Opinion on Ethics and xenotransplantation

N°61 - june 11, 1999

Sommary

I. THE PROMISES OF XENOTRANSPLANTATION

The considerable interest in xenotransplantation at present is linked to the persisting imbalance between the need for organ substitutes and their availability, which has no chance of being righted in view of the growing scarcity of so-called " brain deaths " which represent less than 1% of hospital deaths. Because of progress achieved by modern medicine and biology, techniques for transplanting human organs have become routine and have now amply demonstrated their life-saving and quality-of-life improvement value. The limit which constricts the use of these techniques in all the countries in which they are practised is the shortage of organs for transplantation.

Turning to xenotransplantation would alleviate that scarcity and make healthy organs available at the exact time when they are wanted. It would be possible to eliminate the months of waiting during which health deteriorates, and obviate the need for emergency surgery on an ill-prepared patient simply because an organ suddenly becomes available.

Furthermore, if it could confidently be said that xenotransplantation was devoid of risk, a large number of ethical issues raised by the use of human organs could be solved.

The thought that one's life is saved by the death of a fellow human being, or the implicit gratitude, which a recipient owes to a living donor, are in some cases a major psychological handicap abundantly demonstrated by various studies. In most cases, this should not arise with xenotransplantation. The use of xenografts should eliminate the ever-present risk of illicit trading of organs in spite of laws of prohibition in most countries, since carefully controlled commercial enterprises for the rearing of organ-donor animals could be started. Finally, the possibility of using embryonic animal tissues instead of human ones would solve a great many ethical problems about the appropriateness of using human foetuses.

If the main technical difficulties of xenografting were solved, there would still remain a whole set of ethical matters to discuss before proceeding to the first clinical tests : How do you select the first patients for xenotransplantation ? How do you inform these patients, and go about understanding and overcoming legitimate reluctance on their part ? How can public debate on a large scale be initiated to find out whether a society is ready to accept a technique which may represent a potential danger of infection precisely for those members of society not concerned by the graft ? How can market forces be prevented from disturbing the present system in which human organs are voluntarily donated ? These issues are the main subjects for reflection in this report.

The idea of using xenotransplantation to alleviate a shortage of human organs is not new. Pig heart valves have been used in humans for nearly thirty years. They are processed so that they react like an inert material instead of like a living tissue. More than eighty kinds of

connective tissue (skin, bone), of porcine or bovine origin, are commonly used in human medicine. However, they only play a transitory role, as is the case for skin of porcine origin which is rejected very quickly. It should not be forgotten that an animal product, such as bovine or porcine insulin which was used for almost a century **((1)1)** , saved the lives of millions of diabetics.

Xenotransplantation can mean tissue or cells, not only solid organs. Various uses have been suggested for islets of Langerhans of the pancreas, neural foetal tissue, or bone marrow. Using small masses of tissue devoid of blood vessels is less risky for a patient than using whole organs (6), and the psychological acceptance of tissue xenotransplantation is much better than for an organ to which the patient attaches symbolic value, such as the heart. However, it must be recognised that there is less of a shortage of tissue than of whole organs and that there is therefore more cause for ethical concern about the latter.

The technical problem is that a living tissue of animal origin is rejected very quickly, frequently before even the surgical procedure has been completed. It was only as late as the 90s, and mainly in the last five years, that the causes of such rejection began to be better understood with the attendant hope of overcoming the difficulties in the near future. This explains the recent renewal of interest in the techniques of xenotransplantation (12).

II. SCIENTIFIC ASPECTS

1°) Rejection phenomena

When a human being receives a graft originating from another species, for instance pigs, there are three reject processes to overcome : hyperacute rejection which occurs within minutes or hours, acute rejection which takes place seven to ten days later, and finally chronic rejection with long-term therapeutical consequences, (which are largely uncharted because of the brevity of survival of transplants observed in animals).

Let it be said at once that acute rejection is of the same kind as occurs in the case of allotransplantation, and that its effects can be limited with drugs which depress the immune system. However, recent research suggests that the immune T-cell connected response against the xenograft differs slightly from what is observed with allografts, so that new immunosuppressants (6, 12) may need to be developed. But this is also an incentive for new research on this problem.

Presently, most of the work is focused on hyperacute rejection. This occurs because of the presence of what are called natural antibodies which are constantly present in the blood stream. Throughout our lifetimes, we produce and store antibodies directed against epitopes carried by the very great number of molecules that we encounter, breathe, or eat. Furthermore, certain antibodies, described as preformed, are coded by our genes and protect us from our birth against foreign agents in our environment. Thus, without the immune system needing any induction, we already possess antibodies directed against antigenic structures from other species. As soon as a graft, of porcine origin for instance, is irrigated by the blood stream, antibodies recognise antigen receptors located on the endothelial cells which line the blood vessels of the graft and the endothelial cells are activated. A complex set of proteins called the "complement" circulate constantly in the blood stream, and one of its functions is to destroy cells which the immune system recognises as foreign. Systems which protected the graft's epithelial cells from the complement's attack cease to operate and the complement destroys the epithelial cells. Simultaneously, systems which prevented intra-vascular clotting cease to function, and micro-thrombosis sets in (7, 15).

To control hyperacute rejection, researchers adopt several approaches : they can modify the natural antibodies of the recipient, the graft antigens, the complement, or the cascade of clotting reactions in the recipient (2, 28).

