD4 were found in sperm extracts (73 kDa) and seminal fluid (90 kDa). These data indicate that sperm express a molecule similar to the receptor for HIV described on mononuclear cells.

Human sperm; CD4; Monoclonal antibody; Flow cytometry; Western blot

1. INTRODUCTION

Sperm express a large number of membrane antigens that can elicit immune responses both in men and women. Some of these antigens are involved in antibody-dependent infertilities and can also be expressed on other cells [1]. Among them, molecules initially defined as T-lineage antigens have been demonstrated on the surface of sperm [2]. The description of CD4 as the ligand for HIV gp120 [3] prompted the investigation of this molecule on non-T cells. The expression of CD4 was confirmed on such suspected HIV targets as cells of the monocyte/macrophage lineage, B-cell lines, and non-haematopoietic cells [4,5]. The high infective potential of sperm, supported by the rate of infection in man–man or man–woman transmission of the disease suggests that high concentrations of HIV can be present in this fluid. The expression of the CD4 molecule as a potential HIV-receptor was investigated in human testes, sperm and seminal fluid.

2. MATERIALS AND METHODS

2.1. *Materials and reagents*

Normal sperm from 14 HIV-seronegative healthy males enrolled in a sperm-bank was studied. All samples contained 1.1 to 1.3 × 10^8 sperm per ml, with high vitality and motility, and less than 20% morphological abnormalities. There were no spontaneous agglutination, auto-antibodies, microbial agents or red blood cells. The samples were stored at −20°C and treated separately. Testes biopsies were obtained at autopsy from 4 individuals, within 24 h after death. They were snap frozen in liquid nitrogen and kept at −70°C until use.

Correspondence address: B. Gobert, Lab. Immunologie, Faculté de Médecine, BP 184, 54500 Vandoeuvre, France

A series of CD4 monoclonal antibodies (MoAbs) (T4, Coulterclone, Hialeah, FL, USA; Leu3a, Becton Dickinson, Mountainview, CA, USA; ST4, Biosys, Compiègne, France; OKT4A, OKT4B, OKT4C and OKT4D, gift from G. Goldstein, Raritan, NJ, USA) was used as immunological reagents.

2.2. *Flow cytometry*

Two million extensively washed sperm were incubated for 30 min at +4°C with CD4 MoAbs, washed, stained with sheep anti-mouse Ig fluorescent antiserum (Institut Pasteur Productions, Paris, France), washed again and finally fixed in 0.5 ml paraformaldehyde. Control included incubation of the same amount of sperm with fluorescent immunoglobulins alone. A Coulter Profile flow cytometer (Coultronics, Hialeah, FL, USA) was used with similar electronic parameters for sperm and lymphocyte studies.

2.3. *SDS-PAGE, 2D electrophoresis and Western blot*

Testes biopsies were thawed in Tris-EDTA buffer, minced and disrupted in a Potter grinder, in order to obtain a cell suspension, which was then freeze-thawed three times. Membrane extracts were spun down after removal of nuclei, treated with SDS and β-2-mercaptoethanol (b2ME), and then used for electrophoresis as stated below.

Seminal fluid was removed by centrifugation. Sperm was treated overnight in Tris-HCl 0.1 M pH 7.4, 0.1% Nonidet P-40, 1% Triton X-114 and 2.5% b2ME, sonicated 5 min at 1500 W, spun down at 10000 rpm for 20 min and resuspended in 100 μl phosphate buffered saline (PBS). Protein A Sepharose precipitation of CD4-like material was carried out on disrupted sperm and acetone-precipitated seminal fluid proteins. CD4 20μl were added to 1 ml of the relevant protein preparation and the mixture gently stirred overnight at +4°C. Protein A Sepharose treated with 50 mM Tris pH 7.4, 150 mM NaCl, 2 mM methionine, 0.5% Nonidet P-40 and 0.02% NaN_3 was then added to the preparation and incubated for 3 h at 4°C on a slow rotative plate. After centrifugation and removal of the supernate, bound material was eluted from Sepharose. Protein contents were adjusted for all preparations. SDS-PAGE was performed, after treatment of all samples for 5 min at 100°C in SDS-b2ME, using ultrathin microgels and a PhastSystem (Pharmacia, Uppsala, Sweden). Ampholite-loaded Phast gels establishing a 3–8 pH gradient were used for the second dimension electrophoresis of a polyacrylamide gel first run under similar conditions. Western blotting was carried out immediately after the electrophoresis or 2D electrophoresis with

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

nitrocellulose sheets pretreated in Tris-HCl 0.1 M, methanol and glycine. The same nitrocellulose was used to perform dot-blot identification of CD4 on sperm extracts with the panel of MoAbs to CD4. In both methods, protein free sites were saturated after drying with PBS supplemented with 1% BSA. After three washes in PBS-Tween, the sheets were incubated with CD4 1:100 in PBS for 1 h at 37°C, washed, and reincubated for 1 h with peroxidase labelled sheep anti-mouse IgG (Miles, Napperville, USA). Positive bands were finally developed with 0.1 M H_2O_2 in Tris-HCl 0.1 M, pH 7.4, containing 0.05% diethylaminobenzidin. The gels were stained with silver nitrate.

