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IMMUNOGENICITY OF HUMAN AMNIOTIC EPITHELIAL CELLS AFTER TRANSPLANTATION INTO VOLUNTEERS

C. A. AKLE M. ADINOLFI
K. I. WELSH S. LEIBOWITZ
I. MCCOLL

Department of Surgery, Paediatric Research Unit, Tissue Typing Unit, and Department of Pathology, Guy's Hospital and Medical School, London

Summary Human amniotic epithelial cells do not express on their surfaces HLA-A, B, C, and DR antigens, or β_2-microglobulin. In vitro these cells synthesise the enzymes lacking in patients with selected enzymatic deficiencies: the survival of a transplanted monolayer of human amniotic epithelial cells was therefore investigated in seven volunteers. None of the volunteers showed clinical signs of acute rejection, and amniotic epithelial cells were demonstrated by biopsy up to 7 weeks after implantation. HLA antibodies were not detected in samples of serum from four volunteers thoroughly investigated, and there was no in-vitro lymphocyte reaction to the amniotic cells in two of them. The results suggest that acute immune rejection does not occur after the transplantation of human amniotic epithelial cells.

Introduction

THE human placental chorionic syncytiotrophoblast does not appear to express the HLA-A, B, and C antigens or β_2-microglobulin (β_2m).[1,2] It can survive ectopically in the potentially hostile environment of the maternal lung.[3] There has been little study, however, of the expression of these antigens on the surface of the amniotic epithelium, despite many reports of its use as a graft to replace deficient tissue, to cover open wounds, or to improve the vascularity of ischaemic legs.[4,5] In most of the reported cases, the tissue apparently served its purpose well and caused very little clinical reaction, even after subcutaneous or intraperitoneal implantation[4,5] (F. G. Ellis and R. Pringle, personal communication). However, there is no objective evidence for the survival of the implants since biopsies, to establish whether the amniotic epithelum had survived or endogenous tissue had eventually replaced it, were rarely carried out.

Human amniotic epithelial cells do not express HLA-A, B, C, and DR antigens or β_2-m on their surfaces as judged by immunofluorescence techniques,[6] although in-vitro radiobiological studies suggest that small quantities of these substances are synthesised. Furthermore, we have demonstrated[6] that substantial amounts of certain lysosomal enzymes are produced by the amniotic epithelial cells, and that they are capable of correcting in vitro the enzymatic defects of patients with Hurler's syndrome (mucopolysaccharidosis type I).

These results, together with the evidence of successful human transplants of amniotic epithelium, encouraged us to assess the immunogenicity and survival of amniotic epithelial membrane implants in several volunteers with a view to treating patients with appropriate enzyme defects by such implants.

Subjects and Methods

Preparation of Membrane

Fetal membranes were obtained after an elective caesarean section for cephalopelvic disproportion. Both mother and full-term male infant were healthy, and there was no evidence of rhesus incompatibility. The mother's serum was negative for syphilis and Australia antigen. The sterile membranes were washed in sterile Hartmann's solution, cut into strips, and laid flat in petri dishes containing RMPI 1640 culture medium (Flow Laboratories Ltd., U.K.) with 100 U/ml penicillin and 0·5 mg/ml streptomycin. The dishes were incubated overnight at 37°C in a humidified atmosphere containing 5% CO_2. The membranes were then collected and, under strictly aseptic conditions, the amniotic epithelial cell layer was gently peeled free from the underlying chorion. The amniotic membrane thus obtained consisted of an epithelial monolayer on a basement membrane with an underlying collagen matrix containing a few fibroblasts. This tissue was cut into pieces of approximately 2 cm^2 and stored in tissue culture medium until implantation. Another sample of the same membrane was used to provide cells for tissue culture by digestion with 0·25% (w/v) trypsin (Wellcome Laboratories, Beckenham, U.K.). These cells were plated in RPMI 1640 culture medium supplemented with 20% donor calf serum and used in all the subsequent experiments.

