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Expression of β1 integrin complexes on the surface of unfertilized mouse oocyte

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SUMMARY

Integrins are a family of cell surface receptors that mediate cell-cell and cell-matrix interactions in a variety of different cellular systems. Here we show that unfertilized mouse oocytes express β1 class integrins both at mRNA and protein levels. Using the reverse transcription polymerase chain reaction and oligonucleotide primers based on the DNA sequence of mouse integrins, the RNA transcripts for the β1, α5 and α6 subunits were detected in unfertilized oocytes. The expression of the mRNAs is paralleled by the expression of the corresponding proteins, in fact, the α5/β1 and the α6/β1 complexes can be immunoprecipitated with specific antibodies from ^{125}I-surface-labeled oocytes. Using subunit-specific antibodies we also demonstrate the presence of the α3/β1 at the oocyte surface but α1, α2, α4 or αV subunits were not detectable. Since the mouse α3 DNA sequence is not available, we have not tested for the corresponding transcript. Integrin subunits α6 and β1 were differently distributed on the oocyte surface, as visualized by immunofluorescence staining and by immunoelectron microscopy. α6 antigen was mainly confined to the microvillous area of the oocyte surface, while β1 was more homogeneously distributed over the whole oolemma. These data demonstrate for the first time the expression of three β1 integrin complexes in unfertilized mouse oocytes. Such proteins may have a role in sperm-egg interaction or during very early steps of embryogenesis.

Key words: integrins, oocytes, mouse embryogenesis

INTRODUCTION

Integrins are a family of membrane receptors that mediate the adhesion of cells to the extracellular matrix and to other cells (Hemler, 1990; Ruoslahti, 1991; Hynes, 1992). These receptors are involved in a variety of biological processes including platelet aggregation, leucocyte recognition and adhesion during the immune response and cell migration during embryonal development. Various lines of circumstantial evidence suggest that integrins, or integrin-like molecules, may be present on the surface of mammalian gametes, and might be involved in the interactions between oocyte and sperm at fertilization.

Mouse and human spermatozoa adhere to beads coated with various extracellular matrix molecules, including collagen and laminin (De Felici et al., 1990). Similarly, rabbit sperm quickly adhere by their heads to collagen fibrils (Koehler et al., 1980). Fibronectin has been immunocytochemically detected on the heads of rabbit and human spermatozoa (Koehler et al., 1980; Glander et al., 1987). In the latter species a fibronectin band encircles the equatorial segment of the sperm head (Glander et al., 1987), the region by which the sperm first interacts with the oocyte plasma membrane at fertilization (Yanagimachi, 1988). The same sperm region is also able to bind vitronectin, another adhesive extracellular matrix protein (D'Cruz and Haas, 1991).

Evidence for the presence of integrin-like molecules on mammalian sperm are coming from the work of Lathrop et al. (1990). These authors reported that PH-20, a sperm protein involved in sperm-egg adhesion, has sequence homologies with both α and β subunits of integrin αX/β2. The homology encompasses regions of the α and β subunits that form the ligand binding pocket (Giancotti and Ruoslahti, 1991).

Additional evidence suggests that integrin-like molecules are expressed on the oocyte surface. In fact, adhesion of human and hamster sperm to hamster oocytes and subsequent fertilization can be prevented by addition of soluble peptides (Bronson and Fusi, 1990a, 1990b) containing the Arg-Gly-Asp (RGD) sequence that is a recognition signal for several integrins (Ruoslahti and Pierschbacher, 1987). The effect of the peptides was greatly reduced by changing the D residue into an E (Bronson and Fusi, 1990b), a mutation known to abolish recognition by integrins. Beads coated with RGD-containing peptides were shown to bind to the oocyte, but not to the sperm surface (Bronson and Fusi, 1990b; Fusi et al., 1992), indicating that the oocyte expresses RGD-receptors.

In this study we directly tested the integrin expression in mouse oocytes using DNA probes and antibodies to these molecules. We show that unfertilized oocytes express β1-type integrins at both the mRNA and protein level. Among

the various β1 integrins, oocytes were found to express three major complexes, corresponding to α3/β1, α5/β1 and α6/β1.

MATERIALS AND METHODS

Collection of oocytes

Ovulated oocytes were obtained from the oviducts of 8-10 week old CD-1 mice (Charles River, Como, Italy) that had been superovulated by intraperitoneal gonadotropin injection, 14-16 hours after injection of hCG. Cumulus oophorus cells were removed by hyaluronidase treatment, and the oocytes were finally washed and transferred to a Hepes-buffered culture medium. For polymerase chain reaction experiments great care was taken to collect only oocytes that were completely free of cumulus cells.

