There was a significant decrease in lin+ fraction of Sca-1+ cells compared to both control mice and mice examined 2 to 3 days after cyclophosphamide. Generally, the purity of separation was lower and the apoptotic rate and susceptibility to Fas-mediated cell death was higher in mice recovering from cyclophosphamide damage. These findings demonstrated that not only numbers but also a quality of progenitors changed markedly during regeneration.

226 Tuesday, July 11, 2000 (10:15–12:15)
Session V-5: Stem and Progenitor Cell Transplantation: Experimental II

SKIN-EXPLANT MODEL TO EVALUATE EFFECTIVENESS OF DEPLETION OF HLA-ALLOREACTIVE T-CELLS

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Depletion of host specific alloreactive T-cells from donor lymphocytes is an attractive tool for donor lymphocyte infusions after stem cell grafting. We intended to establish a preclinical method to evaluate effectiveness and safety of this approach. The skin explant model is a powerful method to predict the risk of GvHD in the HLA-identical setting, and we adapted this model to the HLA-haploidentical situation. Unmanipulated donor T-cells are compared with donor T-cells following depletion of host specific T-cells. Prior to depletion donor MNC are incubated in the presence of irradiated recipient MNC and after a period of 5 days activated T-cells, positive for CD25, CD69, CD71 and HLA-DR, are magnetically removed (VarioMacs, Miltenyi). A repeat MLC of unmanipulated donor cells. Fresh skin biopsies are divided into pieces of 1 - 2 mm diameter and incubated with 10^6 cultured T-cells in 20% autologous serum for 72 hours, fixed in formaldehyde and stained with haematoxylin-eosin. Skin alterations are graded according to Learner et al. In 3 separate experiments undepleted cells caused subepidermal cleft formation corresponding to a grade III to IV GvHD reaction. In contrast, depleted cells showed only grade I alterations, as similarly observed in medium control. Our preliminary data suggest that this approach could represent a useful tool to determine effectiveness of magnetic depletion of alloreactive T-cells and be valuable to assess the safety of these cells prior to infusion.

227 Sunday, July 9, 2000 (18:30–19:30)
Poster Session I: Acute Leukemia: Basic Research

INDUCTION OF APOPTOSIS AND GROWTH INHIBITION IN MYELOID MALIGNANCIES BY ARSENIC TRIOXIDE (As_2O_3)

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Clinical efficacy of As_2O_3 has been shown in patients with relapsed acute promyelocytic leukemia (APL). There is evidence As_2O_3 effects not only events specific for APL but also may target mechanism involved in the pathogenesis of other myeloid malignancies. We assessed susceptibility of induction of apoptosis by As_2O_3 (0,01-10 μM) and other agents (e.g. etoposide) in various myeloid and non-myeloid malignant cell lines. Apoptotic cells are measured by staining with annexin-V and 7-amino-actinomycin-D (7-AAD). The cell lines displayed different kinetics of response and different sensitivities of As_2O_3. Minimum concentration of As_2O_3 for induction of apoptosis after appropriate incubation was 1 μM. High concentrations of As_2O_3 (5 μM) induced apoptosis after 72 hours. With 5 μM As_2O_3: > 75% for NB-4, CEM and MV-4-11, 50–75% for PBL-985, ML-2, MV-11, 20–50% for HL-60, HEL, Jurkat, K-562, PBL-985, U-937, KG-1, KG-1a. 1 μM As_2O_3 induced apoptosis in NB-4, HL-60, U-937, CEM, HL-60, KG-1a, PBL-985, ML-2, and MV-4-11 but not in HEL, K-562, KG-1, and Jurkat after up to 35 days of incubation. However, proliferation of HEL, K-562, and Jurkat non-apoptotic subpopulations was reduced when treated with 1 μM As_2O_3. This anti-proliferative effect seems to be independent of apoptosis induction. MDR cell lines CEM-C1 and CEM-C2 were sensitive to 1 μM As_2O_3. 50% of cells were annexin-V positive after 3 day for CEM, 6 days for CEM-C1 and 16 days for CEM-C2. MDR-cell lines HL-60-MX-1 and HL-60-MX-2 were resistant to 1 μM As_2O_3. These data reveal that As_2O_3 induced apoptosis is not restricted to cell lines with the translocation t(15;17). The molecular mechanisms involved in apoptosis induction by As_2O_3 are under investigation. Preliminary results indicate an upregulation of p53, Caspase 3 and an alteration of the Bcl-x expression in CEM after 4-6 hours of incubation with 10 μM As_2O_3. In non-APL cell lines, apoptosis was induced in vitro by concentrations of As_2O_3 that are achievable also in vivo after i.v. infusion of well-tolerated As_2O_3 doses. Thus, As_2O_3 might be a suitable therapeutic agent for myeloid malignancies other than APL and non-myeloid malignancies.

