42. Corte cost., 26 febbraio 1998, n. 27. Foro It. 1998; 123:I, 1370-1374. La Corte spiega, infatti, che anche quando l'Amministrazione sanitaria non impone, ma si limita semplicemente a consigliare di sottoporsi ad una vaccinazione, essa induce il singolo ad affidarsi incolpevolmente ai moniti della pubblica autorità, la quale dovrebbe garantirne pienamente la salute, pur nei limiti delle conoscenze scientifiche del tempo.
43. Corte cost., 26 novembre 2002, n. 476. Foro It. 2003; 128, I: 330-333.
44. Il donatore viene sottoposto secondo le necessità a due trattamenti: l'aferesi cellulare e la plasmaferesi. La prima consiste nella sottrazione di elementi cellulari, normalmente piastrine o globuli bianchi, e nella restituzione del plasma e dei globuli rossi. La plasmaferesi, invece, è la sottrazione del solo plasma, con restituzione al donatore di tutti gli elementi cellulari e sostituzione del plasma sottratto con altri liquidi, normalmente soluzione fisiologica. Questi procedimenti si attuano in due modi: o sottoponendo il donatore ad un prelievo particolare per mezzo di una macchina denominata "separatore cellulare", che provvede ad aspirare il sangue da una vena di un braccio del donatore stesso, a centrifugarlo, a separarne la componente richiesta e a reimmettere nella vena dell'altro braccio il residuo, oppure (ed è il metodo più usato) prelevando il sangue in una sacca di plastica, alla quale sono collegate altre piccole sacche cosiddette "satelliti", e l'operazione di separazione avviene in laboratorio in un tempo successivo alla donazione.

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Articoli/*Articles*

BLOOD: THE LAST 20 YEARS OF DISCOVERY

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SUMMARY

Throughout the last few years, significant attention is being paid to progenitor cells, whose isolation has enhanced the understanding of biology of tissue formation and regeneration. Hematopoietic stem cells are historically the most well studied, they have been shown to provide the best chance for patients who undergo bone marrow transplantation, peripheral blood cell transplantation and cord blood transplantation. To date, the therapeutical potential of stem cells has become increasingly evident in many diseases, leading, beyond the classical procedures, to the implementation of non-haematological trials.

At the beginning of the 20th Alexander Maximow said that in the peripheral blood a small number of cells circulated that might be capable of reacquiring pluripotentiality; he called these cells "gemeinsame Stamzellen".

After several decades of attempts to confirm the existence of these stem cells, Hematopoietic Stem Cells (HSC) transplantations started in the late 1940's with experiments in mice. Starting in the late 1950's several groups tried to apply these concepts to the treatment of patients with leukaemia, and in the late 1970's these concepts gained acceptance. All clinical transplantations used allogeneic or autologous bone marrow as source of stem cells; since peripheral blood as a source of stem cells was still considered inadequate to permanently reconstitute hematopoiesis[1].

Key words: Stem cells - Differentiation -Reparative medicine - Self-renewal.

Three basic categories of cells make-up the human body: somatic cells, germ cells and stem cells. Somatic cells are cells that make-up the human adult; each of these cells, in its differentiated state, has its own copy of the genome (with the only exception of cells without nuclei, i.e. red blood cells). Germ cells are cells that give rise to gametes (eggs and sperm). Stem cells are have the capacity to self renew and the potential to generate mature specialized cell types; they are able to differentiate into cell types within the tissue in which they reside ("stem cell plasticity").

Stem cells generally represent a small percentage of the total cellular make-up of a particular organ. When a stem cell divides, the daughter cells can either differentiate in a specialized cell or self-renew to remain a stem cell, so ensuring that the pool of stem cells is constantly replenished; this is a necessary physiological mechanism for the maintenance of the cellular composition of tissues and organs in the body. Stem cells can also be classified as totipotent, pluripotent and multipotent. Totipotency is the ability to form all cell types; totipotent cells can basically form the whole organism because they have unlimited capability.