Polymerase chain reaction (PCR)

Total RNA was prepared from about 1000 unfertilized oocytes with the guanidinium thiocyanate, phenol-chloroform method (Chomczynsky and Sacchi, 1987). About 500 ng of total RNA was used to synthesize cDNAs using Mu MLV reverse transcriptase (20 U, Boehringer Mannheim, Biochemica) and 250 ng of oligo(dT) (Boehringer). 5 μl of a 20 μl reaction were used for PCR. Primers for β1 (AATGTTTCAGTGCAGAGCC and TTGGGATGATGTCGGGAC) were based on mouse cDNA sequence (Holers et al., 1989) and placed on two exons at the 3′ gene end (our unpublished data) to give an amplified fragment of 262 bp. Primers for α5 (CTCGGCTTCTTCAAACGTTCC and GATCTCAGACTCATGATCCCG) were based on mouse genomic sequence obtained in our laboratory to generate a 113 bp fragment. Primers for α6 (ATTACAGCGAAGGCAAAAGTGGT and CGGCACAGCAACCTTGAATATAC) generating a 208 bp fragment, were based on mouse genomic sequence kindly provided by Vito Quaranta (Scripps Clinic, La Jolla, CA, USA). The amplification was carried out in 50 μl containing 10 mM Tris-HC1 pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatine, 0.2 mM each dNTP's, 300 ng each primer, 2.5 U of Taq I polymerase (Perkin Elmer). The PCR program consisted of one cycle at 94°C for 4 minutes and 30 cycles of 1 minute at 94°C, 1 minute at 60°C, 1 minute at 72°C. Amplified fragments were detected on a 3% NuSieve Agarose gel after staining with ethidium bromide.

Antibodies

Polyclonal antisera to the β1, α1, α2, α3 α4, α5 and αV integrin subunits were prepared by immunizing rabbits with synthetic peptides reproducing amino acid sequences from the cytoplasmic domains of each subunit. The following peptides, obtained from Multiple Peptide System (San Diego, CA, USA), were used: β1: CTTVVNPKYEGK; α1: KIGFKRPLKKKMEK; α2: KYEKMTKNPDEIDETTELSS; α3: CRIQPSETERLTDDY; α4: KLQEENRRDSWSYINSKSNDD; α5: KRSLPGTAMEKAQLKPPATSDA; αV: KKRVRPPQEEQERE. Peptides were coupled to hemocyanin with glutaraldehyde (approximate peptide/carrier molar ratio of 50:1) and rabbits were injected with 500 μg of the conjugate in complete Freund adjuvant. Antibodies reacted specifically with the peptide sequence used for immunization, as determined by ELISA assay on peptide-BSA conjugates. The antibodies were specific to the appropriate subunit and did not show cross reaction with other integrin subunits as demonstrated by immunoprecipitation assays on different cell lines (Defilippi et al., 1991; Rossino et al., 1991). Due to the higly conserved sequence of the C-terminal region of integrin subunits all these antibodies react with integrins of several animal species. The monoclonal antibody GoH3 to integrin α6 subunit was a kind gift from Dr A. Sonnenberg (Central Laboratory of the Netherlands Red Cross, Amsterdam, The Netherlands). This antibody, although directed to the human α6, reacts with the mouse protein as well. An Ig fraction of a polyclonal antiserum to the murine fibronectin receptor was kindly provided by P. Bernardi, University of Padova, Italy (Bernardi et al., 1987). The latter antiserum reacts predominantly with the β1 integrin subunit.

Immunoprecipitation of integrins

Lactoperoxidase-catalyzed ^{125}I surface labeling was performed as described previously (Defilippi et al., 1991). About 500 oocytes were briefly incubated in acidic Tyrode solution (pH 2.5) to remove the zona pellucida, washed in PBS (10 mM phosphate pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$ and 1 mM $MgCl_2$) containing 10 mg/ml polyvinylpyrrolidone (PVP) and eventually transferred to 100 μl of PBS-PVP. For each reaction 2 mCi of carrier-free $Na^{125}I$ (Amersham) were used. Labeled oocytes were extracted for 20 minutes at 4°C with 0.5% Triton X-100 (BDH Chemicals, England) in TBS (20 mM Tris-HCl, pH 7.4, 150 mM NaCl) with the addition of 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mg/ml leupeptin, 4 μg/ml pepstatin, and 0.1 TIU/ml aprotinin (all from Sigma Co). After centrifugation at 10,000 ***g*** for 10 minutes, extracts were incubated with the specific antibodies for 1 hour at 4°C with gentle agitation. Soluble immunocomplexes were bound to Protein A-Sepharose beads (Pharmacia, Uppsala, Sweden) and recovered by centrifugation. Since the integrin α/β complexes are not dissociated under the experimental conditions used, antibodies, although specific for one subunit, immunoprecipitate both of them. After washing, bound material was eluted by boiling beads in 1% sodium dodecyl sulfate (SDS; Pierce, Rockford, IL, USA) and analyzed under non-reducing conditions by 6% polyacrylamide electrophoresis in the presence of SDS (SDS-PAGE; Laemmli, 1970) and fluorographed (Chamberlain, 1979).

Immunofluorescence studies

The zona pellucida of the oocytes was dissolved by incubation in acidic Tyrode (see above), followed by a rapid wash in culture medium. The oocytes were fixed (4% paraformaldehyde in PBS, 15 min), washed, and incubated (30 minutes, 37°C) in TBS containing 10 mg/ml BSA and 20 mM glycine, to block free aldehyde groups. The oocytes were then exposed (30 minutes, 37°C) to undiluted hybridoma supernatant GoH3 or to 25 μg/ml anti-murine β1 rabbit IgG. Incubation with the first antibody was omitted in control oocytes. Primary antibodies were detected by rhodaminated anti-rat or anti-rabbit IgG, respectively. In some experiments the smooth region of the oocyte surface was positively identified by staining the chromosomes of the underlying meiotic spindle with Hoechst H33258 fluorochrome.

