術野付近で多量の細胞が傷害を受けており、それらの細胞は活発に組織修復に必要なサイトカインを産生している。一般の創傷治癒過程においてはこれらのサイトカインは周辺のコラーゲン等の細胞外マトリックスに一時的に吸着され、手術後に徐放出されて細胞侵入や分裂、遊走、毛細血管誘導、組織構築等に貢献する。しかし臨床で使用されている布製人工血管にはその様な細胞外マトリックスが無いので、折角産生されたサイトカインも流れ去り、失活する。これはちょうど布製人工血管が禿げ山の様な状態であり、雨水を蓄え、徐々に放出する事ができない状況にあると理解される。私どもの研究で用いたコラーゲン被覆人工血管ではコラーゲンに bFGF 等のサイトカインが自然に吸着され、その後サイトカインはコラーゲンから徐放出されてゆくと思われる。またコラーゲン自身も徐々に吸収されるので、吸収の過程でさらにサイトカインが放出されるであろう。そのためコラーゲンに沿って宿主の細胞が侵入し、それらの細胞がさらにサイトカインを産生して毛細血管を呼び込み、Tissue Engineering に必要な細胞とサイトカインを人工血管が持つこととなったと考えられる。これはちょうど樹木の生い茂った山が雨水を一時的に蓄え、生物の成長を助ける状態に例えられよう。

すなわち、人工血管植え込み時においては、局所にはサイトカインも細胞も人工血管周囲に存在しているが、それを利用できる状態に無いだけのことであると考えられる。そこで適切な細胞外マトリックスを人工血管に賦与することで良好な Tissue Engineering のサイクルに持ち込むことができる事を示している。ここで注目すべき事は従来コラーゲンは細胞に良好な足場を提供すると考えられていたが、実際にはコラーゲンは足場を提供したのみならず、サイトカインの吸着、徐放出のためのリザーバーとしても活躍していたことであった。この様なことから内膜形成において Tissue Engineering が無理なく行われたと推測される。

アイソトープでラベルした bFGF を用いた我々の基礎研究では市販のコラーゲン被覆人工血管のコラーゲンも bFGF を吸着できた。しかし細胞毒性がコラーゲンにあればそれが前面に出て細胞を誘導することができなかった。したがって、市販の人工血管も少しの改良で Tissue Engineering の理論を活用できる状態にあると思われる。

9、小口径人工血管開発と Tissue Engineering

文頭に述べたように小口径人工血管には内皮細胞被覆が必要であり、そのためには新生内膜形成を Tissue Engineering 的な考えで進めるべきであって、Tissue Engineering における４要素を考慮すれば新しい考え方にもとずく小口径人工血管が誕生すると期待される。

in vivo 環境は Tissue Engineering を行う上でとても恵まれている。in vivo の特性については前述したが、局所のみならず遠隔地からもサイトカインによる応援が受けられる長所がある。そしてそれらを活用することも、意図的に排除する事も可能である。また、幼弱な細胞、たとえば骨髄細胞や各種幹細胞などを働かせることもできる。そしてそれらの分化をも誘導可能という利点も出てくる。

従来の人工血管の考え方は血栓を付着させないこと、血液をもらさないこと等であった。それはそれで必要であるが、一時的な抗血栓性は抗凝固療法を併用したり、適切なシール材で被覆すれば獲得

可能であろう。そして天然の、しかも永久的な抗血栓性をもつ内皮細胞は細胞活動を前述した種々の手段で誘導したり制御することがTissue Engineering的に考えると可能となった。細胞の活動する場は素材、細胞、サイトカイン等の特性を生かし、組み合わせて考えることで人工血管として与えた基材の上で新生内膜を形成させるための作戦が練られるようになってきた。この様な考え方で小口径人工血管の設計を行うと従来考えられなかった幾通りもの設計が可能となってくる。その様な工夫の中で最も素直にTissue Engineeringが進められる方法が小口径人工血管をいち早く実用化に導いてくれると思われる。