Surgical Technique

Seven volunteers, six men and one woman, ranging in age from 28 to 80 years, received implants. A weal was raised in the skin of the upper arm with 2 ml of 1% lignocaine. A small cut was made into the skin with a no. 15 Swann Morton scalpel blade, and a small subcutaneous pocket was created by blunt dissection with a pair of fine artery forceps. A piece of amniotic membrane was placed in the subcutaneous pocket, and the wound was closed with one 6/0 nylon suture and dressed. The wound was examined regularly for any evidence of reaction as judged by pain, redness, or the formation of a nodule. The suture was removed after 10 days. The implanted areas were completely excised under local anaesthetic at varying times after implantation, and the tissue was prepared for cryostat and paraffin-embedded sections. The tissue was harvested after 16 days in one volunteer, 30 days in four volunteers, and 33 days and 54 days in the remaining two. All volunteers were apparently healthy with no evidence of immune supression. The female volunteer was postmenopausal because we thought it unwise at this stage to risk immunisation of a woman of childbearing age. Two volunteers (volunteers A and B) were previously sensitised to multiple allogeneic skin grafts.

Permission for the experiment was obtained from the Guy's Hospital Medical School ethical committee.

Histology

5 μm sections were prepared by standard procedures from paraffin-embedded blocks and were stained with haematoxylin and eosin. 4 μm cryostat sections were cut from unfixed tissue and stained by indirect immunofluorescence with a specific immune serum directed against human amniotic epithelial cells.[6,7]

Immunology

Blood samples from the mother and four of the volunteers were typed for ABO, and HLA-A, B, and DR antigens as described previously.[8] The volunteers were tested 2, 4, and 6 weeks after implantation for cytotoxic antibodies against HLA antigens by the microcytotoxic test.[8] 2 months after implantation, 2 of the volunteers (volunteers C and D) were bled and their lymphocytes tested, together with those of control subjects, for their ability to respond to the amniotic epithelial cells cultured in vitro.[9] Cells from the two volunteers and two normal controls were incubated for 10 days with (a) a confluent layer of amniotic cells and (b) irradiated allogeneic cells. Cultures were checked for the generation of blast cells with an inverted microscope. After 10 days, equal numbers of live lymphoid cells from both types of culture were tested at three different effector:target cell ratios for their ability to lyse phytohaemagglutinin-induced target cells.

Serum samples were also taken from the four HLA-typed volunteers 4 weeks after implantation. The samples were heat-inactivated, diluted 1:5 in phosphate buffered saline and incubated with a suspension of amniotic epithelial cells for 45 min. After thorough washing the amniotic epithelial cells were stained for human immunoglobulin with fluoroscein-conjugated goat antisera against heavy and light chains of human immunoglobulin (Behringwerke A.G., West Germany). A specific antibody against human amniotic epithelial cells was also used.[6,7]

Results

There was no evidence of acute reaction in any of the volunteers; in particular, there was no pain or redness of the skin. None of the implants showed symptoms, except that of volunteer C, which formed a nodule and itched at 3 weeks but settled by the time of excision at 4 weeks. Volunteer E developed a severe maculopapular rash in response to a course of ampicillin prescribed for a lower urinary tract infection during the 7th week of implantation. At this time, a nodule appeared at the site of implantation. The rash settled over the next few days and the implant was removed.

Examination of cryostat sections by immunofluorescence with the specific antiserum confirmed the presence of amniotic cells in all seven biopsies. The paraffin-embedded sections showed a full thickness of skin, including epidermis and dermal connective tissue, with portions of the tightly rolled amniotic epithelial membranes lying in the most superficial layer of adipose tissue. The connective tissue of the amniotic epithelium could be clearly identified and consisted of faintly staining eosinophil material. The amniotic epithelial cells survived as a layer of epithelium at the surface of the inplant or as the lining of alveolus-like slits between the folds of a tightly packed membrane. In some cases the layer was unicellular, whereas in others it was several layers deep, with the debris of desquamated cells lying in the "lumen". The graft was clearly demarcated from the tissue reaction which surrounded it.