Immunoelectron microscopic studies

Zona-free oocytes were fixed as above, blocked (30 minutes, room temperature), and incubated (60 minutes, 37°C) in the presence of undiluted GoH3 or 50 μg/ml anti-β1 antibodies. Incubation with the first antibody was omitted in control oocytes. GoH3-treated and their control oocytes were then sequentially exposed to anti-rat IgG (5 μg/ml, 30 minutes, 37°C) and protein G-gold (BioCell, UK, 5 nm, 1:10 dilution, 2 hours, 37°C), while anti-β1-treated and their control oocytes were directly transferred to protein G-gold. At the end of each treatment the oocytes were washed with TBS containing 2 mg/ml BSA. Oocytes were eventually additionally fixed with 1% glutaraldehyde and postfixed with OsO_4. Dehydration and Epon embedding followed standard procedures. Ultrathin sections (80-90 nm), stained with uranyl acetate and lead citrate, were studied using a Philips EM 301 electron microscope. Morphometric analysis of immunoelectron microscopic pictures was

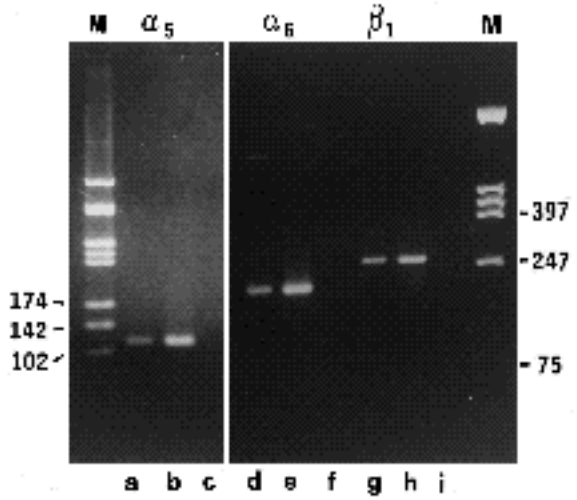

Fig. 1. Detection of integrin mRNAs in unfertilized mouse oocytes by RT-PCR. Total RNA from mouse oocytes was reverse transcribed and amplified with the three different pairs of primers (a,d,g). The same amount of RNA was amplified as a negative control (c,f,i). cDNA prepared from mouse fibroblast was used as positive control (b,e,h). The cDNA was amplified by PCR using oligonucleotide primers based on the sequence of mouse α5 (a,b), α6 (d,e) and β1 (g,h). Size markers are displayed at both sides.

performed with a Vidas image analysis system (Kontron-Zeiss). The number of gold particles per linear micrometer of plasma membrane was determined by analyzing seven randomly selected immunoelectron microscopic pictures for each sample.

RESULTS

Oocytes express mRNAs for integrin subunits

To detect integrin mRNAs we used the polymerase chain reaction, since a classical northern analysis would require more than 10^4 oocytes to collect enough RNA. Total RNA from 10^3 unfertilized oocytes was first reverse transcribed and the resulting cDNA was used as template for amplification. RNA from mouse fibroblasts was used as positive control. Oligonucleotide primers for the β1, α5 and α6 subunits were used, since the sequence of these molecules has been determined in the murine species (see Methods). As shown in Fig. 1, DNA fragments were amplified on oocyte cDNA with all three primer combinations used. The amplified DNA segments had sizes identical to those predicted from the mRNA sequence and comigrated precisely with the DNA fragments amplified on mouse fibroblasts cDNA (Fig. 1). Furthermore, the specificity of the amplification reaction was confirmed by hybridization with the corresponding probes (not shown). The possibility that amplified products are derived from genomic DNA contaminating the RNA preparation was ruled out since no amplification was observed when the reverse transcriptase reaction was omitted (Fig. 1 lanes c,f,i). In addition the primers used for β1 cDNA amplification correspond to sequences separated by introns at genomic level, and thus the amplification of contaminating genomic DNA would have generated a much larger fragment than that actually obtained. These results indicate that unfertilized oocytes express the transcripts for the β1, and at least two α integrin subunits, α5 and α6.

Integrin complexes are expressed at the oocyte surface

Unfertilized mouse oocytes were surface labeled with ^{125}I and subjected to immunoprecipitation, to verify whether these cells, in addition to the integrin transcript, also express the corresponding protein molecules. Polyclonal antibodies raised against synthetic peptides corresponding to COOH-terminal sequences of each integrin molecule were used since these reagents show a wide cross reactivity among integrins of different species. Monoclonal antibody GoH3 was used to detect the α6 subunit since it reacts with both human and mouse proteins. As shown in Fig. 2A (lane c),