おわりに

Tissue Engineering の考え方は始まったばかりであるが医学領域のみならず産業界を含めて広い分野で活用される技術としてとらえられており、おそらく２１世紀には多くの領域で当たり前のように用いられるようになっていると考えられている。ここに述べた考え方は今日の時点での最も進んでいると思われる考え方であるが、サイトカインや接着蛋白等の新しい情報が入ればそれらを参考にしてさらに改良可能と思われる。ここに示したことを参考にした新たな工夫が人工血管のみならず、広い範囲で生まれることを期待している。

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The Concept of Tissue Engineering in the Development of Small Diameter Vascular Prostheses

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Key words: Small diameter vascular prosthesis, Tissue engineering, Neointima, Cytokine, Extracellular matrix

In order to obtain rapid neointima formation, a way of thinking of tissue engineering concepts were introduced in the field of vascular prostheses. Our hypothesis is as follows. Neointima formation is a product of tissue engineering in vivo, and four major factors, i.e., cells, extracellular matrices, cytokines, and in vivo environment should be arranged for effective tissue engineering. Vascular prostheses reported previously lack one or other of these factors. Therefore, endothelial cell seeding alone is not sufficient even if the cells are treated carefully. Recently, we obtained rapid neointima formation with endothelial cell lining throughout the graft surface by using autologous bone marrow transplantation. The key to success is as follows. Firstly, young and primitive cells such as stem cells should be used. Previously, highly differentiated cells such as endothelial cells have been used. The combination of different cell types is also useful. For example, a new skin can not be produced by cultured epidermal cells alone. However, with cultured fibroblasts underneath the epidermal cell layer as feeder cells, a skin-equivalent graft can be produced. Bone marrow contains both young, multipotential cells and highly differentiated cells such as endothelial cells. The second point is separation of cells. We do not use enzymatic separation of cells in order to avoid destruction of biological extracellular matrix in the case of bone marrow. Thirdly, a system for continuous cytosine synthesis is important. We obtained continuous synthesis of cytokines from transplanted marrow cells. Finally an in vivo environment is helpful for cell activity. With this hypothesis, we produced a simple collagen

coated prosthesis, which can adsorb endogenous cytokine, resulting in acceleration of fibroblast migration and proliferation, and capillary ingrowth into the graft wall. From these examples, we can recognize the importance of the combined use of these factors in vascular grafts for effective tissue engineering. Vascular prostheses designed with tissue engineering will become available for clinical use in the near future.

Vascular prostheses transplanted with bone marrow

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Abstract

We obtained natural cytokine synthesis by bone marrow transplanted autologously in a synthetic vascular prosthesis. Bone marrow contains young, primitive cells with a strong survival potential which can differentiate into many kind of mesenchymal cells and can produce many kind of cytokines. These cytokines are useful if we can employ them in the right way at the right time. As an example of the use of natural cytokines, bone marrow transplantation into a synthetic vascular prosthesis will be described. Transplanted marrow cells survived in the prosthesis and accelerated neointima formation on its luminal surface. Bone marrow contains numerous undifferentiated cells which can differentiate into various kinds of cells depending on thier environment. We expected the marrow cells to differentiate into cells such as fibroblasts, endothelial cells, and smooth muscle cells. But we could not find any signs of differentiation of the transplanted marrow cells.

Introduction

We have recently achieved natural cytokine synthesis by bone marrow transplanted autologously in a synthetic vascular prosthesis[1]. Bone marrow contains young, primitive cells with a strong survival potential which can differentiate into many kind of mesenchymal cells and can produce many kind of cytokines[2]. How to use cytokines and growth factors efficiently in clinical medicine is one of the most interesting fields of research[3,4]. Gene transfer technologies using viruses, liposomes, DNA-coated micro projectiles, and infected cells are major methods for this purpose[5,6]. Some basic experiments have shown promising results, but there are also undesirable side effects[7,8]. Instead of genetically modified cells, we used autologous bone marrow.

