Embryonic Stem Cells: From Research to Therapy

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At the turn of the 20th century, biology brings us a series of new debates, based on recent technological advances, which concern our society. After the genetically modified organisms (GMO's) came Dolly and other cloned mammals, and then the embryonic stem cells of human origin. These three advances, eventually combined together, have great prospects to increase our well-being.

This review will focus on the new discoveries regarding embryonic stem cells by summarizing the scientific advances, outlining the potential benefit for our society and finally raising a few ethicalissues.

WHAT ARE EMBRYONIC STEM CELLS ?

What is a Stem Cell ?

The classic definition of a stem cell is a cell that can divide indefinitely and give rise to differentiated cells of various types. Its role is to replace dead cells, maintaining a functional tissue. To achieve this, a stem cell, when dividing, gives rise to two daughter cells, one being a stem cell, the other differentiating into a precursor cell or a functional cell type. Not all stem cells will conform perfectly to this stereotype since one could give rise to two differentiated cells while the neighboring could generate two stem cells. The operational unit is thus the stem cell compartment.

The concept of the stem cell is being used with some flexibility because the formal identification of this kind of cells in an organism is extremely difficult. It cannot be easily recognized by the usual techniques since it does not have the characteristics of a differentiated cell and generally it does not divide very frequently in the organism. Moreover, the behavior of a cell in culture is not always the same as *in vivo*. As we shall see, embryonic stem cells divide indefinitely *in vitro* but exist only for a few days during the normal development of an embryo. On the contrary, many stem cells in the adult divide during the individual lifetime but can not be isolated and cultivated *in vitro*. A final characteristic of a stem cell is its "potency", that is the array of different cellular types that it can form. Again, it is very difficult to assess potency *in vivo* and most of our knowledge is derived from *in vitro* experiments.

The most potent cell is the fertilized egg. It is totipotent since it gives rise to all tissue types necessary to the formation of an adult. No stem cell is totipotent, the closest to it are embryonic stem cells and embryonic germ cells which are pluripotent, giving rise to many very different cell types from gut to brain ones. Other stem cells are said to be multipotent, giving rise to several related cell types, for example the various blood cells. The concepts of pluripotency and multipotency are convenient to use but the distinction between the two is being challenged by recent experiments showing, for example, that some blood stem cells can also give rise to very different cell types such as muscle or neurons, albeit at a very low frequency (Ferrari et al., 1998). This phenomenon of a stem cell being able to generate cell types other than the ones it normally forms is called plasticity or transdifferentiation.

WHERE DO WE FIND STEM CELLS *IN VIVO?*

A stem cell population can be found in numerous adult organs and plays a crucial role in the maintenance of those organs during the entire lifetime. In general, organs in which cells divide, differentiate and die rapidly, have stem cells. These organs are for example the hair, the skin or the gut.

In the case of the intestine, the inner surface is covered by very small folds of a cell layer, the intestinal epithelium. Each fold, called a crypt, contains about 250 cells including 4 differentiated cell types necessary for the digestive and absorption functions of the gut (Stappenbeck et al., 1998). These differentiated cells are located in the top part of the crypt. Below them are a series of fast dividing cells, the progenitor cells. The stem cells are few and can be found at the bottom of each crypt. In each division, the stem cell gives rise to one new stem cell and one progenitor cell which continue to divide, leading to differentiated cells that a few days later will die and be released into the gut cavity In the mouse gut, it takes only 3 to 5 days for a newborn progenitor cell to complete its program. The stem cells are responsible for this rapid turnover. One critical role

of the stem cells in the gut is to precisely maintain the equilibrium (homeostasis) between cell formation and cell death.

This role of the stem cells is illustrated when chemotherapy is administrated to a patient with cancer. The treatment generally targets rapidly dividing cells, not only the cancer cells but also the hair and gut progenitor ones. Thanks to the stem cells which divide slowly and are thus unaffected by the treatment, hair grows back and the intestine regains its functions. If a deregulation occurs in a stem cell so that it does not stop dividing, it can lead to cancer. In human colon cancer, nearly all the tumors carry a mutation in the APC (Adenomatous polyposis coli) gene or in the beta-catenin gene leading to an overactivation of the Wnt signaling pathway. On the contrary, a mutation in the TCF4 gene of the mouse, that switches the Wnt pathway off, leads to a lack of intestinal stem cells. This shows that a precise regulation of a signaling pathway controlling stem cell proliferation (here the one triggered by the Wnt secreted factors) is necessary to maintain homeostasis in an organ.