228 Monday, July 10, 2000 (9:45–11:15)
Session III-1: Acute Leukemia: Clinical and Basic Research

IMPROVEMENT IN CYTOPENIAS OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS) IN RESPONSE TO THALIDOMIDE

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Recent studies investigating the pathogenesis of MDS have yielded two important biological insights, both of which can be exploited for therapeutic purposes. The first is that cytopenias may be the result of excessive cytokine-induced apoptosis of hematopoietic cells, and second that the bone marrows (BM) of MDS patients are highly vascular. Thalidomide was chosen as a potentially useful agent since it has both anti-cytokine and anti-angiogenic properties. The protocol provided for a starting dose of 100 mg po qhs of thalidomide, increased as tolerated to 400 mg. Patients belonging to all FAB categories were eligible. Response meant an increase in Hb by 2.0 Gm/dL and/or a 50% reduction in packed red blood cell (PRBC) transfusions, increase in platelets by 30,000/μl or increase in absolute neutrophil count (ANC) by 500/μl. 83 patients with a confirmed diagnosis of MDS have been accrued on the study and 31 have completed 12 weeks and are available for response evaluation. The median age was 68 years; there were 19 males and 12 females. 18 had RA, 6 RARS, 6 had RAEB, and 1 had CMMoL. 29 had primary MDS, while 2 had received prior therapy for breast cancer. Twenty-four patients were dependent on PRBC transfusions, while 6 were platelet dependent. The most frequent side effects were constipation, fatigue, and fluid retention while neuro-toxicity was avoided to a large extent because of pro-

phylaxis with pyridoxine. 21 patients experienced rather significant partial response. The most striking responses were seen in the erythroid series with 8 patients achieving complete transfusion independence and 13 increasing their hemoglobin by more than 2 Gm/dL. Among the 13 platelet responders, there were 3 who increased their counts by >2000,000/μl and 4 by 100,000/μl. The best responses were seen in the RA/RARS patients, while 3/6 RAEB patients showed some disease evolution. Interestingly, 2 patients showed a response only after thalidomide was stopped. One of these in fact improved his hemoglobin from 7 to 16 Gm/dL, his platelets from 18,000/μl to 71,000/μl and normalized his ANC a full THREE months after stopping thalidomide, mimicking the effect of ATG and suggesting an immune-modulatory role of thalidomide in addition to its ant-cytokine and anti-angiogenic actions. In summary therefore, thalidomide is an exciting new addition to MDS therapeutic armament and needs further investigations.

229 Sunday, July 9, 2000 (14:15–16:00)
Session II-3: Signal Transduction

OVEREXPRESSION OF THE H-RAS ONCOGENE IN PRIMARY PRIMITIVE HUMAN HEMATOPOIETIC CELLS ALTERS PROLIFERATION AND DIFFERENTIATION

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The Ras pathway plays a central role in the control of cellular proliferation and differentiation. In order to examine the role of Ras signaling in hematopoietic regulation, we transduced $CD34^+$-enriched human cord blood cells with an MSCV-based retroviral vector encoding R12 activated H-Ras. The effects upon proliferation and differentiation were then examined in vitro. Compared to cells transduced with a control vector, H-Ras expressing cells had an elevated frequency of CFU-M (76 ± 16% vs. 22 ± 13%) and increased monocyte/macrophage cell frequency in long-term suspension cultures (69 ± 10% $CD14^+$ vs. 28 ± 8% $CD14^+$). H-Ras expression also impaired CFC survival/expansion in suspension culture relative to controls. To determine the effects of decreasing the levels of H-Ras signaling, the farnesyltransferase inhibitor 66177 (Schering-Plough) was used. At low doses, the inhibitor did not significantly reduce the frequency of monocyte/macrophage lineage cells, but led to the appearance of primitive/blastic monocytic colonies and cells with extended proliferative and self-renewal capacities. Upon removal of the inhibitor, these cells rapidly differentiated into large, adherent macrophages. These results suggest that the level of Ras pathway signaling is an important determinant of myeloid cell fate, and may illustrate a manner in which Ras activation in primitive hematopoietic cells contributes to leukemogenesis.

