— 189 —

T Cell Stimulation by Retrovirally p53 Transfected Dendritic Cell(DC) from Mobilized CD34+ Blood Progenitors for Prevention of Post PBSCT Relapse

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목적:Dendritic Cell(DC)은 항원 제공(antigen presenting)세포로 T-세포 매개 면역 반응 유도에 중요한 역할을 한다. p53 유전자 치료는 p53 돌연변이를 교정하는 효과 외에도 암세포제거(tumor purging)및 interferon regulatory factor-1(IFNRF-1)에 의한 class-II 단백형성을 자극하여 면역작용을 증강시킴이 최근에 밝혀졌다. p53 유전자를 이입시킨 DC 이 T-세포 자극(T-cell stimulation) 상승효과가 있는지를 관찰하고자 하였다.

방법:CD34 column 을 이용하여 조혈모세포채집물을 분리한 후 TNF-α와 GM-CSF 를 처리하여 DC 를 체외에서 배양하였다. 조혈모세포채집물의 일부를 overnight 배양하고, 부착세포를 제거하여 환자의 T-세포를 얻었다. 5 일간 IL-2 로 자극시킨 T-세포와 14 일동안 배양한 DC 를 5 일간 함께 반응시켰으며, 마지막 18-24 시간에는 well 당 1uCi/ml ^{3}H-thymidine 을 첨가한 후 beta count 를 측정하였다. LXSN/p53(10^5/ml)과 LXSN(mock infection)을 DC 에 이입한 후 환자의 T-세포 및 정상인의 말초혈액 단핵세포에서 채집한 T-세포를 대상으로 T-세포 활성 정도를 비교 측정하였다.

결과:1) CD 34 양성 조혈모세포로부터 TNF-α와 GM-CSF 를 사용하여 DC 을 얻을 경우 배양 7 일째부터 수지상 모양의 세포를 관찰할 수 있었고, CD1a 와 형태학적 분석시 14 일 배양한 세포의 약 40%가 DC 에 해당하였다. 2)조혈모세포 채집물 $3.59x10^8$ 단핵세포에서 $1.7x10^6$CD34 양성세포를 얻을 수 있었고, TNF-α와 GM-CSF 로 14 일 배양하여 $6.3x10^5$ 세포를 얻었다. 3) 환자의 T 세포 자극효과는 환자의 DC, p53 이입된 DC 처리시 4.8 배, 10.7 배 각각 증가하였으며, 통계적으로 유의한 차이를 보였다($p=0.026$). 4) p53 유전자이입이 T-세포 자극능력을 감소시키지는 않았다.

결론: DC 에 p53 유전자이입은 T-세포 자극면에서 상승효과가 있었다. 좀 더 많은 수를 대상으로 한 전향적 연구결과가 뒷받침된다면 DC 과 p53 을 이용한 면역-유전자요법이 자가 조혈모세포이식 후 재발 방지에 기여할 것으로 기대된다.

— 190 —

Anti-A Isoagglutinin as a Risk Factor for the Development of Pure Red Cell Aplasia(PRCA) Complicating Major ABO-Incompatible Allogeneic Bone Marrow Transplantation(BMT).

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Background. Major ABO incompatibility between donor and recipient is not a barrier for successful BMT. However, several reports have described delayed hemolytic anemia, delayed onset of erythropoiesis, and pure red cell aplasia(PRCA) after major ABO-incompatible BMT. We made an attempt to find risk factors for the development of PRCA in patients who received a major ABO-incompatible bone marrow graft.

Patients & Methods. From December 1993 to May 1998, seventy adult patients underwent allogeneic BMT in Asan Medical Center. Among these, 35 patients(50.0%) received ABO-incompatible bone marrow graft; 10(14.3%) with major difference, 13(18.6%) with major and minor difference, and 12(17.1%) with minor difference. Three of 23 patients who underwent major or major and minor ABO incompatible BMT, were not analyzed each for the following reasons; early death, generalized engraftment failure, and inadequate data. Remaining 20 patients were transplanted for AML(10), ALL(2), CML(3), MDS(2), NHL(1), and SAA(2). Conditioning regimens were BuCy for leukemia/MDS/NHL patients and Cy-ATG for aplastic anemia patients. For prophylaxis of GVHD, cyclosporin plus methotrexate was given. In all patients, the donor marrow was depleted of RBC by CS-3000 collection device before infusion. In 15 patients, anti-A and anti-B isoagglutinins were determined once a week until disappearance of isoagglutinins.

Results. In six(30.0%) out of 20 patients, peripheral blood reticulocytpenia persisted over 60 days after BMT and bone marrow biopsy showed the findings of PRCA. In an attempt to find risk factors for the development of PRCA, we analyzed various variables including age, sex, diagnosis, disease status at BMT, history of pregnancy, pre-BMT transfusion amount, level of AST/ALT at BMT, HBV/HCV status, donor sex, bone marrow cell dose(MNC and CD34+ cells), isoagglutinin type against donor RBC, and pre-BMT isoagglutinin titers. Isoagglutinin type was only risk factor and patients with anti-A isoagglutinins against donor RBC developed PRCA more frequently than patients with anti-B (6/12 vs. 0/7, P=0.024). Median days to reticulocyte over 1% delayed about 2 months in patients with PRCA compared to patients with timely RBC engraftment (96 vs. 24 days, P=0.001). Median days to disappearance of isoagglutinins against donor RBC had trends to delay in patients with PRCA or anti-A isoagglutinins (PRCA vs. non-PRCA 200 vs. 107 days, P=0.075; anti-A vs. anti-B 160 vs. 66 days, P=0.072). Median days to initial appearance of donor type RBC also delayed in patients with PRCA or anti-A isoagglutinins (PRCA vs. non-PRCA 135 vs 33 days, P<0.001; anti-A vs. anti-B 66 vs. 31, P=0.054). Times to disappearance of isoagglutinins against donor RBC were significantly correlated with times to reticulocyte over 1% and initial appearance of donor type RBC (R^2=0.697 and 0.685).

Conclusion. RBC engraftment following major ABO-incompatible BMT was dependent on disappearance of isoagglutinins against donor RBC, and anti-A isoagglutinin was a risk factor for the development of PRCA complicating major ABO-incompatible allogeneic BMT.