Yair Reisner

Esther B.Lustig
Eli Shezen
Benjamin Dekel
Rinat Goren
Shlomit R.Zeliger
Shraga Aviner
Smadar E.Friedman
Gil Hecht
Helena Katchman
Dudi Steiner
Oren Milstein
Lior Zangi
Anna Aronovich
Noga Bronitsky
Dalit Tchorsh

Induction of Immune tolerance by adult bone marrow stem cells and by committed embryonic stem cells

Department of Immunology

Tel. 972 8 934 **4023** **Fax.** 972 8 934 **4145**
E-mail: yair.reisner@weizmann.ac.il
Web page: www.weizmann.ac.il/immunology/ReisnerPage.html

Our group has been involved for more than two decades with questions relating to stem cell transplantation. In particular, two major fields of investigation are being pursued, namely, transplantation of hematopoietic stem cells for recipients without a matched donor and, more recently, the use of embryonic committed stem cells as a new source for organ transplantation.

'Mega dose' stem cell transplantation in leukemia patients

Despite the supra-lethal radiochemotherapy used in the conditioning of leukemia patients, a vigorous graft rejection mediated by cytotoxic T cells was documented. This observation led us to focus our attention on the problem of residual immunity remaining after lethal radiation and during the 1980's we described different mechanisms for rejection of bone marrow allografts and several approaches to overcome them. In particular, we emphasized in our studies the concept of stem cell escalation and showed that there is a quantitative relationship between the level of remaining host T cells and the number of donor stem cells required to overcome the former cells.

In 1993 it first became possible to test the concept of stem cell dose escalation in humans by supplementing BM with peripheral blood progenitor cells (PBPC) collected after administration of granulocyte colony stimulating factor (G-CSF) to the donor. Thus, together with Prof. Massimo Martelli in Perugia, Italy, we embarked on a project to treat the donor, rather than the recipient, with G-CSF, aiming to collect 1 log more stem cells, in accordance with the prediction of the mouse model. To date, we have treated in Perugia more than 150 patients with acute leukemia and the results clearly show that the difficult goal of achieving a high rate of engraftment with minimal GVHD, in the absence of any post GVHD prophylaxis, has finally been attained. The results have been confirmed by many centers in Europe (including Israel) and in the US.

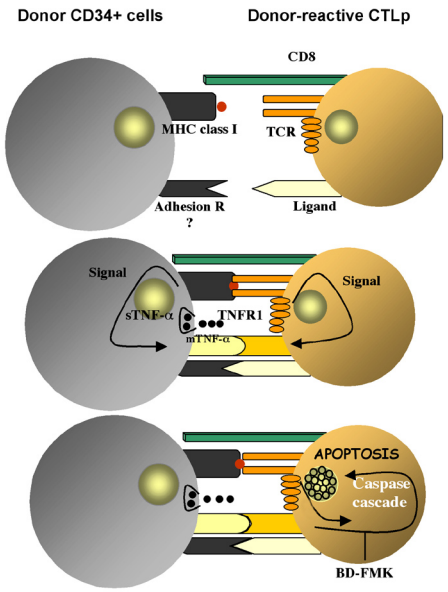

Fig. 1 *Working model describing the mechanism of deletion of donor-reactive CTL-p by donor regulatory stem cell.*

The immune regulatory role of early hematopoietic stem cells

The issue regarding the mechanism by which the megadose stem cells overcome rejection is the subject of intense research in our laboratory. We have demonstrated that CD34 hematopoietic stem cells are capable of tolerizing specifically cytotoxic T cells directed against their own antigens, but not against the antigens of a third party. Subsequent studies revealed the importance of death molecules such as FasL and TNFα. (Fig. 1). By using this machinery the stem cells and other

veto cells can induce apoptosis in specific host anti-donor T cell clones which recognize them. The 'megadose' concept was also shown recently to be useful for tolerance induction in sublethally irradiated mice, helping to overcome the resistance of the large number of lymphocytes surviving the sublethal conditioning. Thus, the recent success in overcoming genetic barriers so as to enable every leukemia patient the benefit of a bone marrow transplant, if indicated, could be further extended in the future to cure diseases other than leukemia, for which the application of lethal radiochemotherapy used in cancer patients is not justified. These include sickle cell anemia, enzyme deficiencies, and autoimmunity. In addition, induction of chimerism following sub-lethal conditioning could be used for tolerance induction as a prelude for organ transplantation or for cell therapy with allogeneic cells in cancer patients.