It would seem that the immuno-absorption of all natural antibodies will never suffice. However, some proteins, which differ from one animal species to another, are now known to be present on the surface of many cells, including endothelial cells, and they are able to inhibit the complement. The better known of these are called CD35, MCP (Membrane Cofactor or CD46), DAF (Decay Accelerating Factor or CD55), protectin (CD59), HRF (Homologue Restriction Factor). *In vitro* , it was found that these molecules protect the cells of another animal species from lysis by the human complement (7).

In practice, immuno-absorption of natural antibodies, or massive administration of drugs to prevent the complement's action, would lead to therapeutic protocols which would be far too burdensome to be usable, probably not totally effective, and possibly dangerous since they would expose patients to infectious diseases once the essential components of natural immunity had been weakened.

2° Physiological compatibility

Knowledge about physiological compatibility between human and animal organs (differences in enzymatic specificity, incompatibility between ligands and receptors, sensitivity to neurogenic stimulation, chronobiological cycles, etc. ...) is still very tentative.

3° Selection of animal donors and immunology

The two animal species which have mainly been used so far are swine and non-human primates. The main argument in favour of primates is immunological. As noted above, hyperacute rejection begins by the fixing of natural antibodies on the corresponding antigenic epitopes. Epitope Gal-a-1-3-Gal, major epitope involved in the pig, is expressed over the whole surface of vascular endothelium. This epitope does not exist in primates. Through the process of evolution, the a-1-3-galactosyltransferase gene, which is the transport enzyme of the epitope concerned, has been de-activated by two deletions of the coding fraction in humans, chimpanzees, and baboons (15, 28).

However, we find that the simple absence of epitope Gal-a-1-3-Gal is not sufficient in itself to preclude any risk of rejection. The first neonate to receive a baboon heart in 1985 only survived twenty days (4). Primates also present other drawbacks. They are slow to develop and have a low reproductive rate, and they are difficult to rear in captivity. Chimpanzees are severely protected by law as they are an endangered species. It would be difficult to cover all requirements with baboons only. Above all, risks of infection by viral disease transmitted by graft to recipients are very high (see below). Finally, the closeness in evolution between humans and non-human primates, the chimpanzee in particular, raises particular ethical objections.

The scientific community is therefore directing its thinking to using transgenic pigs. The aim is to obtain pigs whose endothelium is no longer activated by human blood. Several strategies are possible : non-expression of antigens recognised by natural human antibodies, or express on the endothelium one or several molecules which inhibit either complement or clotting (7, 15).

These various approaches have been explored. As regards complement inhibitors, transgenic pigs have been produced which express human DAF (CD55), or protectin (CD59), or both together . Transgenic pigs expressing DAF have raised great hopes.

Since human DAF also inactivates the complement of other primates, transgenic pig hearts expressing this molecule have been used to transplant Cynomolgus monkeys. The graft was attached in an abnormal site (the neck) to monkeys retaining their own hearts. Non transgenic transplants survived on average 1.6 days, whereas average survival was 5.1 days for the transgenic transplant in recipients not treated with immunosuppressants, and 40 days on monkeys treated with a combination of cyclosporin, cyclophosphamide, and corticosteroids, which are active in suppressing acute rejection (15).

These results were found sufficiently encouraging initially to warrant moving on to human tests, but the authors seem to have given up the idea in the meantime (6, 17, and 29). Protocols including both DAF and protectin genes do not seem to have produced better results so far, than when only the DAF gene is used.

A second strategy, aiming to suppress the Gal-a-1-3-Gal epitope is now being explored. For example, it should be possible to invalidate the a-galactosyl-transferase gene, suppress its expression using an antisense messenger RNA, or use other strategies (22), but none have as yet proved effective.

Therefore, even at the level of animal experimentation, research to produce transgenic donors for transplants which would be acceptable to the host, is still in its infancy (26).

With pigs, gene transfer is achieved with micro-injections directly into the embryo pronucleus. The operation is not highly productive : 100 injected embryos are necessary to produce only one transgenic pig. However, it is possible to produce lineages of transgenic pigs , which develop more or less as do ordinary pigs. Cost in 1999 remains high however : about 150 000 FF per pig, not taking into account depreciation of the special pig-rearing facilities required.

4° Animal selection and risk of infection

The characteristic aspect of xenotransplantation is putting into close and prolonged contact an animal organ and an entire recipient human organism which, at the time of transplant, is treated with powerful immuno-suppressor drugs. These are ideal conditions for a micro-organism in the graft to multiply in the host. Clearly, with allotransplantation, contamination from donor to recipient is also a risk, but within the same species so that there is a chance of being able to control infectious pathologies.

The xenotransplantation situation is more complex (18). It is well known that many animals may harbour in their organs, cells, and genome, micro-organisms for which they are healthy carriers because, in the course of evolution, they have developed protective mechanisms, which render them resistant. Some of these micro-organisms are capable of crossing the species barrier and so of expression in the immuno-depressed host. **The appearance of "new" diseases after crossing the species barrier is not - alas - a myth** : the HIV virus for instance, is very probably of simian origin, and is the cause of a pandemic in which the animal has ceased to play any part, but which we are still incapable of controlling as long as there is no vaccine. The most recent example and one of the most alarming at this time, is the probable passage of prions causing Bovine Spongiform Encephalopathy from cattle to humans.

This infectious danger is therefore sufficiently serious to induce physicians and biologists to publicly raise the question of whether it is ethical to allow mankind to run the risk of devastating and uncontrollable pandemics whereas xenotransplant techniques will never concern more than a limited group of patients (6).