Recent results show that even organs such as the brain, which were thought not to have stem cells, do have them. It is fully possible that all organs have stem cells, maybe dividing very slowly and/or being extremely rare.

The recent discoveries of human embryonic stem cells and their regulation pathways have not yet yielded therapeutic applications. One type of stem cells is however being used routinely in the hospitals during bone marrow transplantation. Indeed, cells from the bone marrow obtained directly from the bone cavity or isolated from blood after a specific treatment contain precursor cells for all types of blood cells such as red blood cells ones and lymphocytes. Moreover, it is likely that only 1 in 10,000 cells from the bone marrow is a long-term stem cell (Berardi et al., 1995). This multipotential hematopoietic stem cell is probably the best-characterized stem cell to date. It can self-renew as well as give rise to lineage restricted stem cells, which in turn generate precursor cells that will differentiate into specific blood cell type. *In vivo*, this stem cell is able, when transplanted, to recolonize a deficient bone marrow and regenerate a complete hematopoietic system. This property is used therapeutically in patients with affected blood cells (e.g. leukemia).

The discovery and isolation of other stem cell types have raised the possibility of new cellular therapies being as successful as the bone marrow transplantation. This is especially true for embryonic stem cells since they represent the most potent stem cells.

BRIEF DESCRIPTION OF EARLY DEVELOPMENT OF THE HUMAN EMBRYO

Before discussing the exciting new findings on embryonic stem cells, it is necessary to describe early human development; particularly since many of the ethical problems about this cells arise from their origin.

After ovulation, the oocyte will travel down the Fallopian tube where fertilization occurs. In the following 2 days, it will divide 3 times to give rise to the 8-cell morula. Based on research on model animals, it is proposed that these 8 cells are totipotent (Gilbert, 2000). They could, in theory, be separated from each other and give rise to 8 identical embryos and later adults. This is the last stage where totipotent cells are present. In the following days (4-5 days after fertilization), a cavity, the blastocoel, will develop in the embryo and it will form a blastocyst, which is composed of three cell types. The outer layer of cells is the trophoblast which will form some extraembryonic tissues and participate in the formation of the placenta. The mass of cells located inside the blastocyst is called the inner cell mass (ICM) and is composed of 2 cell types. One sheet of cells, the primitive endoderm, separates the cell mass from the blastocoel. It will give rise to another extraembryonic tissue, the yolk sac. The core of the ICM is the epiblast, which is isolated from the environment by the trophoblast and primitive endoderm. All the tissues forming the embryo proper and therefore the adult are derived from this epiblast, which consists of 20 to 40 cells. About 5 days after fertilization, the blastocyst will implant in the uterus (Drews, 1993). During the second week of gestation, the embryo will continue to grow. At the onset of the third week, the gastrulation process begins, during which the epiblast generates the three germ layers, the endoderm, mesoderm and ectoderm that constitute the first segregation of tissues participating to the embryo. The endoderm will give rise to the gut, lungs and some other organs, the mesoderm forms mostly the muscle, heart and skeleton, while the ectoderm will form the skin as well as the nervous system. During gastrulation, the epiblast will also give rise to a very small population of cells, the primordial germ cells which will migrate during the fifth week into the forming gonads (future testis or ovary) to produce the germ cells. These germ cells

will give rise much later to spermatozoids or ovules.

The growth and differentiation of the embryo will continue but all the tissues that will give rise to embryonic or germ cells are already in place.