In an ordinal environment, marrow cells works as a physiological tissue synthesizing their usual cytokines[9,10]. In unusual conditions, however, they synthesize various other cytokines for survival. Therefore, if we transplant them ectopically, they will synthesize cytokines actively and continuously. These

cytokines are useful if we can employ them in the right way at the right time. In this communication, as an example of the use of natural cytokines, bone marrow transplantation into a synthetic vascular prosthesis will be described. Transplanted marrow cells survived in the prosthesis and accelerated neointima formation on its luminal surface.

Problems with synthetic vascular prostheses

Vascular prostheses used in clinical applications have a number of problems, such as lack of anti-thrombogenicity, lack of protection from infection, less compliance than natural arteries, and low affinity for host tissues[11,12]. The most undesirable problem is the delay of neointima formation with a natural anti-thrombogenic endothelial cell lining. In fact, most of the prostheses implanted are not endothelialized, and the inner surfaces remain thrombogenic long after implantation[13,14]. Dacron fibers do not cause foreign body reaction[15], but have no anti-thrombogenicity[16,17]. Therefore, when the prosthesis is exposed to the blood, the coagulation system works against the Dacron fibers and red thrombi adhere to its luminal surface. Usually, from the anastomotic sites, approximately 2 cm areas are healed with endothelial cell lining. There was no thrombus deposition in these areas. These areas are covered with endothelial cells which have crept in from the host arterial wall at the anastomotic sites. Endothelial cells prevent thrombus formation and adhesion on the luminal surface of natural blood vessels[12]. On the areas far from the anastomotic sites, however, thick thrombi adhere to the surface because there is no endothelial cell lining. In clinic, vascular prostheses are not endothelialized except at anastomotic sites.

Reasons for the delay and efforts to overcome the problem

There are several reasons for the delay in healing, i.e., foreign body reactions of the prosthesis, inflammation, hypercoagulation, cell aging, and unusual blood flow dynamics. The major reason is the aging of endothelial cells. After more than 70 cell divisions, endothelial cells form another generation due to aging[18,19,20]. The maximum area covered by 70 endothelial cell divisions is less than 2 cm from the suture lines. In clinical practice, we used 30 to 60 cm long vascular prostheses in a patients with peripheral arterial occlusive disease. Therefore, most of the luminal surface except very limited areas of anastomotic sites are not covered with endothelial cells. As a result, the vascular prosthesis cannot acquire natural anti-thrombogenicity of endothelial cells even after a long period of time. This is the one of the most

difficult problems to overcome in a small diameter arterial regions. Details of pathological observations have been described elsewhere[21].

Background of our new trial

As explained, vascular grafts in humans do not endothelialize[13,21]. This is a protracted ulcer in the blood vessel wall. Transplantation of autologous tissue fragments has been used effectively to accelerate the healing process of protracted skin ulcers and prolonged bone fractures[22,23,24]. Tissue fragments contain various kinds of cells. In the skin, fibroblasts act as feeder cells to epidermal cells[25]. In equivalent experiments, smooth muscle cells and fibroblasts were shown to enhance endothelial cell growth[26,27]. We have applied a similar technique to vascular prostheses, since endothelial cell proliferation is greatly improved with an underlying base of feeder cells. Satisfactory results in both animal experiments[28,29] and in clinical practice[30] were obtained with venous and adipose tissue fragments in prostheses. Capillaries for endothelialization originated from the transplanted fragments and complete endothelialization was observed in a canine study[28]. We also showed that tissue fragments transplanted into the fabric prosthesis wall synthesized high amounts of cytokines such as bFGF from the early stage of implantation, resulting in rapid capillary ingrowth into the prosthesis wall[31].

A new trial

From theses evidences, the procurement of autologous tissue containing a satisfactory quantity and quality of proliferate feeder cells become desirable to accelerate endothelialization. In order to regulate the healing process, we designed a vascular prosthesis which would have growth factors during endothelialization. Autologous bone marrow tissue containing various proliferative and differentiative cells with feeder cells and cytokine secretion[32] was chosen for the source of fragments and was transplanted into the vascular prosthesis wall[21]. With this background, we applied bone marrow transplantation technology to the field of vascular prosthesis as shown in the following animal experiment.