The real issue is whether the risk can be reduced to an acceptable threshold. For pigs, animal husbandry techniques have for some considerable time been able to eliminate bacteria, parasites, and viruses propagated by the environment. Animals are obtained in axenic state (totally devoid of micro-organisms) and their digestive tract is inoculated with known flora. They are kept in sterile rooms which are ventilated by overpressure, are given sterile feed, and have minimum contact with keepers. Batteries of multiple tests are used to check on their specific pathogen free status (*EOPS = exempt d'organismes pathogènes spécifiés)* (10). There remains the problem of viruses and viral genomes which are transmitted vertically from dam to offspring.

Those for which there has been the most extensive investigation are retroviruses. Many

retroviral type sequences are to be found in the genome of most animals. They were incorporated in the course of evolution following viral infection or transposition mechanisms not concerned by infectious processes. Various mechanisms prohibit permanently any expression of such retroviral sequences. However, reactivation by some stimuli is possible. Some animal genomes harbour a great variety of retroviral sequences : this is the case for mice and primates. Recent information has come to light concerning the presence of proviral sequences in the pig genome which can be transmitted to cultured human cells (27). However, on the other hand, there is no evidence of any identified swine pathology triggered by retroviral infection (10).

Finally, since pigs and humans are two relatively distant species, transmission of a porcine retrovirus to human beings seems improbable. This is demonstrated by the history of the two species in parallel : wounds inflicted on humans by pigs, widespread consumption of pork, medication of porcine origin given to human beings (insulin, for example). None of these practices have ever led apparently to the transmission of infectious diseases to humans. There is already some experience of pig skin used as a graft for human beings, of porcine pancreatic islets, or of blood infused in porcine kidneys. Antibodies against porcine viruses have been found in the blood of some patients, but no new disease has been observed. However, not nearly enough time has elapsed for firm conclusions to be drawn about these ongoing observations (6, 24).

However, the considerable capacity for mutation of these proviral sequences should not be underestimated, and this could change their tropism or their mode of action. Furthermore, their capacity to integrate oncogenes or to settle close to an oncogene could trigger cancer in recipients (10).

For all of these reasons, pigs devoid of such retroviral sequences would be ideal. This is a difficult achievement, but not impossible since there has been success with chickens for a certain type of retrovirus. Identification of the retroviral sequences in swine has begun and must continue to eliminate carriers. However, the number of copies of these retroviruses and time between generations are such that this is a long, costly, and uncertain approach.

There is also one last problem with transgenic pigs which do not express the Gal-a-1-3-Gal porcine epitope. In this case, viral particles from the cells of these animals will no longer express the antigen and will therefore no longer be recognised as targets by the human complement (25). In the same way, particles from pigs expressing the human DAF may be resistant to the human complement. This is why some authors have stated that transgenic pigs could be more dangerous than ordinary pigs as regards infectious disease transmission (28).

Altogether, it can be said that the known risk of transmission and the number of proviral sequences in their genomes preclude the use of primates as a source of xenografts for the time being. The second point also argues against the use of murine cells. A transgenic pig seems to be the least risky animal as regards infection as long as they are reared in strict specific pathogen free conditions. However, research must continue to gain a better understanding of proviral sequences existing in the animal, and to eliminate them to the greatest extent. It is therefore possible to subscribe to the opinion expressed by INSERM's Intercommission II which considers that bio-risks connected to the xenotransplant of porcine organs can be limited by recent research results and that they should not, *a priori*, prohibit all therapeutic testing in human beings (10), but we must be aware that the reliable biological material that we should be able to expect is not available as yet.

5° Xenogenic cell therapy for human beings

Using cells or cell masses of animal origin devoid of blood vessels as grafts for human beings is only partly affected by the drawbacks described above for solid organs. Cells which have already been used in this way are : pancreatic islets of Langerhans, hepatocytes, cells from the nervous system and the skin. Cells from muscles, cartilage, endocrine glands, the

heart, kidneys, and blood vessels are also being considered. In some cases, these cells are placed outside the body on inert supporting structures, in semi-permeable pockets, and put into contact with the blood in a cardio-pulmonary bypass system. These are in fact bio-artificial organs for which there is no immune rejection problem, but such systems can only be used for a brief period.

When these cell xenografts are put into the body, they do not give rise to hyperacute rejection since they are not vascularised, but they are subject to the usual cellular rejection which is controlled more or less competently by immunosuppressants.

In both situations, however, the risk of infectious contamination is identical to the one described for organs which is why even in the case of cell xenotransplantation, the pig is considered to be the best donor animal, and better than primates.

III. ETHICAL PROBLEMS ARISING OUT OF CLINICAL EXPERIMENTATION

The earliest xenotransplants to be performed on human beings are a topical controversy (9). On the one hand, there is the English team from the Imutran pharmaceutical company (David White) who had announced their intention of performing transgenic pig organ and tissue xenotransplants in 1996 (6, 17, 20, 29) (but did not do so...) and on the other hand, Thomas Starzl's team in the USA who were the first to dare implant a baboon liver to a human being in 1992 and who, when it failed, requested a moratorium to provide enough time to gather new scientific data (23). After the wave of enthusiasm raised in 1995 by the publication of D. White's initial results (grafting a transgenic pig heart into the neck of a monkey) in early 1998 there was an energetic call for caution orchestrated by the magazine "Nature" and echoed by mass media, in France particularly (14). Several scientists, following in the steps of the American researcher Fritz Bach, now consider that to proceed to clinical trials should be regarded more as an ethical issue than a technical problem. It is indisputable that for a particular critically ill patient, **the benefits to be expected from xenotransplantation when rejection can be controlled, outweigh any risk of infection.** However, for the population as a whole, it is not possible to exclude completely the pandemic risk. Therefore, Bach is in favour of a moratorium for any kind of clinical xenotransplantation until such time as public debate on a large scale gives society a chance to say whether the non-zero risk of a new viral epidemic is acceptable (3, 6, 28).