ORIGIN OF EMBRYONIC STEM CELLS (ES) AND EMBRYONIC GERM CELLS (EG)

In 1981, it was discovered that blastocyst embryos from a mouse could give rise to pluripotent cell lines, the embryonic stem cells (ES cells) (reviewed in Hadjantonakis and Papaioannou, 2001; Smith, 2001). To achieve that feat, it is necessary to recover blastocysts before their implantation, remove the trophoblast layer of cells that surrounds the ICM and place it in culture. The isolated ICM is cultivated on top of a layer of fibroblast cells (called feeder cells) that provide factors necessary for the proliferation of the ICM-derived cells without their differentiation. After several days, colonies of proliferating cells can be isolated and further cultivated to give rise to ES cell lines (Evans and Kaufman, 1981; Martin, 1981). The embryonic stem cells are thus isolated from the inner cell mass of the blastocysts and have been shown to have properties equivalent to epiblast cells. ES cells can thus be considered as epiblast cells in a "frozen" state that can be perpetuated *in vitro*. Since their initial discovery, it has been shown that ES cells can be cultivated without feeder cells provided that some growth factors are added to the culture medium.

It is noteworthy to point out that most ES cell lines are derived from one single inbred strain of mouse (strain 129). Attempts to obtain ES cells from other strains were until recently unsuccessful and even today represent a challenge (Brook and Gardner, 1997). Therefore most of our knowledge comes from ES cells from that particular inbred strain. The fact that each strain has a different capacity to form ES cell lines may be particularly significant when considering human sources of ES cells. Indeed, each human has its own genetic identity while all mice from an inbred strain have identical genes (except for the male/female difference) and can therefore be considered clones. Another point to note is that the frequency of ES cell line formation is not high. In the original article from Evans and Kaufman (1981), only 30% of the blastocysts gave rise to ES lines.

In 1998, Thomson and collaborators succeeded in transposing this technique to humans (Thomson et al.1998). First they started with 36 fertilized embryos obtained from *in vitro* fertilization laboratories. They were cultivated and 14 developed to the blastocyst stage. The ICM was then isolated and placed in culture with mouse fibroblasts as feeder cells. After isolation and further cultivation, they obtained 5 human cell lines. For ethical reasons, it is not possible to demonstrate *in vivo* the pluripotentiallity of human stem cells but *in vitro* experiments indicate that these human cell lines are as pluripotent as the mouse ES cells. Even if formally the human cell lines have not been shown to meet all the criteria to be *bona fide* ES cells, they are considered equivalent and are generally called human ES cells (but also sometimes human pluripotent stem cells). Up to now, ES cells can only be obtained from mouse, humans and monkeys (Thomson et al. 1995).

Another source of pluripotent stem cells from the embryo are the primordial germ cells which are fated to give rise to male or female gametes after migrating into the gonads. In 1992, two groups were able to isolate primordial germ cells from mouse embryos before or just after their migration to the forming gonads (Matsui et al., 1992; Resnick et at., 1992). By adding several growth factors to the medium, they noted that the primordial germ cells had many characteristics of embryonic stem cells including the possibility to differentiate in cell types derived from the three germ layers, endo-, meso- and ectoderm. Such cell lines derived from primordial germ cells are called embryonic germ cells (EG). One important difference between ES and EG cells is that genes in EG cells show some parental imprinting. The mechanism of imprinting is not yet completely understood but is an important parameter in the EG cell differentiation that can lead to uncontrolled variations.

The human cells equivalent to EG ones have been isolated by Gearhart and coworkers in 1998 (Shamblott et al., 1998). They isolated primordial germ cells from 38 embryos obtained after therapeutic termination of pregnancy between the 5^{th} and 9^{th} week of development. This led to the generation of several lines of pluripotent cells, equivalent to the mouse EG cells, which could differentiate into tissues coming from all three germ layers (Shamblott et al., 2001). Despite a risk of higher variation (in the present state-of-the-art knowledge), these EG cells are particularly important from an ethical point of view since they can be obtained from aborted fetuses instead of "unused" blastocysts for ES cells.

Since much less research has been done on EG cells, we will focus mainly on ES cells in the remainder of this review.

PLURIPOTENTIALITY OF ES CELLS

Formation of Teratoma by ES Cells *in Vivo*

In the 1970s, a series of experiments showed that pre-gastrula mouse embryos could be grafted into adult mice and that they generated teratocarcinomas (see reviews of Hadjantonakis and Papaioannou, 2001; Smith, 2001). Similarly, when a group of mouse ES cells is grafted into an immunocompatible mouse, the ES cells will form a teratocarcinoma. These tumors contain differentiated cells of clearly identifiable types such as muscle or cartilage. The teratocarcinomas can form various cell types belonging to any of the three layers. Similar grafting experiments have shown that mouse EG cells could also form teratocarinomas.