The tissue reaction was composed of three separate elements. First, there was newly formed connective tissue with fibroblasts and a few thin-walled small blood vessels. The second element was a granulomatous reaction consisting of macrophages, foreign-body giant cells, and a few epithelioid cells and eosinophils. The granulomata formed discrete nodules whose distribution suggested a response to the suture material. Thirdly, there was an infiltrate of chronic inflammatory cells, predominantly lymphocytes and macrophages, with varying numbers of plasma cells and small numbers of eosinophils. Inflammatory cells were present in the newly formed granulation tissue and also as discrete perivascular cuffs in the connective tissue of the dermis.

The reaction was essentially the same in all the volunteers, although the extent and intensity of the inflammatory cell infiltrate varied greatly from a few lymphocytes in the adipose tissue to more florid lesions with massive lymphocytic infiltration and active plasma-cell formation. Nevertheless, there was no evidence of vasculitis or necrosis and polymorphonuclear cells were absent.

None of the four volunteers intensively tested shared HLA-B or HLA-DR series antigens with the mother; they were, therefore, incompatible with at least one HLA-B and one HLA-DR antigen of the child. Three of the four were also incompatible with at least one HLA-A antigen of the child. There was no evidence of cytotoxic antibody production in any of the four volunteers. Two of them (volunteers A and B) had previously been sensitised to multiple allogeneic skin grafts, and both had produced antibodies which were active against the mother's cells.[10] They were not positive for HLA antibodies at the time of implantation, nor did they produce HLA antibodies after the implant.

In the two volunteers whose lymphocyte response to cultured amniotic epithelial cells was tested 2 months after implantation, no lymphoblasts were observed with the amnion cell monolayers, in contrast to the allogeneic lymphocyte-stimulated cultures. Thus, cytotoxic cells were not generated when amniotic epithelial cells were co-cultured with allogeneic lymphocytes.

When the serum samples from the four volunteers were tested by immunofluorescence against the amniotic epithelial cells, there was no specific staining, yet the epithelial cells, even after 12 weeks in culture, continued to stain with the specific immune serum.

Discussion

The tissue reaction in the seven volunteers may be regarded as the result of three separate processes: the repair of a surgical wound; the reaction to a foreign body (in this case, the suture material); and a low-grade immune response to an allogeneic graft. The fibroblastic granulation and the giant-cell granulomata are, respectively, part of the repair process and the reaction to the suture material. It is more difficult, however, to assess the significance of the inflammatory cell infiltrate surrounding the embedded tissue and to establish whether this represents rejection, particularly since it is not possible to compare this reaction with that promoted by an isologous amniotic graft in the absence of any histocompatibility difference with the host. If there is an immune response to the graft, it is certainly low grade and chronic rather than active, and it is clearly ineffective, since the amniotic epithelial cells appear to survive and even, in some cases, to proliferate.

Further evidence that there is little or no immune response to the graft is the absence of detectable immunological reaction. In fact, four of the volunteers were shown not to produce anti-HLA antibodies even though two of them had been previously immunised against some of the maternal HLA antigens.[10] In addition, although lymphocytes from two of the volunteers were readily sensitised in vitro against HLA antigens presumably expressed on the amniotic

epithelial cells, sensitisation could not be induced by co-culturing with amniotic cells from the original donor.

Furthermore, serum samples collected 1 month after implantation from these four volunteers were found by immunofluorescence not to react against the amniotic cells cultured in vitro, although the cells continued to express the specific antigen.

Although our results show no acute rejection of the amniotic epithelial cells, further studies are needed to establish whether these cells can be successfully transplanted without immunosuppression to treat patients with appropriate inborn errors of metabolism.

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Requests for reprints should be addressed to M. A., Paediatric Research Unit, Guy's Hospital Medical School, Guy's Tower, London Bridge, London SE1 9RT.

