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Immunology

Lymphocyte major histocompatibility complex-encoded class II structures may act as sperm receptors

(acquired immunodeficiency syndrome/fertilization/immunoglobulin superfamily)

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ABSTRACT **Human sperm and blood cells were cocultured *in vitro* to determine whether specific interactions occur between gametes and blood cells. Evidence for cell type-specific sperm binding and penetration of lymphocytes is presented together with findings that suggest that either or both events involve major histocompatibility complex-encoded class II molecules on lymphocytes and a sperm ligand that is immunoreactive with antibodies to T-cell surface antigen T4. Involvement of HLA-DR is suggested by the pattern of sperm interactions with HLA-DR-positive and -negative cells and by inhibition of sperm binding to HLA-DR-positive cells by a monoclonal antibody that identifies a nonpolymorphic determinant on the HLA-DR molecule. That the complementary sperm ligand may be a T4-like structure is suggested by specific inhibition of sperm–lymphocyte binding with monoclonal antibodies OKT4 and OKT4A. The results are discussed in terms of possible roles for immunoglobulin-related structures in human fertilization and in the sexual transmission of the acquired immunodeficiency syndrome.**

The major histocompatibility complex (MHC) antigens and related members of the immunoglobulin superfamily play central roles in immune functions in vertebrates (1). Recent evidence suggests that polymorphic MHC-encoded elements are involved in reproductive behavior and physiology as well (2, 3). The possibility that immunoglobulin-related recognition structures may participate directly in fertilization is suggested by studies with the marine protochordate *Botryllus*, whose transplantation genes control both blood cell-mediated allorecognition and the specificity of sperm–egg interactions (4). The present study was undertaken (*i*) to determine whether direct interactions between human sperm and lymphocytes occur and (*ii*) to examine the possibility that these reflect structural similarities between lymphocyte and gamete cell surface recognition molecules in mammals.

MATERIALS AND METHODS

Reagents. 4,6-Diaminidino-2-phenylindole (DAPI), 2-(2-aminoethyl)isothiouronium bromide hydrobromide (AET), fluorescein isothiocyanate (FITC), and poly(L-lysine) were from Sigma. RPMI 1640 medium was from Flow Laboratories; penicillin/streptomycin and L-glutamine were from GIBCO.

Cells and Monoclonal Antibodies. Peripheral blood leukocytes (PBLs) were obtained from the blood of type O+ healthy adult male volunteers by Ficoll-Hypaque centrifugation (5). Sperm from the same donors were washed three times before use. Monocytes were prepared as described (6). Nonadherent cells were separated into T- and B-cell fractions by rosetting with AET-treated sheep erythrocytes (7). The T-cell lines JM, CEM-CCRF, MOLT4, and MT2 (8) were provided by M. McGrath (University of California, San Francisco). A human T-cell lymphotropic virus type II (HTLV-II)-infected B-cell line, 729/neo pH 6 (9, 10), and a HTLV-I-transformed T-cell line, MT4, were supplied by I. Chen (University of California, Los Angeles). The monocytic cell lines I937 and U937 were gifts from O. Finn (11). Monoclonal antibodies OKT4, OKT4A, and OKT8 were from Ortho Diagnostic Systems. Anti-HLA-DR (L243, a reagent that identifies α- and β-chain joint public determinants) was from Becton Dickinson. Anti-MHC class I (W632, an antibody against HLA-A, -B, and -C shared public determinants) was from Accurate Chemicals. Isotype control reagents (IgG2a and IgG2b) were from Coulter. Biotinylated goat anti-mouse IgG and avidin-D fluorescein were from Vector Laboratories (Burlingame, CA).