Preparation of the graft

As a basic matrix, an expanded polytetrafluoroethylene prosthesis (e-PTFE prosthesis) with fibril length of 60 to 150 μm (average 90 μm) was used. The prosthesis was donated by the Vascular Group, Baxter

CVS Division (Irvine, CA, U.S.A.). Approximately 0.5 ml of bone marrow was obtained and stirred into 20 ml of lactated Ringer solution to create a bone marrow suspension. This suspension was sieved through the prosthesis wall by repeated and pressurized injections into the closed prosthesis with a syringe. The residual suspension that passed through the prosthetic wall was then injected again. During the repetition of this sieving procedure, bone marrow tissues were trapped in the pores of the prosthesis wall. Then the prosthesis was washed several times with lactated Ringer solution to remove the free remnants of tissue from the luminal surface, and it was implanted as a vascular substitute in the same dog from which the bone marrow was taken. As a control, a similar E-PTFE prosthesis without any bone marrow transplantation was used.

Prosthesis implantation

Twenty-four adult dogs of both sexes, weighing 7 to 12 kg, were used for implantation of the vascular prostheses (inside diameter 6 mm; length 6 to 8 cm) in the abdominal aorta. Twelve of them were used for the implantation of the treated prostheses, and 12 for the control prostheses. The abdominal cavity was entered via a midline incision in the abdomen. The abdominal aorta was exposed and mobilized by sacrificing several branches between the renal arteries to the trifurcation. A 5 cm segment of the aorta was resected and replaced by the prosthesis. During the operation, an antibiotics (1 gm Cefazolin sodium, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) was administered into the abdominal cavity, and no anticoagulants were used at any time.

All animal care was in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 80-23, revised 1985).

Prosthesis removal

The prostheses were removed from the animals at 3 days, 3 weeks and 3 months. Before harvesting, heparin sodium (100 IU/kg) was administered intravenously to prevent clotting. All the removed specimens were rinsed with saline solution to remove excess intraluminal blood and inspected macroscopically and microscopically.

All of the animals with prosthesis implantation looked healthy during the observation period. Chronic ileus symptom with a distended abdomen due to intestinal adhesion was noticed in one test animal, but

it survived the observation period. In the treated group, 2, 4, and 6 prostheses were retrieved at 3 days, 3 weeks, and 3 months, respectively. One prosthesis out of 4 at 3 weeks was occluded, but all the other prostheses were patent. In the control group, 2, 4, and 6 prostheses were retrieved at 3 days, 3 weeks, and 3 months, respectively. All of them were patent.

Macroscopic appearance of the removed grafts

The treated prostheses removed at 3 days were covered with fresh, thin, red thrombus, and were colored red. There was no thick thrombus deposition on the surface. At 3 weeks, all the prostheses were dark red in color along the entire luminal surface, and there was no fresh thrombus at all. The prosthesis wall was soft and pliable. There was no unusual adhesion, hematoma, seroma formation, inflammatory reaction or scar tissue around the prosthesis. The one occluded prosthesis was hard along its entire length and dark brown in color. Its lumen was completely occupied by connective tissue. But there was no inflammatory reaction either inside or outside. At 3 months, the luminal surfaces were still red in color, but the degree of redness was less than at 3 weeks. There was also no thrombus in the luminal surface. On the adventitial side, there was no unusual response at all. The prostheses were soft and pliable. Some areas of the luminal surface were red like thrombus deposit, but they had no thrombus deposition at all. The redness had arisen from the inside of the wall. The adventitial side was white in color composed of very loose connective tissue without unusual reactions.

In the control group at 3 days, thin red thrombus covered the luminal surface. No thick thrombus was present. At 3 weeks, the luminal surface looked slightly red in color due to a thin layer of red thrombus . Near the anastomotic sites, however, the lumen was white and glistening. There were no unusual reactions around the prosthesis. There was no hematoma, scar formation, seroma formation, or inflammatory reaction. At 3 months, the prostheses were surrounded with very loose connective tissue without any unusual responses. The luminal surface was red in color with thin red thrombus adhesion except for the anastomotic sites, where the surface was glistening and white for about 10 mm from the suture line. At the adventitia side, the prosthesis was surrounded with loose white connective tissue without unusual reactions.