Since it is assumed that human and mouse ES and EG cells are equivalent, this ability to give rise to teratomas is a mixed blessing for their potential therapeutic use. Indeed, it shows that the ES and EG cells are highly pluripotent, but also that undifferentiated stem cells, when placed in an organism, can develop into tumors.

DIFFERENTIATION OF ES CELLS *IN VITRO*

ES cells can be indefinitely cultivated *in vitro* in an undifferentiated state provided that some extrinsic factors are added to the culture medium. In the early days of ES cell research, the ES cells were always cultivated on a feeder layer of fibroblasts that provided the necessary trophic factors. Later, it was found that the cytokine leukemia inhibitory factor (LIF) could be added to the medium to maintain the ES cells in an undifferentiated state without feeder cells (Smith et al., 1988). One gene, Oct-4 has been shown to be expressed specifically in the undifferentiated ES cells and may participate in keeping the cells in that undifferentiated state (Nichols et al. 1998).

The withdrawal of LIF or feeder cells leads to the differentiation of ES cells. This differentiation process is enhanced when the ES cells are cultivated in suspension. In that situation, the ES cells cannot attach to the culture dish and they will aggregate with each other, forming clumps of cells. These clumps, called embryoid bodies (EB) will start differentiating into various cell types. After several days, the EB can be transferred into other culture dishes where the cells can again reattach and further differentiate. By fine tuning these manipulations, a wide range of cell types can be obtained such as blood cell, heart muscle cell (even beating EB can be seen), cartilage cell and neuron (see for exemple Wiles and Keller, 1991). However, there is generally a mixture of various cell types in one single dish and one procedure will not always give the same results from experiment to experiment. Only those "skilled in the art" can obtain a specific cell type with moderate efficiency and frequency.

It was discovered that the addition of retinoic acid (RA) or dimethylsulfoxide (DMSO) to the EB culture could alter the differentiation path taken by the cells. With RA, neurons were preferentially formed while DMSO induced mostly the formation of muscle cells (Bain et al., 1995; Fraichard et al., 1995).

This indicates that it is possible to guide, at least to some extent, the differentiation of ES cells. The recent generation of human ES cells and their therapeutic potentialities has reinvigorated this research field with the aim to guide the differentiation of ES cells along specific paths to obtain an homogenous population of cells that could then be safely used in patients. The most promising approach to achieve this goal is based on the application of our knowledge of embryonic development to the differentiation of EBs. Indeed, it was shown that the progressive differentiation of EBs follows the sequence of events normally occurring in an embryo. Soon after the aggregation of the ES cells into a cluster, the outer layer differentiates into primitive endoderm cells and later, mesoderm cells appear inside the EBs. This sequence of event as well as the panoply of genes expressed is reminiscent of gastrulation during which the three germ layers are produced. Later differentiation processes in the EBs are also likely to parallel the normal embryonic development (see for exemple Gratsch and O'Shea, 2002).

Our knowledge and understanding of the mechanisms that are responsible for normal embryonic development has enormously increased these last 15 years. This gain has been mostly achieved by identifying and studying the genes and proteins involved in the development of the mouse but also in other model organisms such as the fruitfly, the frog or the nematode worm. This understanding obtained by "basic" research can now be used in "applied" research to solve the problem of guiding ES cells differentiation along chosen paths.

Intensive research continues to recapitulate *in vitro* the sequence of events occurring in the embryo by supplying combinations of growth and differentiation factors to ES cells at various stages of

their differentiation. This approach would lead to the reproducible generation of a specific cell type according to the differentiation protocol used.