Sperm–Lymphocyte Interaction Assay. Aliquots (50 μl) containing 2×10^5 blood cells [in RPMI 1640 supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), and L-glutamine (0.3 mg/ml)] were cocultured in triplicate in round-bottom 96-well microtiter plate wells with 2×10^6 washed sperm in an equal volume (50 μl) of Tyrode's solution. The cocultures were incubated at 37°C in 5% CO_2 for 2–4 hr. In some experiments, cells or sperm were preincubated with antibody (8 μg/ml) for 30 min prior to washing and coculture. For quantitation of sperm binding and penetration, 5-μl aliquots from each well were lightly fixed with glutaraldehyde, mixed with DAPI (a blue fluorescent DNA-binding dye; 2-μg/ml final concentration; ref. 13) and examined under simultaneous ultraviolet and bright-field Nomarski illumination. Cells scored as positive had one or more attached or penetrated sperm heads (visualized by blue DAPI fluorescence staining of their chromatin). Data are expressed as the mean percentage (± SEM) of positive cells (of 100 cells counted in adjacent fields) for triplicate samples.

Activated T Cells. Peripheral blood T cells incubated with phytohemagglutinin (PHA, 0.5 μg/ml; Wellcome Research Laboratories) for 48 hr were washed before addition of sperm. For mixed lymphocyte reactions (MLRs), mixtures of 10^5 irradiated (3000 rads; 1 rad = 0.01 Gy) or untreated T cells per ml from each of two donors were cultured in pairwise combinations (one irradiated and one untreated, for one-way MLRs, or both untreated, for two-way MLRs). The cultures were incubated for 5 days at 37°C in 5% CO_2 before addition of sperm. T-cell stimulation by mitogen or MLR was assessed, in parallel cultures of the same cells, by [^{3}H]thymidine incorporation. [*methyl*-^{3}H]Thymidine (1 μCi; 6.7 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq) was added to each well 8 hr before harvest (on day 3 for PHA and on day 5 for MLR). Stimulation indices were calculated as the mean

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Abbreviations: DAPI, 4,6-diaminidino-2-phenylindole; FITC, fluorescein isothiocyanate; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; PHA, phytohemagglutinin; PBL, peripheral blood leukocyte.

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cpm for triplicate PHA- or MLR-stimulated cultures divided by the mean cpm for control unstimulated cultures of T cells from the same donor.

ELISA Assays. Washed cells (10^5) placed in triplicate wells of poly(L-lysine)-treated Immulon Microtiter plates (Dynatech) were incubated with anti-HLA-DR antibody (5 μg/ml) followed by β-galactosidase-conjugated anti-mouse IgG secondary reagent and were developed with *p*-nitrophenyl β-D-galactoside (Bethesda Research Laboratories). The plates were read on a Bio-Tek plate reader at 405 nm. Data are expressed as the mean absorbance (± SEM) for triplicate wells.

Immunofluorescence. Sperm were incubated for 30 min each, followed by washing, in solutions or (*i*) primary antibody (from those listed above), (*ii*) biotinylated goat anti-mouse IgG, and (*iii*) avidin-D fluorescein (Vector Laboratories). Fluorescence microscopy was done as described above for DAPI fluorescence, using appropriate filter sets.

Electron Microscopy. MT4 cells cocultured with sperm were fixed in glutaraldehyde and postfixed in 1% OsO_4. The pelleted cells were dehydrated in ethanol and embedded in Spurr resin for sectioning. The uranyl acetate- and lead citrate-stained sections were examined with a JEOL JEM-100CX electron microscope.

RESULTS

Sperm Bind and Penetrate PBLs. Fig. 1 *A* and *B* are light-microscope fields of sperm mixed with peripheral blood B cells and monocytes, respectively, and sampled 2–4 hr after establishment of cocultures. Sampling over a 24-hr period revealed that most bound sperm had lost their tails by ≈2 hr after binding (Fig. 1*A Inset*), and had penetrated the blood cells by 4–12 hr. The total percentage of cells scored as positive did not change after the first 15 min (data not shown). In contrast to the B-cell and monocyte preparations, T cells in general showed considerably less sperm binding and penetration (Fig. 1*B Inset*). Compiled data showing percentages of bound or penetrated cells of all three types from 15 different donors are presented in Fig. 2. Fig. 3 is an electron micrograph of a section from a pelleted coculture of sperm and MT4 T cells (see below). The cell in the section contains a sperm head (black arrows).