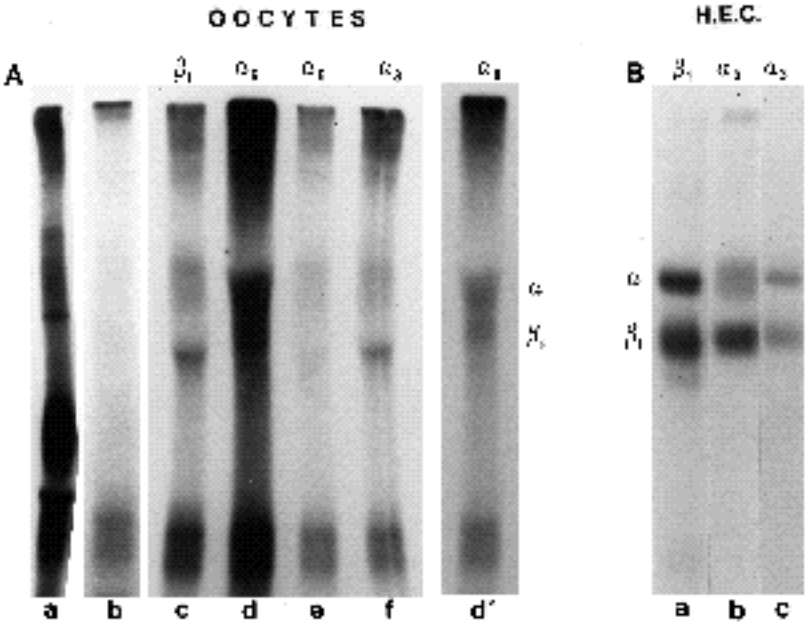

Fig. 2. Expression of integrin complexes at the surface of unfertilized oocytes. (A) Surface proteins of mouse oocytes were labeled with ^{125}I and integrin complexes were identified by immunoprecipitation with specific antibodies. Proteins are separated by SDS-PAGE under non-reducing condition and visualized by fluorography. Lane a: total ^{125}I-labeled, Triton-soluble oocyte proteins; lane b: polyclonal β1 antibodies incubated with 1 mg/ml of β1 C-terminal peptide; lane c: polyclonal β1 antibodies; lane d: monoclonal α6 antibody GoH3; lane e: polyclonal α5 antibodies; lane f: polyclonal α3 antibodies; lane d′: shorter exposure of lane d. (B) Integrin complexes of human endothelial cells are shown for reference. ^{125}I-surface-labeled human endothelial cells were detergent extracted and integrins were immunoprecipitated with specific antibodies; lane a: polyclonal β1 antibodies; lane b: polyclonal α5 antibodies; lane c: polyclonal α3 antibodies. The position of αs and β1 subunits are indicated.