FROM ADULT TO ES CELLS AND BACK TO THE ADULT

Integration of ES Cells into the Embryo

A remarkable property of murine ES cells is their capacity to participate to all tissue types *in vivo* (Beddington and Robertson, 1989; Hogan et al., 1994). It is possible to inject a few ES cells into the blastocoel of a blastocyst embryo and then to reintroduce this embryo into a female. It can then implant and develop normally. The resulting pup is a chimera containing cells derived from the injected ES cells and from the blastoyst. The chimerism can easily be visually detected if the ES cells carry the genes for one coat color (e.g. brown/agouti) and the host blastocyst for another color (e.g. white/albino), resulting in a mottled mouse. The progeny of the ES cells can contribute to all tissues of the chimera including the germ cells. In this case, half of the chromosomes from the offspring of the chimera will come from the ES cell derivatives and the second half from the other parent. Similar results have been obtained with EG cells. By manipulating the embryo receiving the ES cells, it has even been possible to obtain mice entirely derived from the ES cells without any contribution from the host embryo. Albeit it requires difficult manipulations, specific ES cell lines and is a low frequency event, it demonstrates, however, that the ES cells can form all adult tissues. The ES cells however are not able to form extraembryonic tissues such as the trophectoderm and placenta, which are derived from the host embryo.

There is one caveat; during prolonged culture periods ES cells may progressively lose their competence to participate in all tissues and in particular to the germline. This loss of potentiality probably results from some partial, yet undetectable, differentiation of the ES cells due to non-optimal culture conditions and manipulations.

GENETIC ENGINEERING OF ES CELLS

A unique approach to specifically alter genes in the ES cells was developed in the laboratory of M.Cappechi and others (Thomas and Capecchi, 1987; McMahon and Bradley, 1990). They showed that it was possible to engineer a gene *in vitro* using the tools of molecular biology and to reintroduce that DNA fragment into the ES cells. At a low frequency, the introduced, engineered, fragment will replace the homologous gene present in the ES cell by a process called homologous recombination. This leads to an ES cell that carries in its chromosomes one normal gene and one engineered gene in place of the normal one. That ES cell can be further cultivated to give rise to a clone (a group of cells of the same genetic composition). Cells of that clone can then be injected into a blastocyst, giving rise to a chimeric mouse. Provided that the chimerism extend to the gonads, it is possible to cross that chimera to obtain heterozygote offspring that carry the altered gene (originally from an ES cell) and a normal gene from the other parent. Further crossing between heterozygote carriers will generate homozygote mice in which both copies of the gene are altered according to what has been engineered *in vitro*.

This long and tedious technique is called targeted mutagenesis since it allows one to design a specific mutation for any chosen gene on the blackboard and then to create a mouse strain that will carry it. It is now possible to completely delete a gene; to remove part of its function; to replace it by another gene; to activate or inactivate it in a specific tissue or at a specific time; etc. Recent advances in molecular biology have removed most of the technical barriers of the DNA engineering such that the principal limiting factor for the mouse genetic engineering is the researcher's imagination and his/her financial possibilities.

FROM AN ADULT CELL TO AN ES CELL

The making of Dolly, the cloned sheep, was an earthquake not only for our society but also for the community of biologists because it opened new doors. We will not discuss the "clone-a-dictator" kind of applications, usually called reproductive cloning, but rather how cloning could be used to generate stem cell lines from a chosen individual such as a patient. Such techniques have not yet been accomplished in humans but should be at hand in the nearby future.

The general procedure is to recover some cells from the patient, for example by scraping slightly the inside of the mouth. At the same time, it is necessary to obtain some eggs from a woman, fertilize them *in vitro* and remove their nucleus before they divide. The nucleus is then removed from a cell of the patient and transplanted into the recipient fertilized egg. This forms a new zygote that will be cultivated *in vitro* up to the blastocyst stage. That

blastocyst is then placed in culture on a layer of feeder cells where it will have the possibility to produce pluripotent ES cells that can be further grown, engineered and frozen.

Voilà! These ES cells will carry in their nucleus the same genetic information of the patient. There is one caveat; not all the genetic information will be the same since mitochondria, small organelles located in the cytoplasm of the cell, also contain DNA coding for a few genes and these mitochondria will be coming from the egg. One technical modification with important ethical consequences is that it may be possible in the very near future to use an unfertilized egg as recipient instead of a one-cell embryo.

This technique, called therapeutic cloning, has not yet been done but is well within reach of the present technology. It is expected however to have a rather low rate of success since all these manipulations require highly trained scientists and optimal conditions.