230 Monday, July 10, 2000 (16:00–17:00)
Poster Session II: Stem and Progenitor Cell Transplantation: Clinical Research

ALLOGENEIC BONE MARROW TRANSPLANTATION FOR ADVANCED SOLID TUMORS

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Pts with metastatic solid tumors, refractory to chemotherapy, present, a poor prognosis. Allogeneic BMT delivers the best disease control in leukemia. Thus we proposed this strategy to 12 pts (age: 37(21–45); M/F = 3/9) with refractory solid tumors (Breast: 3, Kidney: 3; Melanoma: 3; Ovarian: 2; Sarcoma: 1). All received an allo BMT from a identical sibling. The first 6 pts were prepared with Buscy200 regimen and a GVHD prophylaxis associating CSA and MTX. The 6 others received Busulfan (8 mg/kg) + Fludarabine + ATG (Mini) and CSA as GVHD prophylaxis. Only 3 pts received post graft GCSF. One patient (ovarian) died on day 5 from disease progression. All others had full hematological recovery. Among 9 studied pts, 5 had mixed donor chimerism (90% (90–99.7)) and 4 full DC. 4 pts presented grade 2 AGVHD. 3 pts received DLI prior day 100. Only 1 pt (refractory ovarian carcinoma) experienced an objective response: this was correlated with GVHD evolution : 20 mths after transplant, after a new progression, she is receiving DLI. 3 pts are alive at 8, 10 and 20 mths with measurable disease. 9 pts decreased at 3 mths (1-10) (progression: 8; TRM: 1 (Buscy regimen)). These data indicates that in this situation allo BMT is feasible notably using mini regimen. However the speed of disease evolution limits the possibility to obtain an allo effect inviting to treat these pts sooner. Based on these data, a multicenter trial is presently ongoing in France.

231 Tuesday, July 11, 2000 (8:15–9:45)
Plenary Session VI: Presidential Symposium

CHRONIC GVHD IS INCREASED AFTER ALLO BLOOD CELL TRANSPLANTATION BUT IS ASSOCIATED WITH A REDUCTION OF RELAPSE RATE: RESULTS OF A RANDOMIZED STUDY

D. Blaise, M. Kuentz, J. H. Bourhis, N. Milpied, L. Sutton, J. P. Jouet, M. Attal, P. Bordigoni, J. Y. Cahn, J. M. Boiron, M. Michallet for the SFGM

We recently reported the results of a randomized study comparing allo BMT (N = 53) and BCT (N = 48) (JCO, Vol. 18, N.3, Feb 2000) for early stage leukemia: this study establishes that BCT is associated with better hematological recovery, lower costs, no more AGVHD and identical outcome. In the present analysis, with a longer follow-up (27 mths (13-41)), we focus on cGVHD. cGVHD is more frequent after BCT (28/48 (58%) vs. 16/53 (30%) ($p < 0.02$)) and more severe (extensive cGVHD = 61% after BCT vs. 25% ($p < 0.05$)). In addition there is a trend for late cGVHD in BCT (43% started after 6 months vs. 19% in BMT). No causal factor has been found so far. cGVHD has a major effect on relapse control : only 1 pt relapsed of the 39 with cGVHD vs. 7 of 55 without (2 year relapse KM estimate by : 4% (1-18) vs. 18% (9-34) : $p < 0.03$). This effect persisted when the analysis was restricted to BCT population (patients with c GVHD : 0 relapse out of 24 patients; without cGVHD : 3 of 20 (2 year KM relapse estimate = 22% (7-51)) : $p < 0.04$). However this did not equate so far into a