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APPLICATION OF SPECIFIC EXTRACORPOREAL REMOVAL OF LOW DENSITY LIPOPROTEIN IN FAMILIAL HYPERCHOLESTEROLAEMIA

W. STOFFEL H. BORBERG
V. GREVE

Institut für Physiologische Chemie und Medizinische Klinik der Universität zu Köln, West Germany

Summary A highly selective method for the removal of apolipoprotein-B-containing serum lipoproteins (low density lipoproteins [LDL] and very-low-density lipoproteins [VLDL]) from hypercholesterolaemic patients by immunoadsorption in an extracorporeal system consists of separation of plasma from the blood cells by a blood-separation centrifuge, followed by the selective adsorption of LDL from the plasma on anti-LDL-'Sepharose'. The LDL-free plasma effluent, from which high-density lipoproteins (HDL) and other plasma proteins are not removed, is returned to the patient with the blood cells. Three patients with familial hypercholesterolaemia, one homozygous and two heterozygous, were treated repeatedly during a period of 9 months. No undesirable side-effects or changes in clinical, chemical, haematological, or immunological parameters have yet been observed. The new procedure has several advantages over treatments currently used; it is non-invasive, more specific, and less costly and lowers LDL to a greater degree.

Introduction

WE have demonstrated that apolipoprotein-B-containing lipoproteins (low-density lipoproteins [LDL] and very-low-density lipoproteins [VLDL]) can be removed specifically and efficiently in vitro and in vivo from pig blood by a combination of extracorporeal plasma separation and LDL immunoadsorption.[1] This technique has been applied over the past 9 months to three patients with familial hypercholesterolaemia; one patient was homozygous and two were heterozygous.

The efficiency and safety of extracorporeal removal of LDL are described here, and its advantages over plasma exchange (plasmapheresis) in the treatment of patients with familial hypercholesterolaemia are summarised. Plasma exchange treatment has been discussed by Thompson.[2]

Patients and Methods

Patients

Patient 1 was a 28-year-old male heterozygote. His body weight was 73 kg and he underwent treatment for 3 months. Patient 2 was a 37-year-old female heterozygote who weighed 68 kg and underwent treatment for 9 months, and patient 3 was a 15-year-old homozygous boy whose body weight was 47 kg. His period of treatment was 3 months.

Methods

The chemical procedures for the measurement of lipids and lipoproteins have been described previously.[1] Human LDL was purified by standard procedures;[3] it was proved to be homogeneous by agarose electrophoresis,[4] immunodiffusion,[5] and immuno-electrophoresis.[6] Human LDL was covalently bound to 'Sepharose' C1-4B for mass production of monospecific antibodies against human LDL from sheep immune serum. Anti-LDL was coupled to cyanogen-bromide-activated sepharose C1-4B as previously described.[1] For the treatment of patients the following changes to the published procedure were made: two silanised, glass columns (internal diameter, 10 cm; length, 8 cm) with sintered glass filters (pore size, 20–40 μm) were each packed with 400 ml anti-LDL-sepharose. The blood flow-rate was adjusted to 60–80 ml/min, and the plasma flow-rate during treatment was adjusted to approximately 30 ml/min. Coagulation was prevented by treatment with heparin (input rate, 40 units/min; not more than 10 000 units per treatment) or a combination of heparin (2500 units before the run) and citrate (acid-citrate-dextrose, United States Pharmacopeia formula B) added to blood in a ratio 1:8. The two columns were used in rotation; while LDL was being desorbed from one column (out of the circuit) the other (in circuit) served as immunoadsorbent. Carrot juice was given to patients the evening before treatment so that movement of LDL through the column could be monitored by the orange colour of carotenes in the LDL. The dead volume of the entire system, consisting of an IBM continuous-flow blood-cell-separator centrifuge (model IBM 2997), tubing, and anti-LDL column volumes, was about 450–500 ml. Before switching columns from adsorption to desorption, the plasma in the dead volume of the LDL-loaded column was displaced into the patient with 400–500 ml saline. LDL-desorption was performed with 1 mol/l glycine/HCl buffer pH 2·8–3·0, as previously described. The working capacity of each column was about 2 g total LDL-cholesterol (or about 1 g apolipoprotein-B or 4 g LDL). During treatment the first immunoadsorbent column was saturated within about 30 min, the second and third in about 45 min, and the fourth in 60 min.

Two columns were assigned to each patient and were used in successive treatments; some have been in use for 9 months without any loss of LDL-binding capacity. Between treatments the columns were stored at 4°C under 0·02% NaN_3 in saline saturated with chloroform. The columns were rinsed thoroughly with saline (3–4 litres) before use.