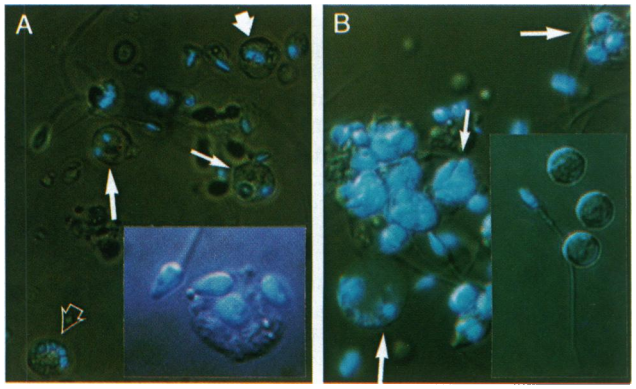

FIG. 1. Interaction between sperm and PBLs cocultured for 2–4 hr. Sperm heads and some leukocyte nuclei are visualized by the bright blue fluorescence of the DNA-binding dye DAPI. (*A*) B-cell fraction. Several cells in this representative field have one or more bound or penetrated sperm (arrows). In some cases, multiple penetrated sperm heads seem to have displaced the cell nucleus (open arrow). (×160.) (*Inset*) Binding of sperm to lymphocyte surfaces is followed by loss of the tail. (×400.) (*B*) Monocyte fraction. Both intact and tail-less surface-bound sperm are visible; many cells contain penetrated sperm heads (arrows). (×160.) (*Inset*) T-cell fraction. Few sperm–lymphocyte conjugates are found in sperm/T-cell cocultures. (×160.)

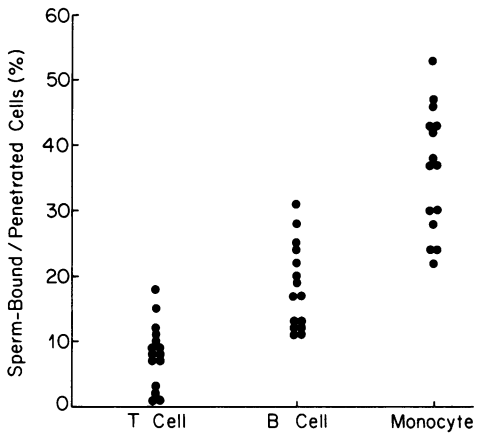

FIG. 2. Quantitation of sperm–lymphocyte interactions for PBLs from 15 different donors. Percentages of cells with one or more bound or penetrated sperm (designated "bound/penetrated") for separated peripheral blood T cells, B cells, and monocytes were determined by the sperm–lymphocyte interaction assay.

Sperm Binding and Penetration Is Specific for Cells with Surface HLA-DR Structures. Because MHC class II structures are activation antigens for peripheral blood T cells, which are largely negative for surface expression of these antigens in the resting state, we next examined the possibility that differences between the three cell types tested directly involved class II molecules.

Activated T cells. The percentage of HLA-DR-positive T cells from freshly drawn peripheral blood (determined by immunofluorescence) ranges from 5% to 10% (data not shown); this proportion is similar to the numbers of peripheral T cells that are bound and penetrated by sperm (Fig. 2). After activation with PHA (Fig. 4*A*) or by allogeneic T cells in a one-way or two-way MLR (Fig. 4*B*), T cells showed a dramatic increase in sperm binding and penetration (Fig. 4 *C* and *D*) as compared to control cells from the same donors that had been cultured without stimulation for the same period of time (see Fig. 1*B Inset*). In each case, the percentage increase in cells bound or penetrated by sperm was similar to the percentage increase in cells expressing HLA-DR markers, as determined by immunofluorescence; and positive cells, without exception, were from that population of stimulated T cells (≈30%) which also were positive for surface HLA-DR (data not shown).