Light microscopic appearance

Before implantation, the treated prosthesis had numerous bone marrow tissue including megakaryocytes and erythroblasts in the interstices of the Teflon fibrils of the E-PTFE prosthesis. Stem cells were not identified. The cells, including small pieces of bone marrow tissue, were enmeshed and accumulated in the interstices of the Teflon fibrils near the luminal surface. But numerous cells including peripheral red blood cells were present all over the prosthesis wall.

At 3 days after implantation, the prosthesis wall contained numerous cells including bone marrow cells. These marrow cells were immunohistochemically reactive. At 3 weeks, the luminal surface of the treated prosthesis was completely lined with a continuous layer of endothelial-like cells. These cells could be stained with the PAP method, indicating that they had factor VIII, and were identified as endothelial cells. There were many capillary blood vessels near the luminal surface. The thickness of the neointima was about 20 μm. A small pannus about 30 μm thick extended approximately 2 mm from the proximal anastomosis. There were no giant cells around the prosthesis, which was surrounded with connective tissue containing a large number of fibroblasts and collagen fibers. Inside the prosthesis wall, numerous macrophages could be seen. Many capillary blood vessels were also observed inside and outside the wall. Hemopoiesis areas with erythroblasts were present sporadically inside and outside the graft wall. Around these areas there was always some capillary blood vessel ingrowth. Because of the hemopoiesis and the numerous capillary blood vessels, the wall contained a great amount of endothelial cells and blood components.

At 3 months, the luminal surface of all the prostheses was covered with a layer of endothelial cells. The thickness of the neointima was between 10 to 100 μm. There was no thrombus on the luminal surface. At the anastomotic sites, small panni from 1 to 3 mm in width along the suture line and 20 to 100 μm in thickness were seen, but there was no intimal hyperplasia at all. These panni were formed to make the surface smooth between the host aorta and the prosthesis surface. There were some areas with hemopoiesis associated with capillary blood vessels. But the number of these areas was less than at 3 weeks. Inside the neointima, there were small pieces of bone fragments. They were embedded in the neointima without any inflammatory reactions or intimal hyperplasia. Hemopoietic activity was still present at 3 months. We observed some erythroblastic islands which are typical sites of hemopoiesis in the interstices of prosthesis fibers. Around the erythroblastic islands, numerous capillary blood vessels were present.

In the control group at 3 days, the prosthesis was covered with a fibrin layer with numerous erythrocytes. Inside the prostheses wall, a fibrin layer containing erythrocytes and leukocytes occupied all

areas. At 3 weeks, the surface near the proximal anastomotic site had a pannus. It was between 0.5 to 1.5 mm in width and 20 to 50 μm in thickness. The areas about 5 mm from the anastomotic site were endothelialized. The other areas far from the anastomotic sites were not endothelialized, but were covered with a thin layer of fibrin. Inside the prosthesis wall there were numerous macrophages. On the adventitia side, fibrin layers of 50 to 200 μm in thickness were attached sporadically to the outer surface. Connective tissue composed of fibroblasts and collagen fibers was also seen. There were some capillary blood vessels inside the connective tissues on the adventitia side. But there were no capillary blood vessels inside the prosthesis wall, i.e., in the interstices of the PTFE fibrils. In immunohistochemical examination, the control graft showed no bFGF reactive in any areas of the graft wall.

At 3 months, the panni at the anastomotic sites of the control prostheses were about 1 to 3 mm in width and 20 to 50 μm in thickness. A continuous endothelial cell lining from the suture line was noticed beyond the pannus edge, but the other areas far from the anastomotic sites were covered with a thin layer of thrombus without endothelial cell lining. Inside the wall, numerous macrophages were seen. But there was only a very small number of capillary blood vessels inside and outside the graft wall. On the adventitial side, connective tissue layer composed of fibroblasts and capillary blood vessels could be seen. There was a fibrin layer on the adventitia side.