POTENTIAL THERAPEUTIC AND PHARMACEUTICAL APPLICATIONS

Up to now, the ES cell technology is not applicable to human use. There is however great hopes to relatively soon be able to cure numerous conditions, from heart attacks to Parkinson disease, with stem cell therapies. Since the ES cells are the most pluripotent stem cell type existing, they could be viewed as the ultimate panacea.

It is important not to forget that other stem cell types, eventually more specialized and less pluripotent, could also help in improving human health. Such stem cells could be isolated and amplified from the bone marrow or the placental chord blood and then differentiated *in vitro* into specific cell types.

POSSIBLE THERAPEUTIC APPLICATIONS OF ES CELLS

Since no therapeutic application of embryonic stem cells exists yet, I will review briefly the differentiated cell types that have been obtained by manipulating mouse or human ES cells *in vitro*. Most of these manipulations have been carried out by changing the culture conditions and adding or removing specific growth factors to the medium without any genetic manipulation of the cell DNA (Dinsmore et al., 1996; Schuldiner et al., 2000). This is a great advantage for cell-based approaches such as the ones considered here, compared to gene therapies which require adding or modifying one or more genes in a patient.

The ES cells can be differentiated into cardiomyocytes, cells that are the building blocks of the heart (Kehat et al., 2001). These cardiomyocytes can even be seen beating at their own rhythm in the culture dish. A promising application is to graft cardiomyocytes in the damaged region of the heart in patients with a heart disease. The grafted cells would then be able to replace the dead cardiac cells and restore the function of the heart.

Another type of cell that can be obtained is the pancreatic, insulin-secreting type (Lumelsky et al., 2001). These cells have a tremendous potential to provide a cure to diabetes. Diabetes is one of the most prominent health problems in the Western world with a very high financial cost for the patient as well as health care organizations.

ES cells have also been differentiated into muscle cells, cartilage cells, bone cells, neuronal cells, fat cells, skin cells and blood cells, all of which could potentially be used in a myriad of applications (see for exemple Kim et al., 2002).

The best therapeutic solution may not be to transplant already differentiated functional cells (e.g. beating cardiomyocytes) but rather committed precursor cells (cardiomyoblasts). The precursor cells, once inside the body, would then differentiate accordingly to their micro-environment. This ensures that it is the environment inside the host tissue that triggers the differentiation of the grafted precursor cells only where it is necessary.

Another promising application of differentiated stem cells is in the pharmacology field. The cellular functions of a wide array of the specific cell types that can be generated could be studied in details. Thanks to the availability of panels of differentiated cells, the pharmaceutical industry could test the effects of newly synthesized compounds on them. Such large scale screening could lead to the identification of new active pharmaceutical agents while reducing the time, costs and animal testing usually required.

These results as well as the future discoveries of ES cell differentiation will probably lead to a large scope of therapeutic tools able to treat numerous diseases.

LIMITS IN THE USE OF ES CELLS

To temper this rosy view of ES-based therapy, it is necessary to discuss several of its limitations.

The first problem that affects any transplantation

approach is the immunological compatibility between the graft and the host. Just as for an organ or bone marrow transplantation, the grafted ES cells will be targeted for destruction by the immune system of the host. The clinical answers to this problem will be to suppress the immunological response in the host by the use of specific drugs. This solution is however far from optimal as seen everyday in transplant patients. Another solution is to generate a large number of ES cell lines of different immunological types so that the probability of finding a compatible donor will be high. This registry system is very similar to what exists for bone marrow transplantation. A long-term solution, based on targeted mutagenesis would be to genetically engineer one or a few ES cell lines so that they become universal donors. This involves deleting a series of genes that are involved in the immune recognition system to generate an ES cell equivalent to the O negative blood used in blood transfusions.

Another major difficulty is to be able to graft a homogenous population of differentiated cells. Indeed, it often happens, at least in the present state of the art, that a mixture of cell types is obtained after the differentiation procedure. It will then be necessary to eliminate all cell types other than the one needed. This requires sophisticated cell purification and cell sorting tools. Not only it is better to remove unwanted differentiated cells but it is of the utmost importance to eliminate any residual undifferentiated ES cells since it is well known that they will give rise to teratocarcinomas. This may be done by developing protocols to eliminate any cells expressing genes specific for the ES undifferentiated state such as Oct-4.