3. RESULTS

CD4-stained sperm were observed both in flow cytometry and UV-light microscopy. CD4+ mononuclear cells were detected in small numbers. Light scattering parameters allowed to separate the mononuclear and sperm populations in cytometry (fig.1a). A significant fluorescent signal (fig.1b) was recorded on sperm stained for CD4. Using arbitrary

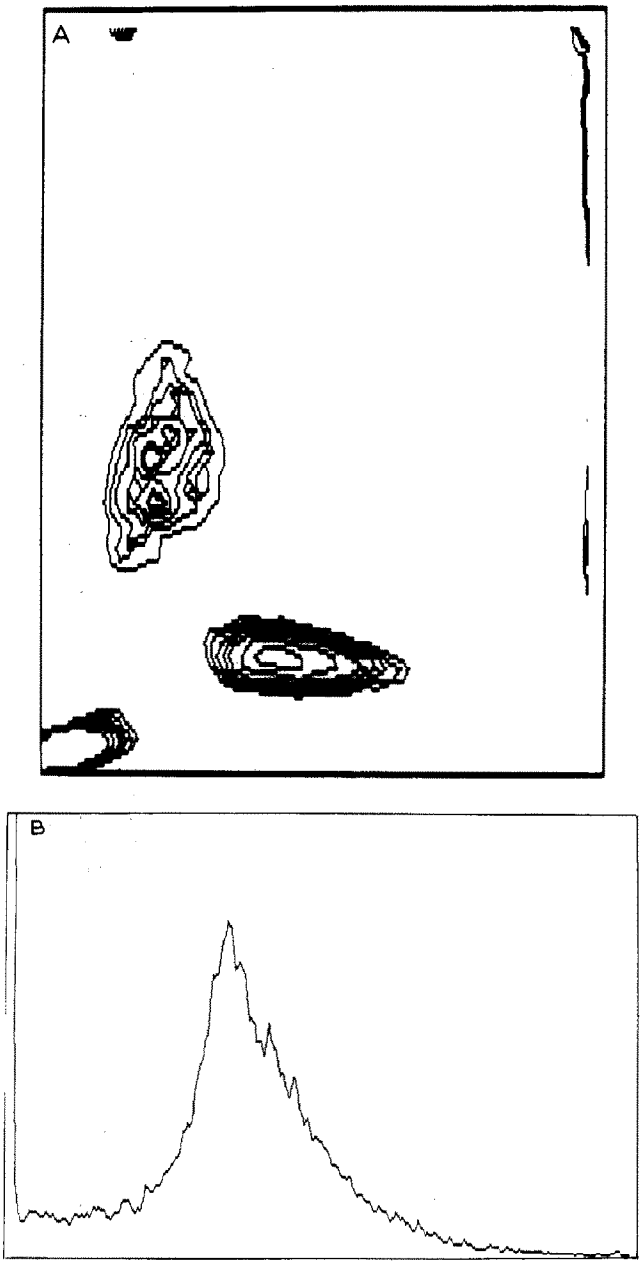

Fig.1. (A) Superimposed cytometry scattergrams obtained with peripheral blood lymphocytes (upper left spot) and sperm cells (lower right spot). X-axis: side scatter, Y-axis: forward scatter. The two populations are clearly distinct. (B) CD4+ histogram obtained with sperm in flow cytometry. Fluorescence analysis was gated on the lower right spot shown in (A). X-axis: log green fluorescence.