Pluripotency is the ability to form several cell types but not the whole organism. There are four classes of pluripotent stem cells: embryonic stem cells, embryonic germ cells, embryonic carcinoma cells and the adult progenitor cells from bone marrow.

Multipotency is the ability to generate a limited range of differentiated cell lineages appropriate to their location, e.g. blood stem cells which give rise to red blood cells, white blood cells and platelets.

The fundamental dogma that somatic tissue-derived adult stem cells may differentiate only into mature elements of the tissue in which they reside was undermined in 1998, when it was first demonstrated that adult stem cells, (such as haemopoietic stem cells, neuronal stem cells and mesenchymal stem cells), could cross boundaries and differentiate into cells of a different tissue. This phenomenon of adult stem cell plasticity has been termed "transdifferentation". Differentiation is the process whereby an

unspecialized early embryonic cell acquires the features of a specialized cell such as heart, liver or muscle. The best studied adult stem cell is the hematopoietic stem cell (HSC).

The adult hematopoietic system is composed of a series of pluripotent, multipotent and unipotent cells leading to functionally distinct mature blood cell types[2]. Rare pluripotent hematopoietic stem cells (HSCs) are at the foundation of the adult hematopoietic system and are maintained in constant numbers in the adult bone marrow in a relatively quiescent state; they both self-renew more stem cells and give rise to clonal progeny that continue to differentiate. Stem cell survival and proliferation in vivo depend on a close connection with bone marrow stromal cells. These stromal cells include cells of mesenchimal origin (e.g. fibroblasts, osteocytes, adipocytes and vascular smooth muscle cells) that are known to constitute a supportive microenvironment. The specific role of each stromal population is still unknown, but recent studies clearly revealed that osteoblasts are an important component of the bone marrow niche.

HSCs are entirely responsible for the development, maintenance, and regeneration of blood forming tissues. Furthermore, HSCs are the most important, if not the only, cells required to engraft in hematopoietic tissue transplantations. HSCs have been used widely in clinical settings for over 40 years and form the basis of bone marrow transplantation success.

HSC transplantation is an effective therapy for a wide variety of neoplastic diseases, in addition to congenital and autoimmune disorders. The two major types of HSC transplantations, autologous and allogeneic, are defined by the donor graft source[3]. Both autologous and allogeneic HSC transplantations are used, depending on donor availability and the type of disease being treated. In autologous HSC transplantation, chemotherapy and/or radiation are administered to the patients, so, prior to HSC transplantation, patients undergo harvesting of their hematopoietic cells from bone marrow or from peripheral blood. The principle behind allogeneic HSC transplantation differs, as do the spectrum

of diseases treated and the potential benefits and complications. For hematologic malignancies, thought to evolve from early hematopoietic progenitors (i.e., chronic myelogenous leukaemia) or from bone marrow failure states (i.e., aplastic anemia), replacement with healthy donor cells is required. Compared to autologous HSC, allogeneic HSC transplantation has a higher incidence of treatment-related morbidity and mortality, particularly because it contains immune cells that can respond against host-specific antigens and causes the syndrome called graft-versus-host disease (GVHD). Despite the prophylaxis with immunosuppression, ~20-30% of allogeneic HSC transplantation patients develop an acute form of GVHD, and ~50% develop a chronic form of the disease. If we put together the risk of GVHD, immunosuppression, and the potential failure engraftment, the transplantation-related mortality for an allogeneic HSC transplantation is ~10-15%.

Unfortunately, HSCs, like many other adult stem cells, are rare in bone marrow and difficult to isolate in large numbers. For example, only approximately 1 out of 10000 bone marrow cells is an HSC. In a steady state, HSCs are even rarer in peripheral blood, since a low number of HSCs exits from the bone marrow and circulates in peripheral blood.

Little is known about the physiological roles of such trafficking, however the role of the so-called cytokines (or growth factors) is clear; they may influence this process increasing both the number of HSCs exiting the bone marrow and their lifespan. Compared to bone marrow harvest, a significantly higher number of stem cells can be so obtained from the blood, after a growth factor injection; a process called "mobilization".