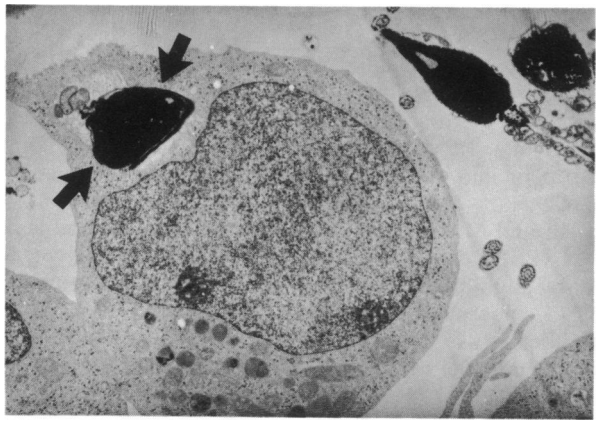

FIG. 3. Electron micrograph of an MT4 cell containing a penetrated sperm. The cell contains a sperm head (arrows) to the left of the cell nucleus. (×5000.)

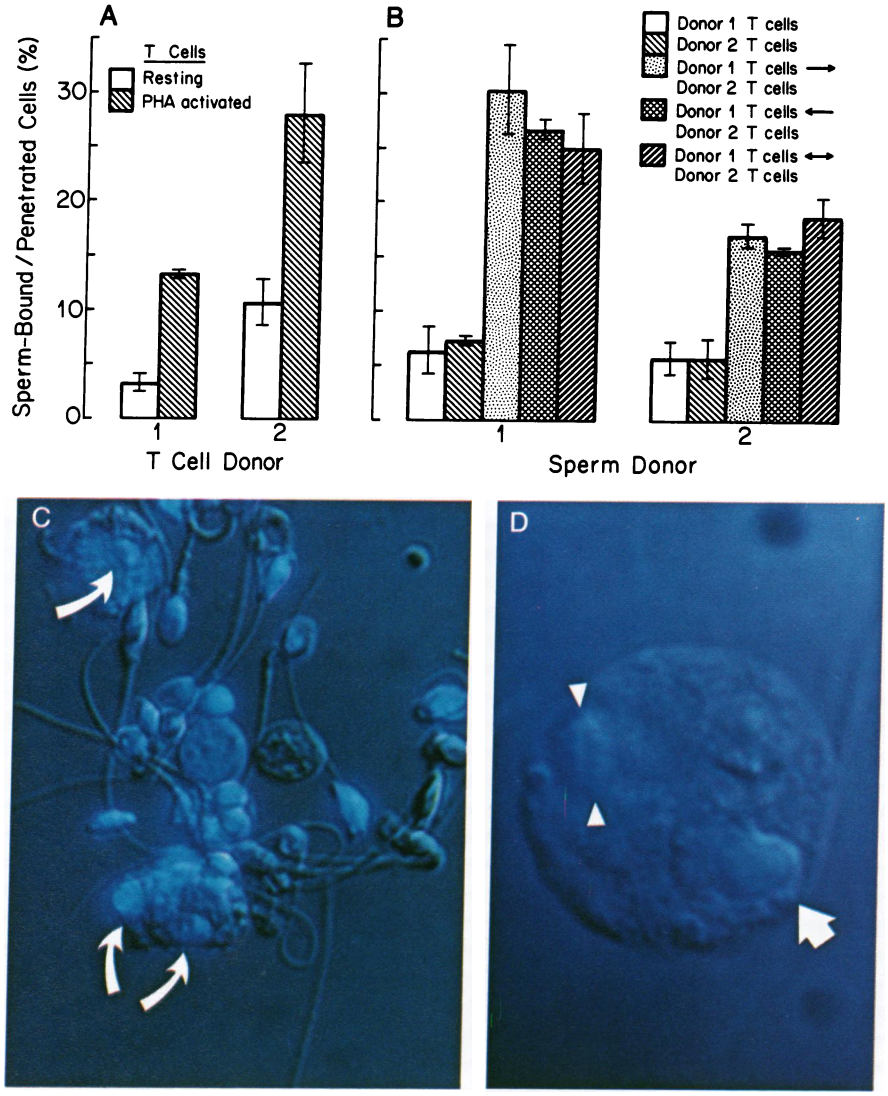

FIG. 4. Sperm-interaction characteristics of resting vs. activated peripheral T cells. (*A*) Percentages of cells with bound or penetrated sperm for donor T cells cultured with or without PHA. Stimulation index (assessed in parallel cultures by [^{3}H]thymidine incorporation) was >100 (data not shown). (*B*) Percentages of cells with bound or penetrated sperm from donor T cells cultured either alone or with allogeneic cells from a second donor in one-way (both combinations, ← and →) and two-way (↔) MLRs. (*C*) MLR-activated T cells cocultured with sperm. Many of the cells in this field have attached or penetrated sperm (arrows), whereas unstimulated T cells show background levels of sperm interaction (see Fig. 1*B Inset*). (×300.) (*D*) Surface-bound and penetrated sperm heads on the same cell. Bound (arrow) and penetrated (arrowheads) sperm heads on this cell (from a PHA-activated peripheral T-cell preparation) are typical of late stages of binding/penetration sequence. (×750.)

Cell lines. T-cell, B-cell, and monocyte lines were tested in parallel for their ability to interact with sperm and for their relative amounts of surface HLA-DR. The HLA-DR-negative T-cell lines JM and CEM showed background levels of sperm interaction similar to or lower than those for resting peripheral T cells, whereas the T-cell lines MOLT4 and MT2 were strongly positive for sperm binding and penetration, as was the B-cell line 729/neo (Fig. 5*A*). The HLA-DR-negative cell lines, like the peripheral T cells, showed