It is therefore clear that the present status of scientific competence, and also good clinical practices prohibit any intention of applying these techniques directly to human beings at this time. In the circumstances, three kinds of issues arise :

At which point in scientific progress will it become ethical to propose xenotransplation to a patient ?

Who will the first xenotransplant patients be ?

What precautions should be taken and what information should be given to these early patients ?

Using xenotransplantation for human patients will require progress in at least the three directions listed above. The first requirement is overcoming acute rejection. Since we have a usable animal model, grafting transgenic pig organs to monkeys, it is essential that convincing results with graft survival times of more than just a few hours, or for that matter a few weeks, should be available and submitted for peer review in publications.

Quite obviously, this will not constitute absolute proof that a kidney or liver graft will subsequently function exactly as would a human organ. But at least, there will be potent

arguments in favour of patient survival extension. At this point there will arise the still unsolved problem of controlling chronic rejection in the long term.

The second field of necessary preliminary endeavour is potential patient infection by graft micro-organisms. As previously mentioned, even defective retroviruses integrated in pig genomes can be detected. As detailed as possible mapping of integrated viruses of porcine genomes is needed before animals are used, but this has not yet been done. Animals selected after testing must be reared in specific pathogen free conditions under supervision by independent health authorities who would have exclusive rights to deliver identification permitting their use for xenotransplantation.

Arguments in favour of choosing one or other type of graft as the first to be used in human experimentation are still inconclusive. Some opinions would prefer to begin with so called "mechanical" organs like the cardiac pump, rather than "personal" ones like neural tissue. In fact, using neural foetal pig cells to treat for Parkinson's disease is akin to introducing into the cranium a pump for the production of neurotransmitters such as dopamine. Xenotransplants of nerve cells potentially capable of modifying the behaviour and therefore the personality of a functionally impaired patient (Parkinsonian syndrome, for example), if the procedure did become a working possibility, would only be ethically acceptable if the result was to reinstate a previously existing function. If, for whatever reason, the aim was to modify a personality, it would be totally unacceptable.

Liver xenotransplants should probably not be the first to be attempted. The extraordinarily complex functions of this organ differ slightly from one animal species to another. It is difficult to predict with any accuracy what functions pig liver will carry out in a human body. Furthermore, we know absolutely nothing about human receptors to animal proteins.

In fact, a totally transgenic liver still seems rather a utopian project.

The technical difficulties inherent to initial clinical trials are such that of necessity they will be performed in one of a very restricted group of centres approved by national health authorities.

Once the necessary scientific progress has been made, it would seem logical that the first patients would be those for whom no other form of therapy is currently available : patients in a critical state and unable to wait for a human organ to be available or other patients who cannot be put on a waiting list for whatever reason. It must therefore be clear that the technique's effectiveness will have to be judged at first on the results of particularly difficult cases.

A major ethical problem will frequently arise : the choice will not be so much "xenograft or death", as "xenograft now, or wait for a human organ for an indefinite length of time". As it seems likely that at first the reliability of a human organ will be considerably greater than that of a xenotransplant, this will be a difficult decision to take. The choice must therefore be perfectly unencumbered. If a patient turns down a xenograft, the possibility of obtaining a human graft must be retained with identical chances.

In the same way, it is likely that in the initial phases, xenotransplants will be offered to patients in the acute phase of their sickness as a possibility of gaining time before a human organ becomes available. There again, it is important that the fact of having had the benefit of a xenotransplant does not in any way spoil one's chances of human organ transplantation at a later date (1).

Patients' consent to xenografting, even initially, does not appear to be very different from what is generally practised now for human organs. Patients must be completely informed about the experimental nature of the procedure, the successive phases, the risks, and the alternatives. Such information must definitely be given by qualified personnel capable of coping with psychological, scientific, and ethical issues. Consent should be requested in the same manner as for allografts. During the experimental clinical phase, it would certainly be

preferable to steer clear of all those who cannot give free and informed consent, such as children and unconscious patients, although the very young (less than one year old) have lower natural antibody counts than adults so that hyperacute rejection is likely to be less severe.

One special problem in connection with consent may well arise during the therapeutic trial phase. It will be absolutely essential during this phase to engage in detailed and prolonged epidemiological monitoring of these patients. It is also very likely that special precautions will be demanded of such graft patients to prevent any dissemination of some new pathogenic organism. This means of course that patients will have to consent and even commit themselves in writing for this monitoring procedure to take place for some considerable period of time during which they would be obliged to accept the possible constraints of quarantine measures of the kind which are imposed on carriers of a risk of dissemination of an epidemic disease.

Finally, it should be noted that even for those facing death, xenotransplantation should not be presented in over enthusiastic terms. In the event of defective xenotransplant function, burdensome therapy will be needed to prolong life to some extent, but quality of life will be very poor.