Finally, the flip side of the coin with a custom job like the culture and differentiation of ES cells into a specific cell type is that it will take quite a long time, probably several weeks, to obtain transplantable cells. An ES-based therapy is therefore not a fast response solution to sudden health problem but would be more applicable in case of slowly degrading conditions.

The recent progresses in ES cell technology have brought the scientific and medical community to a new frontier that remains to be crossed, the formation of organs *in vitro*. Indeed, a mass of differentiated cells cannot replace an organ that contains multiple cell types organized in a functional unit. In the future, it will be necessary to find solutions to transform the ES cell-based therapy into an organ-based therapy.

In conclusion, the discovery of human embryonic stem cells, giving rise *in vitro* to a large variety of functional cell types, has raised hopes of finding therapeutic solutions for many diseases and health problems. Several difficulties have already been identified and more will undoubtedly arise but they will most likely be ironed out thanks to the intensive research going on. It is also certain that it is not a "cure-all" solution and that more medically-oriented research as well as basic research in new domains will be necessary.

ETHICAL QUESTIONS

As any change brought around by new technological developments, the use of embryonic stem cells and embryonic germ cells has to be considered not only from the scientific and medical side but also from an ethical point of view. After describing the scientific basis of the ES cell technology and the pros and cons of ES-based cell therapies, it is necessary to highlight some points relevant to the ethical debate.

We have divided the discussion into three parts. The first is geared towards the therapeutical uses of ES and EG cells by transplantation. The second focuses on the uses of supernumerary embryos resulting from *in vitro* fertilization and embryos recovered from terminated pregnancies. Finally, the most difficult questions are centered around the generation of embryos for the specific purpose of deriving ES cells. It is also necessary to mention that ES cells could eventually be used for reproductive cloning since in rare occasions some murine ES cells are able to form an entire mouse (but never the placenta and some other embryonic tissues). This is akin to the reproductive cloning à la Dolly and is generally forbidden when applied to humans.

The proposed medical use of ES cells is very similar to classic organ transplantation procedures. Indeed, provided that the technological hurdles are solved, the cells would be prepared *in vitro* and grafted into the patient. The patient would have to follow a preparatory as well as post-operation treatment that in the case of immunosuppression can last a lifetime. For what concerns this type of procedure, it is likely to be accepted or rejected on the same basis as blood transfusions and bone marrow or organ transplantations.

The second debatable point is centered on the origin of ES and EG cells. The embryonic germ cells (EG) are isolated from embryos recovered after abortion between the 5th and 9th weeks of pregnancy whereas the ES cells are isolated from blastocysts derived from supernumerary embryos generated by

in vitro fertilization (IVF). In this case, the embryo used to make ES cells has the potential, if transferred to a recipient woman, to give rise to a human being. Since ES and EG cell isolation requires the destructive use of embryos, further questioning will depend on the answers to the abortion and *in vitro* fertilization problematics. More specifically: Can we discard the superfluous embryos resulting from IVF? And is abortion between the 5th and 9th weeks of pregnancy ethically possible? If yes, the debate can continue with the question: Can we use these embryos to generate ES and EG cells?

On one hand, it can be considered that they should not be touched out of respect for the potential human being. On the other hand, it could be argued that the abortion and destruction of the embryo implies that it is not considered a human being but rather a lump of cells (which, although, has the potentiality of forming a human being). Since the embryos would otherwise be discarded there may not be an ethical problem in using them for research on ES/EG cells.

There is a precedent for a very similar situation. Indeed, some research, for example to optimize the pre-implantation genetic diagnostic techniques used to detect known genetic diseases, is being done on superfluous embryos resulting from IVF. The same rules, such as the informed consent from the parents, could be applied here. In the case of current research on embryos obtained by IVF, the embryos are cultivated only for a short period of time, at most up to the blastocyst stage, and are then discarded. The situation is different for ES cells; the culture period is basically indefinite since it is a stem cell line. However, the embryo proper will only be cultivated up to the blastocyst stage and a cell line will then be generated. The embryo and the ES cells will carry the same genetic information but do not have the same potential. Indeed during the culture process, the embryo, which is totipotent, disappears and a pluripotent embryonic stem cell line is formed.