some sperm binding, which in both cases likewise may reflect a low background of nonspecific adhesion. A significant correlation was seen between the percentages of positive cells and the amount of bound anti-HLA-DR detected by ELISA for each cell line. This pattern was most clear when the sperm-binding characteristics of the HLA-DR-negative monocyte line U937 were compared with those of its HLA-DR-positive variant line I937 (Fig. 5*B*). While the U937 cells showed low levels of sperm binding and penetration similar to those for resting peripheral T cells, JM cells, and CEM cells, the I937 variants were strongly positive for sperm binding.

Preincubation of PBLs with Anti-HLA-DR Antibody, but Not with Anti-HLA-A/B/C or Isotype-Control Reagents, Blocks Sperm–Lymphocyte Binding. Results from two donors are shown in Fig. 6 *A* and *B*. Inhibition by anti-HLA-DR was dose-dependent (data not shown). In contrast, an equivalent concentration of an anti-HLA-A/B/C was without effect (Fig. 6*C*).

Preincubation of Sperm with OKT4 and OKT4A Antibodies, but Not with OKT8 or an Isotype Control Reagent, Blocks

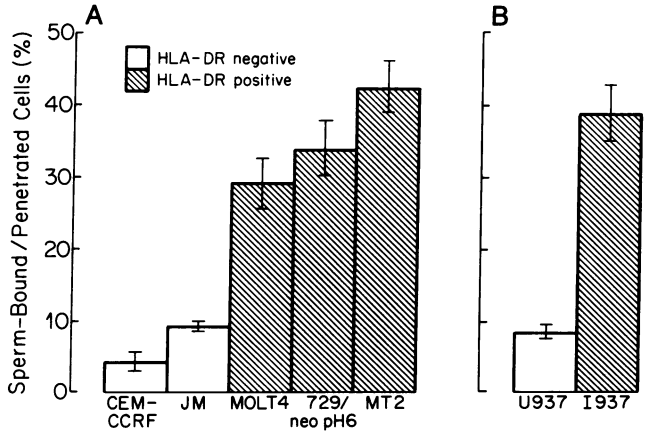

FIG. 5. Sperm–lymphocyte binding percentages for T- and B-cell (*A*) and monocyte (*B*) lines. The amounts of HLA-DR antigens for cells of the different lines as determined by ELISA [absorbance at 405 nm: JM, 0.026 ± 0.006; CEM, 0.077 ± 0.004; MOLT4, 0.266 ± 0.010 (this "MOLT4" subline, whose original parent line was HLA class II-negative, apparently contains contaminating HLA-DR positive cells of another line); 729/neo, 0.701 ± 0.010; MT2, 0.869 ± 0.144; U937, 0.069 ± 0.017; I937, 0.487 ± 0.017] correlated with the percentage of sperm binding (correlation coefficient $r = 0.92$; $P = 0.026$).