IV. SOCIAL ACCEPTANCE OF XENOTRANSPLANTATION

1° Overall attitudes to xenotransplantation

Development of sophisticated therapy techniques such as xenotransplantation depend above all on the social context in which it takes place. It is very difficult to predict the attitudes of individuals when faced with routine use of a technique, which for the time being is still confined, to the laboratory. This is, in fact, the heart of the problem : xenotransplantation is not just a transition solution to the shortage of human organs ; **in the long term it would become a routine procedure**, in approximately the same way as human kidney transplantation.

Part of the reactions of the public to xenotransplantation will in fact be no more than an extension of its reactions to human organ grafts. Replacing a failing organ by another one is part of the ongoing quest for prolonging life. The importance of quality of life versus a simple extension of life is certainly considerable, but the problem is in no way specific to xenotransplantation.

Is it better to owe a "new" organ to a dead human or to an animal bred and sacrificed specifically for that purpose ? The question is bound to arise insofar as this double possibility of transgression comes up against notions of sacrosanct frontiers between the living and the dead on the one hand, and between humans and animals on the other. We have very little data on the impact of the xenotransplantation concept on the public's imagination and very little objective data on the reactions of various segments of the population to such a subject. A survey was recently conducted in the United Kingdom to find out more about young peoples' attitudes to xenografting. Results were as follows : 55% of an 11-18 year old age group considered, some of them enthusiastically, that research on the subject should be pursued, but 45% held an opposing view (1). Questions put to various sections of population reveal that only about 40% of them would accept a xenotransplant. However, the percentage is considerably increased if the individual is personally concerned, and in the case of risk to life, it goes up to 78%. Nevertheless, about 75% of those questioned see xenotransplantation as a possibility for the future. In fact, answers depend very much on how the question was put (5, 11, 16, 19).

So the situation is full of contrasts and it is clear that total transparency on the progress of research will be useful for the idea of xenotransplantation to be acceptable to a majority of people.

2° Aversion to xenotransplantation on the part of recipients

This rejection of the technique, *a priori* fairly massive, can be explained in various ways. Several religions consider the pig as unfit for human consumption but when religious authorities were asked, they were generally rather positive as regards using pigs as graft donors. Some individuals hold philosophical beliefs to the effect that the life of an animal is as valuable as the life of a human being and that we cannot claim for ourselves the right to sacrifice one to save the other. However, such views are too uncommon for them to be the reason why in previous enquiries more than 50% of those polled were reticent.

The main problem is probably connected to the individual's notion of identity as related to the perception of that individual's body. When an individual identifies with all the organs of his body, it is difficult enough to accept the thought of even a human graft. There will also be a tendency to establish a hierarchy in the importance of the organ : a kidney is better than a heart to which is still frequently associated symbolic emotional importance. An animal organ will be thought of as even more destructive of identity.

In fact, a graft of any kind breaks through the usually intact frontier between self and non-self, and the psychological repercussions of the "violation" has been studied with attention in the case of allografts. With xenografts, there is a further violation, that of the frontier between humans and animals, and this is of particular significance. An individual who manages to transcend the purely organic level of his being and who considers that the essence of humanity is thought, which precisely permits transcendence, will have little or no aversion to animal grafts. On the contrary, those who will not, or cannot differentiate between their humanity and their material being, will reject xenotransplantation. They will feel that the graft degrades them to the level of a human-animal chimera in which their humanity is dangerously diluted.

The opposite line of reasoning is easily accessible : a human being deprived of a kidney, a colon, or even a heart while being sustained by extra-corporeal circulation, is no less human. Is a cancerous liver which progressively destroys a man likely to make him more human than the porcine liver which keeps him alive and thereby enables him to retain his human definition ? One could go so far as to say that awareness of the organic animality of humans will enable them to empower their neuronal, cortical, linguistic and relational capacities with more transcendental qualities than their livers, hearts, or other organs, and will teach them to reject any identity between humanity and bodily organs. The concept of human dignity signifies that the respect which must be given to the integrity of the organs of the human body does not *ipso facto* signify that the humanity of a human being is contained in those organs.

There again, much more research is required to grasp the motivations of those who would be among the first to receive xenotransplants, so that the experience gained might serve to better understand, inform, and counsel subsequent patients.

V. LEGISLATION AND XENOTRANSPLANTATION

Once technical progress has made transplantation routine, the market for it will be gigantic although it is difficult to estimate its size at this time. Global evaluations have been made ranging from $1.4 billion (7) to $6 billion (6). Some authors have estimated that in the next few years 50,000 pig hearts and 40,000 pig kidneys could be implanted in human beings every year (13). As a consequence, it appears that most laboratories working in the field of xenotransplantation are supported by powerful biotechnological companies in the United Kingdom and the U.S.A. These same corporations market immunosuppressants which would be used to a much greater extent if xenografts were successful.