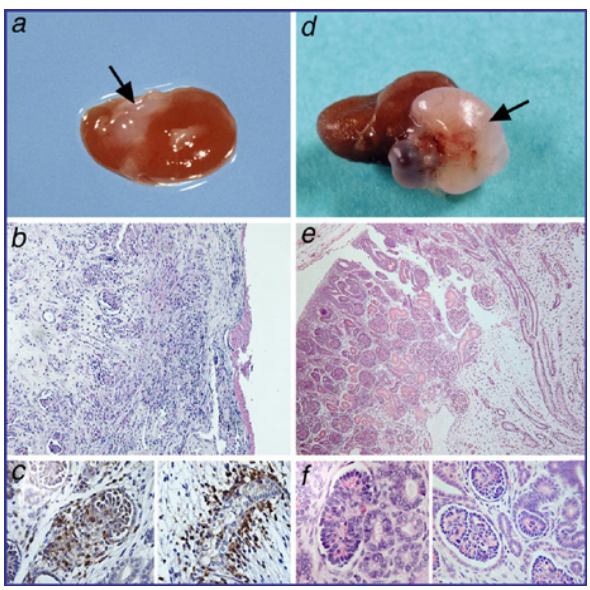

Fig. 2 *Human lymphocytes evade developing grafts of early human kidney precursors (d-f) but not of later gestation kidneys (a-c)*

The role of porcine and human committed embryonic stem cells as a new source for organ transplantation

In a recent Nature Medicine paper our group demonstrated that when kidney precursor tissues are harvested too early, they grow into a disorganized mass; when harvested too late, they provoke an immune response. Thus, we found that kidney precursor cells harvested during a particular time window (7-8 weeks or E28 from human and pig respectively) grew into a functional kidney that integrated with the host's vasculature and provoked little or no immune response. (Fig. 2).

While further studies in large animals and with other embryonic tissues are required, this seminal study strongly indicates that transplant patients would require a reduced quantity of immunosuppressive drugs following transplants of organ-specific precursor cells than are used today after full organ transplants. In particular, the recent advances with the development of SPF born pigs which are virus free, and are in the process of approval by the FDA for transplantation in humans, might pave the way for the use of porcine embryonic tissues or organs as an unlimited source for transplantation. Thus, the use of human or porcine early organ-specific precursor cells may be appropriate for treating many serious diseases. Initial targets include hemophilia and other liver-related genetic deficiencies, diabetes, end-stage renal failure, and liver cirrhosis.

Selected Publications

Bachar-Lustig, E., Rachamim, N., Li, H.W., Lan, F. and Reisner, Y. (1995) Megadose of T cell-depleted bone marrow overcomes MHC barriers in sublethally irradiated mice. *Nature Medicine*, 1, 1268-1273,

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Martelli, M.F., Aversa, F., Bachar-Lustig, E., Velardi, A., Reich-Zeliger, S., Tabilio, A., Gur, G. and Reisner, Y. (2002) Transplants across human leukocyte antigen barriers. *Seminars in Hematology*, 39, 48-56.

Gur, H., Krauthgamer, R., Berrrebi, A., Klein, T., Nagler, A., Tabilio, A., Martelli, M.F. and Reisner, Y. (2002) Tolerance induction by megadose hematopoietic progenitor cells: expansion of veto cells by short- term culture of purified human $CD34^+$cells. *Blood*, 99, 4174-4181.

Dekel, B., Burakova, T., Arditti, F., Reich-Zeliger S., Aviel-Ronen, S., Rechavi, G., Kaminski, N., Passwell, J. and and Reisner, Y. (2003) Human and Porcine Early Kidney Precursors as a New Source for Transplantation. *Nature Medicine*, 9, 53-60.