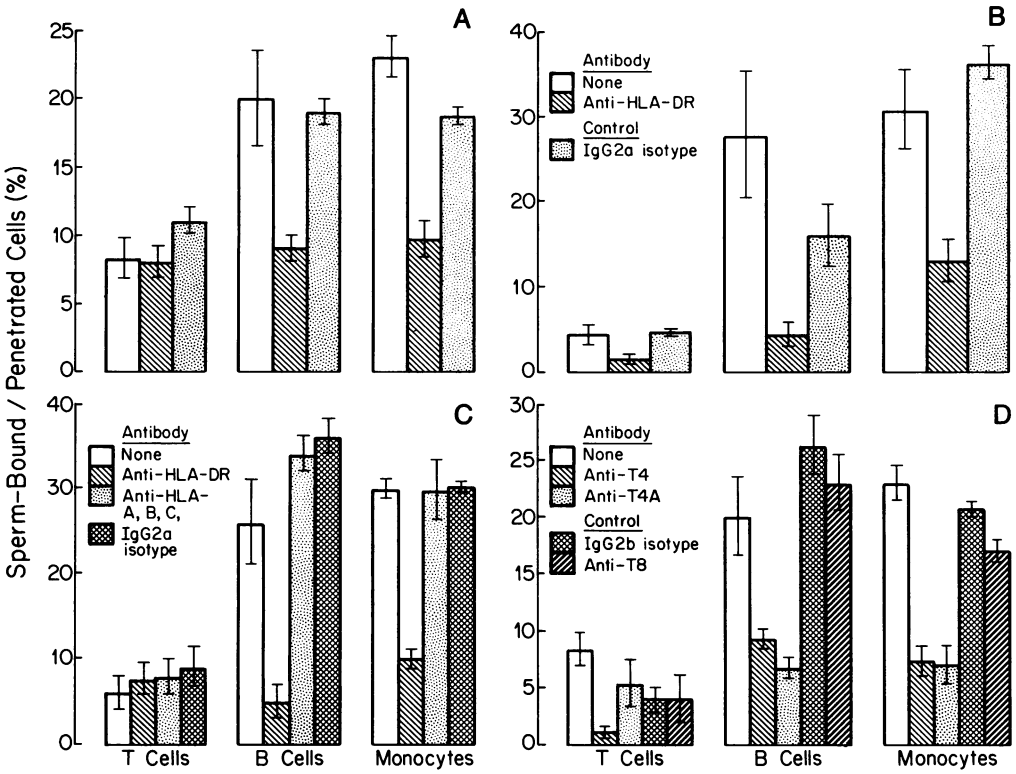

FIG. 6. Blocking effect of anti-HLA-DR and anti-T4 monoclonal antibodies on interaction of sperm with donor T cells, B cells, and monocytes. (*A* and *B*) Percentages of bound and penetrated lymphocytes for sperm–lymphocyte cocultures, where the blood cells were pretreated with no antibody, anti-HLA-DR, or an isotype-control reagent (same isotype as the anti-HLA-DR). *A* and *B* show results for PBLs from two different donors. The key for both appears in *B*. (*C*) Percentages of bound and penetrated lymphocytes (from donor 2, *B*) in sperm–lymphocyte cocultures, where the blood cells were pretreated with anti-HLA-DR, with anti-HLA-A/B/C (IgG2a), or with an IgG2a isotype-control antibody different from the one used in *A* and *B*. (*D*) Percentages of bound or penetrated lymphocytes for cocultures of donor PBLs, where the sperm were pretreated with no antibody, OKT4, OKT4A, OKT8, or an IgG2b isotype-control reagent (to control for OKT4, which is IgG2b; all others are IgG2a).