It is clear that if the technique is a success, the present centralised system for the

collection, allocation, and distribution of human organs which functions in most developed countries would be replaced by a commercial system which would definitely need to be circumscribed by legislation. Although in other concerned countries there is no legislation to control specifically access to transplants (8), France was the first country world wide to have introduced in its law dated July 1, 1998 which bears on the reinforcement of health supervision and sanitary checks on products for the use of human beings, and the use for therapeutic purposes of animal organs, tissues, and cells. This scientific anticipation provided by the law does not create *de facto* a judiciary anticipation, and although CCNE welcomes the *avant-garde* nature of the law, it finds that it is simply a guideline with specific recommendations which do not, obviously, imply the principle of authorisation to practise xenografts. Rules of good practice for the use of animal cells, organs, and tissues are prepared by the French Agency for Sanitary Safety (*Agence Française de Sécurité Sanitaire*), after consulting the French Transplantation Establishment (*Etablissement Français des Greffes*), and approved by the Minister in charge of Health. The Minister's decisions set out rules of good practice as regards selection, production and breeding of animals, sanitary conditions which animals must meet, rules of identification of animals so as to ensure final products can be traced. This is not a system of clinical trials which must comply with the *Huriet-Sérusclat* Law. Instead, the system is based on ministerial authorisation. In the United Kingdom, a working group was tasked with drawing up conclusions which were adopted in 1995 by the Nuffield Council on Bioethics (1). In the United States, experts have drafted a code of good practices as regards xenotransplants which was published by the U.S. Public Health Service, and approved by the FDA, the National Institute for Health, and the Center for Disease Control and Prevention (6). As for the Parliamentary Assembly of the Council of Europe, they have proposed a moratorium, put forward by Mr. Plattner, for decision by the Council of Ministers. As a result, a working group was created.

Xenotransplantation raises the question of animal status. Animals have no judicial *persona*. Although they are considered in law as objects, they nevertheless are granted legal protection as living creatures (laws on cruelty to animals and vivisection). In societies where sacrificing animals for various uses has been accepted since time immemorial, developing a new use, i.e. xenografts, is not likely to be a major problem. However, conforming with decent conditions for breeding and killing the animals implies that these conditions shall be considered as inherent to the practice of xenotransplantation. Respect for humanity implies that humans are duty-bound to treat animals with respect, although not necessarily the existence of animal rights as such. This entails for donor pig rearing facilities, the obvious necessity of complying with European legislation as regards laboratory animals, and in particular with the rules governing the use of GMOs (Genetically Modified Organisms), in confined conditions during the research phase and in dissemination conditions later. Although transgenic animals express human proteins, that does not make them more human and those who breed and use them need do no more, but no less, than comply with these rules.

It is clear that the production of grafts, their use, and clinical trials will have to be strictly controlled by legislation and monitored by health authorities, in compliance with existing law.

- The law on bioethics and the law on sanitary safety will certainly need to deal with avoiding risks inherent to transplantation.

- A health authority such as the French Agency for Sanitary Safety will need to define rules to be followed by donor animal breeding facilities, make sure that good practices are followed when harvesting and transporting grafts, and give official approval to breeding centres.

- The national sanitary and health authorities concerned, in particular the Ministry of Health and the French Transplantation Establishment, will be required to approve centres best able to perform xenotransplants, and at least initially, to validate launching the first clinical trials and selection of early patients.

- Finally, the same bodies will need to initiate and monitor closely the essential epidemiological study of transplanted patients.

VI. GENERAL CONCLUSIONS

Using animal organs to transplant human beings now represents a genuine hope of overcoming the shortage of human organs. The main ethical issues raised by this technique are the following :

1) Is the principle of using animal organs to improve the survival and well-being of humans acceptable ?

The reply to that question is certainly affirmative, although the convictions of those who consider that all life has equal value and that therefore it is unacceptable to sacrifice an animal to ensure human survival, must be respected. CCNE is well aware that this issue is under debate. It considers that it is of sufficient importance to warrant further thought.

Were xenotransplantation to become a general practice, it would also be possible to side-step intricate ethical problems connected to harvesting them from living human donors, or cadavers, in particular as regards sampling embryonic tissue.

2) Is the risk of human contamination by hitherto unknown infectious organisms originating from the graft sufficient to refrain, albeit momentarily, from launching the clinical phase ?

The question has not yet been answered. Some opinions are that the risk of triggering an irrepressible pandemic calls for a moratorium, at least temporarily. Others consider that if effectiveness and tolerance were no longer a problem, using the technique for humans would be acceptable immediately if drastic precautions were observed :

- Exclude murine tissue and particularly non-human primate organs because their cells carry genomes which abound in retroviral sequences which could be activated in humans;

- Prefer the use of transgenic pigs since the species is further removed so that retroviral sequences are less likely to contaminate humans;

- Use swine reared in specific pathogen free environments so as to be able to declare an absence of any transmissible infectious agent other than viral sequences integrated in the genome.

Intensive research must be encouraged to identify retroviral sequences in porcine genomes so as to try and eliminate carriers, but much time and effort will be required to achieve this because it will always be difficult to be certain of the presence or absence of late-appearing non-conventional agents. It could simply be said that, taking into account proximity between humans and pigs since the latter became domesticated and potential dangers involved, although the probability is very small, it is particularly disquieting.

When necessary trials are conducted involving inter-animal species xenografts (pigs/non-human primates), attempts should be made to identify events which trigger the mobilisation of porcine viral sequences and their recombination with primate sequences.

Any new technique is risk-bearing. Clinical experiments can only start once risks have been evaluated and d to expected benefits, with the consent of fully informed patients. However, xenotransplantation is different from allotransplantation insofar as the risk of infection is not confined to a single patient and extends to the population as a whole. This is no longer the classical "patient/physician" situation ; there is a third partner and a major one, **society as a whole**, with an **evaluation to be made of the individual benefit to collective risk balance**. This goes to show that the ethics of

xenotransplantation must be discussed in the most extensive public forum. Moreover, since epidemics do not respect national borders, debate must be international.

3) Is it now possible to control the immune rejection reaction sufficiently for early clinical trials to be allowed ?