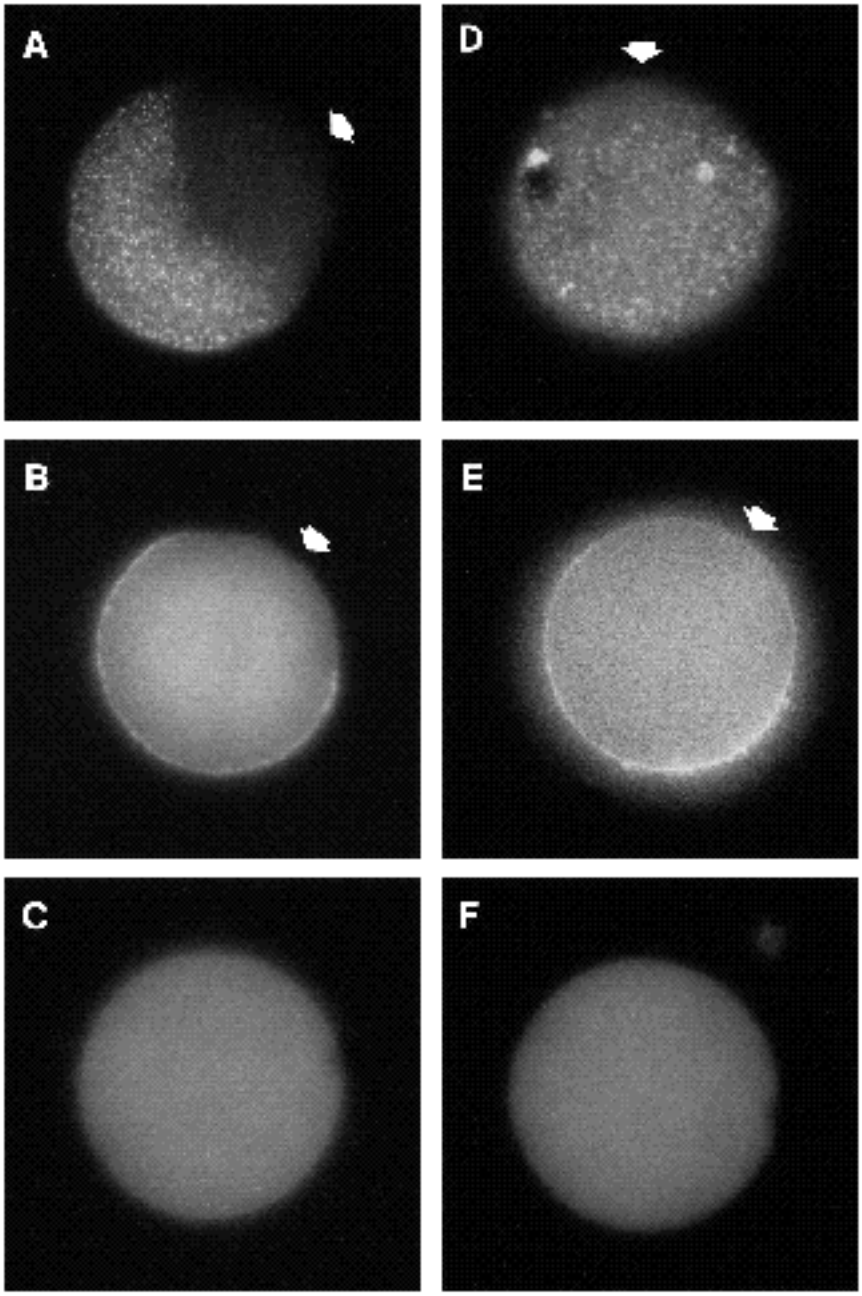

Fig. 3. Immunolocalization of α6 (A, B) and β1 (D, E) integrin subunits on zona-free unfertilized mouse oocytes. The cells were immunofluorescently stained with rat monoclonal antibody GoH3 to α6 (A,B) or rabbit IgG anti-murine β1 integrin (D, E), followed by the appropriate TRITC-conjugated secondary antibody. Control oocytes (C, F) were only exposed to the secondary antibody. A, D: tangential view; B, C, E, F: equatorial view. Notice that immunoreactivity to α6 is essentially confined to the microvillous area, while staining due to anti-β1 antibody can also be seen on the smooth surface of the oocyte. Smooth areas are indicated by arrows. Magnification, 500×.

antibodies to β1 immunoprecipitate two major bands corresponding to the β and its associated α subunits. Using a panel of α-specific antibodies, α6, α5 and α3 (Fig. 2A lanes d-f) were found to be the more abundant subunits, while α1, α2, α4 and αV were not detectable. The electrophoretic bands of oocyte integrins were rather broad and diffuse compared to the corresponding integrins from different cell types (Fig. 2B). The reason for this behaviour is unclear but it seems not to be due to a peculiar glycosilation. In fact, digestion of the immunoprecipitated material with chondroitinases, neuraminidase or N- and O-glycanases did not reduce the broadness of the bands. In addition to integrin molecules, unresolved high relative molecular mass material and an 80×10^3 M_r component were non-specifically immunoprecipitated. In fact, they comigrated with major labeled material in the total oocyte extract (Fig. 2A lane a) and were present in the material immunoprecipitated by antibodies blocked by the specific peptide (Fig. 2A lane b).

Integrins show a discrete localization at oocyte surface

The distribution of integrins at the oocyte surface was investigated by immunofluorescence and by immunoelectron microscopy. As shown in Fig. 3, unfertilized mouse oocytes were strongly stained by monoclonal antibodies reacting with mouse α6 and β1 integrin subunits. α6 antibody staining (Fig. 3A,B) was very finely punctate and essentially restricted to a portion of the oocyte surface corresponding to the microvillous area. On the contrary,