Before 1990, almost all HSCs transplantations were bone marrow-derived. At the end of the 1980's the first case of allogeneic peripheral blood stem cell (PBSC) transplantation was reported. Since the donor was not mobilized by cytokines, he underwent ten apheresis to harvest a sufficient stem cell number. Engraftment was succesful. In 1988, the ability of granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony stimulating

factor (G-CSF) to mobilize HSCs to the blood stream was documented (other hematopoietic growth factors as interleukin-3 and stem-cell factor were not approved as mobilization agents for routine clinical use). Mobilization with either chemotherapy and/or growth factors injection may result in an efflux of HSCs out of the bone marrow into the blood and lead to a concentration of HSCs in the peripheral blood that equals or exceeds the concentration in the bone marrow itself. An HSC-enriched cell fraction can then be collected by apheresis from the blood. In 1995, the first series of G-CSF-mobilized stem cell allogeneic transplantations was published. Now allogeneic transplantations have increased at an average annual rate of 10% and the peripheral blood stem cell (PBSC) allogeneic transplantations have risen at an even higher rate.

Under standard conditions, donors and recipients are matched at the major histocompatibility complex (MHC), which in humans are the genes of the human leucocyte antigens (HLA). However, since the ability to identify suitably matched related or unrelated donors can be difficult in some patients, alternative sources of stem cells have been explored. Cord blood provides a readily available source for such patients[4]. The collection of cord blood cells is relatively easy and the risk of severe acute graft-versus-host disease (GVHD) is lower thus making the cord blood transplantation an appealing alternative to bone marrow transplantation.

The first known attempt took place about 34 years ago when a young 16-year-old boy with acute lymphoblastic leukaemia received cord blood units from 8 different donors. One unit engrafted and lasted for 38 days. But the first successfull allogeneic cord blood transplantation occurred approximately 16 years ago in a child with Fanconi anemia[5]. Data accumulated over the past several years have demonstrated that cord blood is an accepted alternative source for hematopoietic stem cells in children. It offers many practical advantages such as:

1) absence of risk for mother and newborn;
2) relative ease of procurement and availability (as stored cord blood cells are fully tested and HLA-typed, they are available for immediate use);
3) potential reduced risk of GVHD.

While the clinical data is encouraging for pediatric patients, cord blood use can be more problematic in adult patients since the limited number of hematopoietic progenitors and the collection can occur only in a single occasion. Nevertheless cord blood transplantation has recently been explored in an increasing number of adult patients. The reason is that, while the total numbers of mononuclear cells are limited, the progenitor content and the proliferative potential of cord blood cells are high. So some protocols are now available to attempt to use cord blood as an alternative source of hematopoietic stem cells for allogeneic transplantations for adult patients too.

The possibility to purify and expand HSCs has recently led to the implementation of non-hematological clinical trials, aimed at developing tissue repair protocols in chronic-degenerative disorders as Alzheimer's disease, Parkinson's disease and other neurological disorders; and it has opened up new and unexpected therapeutical perspectives also in the treatment of nephropathies (kidney transplantation)[6, 7], and other diseases involving liver, brain and heart. For example the infusion of autologous bone marrow stem cells in the coronary artery has been proposed for regenerating the myocardium after ischaemia[8, 9].

Treatment via stem cell will be a "cell-based therapy". For the first time, treatment will be the administration of cells directly into the body thus delivering stem cells to the tissues where they are needed. The biology of these mysterious cells is yet to be understood and a lot more basic research is needed before new therapies using stem-cells can be applied, problems exist with the manipulation of adult stem cells, in fact stem cells in adult human tissues are difficult to isolate and those that can be isolated with

relative ease are unfortunately difficult to scale-up in culture; nevertheless some of these problems are technical and might be overcome in the future. The therapeutic potential of stem cells has promised to revolutionize the future of medicine and has created a new field of research, called regenerative medicine.

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