There was no intimal hyperplasia in the pannus layer of the anastomotic sites. These endothelial cells were located on the fibrin layer or on the connective tissue containing fibroblasts. Inside the wall, a small number of macrophages were seen. Some capillary blood vessels were also observed. On the adventitial side, a thin layer of fibrin 1 mm in thickness and 5 mm in width directly adhered to the outer surface. Outside the fibrin layer, connective tissue composed of fibroblasts and collagen fibers was seen. A small number of capillary blood vessels were observed here and there.

Acceleration of the neointima formation observed in the results

As shown in the results, neointima formation was obviously accelerated by the bone marrow transplantation. The treated prostheses showed more rapid endothelialization than the controls. Without exception, all of the retrieved prostheses showed extremely rapid healing. Endothelialization of the center area was quicker than at the anastomotic sites. In the control group, endothelialization always started from the anastomotic sites even at 3 months. There was no endothelial cell lining in the center areas of the control

prostheses. The patterns of endothelialization of the treated prostheses and the controls showed great differences.

In humans, endothelialization always starts from the anastomotic sites[13] as in the control prostheses of the current experiment. If it could start from the entire luminal surface at a very early stage after implantation as in the treated prostheses of the current experiments, the prostheses could quickly obtain the natural anti-thrombogenicity of endothelial cells, which would be of great benefit in small diameter vascular prostheses.

In order to obtain complete endothelialization, endothelial cell seeding techniques have been tested during the past two decades. Some of them produced favorable results[33,34,35,36] , but they remain unavailable for general use because they require special cell culture techniques and facilities. They are also not available for emergency use, because the cell culture requires an extended period of time. In endothelial cell seeding experiments previously reported, the survival rate of the seeded cells were extremely low. Almost no seeded cells are available for endothelialization[37,38]. Some experiments showed that the cells in the newly formed neointima did not originate from the seeded cells but from the surrounding host tissue at the adventitia side. These experiments were not successful because the seeded cells were washed away by the circulation. Enzymatically separated cells have difficulty surviving in vivo.

Survival of the transplanted cells

It was obvious that the bone marrow cells survived and maintained their activities during the sieving procedure and after implantation. They created colonies and acted as bone marrow tissue inside the prosthesis wall. This result is greatly superior to the low survival rate of enzymatically derived single cells. We need clumps of endothelial cells to minimize washoff. In the metastasis of cancer cells, it is assumed that a single cell that has migrated from the original tumor does not survive to cause a metastasis. A certain number of cells is required to start a new colony. In the current experiments, the survival rate of the bone marrow cells *in vivo* was not calculated, but bone marrow tissues formed clumps of cells and it is evident that these bone marrow tissues certainly survived and continued their hemopoiesis.

Our hypothesis regarding the cell survival is as follows. The interaction of different kind of cells is probably of crucial importance. In artificial skin grafts, new skin can not be produced with cultured epidermal cells alone. However, with cultured fibroblasts underneath the epidermal cell layer, a skin-equivalent graft can be produced[25]. Fibroblasts are considered to act as feeder cells for the epidermal cells. Fibroblasts

do not suppress the epidermal cell growth in vivo. The combined use of different cell types is important for organ reconstruction. Wildevuur et al. found that seeding of smooth muscle cells enhanced endothelialization of vascular prostheses[26]. Tissue fragments containing endothelial cells, smooth muscle cells, and fibroblasts also enhanced neointima formation of a fabric vascular prosthesis[27,28]. As shown in our in vitro cell culture results, bone marrow contains various cell types. Therefore, the bone marrow in the vascular prosthesis was in a desirable condition for their survival.

Ectopic hemopoiesis of the transplanted marrow cells

It was surprising that the hemopoiesis took placet in the treated prosthesis wall. Colonies of erythroblasts were always associated with capillary blood vessels. During the hemopoiesis, the marrow cells require nutrition for survival and raw materials for hemopoiesis. Therefore, capillary blood vessels are required quickly. There is natural angiogenesis. As a result of this activity, the prosthesis wall can obtain numerous capillary blood vessels. This is the reason why the luminal surface of the treated prostheses looked red without fresh thrombus deposition.