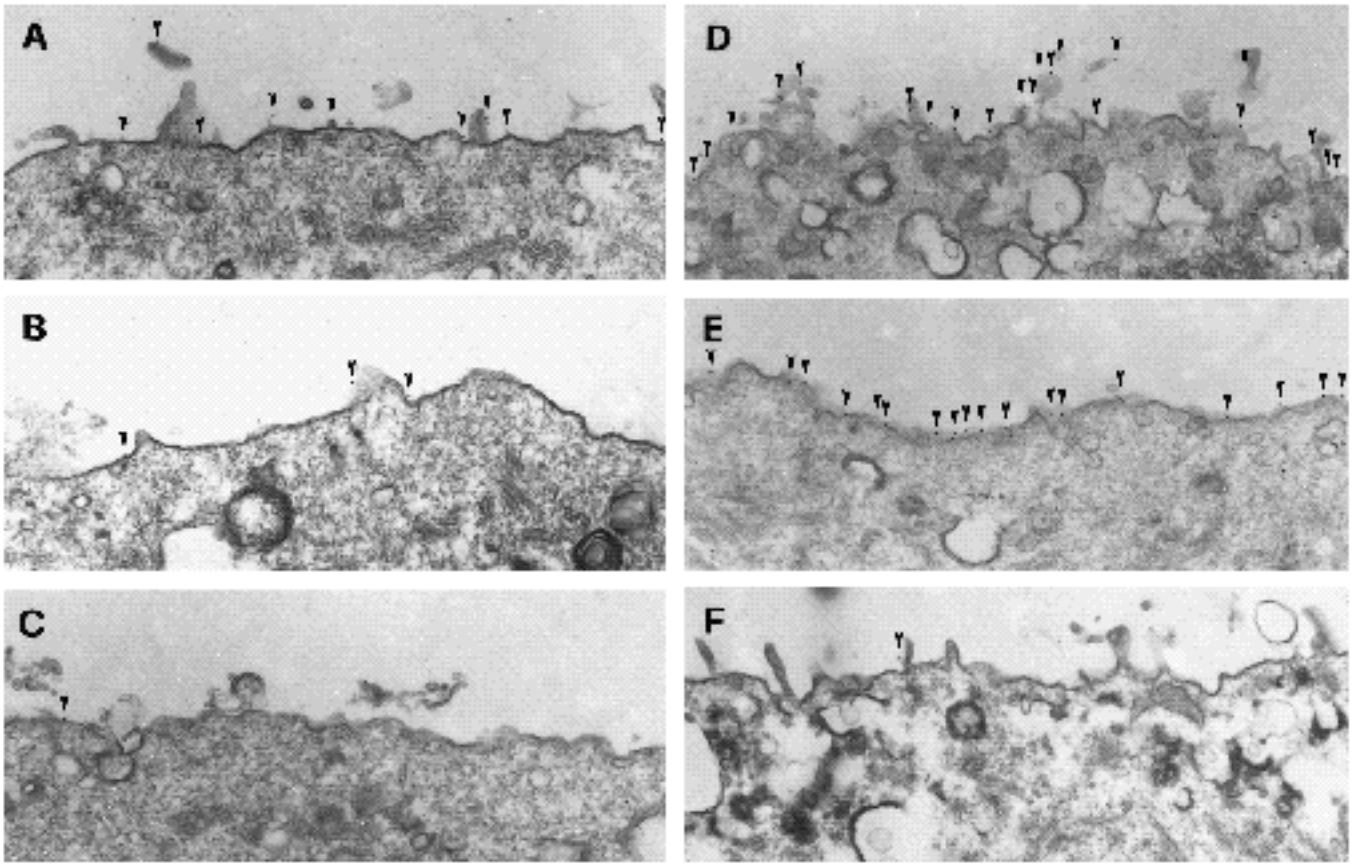

Fig. 4. Electron micrographs of the surface of oocytes labeled with antibodies against α6 (A, B) or β integrin subunit (D, E) and 5 nm protein G-colloidal gold (arrowheads). The density of α6 molecules per unit of surface area is higher on the microvillous (A) than on the smooth surface (B). The density of β1 molecules is very similar on both surfaces (D, E). (C, F) Microvillous surface of control α6 and control β1 oocytes respectively (first antibody omitted). Magnification, 25,000×.

immunoreactivity to β1 was present over the whole cell surface, and with slightly increased staining in the microvillous area (Fig. 3D,E). The immunofluorescence observations were confirmed by immunoelectron microscopy studies. Density of gold particles was calculated using a computerized image analysis system; the data obtained showed that particles corresponding to α6 were fivefold more dense on the membrane of microvilli (Figs 4A, 5B), than on the smooth area (Figs 4B, 5B′). Particles corresponding to β1 showed comparable density on both oocyte surfaces (Figs 4D,E, 5A,A′). Rare gold particles were occasionally seen in control oocytes, withouth specific localization (not shown).

DISCUSSION

In the present study we show that mouse oocytes express three β1 group integrins at their surface, namely α3/β1, α5/β1 and α6/β1. Three subunits, α5, α6 and β1, were identified both at the protein and mRNA level, while the fourth one, α3, was detected at the protein level. Immunofluorescence and immunoelectron microscopy showed that α6 was mainly concentrated on the microvillous region of the oocyte surface, while β1 was uniformly distributed.

The polarized distribution of α6 integrin subunit appears of particular interest in connection with the well known morphological and functional organization of the oocyte surface. Approximately 80% of the mouse oocyte surface bristles with microvilli and overlies cytoplasm containing cortical granules, while the remaining portion, overlying the metaphase spindle, is smooth and supported by a thickened layer of polymerized actin (Nicosia et al., 1977; Maro et al., 1984; Longo and Chen, 1985). These morphological differences correspond to distinct properties of the two regions. In fact, microvillous and smooth regions differ in membrane protein diffusibility (Wolf and Ziomek, 1983), and sperm-egg fusion only occurs in the microvillous area (Yanaginachi, 1988). The data reported here demonstrate for the first time that these two areas also differ in protein composition. The finding that the α6 integrin subunit is fivefold more concentrated in the microvillous portion of the oocyte, suggests a possible role of α6/β1 in fertilization. Preliminary attempts to inhibit in vitro fertilization with the GoH3 monoclonal antibody to α6 were, however, unsuccessful.

The three integrin complexes detected on the oocyte surface are known to be expressed on several mesenchimal and epithelial cell types where they function as receptors for extracellular matrix proteins such as fibronectin, laminin and collagens. α5/β1 is the prototype fibronectin receptor binding to the RGD sequence (Pytela et al., 1985). α3/β1 is a receptor for several extracellular matrix proteins includ-

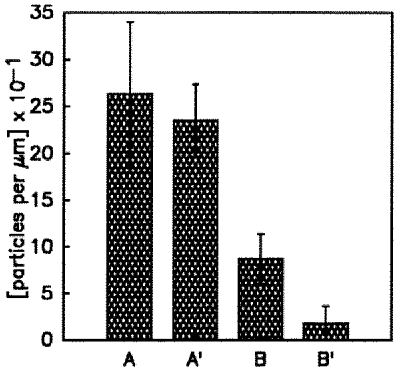

Fig. 5. Asymmetric distribution of the α6 integrin subunit on the oocyte surface. The gold particle density per linear μm of plasma membrane was determined by analyzing seven randomly selected immunoelectron microscopic pictures for each sample. A and A′ are the values for β1 on the micrivillous and smooth surface respectively; B and B′ are the values for α6 on the micrivillous and smooth surface respectively. The vertical bars indicate the standard deviation.

ing fibronectin and laminin. Its binding to fibronectin involves the RGD site (Elices et al., 1991) while binding to laminin is RGD independent (Gehlsen et al., 1992). α6/β1 is a receptor for laminin and it does not recognize the RGD sequence (Sonnenberg et al., 1988). In addition to being extracellular matrix receptors, α5/β1 and α3/β1 can be involved in cell to cell adhesion. Immunocytochemical studies in keratinocytes and in endothelial cells, as well as in other cell types, showed that these integrins are localized in areas of cell to cell contact (Kaufmann et al., 1989; Larjava et al., 1990; Lampugnani et al., 1991). The integrins expressed on the oocyte might therefore function in cell-cell and/or cell-matrix interaction during early embryonal development.