Sperm–Lymphocyte Binding. Fig. 6*D* shows that preincubation of sperm with OKT4 or OKT4A prior to their addition to cocultures blocked their interaction with the blood cells relative to equal concentrations of an IgG2b isotype-control antibody or OKT8 (which acts also as an IgG2a isotype control). As with anti-HLA-DR preincubation of the lymphocytes, the effect was dependent on antibody concentration (data not shown). Neither anti-HLA-DR nor anti-HLA-A/B/C inhibited binding when tested under the same conditions (data not shown).

Results similar to those depicted in Fig. 6 for PBLs were also obtained for cells of HLA-DR-positive cell lines. Fig. 7 shows cocultures of MT4 cells and sperm for which the MT4 "targets" had been preincubated with an isotype-control reagent (Fig. 7*A*) or anti-HLA-DR antibody (Fig. 7*B*) or for which the sperm had been preincubated with OKT4 (Fig. 7*C*).

When similar concentrations of the antibodies and isotype controls listed above were tested by biotin–avidin immunofluorescence for binding to sperm, weak but consistent fluorescence staining was seen for unfixed sperm with OKT4 and OKT4A (Fig. 8 *A–D*). All the other antibodies were negative for live sperm by this sensitive test (results with OKT8 are shown in Fig. 8 *E* and *F*).

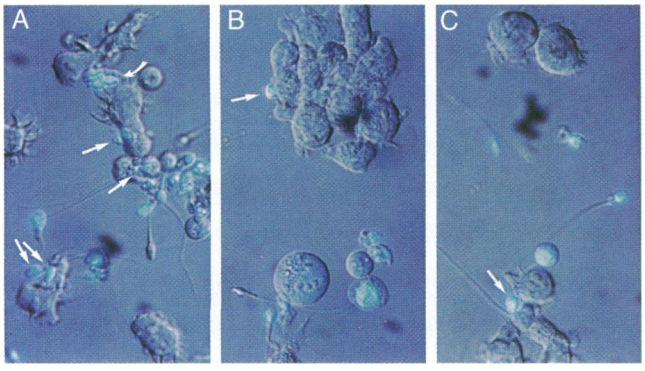

FIG. 7. Cocultures of MT4 T cells and sperm with or without pretreatment with antibody. (*A*) MT4 cells preincubated with IgG2a isotype control reagent (sperm binding/penetration = 62 ± 3%). (*B*) MT4 cells preincubated with anti-HLA-DR antibody (19 ± 5%). (*C*) Sperm preincubated with OKT4 (24 ± 3%). (×110.)

DISCUSSION

Although there have been previous reports of sperm binding and penetration of mammalian somatic cells (14), to our knowledge this is the first report of cell type-specific interactions of this type with blood cells. That HLA-DR structures on lymphocytes and T4-like molecules on sperm are, or are