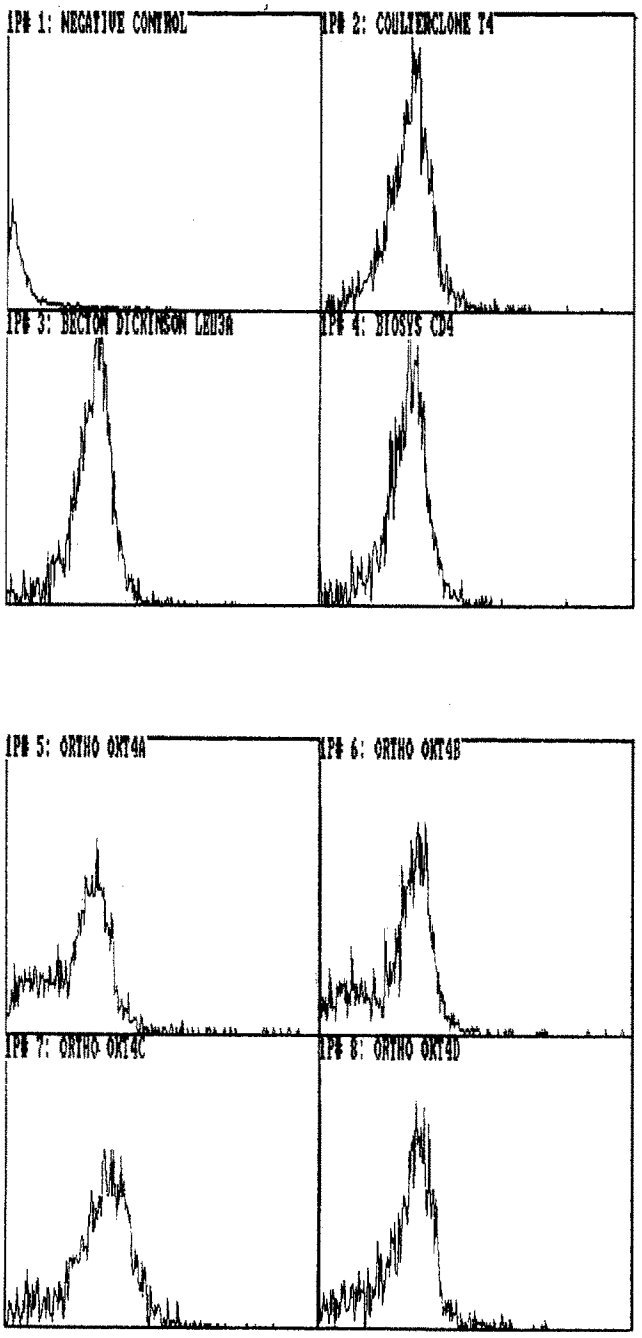

Fig.2. Comparative fluorescence histograms of sperm cells stained with a panel of monoclonal antibodies to CD4. X-axis: log green fluorescence. Negative control: fluorescent anti-mouse immunoglobulin alone.

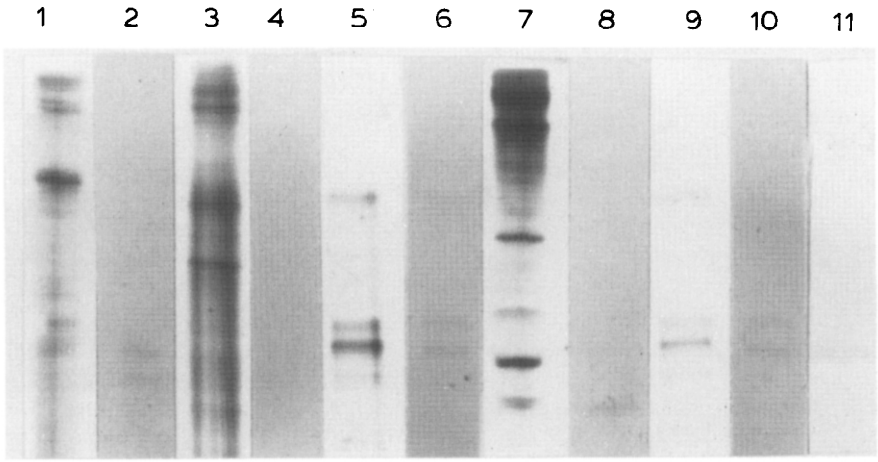

Fig.3. SDS-PAGE and CD4-Western blotting of sperm (lanes 1,2), seminal fluid (7,8), peripheral lymphocytes (11), and protein A-Sepharose isolated CD4-like material from sperm (5,6) and seminal fluid (9,10). Lane 4 is a negative control Western blot from the supernate of protein A-Sepharose-CD4-treated sperm (3). Similar data were obtained with sperm from 14 healthy donors.

units of fluorescence control unlabelled sperm yielded a fluorescence intensity of 1.03 while 99.6% of sperm cells were included in the peak shown in fig.1b, with a mean fluorescence intensity of 95.9. Similar results were obtained with all the MoAbs used (fig.2).