Thrombospondin is the only matrix protein reported to be present on unfertilized oocytes (O'Shea et al., 1990). This protein is stored in intracellular granules and found in several extracellular location at the two cell stage and later on. Thrombospondin contains an active RGD sequence and promotes the trophoblast spreading in vitro more efficiently than other matrix proteins (O'Shea et al., 1990), suggesting that it may have a role during trophoblast implantation. Other matrix proteins become expressed during early embryogenesis: laminin B1 and B2 chains appear at the four cell stage (Cooper and MacQween, 1983; Dziadek and Timpl, 1985) and fibronectin and collagen type IV are first detected at the blastocyst stage, in the inner cell mass (Wartiovaara et al., 1979; Leivo et al., 1980). Thus integrins may be important in mediating the organization of matrix proteins during the early phases of embryo development. In fact α5/β1 and α6/β1 are also expressed on embryonal stem cells (our unpublished data and Cooper et al., 1991).

Another possible role of these molecules on the oocyte may be to mediate sperm-oocyte interaction during fertilization. It has been reported that RGD-containing peptides can competitively inhibit sperm-oocyte adhesion and subsequent egg penetration (Bronson and Fusi, 1990a,b). Using peptide-coated beads these authors also showed that the RGD-binding molecule is localized on the oocyte and not on the sperm surface. These data were further extended by showing that α5 subunit antibodies bind to human oocytes (Fusi et al., 1992). Here we directly identify, on the oocyte surface, two RGD-binding integrin complexes, α3/β1 and α5/β1, that are good candidates to mediate the RGD-dependent sperm-oocyte recognition. Recent data indicate that integrin ligands may indeed be present on the sperm surface. In fact, PH30, a sperm surface protein possibly involved in sperm-oocyte fusion, contains a putative integrin-binding domain highly homologous to disintegrins, a family of integrin ligands found in snake venom (Blobel et al., 1992).

Altogether the data strongly suggest that integrins may play a physiological role in fertilization.

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REFERENCES

Bernardi, P., Patel, V. P. and Lodish, H. F. (1987). Lymphoid precursor cells adhere to two different sites on fibronectin. *J. Cell Biol.* **105**, 489-498.

Blobel, C. P., Wolfsberg, T. G., Turck, C. W., Myles, D. G., Primakoff, P. and White, J. M. (1992). A potential fusion peptide and an integrin ligand domain in a protein active in sperm-egg fusion. *Nature* **356**, 248-252.

Bronson, R. A. and Fusi, F. (1990a). Sperm-oolemmal interaction: role of the RGD adhesion peptide. *Fertil. Steril.* **54**, 527-529.

Bronson, R. A. and Fusi, F. (1990b). Evidence that an Arg-Gly-Asp (RGD) adhesion sequence plays a role in mammalian fertilization. *Biol. Reprod.* **43**, 1019-1025.

Chamberlain, J. P. (1979). Fluorographic detection of radioactivity in polyacrylamide gels with water-soluble fluor, sodium salicylate. *Analyt. Biochem.* **98**, 132-135.

Chomczynski, P. and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analyt. Biochem.* **162**, 156-159.

Cooper, A. R. and MacQueen, H. A. (1983). Subunits of laminin are differentially synthesized in mouse eggs and early embryos. *Dev. Biol.* **96**, 467-471.

Cooper, H. M., Tamura, R. N. and Quaranta, V. (1991). The major laminin receptor of mouse embryonic sterm cells is a novel isoform of the α6-β1 integrin. *J. Cell Biol.* **115**, 843-850.

D'Cruz, O. J. and Haas, J. B. Jr. (1991). Exposure of binding sites for clusterin and vitronectin on human sperm following antisperm IgG-induced deposition of membrane attack complexes (C5b-9) of human complement. *Biol. Repr.* **44** (Suppl. 1), 139.

De Felici, M., Camaioni, A., Russo, M. A. and Siracusa, G. (1990). Selective binding of mouse and human spermatozoa to beads coated with extracellular matrix components. *Mol. Reprod. Dev.* **27**, 337-343.

Defilippi, P., van Hinsbergh, V., Bertolotto, A., Rossino, P., Silengo, L. and Tarone, G. (1991). Differential distribution and modulation of expression of α1/β1 integrin on human endothelial cells. *J. Cell Biol.* **114**, 855-863.

Dziadek, M. and Timpl, R. (1985). Expression of nidogen and laminin in basement membranes during mouse embryogenesis and in teratocarcinoma cells. *Dev. Biol.* **111**, 372-382.

Elices, M., Urry, L. A. and Hemler, M. E. (1991). Receptor function for the integrin VLA-3: fibronectin, collagen and laminin binding are

differentially influenced by Arg-Gly-Asp peptide and by divalent cations. *J. Cell Biol.* **112**, 169-181.

Fusi, F. M., Vignali, M., Busacca, M. and Bronson, R. A. (1992). Evidence for the presence of an integrin cell adhesion receptor on the oolemma of unfertilized human oocytes. *Mol. Reprod. Dev.* **31**, 215-222.