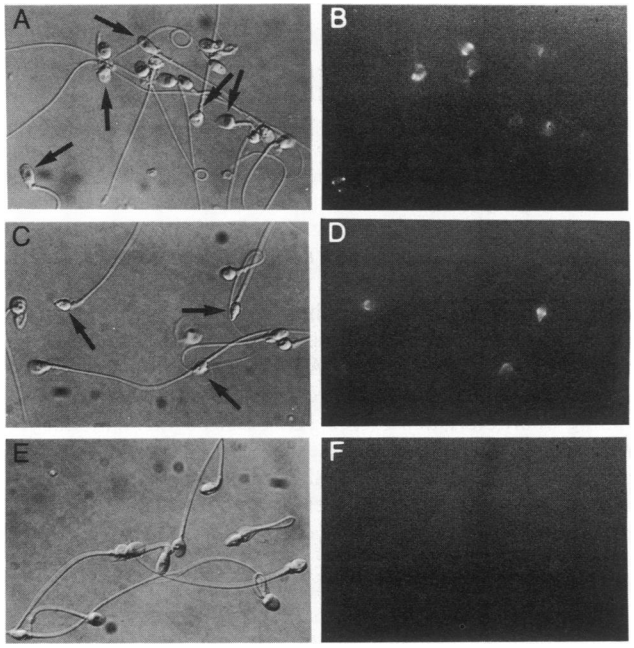

FIG. 8. Binding of OKT4 and OKT4A to the sperm surface, detected by biotin–avidin immunofluorescence: bright-field (*A*, *C*, and *E*) and fluorescence (*B*, *D*, and *F*) photographs. (*A* and *B*) OKT4A. (*C* and *D*) OKT4. (*E* and *F*) OKT8. (×140.)

components of, the recognition structures in these interactions is suggested by (*i*) sperm binding and penetration of HLA-DR-positive cells (peripheral blood B cells and monocytes, activated T cells, and a variety of HLA-DR-positive lines of all three cell types) and (*ii*) inhibition of sperm–lymphocyte interactions by antibody directed against HLA-DR or T4, as compared to antibodies to related antigens.

Adhesion interactions between T-cell surface T4 and class II structures on antigen-presenting cells have been proposed in models for antigen recognition by T cells (15), and other data suggest a regulatory role as well (16). Although T4 was initially described as a T-cell-specific structure in mice and humans, T4 has recently been isolated from rat macrophages (18), and two species of T4 mRNA have been identified from human brain cells (19). The latter observations suggest that T4-related cell surface structures may play recognition roles in phenomena unrelated to immune function (17, 19).

Significance for Mammalian Fertilization. In mice, sperm penetration of the egg investments (20, 21) is coupled to sperm surface recognition of a zona pellucida protein, ZP3 (22). The findings reported here introduce the possibility that sperm–egg recognition and adhesion in mammals may involve molecules of the immunoglobulin superfamily that are related to T4 or HLA-DR. On the other hand, class II involvement in sperm–leukocyte interactions could reflect (*i*) recognition between adhesiotopes shared by class II and/or T4 molecules with otherwise unrelated sperm–egg or tissue adhesion proteins (30) or (*ii*) association of class II expression with leukocyte enzyme systems that may also be involved in fertilization (31, 32).

Significance for Acquired Immunodeficiency Syndrome (AIDS) Transmission. T-cell surface T4 has been identified as the primary cell surface receptor for the human immunodeficiency virus (HIV) that causes AIDS (23, 24). Rapid binding between sperm and blood cells *in vitro*, as described here, may reflect similar events *in vivo* following rectal or vaginal intercourse. If HIV binds to sperm surface T4-like molecules, or even if virus binding to sperm is nonspecific, it is conceivable that initial infection of resident macrophages in these tissues, or of PBLs, could result from direct penetration of class II-positive cells by virus-bearing sperm. It may be significant in this regard that (*i*) local or systemic induction of cell surface class II expression for a variety of cell types may result from concurrent or preexisting inflammation or disease (12); (*ii*) healthy male homosexuals have elevated numbers of allogeneic cytolytic T lymphocytes, which, like T cells stimulated *in vitro* (Fig. 5), express HLA-DR molecules as activation antigens (26, 27); and (*iii*) rectal receptivity is a major AIDS risk factor for male homosexuals (28). If HIV infection of class II-positive cells by sperm penetration *in vivo* is as efficient as is sperm penetration of these cells *in vitro*, repeated alloimmunization or prior infection with other infectious agents would be risk factors also shared by hemophiliacs, intravenous drug users, and sexually active individuals living in areas where other sexually transmitted diseases or pathogens (cytomegalovirus, hepatitis B, parasites) are common (29).

Note Added in Proof. Portis *et al.* (33) have reported that efficient male-to-female sexual transmission of certain murine retroviruses occurs and that virions are associated with mature spermatozoa in the epididymis. Because target cells can be infected *in vitro* by interaction with sperm bearing surface-bound murine type C viruses, both findings support and extend the suggestion by Levy *et al.* (34) that horizontal transmission of murine retroviruses might occur through sperm penetration.

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