It seems thus that the generation of ES or EG cells from discarded embryos may not cause a new ethical challenge in view of the previous debates on embryos.

Finally, two major ethical questions concerning the generation of ES cells must still be raised. These questions result from approaches aiming at generating a better or even a perfect immnunocompatible ES cell line to bypass the rejection problems that plague all transplantation therapies.

First, can we use one-cell embryos (for example remaining from IVF procedures) as recipients for transplanted nuclei to create a transient embryo and generate ES cells? This is tantamount to kicking one egg out of the nest and replacing it with your own like the reproductive strategy of the cuckoo. The technique goes one step further than the ones discussed previously since it destroys the genetic identity of the host one-cell embryo (except for the mitochondrial genes) and replaces it with a new one.

This tough problem may be solved by a new technical development. Preliminary results show that it could be possible to use an unfertilized egg in this nuclear transplantation approach thereby solving the ethical debate on the problem of destroying the genetic identity of a potential human being. The host egg could thus be considered as a womb providing the ideal micro-culture conditions for a nucleus that will later give birth to ES cells.

The last and crucial question is can we produce embryos with the sole aim of generating ES or EG lines? In that case IVF would be carried out, not for reproductive but for therapeutic purposes. The answer to that question has to be weighed in light of the advantage provided by this approach. The generation of ES cells from specific donors is possibly the only valid solution to obtain cells that are immunocompatible with the patient (e.g. a child or relative of the donors). In the case of nuclear transplantation, a perfectly compatible match would be acheived. Since the rejection of a graft and the treatment required to suppress this reaction represent actually an enormous challenge for any transplantation therapy, the generation of ES cells from specific donors offers the ultimate answer to the rejection phenomenon.

Up to now, few countries has passed legislation regulating the use of human embryonic stem cells or embryonic germ cells. Intense debate is however going on in Europe and other countries. In the United States of America, the government decided to allow funding of research concerning already existing ES cell lines but not on the generation of new ones. Because of the rapid progresses in the field of ES cell technology and the general lack of governmental decisions, we are not able to discuss further policies concerning the isolation and use of human embryonic stem cells.

To conclude, we would like to emphasize the fact that embryonic stem cells and germ stem cells have an enormous life-saving potential which no other application has demonstrated to date. We expect that this groundbreaking ES cell technology will be blooming in the coming decade and will deliver numerous therapeutic solutions to health problems.

However, as always in science, it is also possible

that other technologies, for example based on other types of stem cells isolated from the placental chord blood or bone marrow will be improved or discovered and that these will achieve the same effect or complement the ES cell based therapies.

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ABSTRACT This review focuses on the new discoveries regarding embryonic stem cells by summarizing the scientific advances, outlining the potential benefit for our society and finally raising a few ethical issues. The classic definition of a stem cell is a cell that can divide indefinitely and give rise to differentiated cells of various types. No stem cell is however totipotent, the closest to it are embryonic stem cells and embryonic germ cells which are pluripotent, giving rise to many very different cells types from gut to brain ones. Other stem cells, generally adult cells, are said to be multipotent, giving rise to several related cell types, for example the various blood cells. Only one type of stem cells is however being used routinely in the hospitals during bone marrow transplantation. The recent discovery of human embryonic stem cells that are able to give rise *in vitro* to a large variety of functional cells types, has raised hopes of finding therapeutic solutions for many diseases and health problems, from heart attacks to Parkinson disease, but has not yet yielded therapeutic applications. Since the ES cells are the most pluripotent stem cell type existing, they could be viewed as the ultimate panacea. Several difficulties have already been identified and more will undoubtedly arise but they will most likely be ironed out thanks to the intensive resarch going on. A few ethical questions also need to be addressed concerning the therapeutical uses of ES and EG cells by transplantation and the use of supermumerary embryos resulting from *in vitro* fertilization and embryos recovered from terminated pregnancies. Finally, the most difficult question is centered around the generation of embryos for the specific purpose of deriving ES cells. We would like to emphasize the fact that embryonic stem cells and germ stem cells have an enormous life-saving potential which no other application has demonstrated to date. We expect that this groundbreaking ES cell technology will be blooming in the coming decade and will deliver numerous therapeutic solutions to health problems.

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