Transplant rejection occurring in the first few days may be reversed with the help of immunosuppressants, in approximately the same way as is the case for human allografts. However, hyperacute rejection which appears as soon as the host blood irrigates the graft, is still incompletely controlled. Transgenic pigs that no longer express receptors causing rejection, or able to inhibit *in situ* the harmful activity of the complement, would seem to be the solution. Such avenues of research must continue to be explored actively. Furthermore, an intensification of experimental organ transplants from transgenic pigs to non-human primates is certainly desirable, since they are an excellent, albeit very costly, model for human xenografts.

If acute or hyperacute rejections could be suppressed, there would still remain the problem of chronic rejection which could allow infectious agents to make use of therapy to produce new diseases.

4) Does xenotransplantation raise specific social or individual acceptance problems ?

There has been too little research so far to enable us to judge objectively the degree of acceptability of xenotransplantation, but it does seem that there is some widespread aversion. **It is not so much that there is a fear of infection, but there is mental inability to transgress the man-to-animal barrier.** A considerable amount of understanding and counselling will need to be deployed for prospective xenotransplant patients. Once that has taken place, obtaining free and informed consent must proceed on the same basis as for allografts.

Let us also note that for quite a long time, xenotransplantation will remain nothing more than an alternative to the more reliable technique of human organ transplantation. If xenotransplantation meets with growing success, we shall need to have care that its very success does not lead to discouraging and demobilising organ donor volunteers. There is a real risk of moving from a situation where solidarity and a sense of responsibility prevail under the constraint of organ scarcity, with life saving objectives, into a situation of purely economic resource where convenience plays a growing role to the detriment of preserving survival.

5) Are there any new legal repercussions as a result of xenotransplantation?

It is only in France, so far, that the required legal framework has been drawn up to apply the technique to humans. Large biotechnological corporations are expecting a vast potential market (evaluated at 6 billion dollars for 2010) to which should be added the immunosuppressant market. A commercial system, based on market forces using resources strong enough to thwart state regulations, may well replace in the long run the benevolent gift of human organs. Therefore, it will need to be carefully ruled by legislative and health authorities, not just in our own country, but also by international standards because of the effects of globalisation.

The use of xenotransplantation could be included in the review of the law on bioethics and the law on sanitary safety, aiming at :

prior consent by national health authorities before implementing the first clinical trials;

institutions authorised to conduct these early therapies approved by national health authorities;

official approval and sanitary monitoring of donor animal rearing facilities, sampling

procedures, and transport of grafts; monitoring of adherence to European legislation as regards the use of laboratory animals throughout all the phases of the procedure, taking into account legislation on genetically modified organisms when using transgenic pigs.

It must be remembered that the existence of a law is not in itself a condition creating licit procedures. Ethical reflection fills the gap between law and the very principle of clinical trials involving human beings.

6) Is xenotransplantation a vital necessity in medical terms ?

For certain patients awaiting an allograft, supposing its effectiveness has become patent, the answer is yes. However, there will never be many such cases. Were xenotransplantation to become routine, there would be an increase in indications so that the economic impact would be significant.

7) The issue of information

The principle of applying xenotransplant techniques to humans supposes the utmost transparency as regards previous and necessary animal experimentation, and particular vigilance during follow-up. Once a xenograft has been performed, society would find it difficult to accept careless monitoring. Follow-up and pertinent information must be made known to the public without excessive media commotion, but so that it is freely available to anyone expressing an interest.

8) Decision to apply the technique

A ranking of reasons for circumspection must be outlined.

"Conventional" rules of caution :

Lack of knowledge on existing chances of success.

Lack of control over hyperacute and chronic rejection.

Encouraging animal experimentation, but nowhere near sufficiently effective to apply to humans.

"New" rules of caution :

Consideration of the ideology of an absolute boundary between humans and animals, even though such consideration is rejected by certain creeds, populations, or social groups.

The potential risk of infection, which has by no means been validated, but the possible dangers of recombination are out of all proportion to the small number of patients who could benefit from xenotransplants.

CCNE is not requesting a moratorium on pre-clinical xenotransplantation research. However, it is felt that prior and obligatory success with animal models, follow-up of effectiveness, maximum evaluation of the possibilities for protection from the risk of infection, and psycho-sociological research, must be demanded before moving on to the clinical phase, which is unlikely to emerge in the near future. It advocates a continuation of scientific animal experimentation, providing such research is properly conducted, i.e. through transparency, high standards, and founded on the belief that saving human lives cannot be at the expense of presently accepted good practices respectful of the relationship between humans and animals.

The central issue is the ethics of the decision to launch clinical implementation. By assessing the balance between risk and benefit, the principle of caution applies and must first take into account effectiveness. But this must be a principle of caution which rests

more on the notion of responsible practitioners and researchers, than on a rejection of progress. A chimpanzee with a porcine liver graft enjoying a normal life would demonstrate that xenografting is technically possible. Once scientific, infectious, immunological and psychological problems have been solved, opening the way for applying xenotransplantation to humans, consideration would need to be given to the matter of human mobility in Europe and globally. OECD has raised the issue of importing genetically engineered animals and has requested a global communications network on risks involved. It is hard to imagine that one European country could authorise the principle of xenografting without consulting neighbouring countries.

CCNE's present thinking is expressed in a time of anticipation. When moving on to clinical practice becomes based on acceptable scientific criteria, CCNE will wish to restate its position.