In general, bone marrow obtained by needle puncture contains some endothelial cells. The amount of endothelial cells is not high. But these cells could survive to make colonies to promote marrow cell survival, producing the entire luminal surface lining within a short period of time. This activy is quite aggressive in the young, primitive cells of the bone marrow. We can obtain an active bone marrow tissue even from elderly patients. Therefore, bone marrow transplantation into the vascular prosthesis wall can induce this activity in the healing process of neointima very efficiently.

Bone marrow transplantation technology

Exogenic hemopoiesis on these vascular prostheses was characteristic. During hemopoiesis, some cytokines might aid capillary ingrowth into the prosthesis wall. Marrow cells need nutrition for their survival, raw materials for producing blood cells during hemopoiesis, and routes for shipping out their products, i.e. "blood cells". As a result, capillary ingrowth was required. These requirements of the marrow cells might be translated by cytokines and growth factors. Remarkable capillary ingrowth shortly after the prosthesis implantation indicated the existence of strong angiogenic properties. Detection of bFGF in the transplanted marrow cells suggests that its production continued throughout the prosthesis wall as long as those

cells existed. bFGF has gained attention due to its strong angiogenic property and ability to accelerate capillary ingrowth to form collateral circulation for the ischemic heart[39,40]. The dark-red color of the treated prostheses observed macroscopically might have come from the newly formed capillaries and numerous immature blood cells at hemopoiesis . The size of the capillaries decreased in parallel with the regression of the hemopoiesis and at that time the prosthesis turned light pink. These four factors, i.e., bFGF, capillary ingrowth, hemopoiesis, and graft color seem to be interrelated.

The dose of cytokine and growth factors sufficient for angiogenesis and neointima formation might be small, but continuous release would be essential for the cells to endothelialize the prosthesis lumen, since these factors' efficacy lasts a short time[40,41,42]. However, there appears to be an autoregulating system in the bone marrow treated prostheses, since endothelialization was complete within 3 weeks and was arrested without endothelial cell hyperplasia or hemangioma formation, whereas hemopoiesis continued for more than 3 months.

Cytokines synthesized from bone marrow

The activity might be stimulated by cytokines such as bFGF synthesized by the transplanted bone marrow. We have shown that in a vascular prosthesis made with fragmented autologous tissue, a high amount of bFGF was synthesized soon after implantation[31]. There was much capillary ingrowth in the prosthesis wall and the luminal surface was endothelialized within 1 month. In the current experiments, we did not identify any synthesized cytokines other than bFGF. During hemopoiesis, however, marrow cells could continuously produce various cytokines that accelerate the neointima formation. In effect, we transplant a system that slowly releases cytokines and supplies young cells. Such systems could be useful not only in vascular prostheses, but also in various hybrid artificial organs and in surgery in general. Thus we were able to produce neointima formation on vascular prostheses without recourse to the use of gene transfer technology.

Differentiation of cells

Bone marrow contains numerous undifferentiated cells which can differentiate into various kinds of cells depending on thier environment. In the current experiments, we expected the marrow cells to differentiate into cells such as fibroblasts, endothelial cells, and smooth muscle cells. But we could not find any signs of differentiation of the transplanted marrow cells. If such differentiation could be effected with the

aide of some kind of cytokines, bone marrow technology would be useful to design future hybrid artificial organs with the ability of cytokine synthesis .

Conclusion

Active angiogenesis in the bone marrow tested prosthesis and accelerated endothelialization throughout the luminal surface seemed to be the result of co-operation of the transplanted cells and cytokines secreted from them. The prosthesis appears to be an autocrine artificial organ producing growth factors for itself under an autologously controlled system. Based on these observations, we could understand how useful the ectopic transplantation of bone marrow was. We expect that this technology will be applied in all medical fields.

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