Gehlsen, K. R., Sriramarao, P., Furcht, L. T. and Skubitz, A. P. N. (1992). A synthetic peptide derived from the carboxy terminus of the laminin A chain represents a binding site for the α3/β1 integrin. *J. Cell Biol.* **117**, 449-459.

Giancotti, F. G. and Ruoslahti, E. A. (1991). Single-chain integrin? *New Biol.* **3**, 525-6.

Glander, H. J., Herrmann, K. and Haustein, U. F. (1987). The equatorial fibronectin band (EFB) on human spermatozoa: a diagnostic help for male fertility? *Andrologia.* **19**, 456-459.

Hemler, M. E. (1990). VLA proteins in the integrin family: structures, functions and their role on leukocytes. *A. Rev. Immunol.* **8**, 365-400.

Holers, V. M., Ruff, T. G., Parks, D. L., McDonald, J. A., Ballard, L. L. and Brown, E. J. (1989). Molecular cloning of a murine fibronectin receptor and its expression during inflammation. *J. Exp. Med.* **169**, 1589-1605.

Hynes, R. O. (1992). Integrins: versatility, modulation and signaling in cell adhesion. *Cell* **69**, 11-25.

Kaufmann, R., Frosch, D., Westphal, C., Weber, L. and Klein, C. E. (1989). Integrin VLA-3: ultrastructural localization at cell-cell contact sites of human cell cultures. *J. Cell Biol.* **109**, 1807-1815.

Koehler, J. K., Nudelman, E. D. and Hakomori, S. (1980). A collagen-binding protein on the surface of ejaculated rabbit spermatozoa. *J. Cell Biol.* **86**, 529-536.

Laemmli, U. K. (1970). Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Lampugnani, M. G., Resnati, M., Dejana, E. and Marchisio, P. C. (1991). The role of integrins in the maintenance of endothelial monolayer integrity. *J. Cell Biol.* **112**, 479-490.

Larjava, H., Peltonen, J., Akiyama, S. K., Yamada, S. S., Gralnick, H. R., Uitto, J. and Yamada, K. M. (1990). Novel function for β1 integrins in keratinocyte cell-cell interactions. *J. Cell Biol.* **110**, 803-815.

Lathrop, W. F., Carmichael, E. P., Myles, D. G. and Primakoff, P. (1990). cDNA cloning reveals the molecular structure of a sperm surface protein, PH-20, involved in sperm-egg adhesion and the wide distribution of its gene among mammals. *J. Cell Biol.* **111**, 2939-2949.

Leivo, I., Vaheri, A., Timpl, R. and Wartiovaara, J. (1980). Appearance and distribution of collagens and laminin in the early mouse embryo. *Dev. Biol.* **76**, 100-114.

Longo, F. J. and Chen, D. Y. (1985). Development of cortical polarity in mouse eggs: involvement of the meiotic apparatus. *Dev. Biol.* **107**, 382-394.

Maro, B., Johnson, M. H., Pickering, S. J. and Flach, G. (1984). Changes in actin distribution during fertilization of the mouse egg. *J. Embryol. Exp. Morph.* **82**, 211-237.

Nicosia, S. F., Wolf, D. P. and Inoue, M. (1977). Cortical granule distribution and cell surface characteristics in mouse eggs. *Dev. Biol.* **57**, 56-74.

O'Shea, K. S., Liu, L. H., Kinnunen, L. H. and Dixit, V. M. (1990). Role of the extracellular matrix protein thrombospondin in the early development of the mouse embryo. *J. Cell Biol.* **111**, 2713-2723.

Pytela, R., Pierschbacher, M. D. and Ruoslahti, E. (1985). Identification and isolation of a 140 kD cell surface glycoprotein with properties expected of a fibronectin receptor. *Cell* **40**, 191-198.

Rossino, P., Defilippi, P., Silengo, L. and Tarone, G. (1991). Up-regulation of the integrin α1/β1 in human neuroblastoma cells differentiated by retinoic acid: correlation with increased neurite outgrowth response to laminin. *Cell Reg.* **2**, 1021-1033.

Ruoslahti, E. (1991). Integrins. *J. Clin. Invest.* **87,** 1-5.

Ruoslahti, E. and Pierschbacher, M. D. (1987). New perspectives in cell adhesion: RGD and integrins. *Science* **238**, 491-497.

Sonnenberg, A., Modderman, P. W. and Hogervorst, F. (1988). Laminin receptor on platelets is the integrin VLA-6. *Nature* **336**, 487-489.

Wartiovaara, J., Leivo, I. and Vaheri, A. (1979). Expression of the cell surface-associated glycoprotein, fibronectin, in the early mouse embryo. *Dev. Biol.* **69**, 247-257.

Wolf, D. E. and Ziomek, C. A. (1983). Regionalization and lateral diffusion of membrane proteins in unfertilized mouse eggs. *J. Cell Biol.* **96**, 1786-1790.

Yanagimachi, R. (1988). Mammalian fertilization. In *The Physiology of Reproduction.* vol. 1 (ed. E. Knobil, J. Neill, L. L. Ewing, G. S. Greewald, C. L. Markert and D. W. Pfaff) pp. 135-185. Raven Press Ltd., New York: USA.

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