Dot-blot performed on sperm extract yielded similar results.

SDS-PAGE and Western blotting of testes cell-membranes showed a single 56 kDa band.

SDS-PAGE and Western blotting resulted in all sperm samples in the consistent identification of two bands with respective molecular weights of 60 and 73 kDa (fig.3, lane 2), the lighter one being similar to that obtained from lymphocytes (fig.3, lane 11). Cell-free seminal fluid was also submitted to SDS-PAGE and Western blotting, which resulted in the identification of a single 90 kDa band (fig.3, lane 8). SDS-PAGE electrophoresis and Western blotting of CD4-bound material isolated using Protein A-Sepharose retrieved an identical 60 kDa band from sperm and seminal fluid (fig.3, lanes 6 and 10). Western blotting of 2D gels from sperm showed two spots with pI of 6.7 and 6.7, corresponding to the major spots observed for peripheral lymphocytes.

4. DISCUSSION

HIV infection is transmitted by the exchange of virus-containing biological material between two individuals. HIV antigens can be detected in the serum, but the virus is usually integrated in the genome of a host cell [6]. The receptor for HIV has been demonstrated to be the CD4 molecule [3], mostly present on lymphocytes. Peripheral blood lymphocytes are thus deemed responsible for transfusion-related HIV infection. Sexual transmission, however, is the most common form of HIV infection. Although several cases of female-to-male transmission have been reported, the infectivity of semen appears to be superior to that of female genital secretions. Small numbers of lymphocytes are indeed present in semen [7,8] and it was possible for some authors to demonstrate that they contained infectious HIV [9].

Another explanation for the infectivity of semen could be the presence of viral particles in and/or on sperm. This would require the presence of an HIV receptor on these cells. Recently, Bagasra et al. [10] reported in vitro experiments demonstrating the attachment of HIV particles on sperm obtained from healthy seronegative donors, and their penetration in these cells. Immunofluorescence studies suggested the presence of a CD4-like molecule on the membrane of the material they studied.

Our data indicate that sperm indeed express a molecule similar to the receptor for HIV described on human mononuclear cells [11]. We observed this protein on the surface of sperm, and in seminal fluid. It was recognized by a panel of 7 MoAbs directed to various epitopes of lymphocyte CD4. The molecular mass of 60 kDa observed after selective immunoseparation is consistent with a CD4-like structure. The observation of a similar band from testes extracts suggests that CD4 may not be passively acquired by sperm in seminal fluid but appears in early stages of sperm maturation. The previous demonstration of common antigens between T-cells and sperm sustains these data [2]. Comparison of sperm data with lymphocytes indicate a similar molecular weight and compatible pI range. The presence of an additional 73 kDa band in sperm extracts and of a heavier 90 kDa molecule in seminal fluid might reflect various degrees of glycosylation of the molecule.

We postulate that the HIV virus can enter and be transported by sperm, and that sperm may contribute

to HIV infection. The number of possibly infected cells exchanged during sexual intercourse would then be much higher than the mere million of CD4+ lymphocytes contained in one ejaculate [8]. This would be consistent with the high rate of infectivity of sperm, compared for instance to saliva.

REFERENCES

[1] Alexander, N.J. and Anderson, D.J. (1987) Fertil. Steril. 47, 192–205.
[2] Mathur, S., Goust, J.M., Williamson, O. and Fudenberg, H.H. (1981) Am. J. Reprod. Immunol. 1, 113–118.
[3] Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Gluckman, J.C. and Montagnier, L. (1984) Nature 312, 767–769.
[4] Ho, D.D., Rota, T.R. and Hirsch, M.S. (1986) J. Clin. Invest. 77, 1712–1715.
[5] Fauci, A.S. (1988) Science 239, 617–619.
[6] Rabson, A.B., Koenig, S., Daugherty, D.F. and Gendelman, H.E. (1988) Gene Anal. Techn. 5, 41–53.
[7] Olsen, G.P. and Shields, J.W. (1984) Nature 309, 116–117.
[8] Witkin, S.S. and Goldstein, M. (1988) J. Reprod. Immunol. 14, 283–290.
[9] Zagury, D., Bernard, J., Leibowitch, J., Safai, B., Groopman, J.E., Feldman, M., Sarngadharan, M.G. and Gallo, R.C. (1984) Science 226, 449–451.
[10] Bagasra, O., Freund, M., Weidmann, J. and Harley, G. (1988) J. Acquir. Immune Defic. Syndr. 1, 431–435.
[11] Sattentau, Q.J. and Weiss, R.A. (1988) Cell 52, 631–633.