these two cytokines had opposite effects on survival and cardiac function. GM-CSF worsened subacute survival, elongated and thinned the infarcted area, and deteriorated cardiac function. This was explained by the finding that a number of macrophages were mobilized into the infarcted area by GM-CSF at the acute phase. In contrast, G-CSF administration significantly improved survival and cardiac function. This phenomenon was explained by the findings that the recruitment of GFP^+CD45^- cells was markedly increased in the infarcted area of G-CSF-treated mice compared to the saline or GM-CSF group at the chronic phase (60 days). Furthermore, approximately 10% of GFP^+ cells were also $actinin^+$, suggesting a cardiomyocyte phenotype. FISH analysis negated the possibility of cell fusion between donor and recipient cells. (2) A number of HSC-derived GFP^+ cells was observed at the infarcted area at 60 days, but did not express CD45, and considered to be fibroblasts. Only a few GFP^+ cell revealed $actinin^+$. [Conclusion] Appropriate cytokine therapy may therefore augment the repair of damaged myocardium by mobilized bone marrow cells and prevent cardiac remodeling.

4

Regeneration Therapy for Heart Failure due to Nonischemic Cardiomyopathy

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Potential repair by cell grafting or bone marrow-derived cells (BMC) recently holds particular attention in heart disease, since the meager capacity for proliferation of adult cardiac myocytes likely contributes to the difficulty in controlling heart failure. Effectiveness of such regeneration therapies has been reported in ischemic heart diseases, post-infarction heart in particular. However, it is not established whether the therapies are effective on heart failure due to nonischemic cardiomyopathy. We here present two models with heart failure subjected to regeneration therapies: 1) doxorubicin cardiomyopathy in the rabbit treated with autologous BMC transplantation; and 2) hereditary cardiomyopathy in the hamster (UM-X7.1 strain) treated with granulocyte colony-stimulating factor (G-CSF). In both models, the treatments resulted in a significant improvement of the left ventricular function and remodeling compared with the vehicle-treatments. Immunofluorescence analysis documented the formation of new myocytes which were considered to originate from BMC in the treated groups. In addition, cardiac fibrosis was significantly reduced and cardiac myocyte degeneration detected by dye-inclusion was significantly ameliorated in the treated groups. Particularly, in cardiomyopathic hamsters treated with G-CSF, myocardial matrix metalloproteinases (MMPs; MMP-2 and -9) were significantly upregulated, which might contribute to reduction in excessive fibrosis, and myocardial TNF-α was decreased. In summary, regenerative therapies such as autologous BMC transplantation and G-CSF treatment are effective on heart failure models due to nonischemic cardiomyopathy. The mechanisms responsible for the effects likely include not only myocardial regeneration but also the others such as prevention of the myocyte degeneration, increase in MMPs (anti-fibrotic action), and decrease in TNF-α (a cardiotoxic cytokine).

5

Application of Embryonic Stem (ES) Cells for Vascular Regeneration Medicine

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ES cells possess "self-renewal" and "pluripotency" and are attracted as the promising cell source for regeneration medicine. We have identified from mouse ES cells "vascular progenitor cells (VPC)", which can differentiate both into endothelial cells (EC) and mural cells (MC) (vascular smooth muscle cells or pericytes) to construct blood vessel structure in vitro with single cell population (Nature 2000, 408:92-6). We further demonstrated that VPC at proper differentiation stage, when implanted into adult mice, contributed to neoangiogeneis to augment significantly blood flow in tumor-implanted angiogenesis model (Blood 2003, 101:2675-8). Furthermore, implantation of VPC in collagen I gels with bFGF or VEGF effectively constructed blood vessel network in mouse hindlimb ischemia model. The results indicate that VPC are useful stem cells for vascular regeneration and that differentiation stages of VPC and proper scaffold with appropriate growth factors are crucial for stem cell therapy wit ES cells. We demonstrated that differentiation kinetics of VPC derived from monkey ES cells were different from mouse ES cells (Circulation 2003, 107:2085-8), and so, investigation on primate ES cells is indispensable for clinical application of ES cells to vascular regeneration. Now, we are investigating human ES cells to search for VPC and preliminary results on basic characters of human VPC and their differentiation kinetics into EC and MC are being obtained. We have recognized that human ES cells are the good cell source for human vascular regeneration.

6

Myocardial regeneration therapy using autologous cells for the impaired myocardium : A novel strategy for the clinical trial

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However gene therapy or cell transplantaion have been clinically tried to restore the impaired heart, its efficacy, ethical problems and cell sources are still controversial. We have reported the superiority of cell transplantation with angiogenic gene therapy. And most recently, we have developed a tissue-engineered cardiomyocyte sheets for treating the impaired myocardium. In this study, we hypothesized that autologous cell therapy using myoblasts and bone marrow mononuclear cells or a tissue-engineered autologous myoblast sheets may overcome the several problems and promise a clinical trial for the impaired myocardium. (1.Myoblasts and bone marrow cells in ICM) LAD ligated dogs were used for the preclinical trial of ischemic cardiomyopathy(ICM). Autologous myoblast(M), bone marrow cell(B) and both B and M(BM group) or culture medium alone(control) were injected around the scar of infarction after two weeks of ligation. Four weeks later, cardiac function and number of capillaries were significantly improved in the BM group than the other groups. (2.Myoblast sheets in DCM) We constructed autologous myoblast sheets using a temperature-responsive culture dishes grafted Poly(N-isopropylacrylamide) and implanted to the DCM hamster. LV function, wall thickness and survival rate were significantly higher in the myoblast sheet group than the control or the myoblast injection group. In conclusion, cell transplantation using myoblasts and bone marrow mononuclear cells in ICM or a tissue-engineered myoblast sheets especially in DCM regenerated the impaired myocardium and improved cardiac function. Thus, these autologous cell therapies may promise a clinical trial for the impaired myocardium.

7

Three-Year Follow-Up of the Safety and Feasibility of Intramyocardial Bone Marrow Mononuclear Cell Implantation in Patients with Ischemic Heart Disease

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Much recent attention has been focused on the use of cell-based therapy to induce therapeutic angiogenesis and repair injured myocardium. Bone marrow mononuclear cells (BM-MNCs) have been shown to be one of the most viable cell populations for clinical application because the implantation of autologous BM-MNCs is not associated with problems of immunological rejection or ethical conflict. In October 1999, we started a small clinical trial of intramyocardial implantation of BM-MNCs in patients with ischemic heart disease who underwent simultaneous coronary artery bypass grafting (CABG). After completion of the standard CABG procedure, eight selected patients were given an intramyocardial injection of freshly collected BM-MNCs (5-10&times10^7 cells in 0.1 ml RPMI) into targeted ischemic myocardium (5-22 points), where there was no indication for surgical CABG or other intervention. A specific increase in myocardial perfusion was seen in five of the eight patients, and two patients showed improvement of left ventricular wall motion, 1 and 12 months after treatment. No systemic or local side effects related to the intramyocardial injection of BM-MNCs have been detected in any of these patients in 3 years of follow-up examinations. The results of this trial provide evidence that the intramyocardial implantation of BM-MNCs is a feasible and safe treatment for patients with severe ischemic heart disease. Our recently experimental investigations are also presented to discuss several hotly disputed problems of cell therapy. Although further extended clinical trials are required to confirm the safety and efficacy, cell-based regenerative therapy provides a viable new treatment option for inducing therapeutic angiogensis and repairing injured myocardium.