Glossary

- Xenonotransplantation = xenograft : grafting tissue or organs from one animal species to another; e.g. grafting pig organs to a human being, whereas an allograft is a graft taken from an individual and transferred to a member of the same species ; e.g a human organ to another human.

- Immunosuppressive agent : a drug used to diminish or eliminate the immune reactions of an individual. With immunosuppresive agents it is possible to avoid rejection by immunologic reaction of an alien organ, but at the same time, they depress defences against pathogenic microorganisms in the environment and thereby put an immunodepressive individual at some risk.

- Epitope : minimal molecular grouping to which the immune system will respond, for example by producing antibodies. A protein generally contains several epitopes.

- Transgenic animal : an animal carrying in its chromosomes genes from another living cell, animal, vegetable, or a micororganism. These genes may, or may not, be expressed in the cells of the transgenic animal.

- Retrovirus : a retrovirus is a virus in which the genome is formed by a single strand ribonucleic acid (RNA). During its life cycle, this virus must transcribe its RNA into a double strand deoxyribonucleic acid (DNA) which is inserted into the genome of the infected cell. This fragment of DNA can remain in the infected cell for generations without emerging unless it is inducted by various events in the cellular environment. It is then called an endogenic provirus.

- Oncogene : these are genes which are present in most animal cells and which are active in growth regulation. If they mutate or are overexpressed, they may convert the cell into a tumour cell and therefore play a role in the onset of cancer.

Bibliography

1. Animal to Human Transplants. The ethics of xenotransplantation. Nuffield Council on Bioethics (UK). 1995

2. Bach FH, Ferran C., Soares M *et al* . Modification of vascular responses in xenotransplantation : inflammation and apoptosis. Nature Med. 1997, 3, 944-949.

3. Bach FH., Fineberg HV. Call for moratorium on xenotransplants. 1998, Nature, 391, 326.

4. Baily LL. *et al* . Baboon-to-human cardiac xenotransplantation in a neonate. J. Am. Med. Association, 1985, 254, 3321.

5. British Renal Symposium. Abstracts. Harrogate, 1994.

6. Butler D. Last chance to stop and think on risks of xenotransplants. Nature, 1998, 391, 320-326.

7. Ceccaldi P, Millet A. Xénogreffe et transgénèse. Biofutur, 1996, 158, 17-25.

8. Chae JS., Cooper KC. Legal implications of xenotransplantation. Xénotransplantation, 1997, 4, 132-139.

9. Dickinson D. Pig heart transplant "breakthrough" stirs debate over timing of trials. Nature, 1995, 185-186.

10. Intercommission II de l'INSERM : "Utilisation thérapeutique des produits humains et des produits de substitution". Compte rendu du colloque du 2 juin 1997.

11. Julvez J., Tuppin P., Cardoso J. *et al* . Enquête nationale "Population et Xénogreffe", résultats préliminaires. Actes du colloque "Aspects scientifiques et socioculturels des xénogreffes", Etablissement français des greffes, 29-30 octobre 1998, 68-71.

12. lanza p., Cooper KC., Chick W. Xenotransplantation. Scientific American, 1997, 40-47.

13. Lehmar S., Trans-species transplants raise virus fears, Nature, 1995, 376, 8.

14. Le Monde, Edition du 23 janvier 1998.

15. Malassagne B., Tabois F., Houdebine LM. *et al* . Application de la transgénèse à la xénotransplantation. Résultats expérimentaux et perspectives cliniques. Presse Med. 1996, 25, 1247-1250.

16. Mohacsi PJ., Blumer E.C, Quine S. *et al* . Aversion to xenotransplantation, Nature, 1995, 378, 434.

17. Moran M. Pig-to-human heart transplant stated to begin in 1996. Nature Med. 1995, 1, 987.

18. Murphy FA. The public health risk of animal organ and tissue transplantation into humans, Science, 1996, 273, 746-747.

19. NKF survey reveals positive feelings on animal-to-human transplants. Dialysis and Transplantation, 1995, p. 677.

20. Rosengard AM., Cary NRB., Langford GA. *et al* . Tissue expression of human complement inihibitor, decay-accelerating factor, in transgenic pigs. Transplantation, 1995, 59, 1325-1333.

21. Sachs DH. Xenografts, cloning and the immune system. Nature Med., 1997, 3, 951-952.

22. Sandrin MS., McKenzie IEC. Gal a (1,3) Gal, the major xenoantigen(s) recognised in pig by human natural antibodies. Immunol. Rev., 1994, 141, 169-190.

23. Starzl T. Comments made at Institute of Medicine conference on Xenograft transplantation : science, ethics and public policy, 1995, Washington D.C.

24. Stoye J. No clear answers on safety of pigs as tissue donor source. Lancet. 1998, 352, 666-667.

25. Takeuchi Y., Porter CD., Strahan KM. *et al* . Sensitization of cells and retrovirus to human serum by (al-3) galactosyltransférase, Nature, 1996, 379, 85-88.

26. The 4 th International Congress for Xenotransplantation. Book of abstracts. Nantes, France, 1997.

27. Le Tessier P., Stoye JP., Takeuchi Y. *et al* , Two sets of human - Tropic pig retrovirus, Nature 1997, 389, 681-682.

28. Weiss AR., Transgenic pigs and virus adaptation. nature, 1998, 391, 327-328.

29. White D. Letter to Nature, 1995, 378, 434.

Notes

1. **(1)** Nowadays, more than 90% of patients in the West are treated with human recombinant insulin .