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THERAPEUTIC APPLICATIONS OF IMMUNOMAGNETIC CELL SELECTION: A REVIEW

S. S. Farag, M.D. Ph.D.

The Ohio State University Comprehensive Cancer Centre,
Division of Hematology and Oncology, Columbus, OH 43210

INTRODUCTION: With the explosion in the development of monoclonal antibodies and the improved characterization of lineage and tumor specific antigens, it is now possible to separate many specific cell types from mixed populations in blood, bone marrow and other tissue fluids. Immunomagnetic technology has greatly facilitated this process, particularly in the research laboratory where certain cellular subtypes can be separated with high purity for further investigations. Cell selection for therapeutic use, however, is a growing field that places more stringent demands on immunomagnetic technology.

Cellular therapy finds its greatest application in the field of stem cell graft engineering and adoptive immunotherapy of cancer. The main applications include purging of malignant cells from autologous stem cell products, depletion of T cells, and selection of specific lymphocyte subsets with potential antileukemic activity.

PURGING OF STEM CELL PRODUCTS: Autologous bone marrow or peripheral blood stem cells may be used to support high dose chemotherapy for a variety of malignancies. One limitation of this technique is the contamination of the stem cell product with residual malignant cells. Eradication of these cells may reduce the chance of relapse following transplantation. Purging strategies have been investigated in leukemia, lymphoma, neroblastoma and breast cancer. For example, monoclonal antibodies against B lymphocyte specific antigens, such as CD10, CD19 and CD20, have been used with complement for the ex-vivo purging of lymphoma cells from autologous bone marrow prior to transplantation [1,2]. More recently, monoclonal antibodies to the CD34 antigen, present on the surface of hematopoietic progenitor cells, have been captured on solid substrates such as immunomagnetic beads or avidin coated columns and used for the positive selection of CD34+ cells. Positive CD34 selection has been used as a means of negative purging of lymphoma, myeloma, and breast cancer cells from autologous bone marrow and peripheral blood stem cell products [3-8]. Although 2-3 $\log_{10}$ purging can be achieved, the biological and clinical significance of purging in the autologous stem cell transplantation remains controversial.

T CELL DEPLETION : T cells are the major cell type responsible for the development of graft versus host disease (GvHD) following allogeneic stem cell transplantation. Clinically significant GvHD (grade II or higher) occurs in 29-42% of recipients of HLA-identical sibling stem cells and >75% of recipients of HLA-mismatched grafts [9], and is the primary cause of failure of allogeneic stem cell transplantation. Development of severe GvHD is the major obstacle to successful transplantation across the HLA barrier, thereby limiting the procedure to only 40% of patients who can benefit from the treatment by having a suitable HLA-matched donor. Engineering the graft to deplete T cells can completely abrogate GvHD, even in mismatched bone marrow transplants. In the mismatched setting, T cell depletion has been associated with graft rejection, which may, however, may be overcome by the use of large doses of CD34+ cells [10,11], and potentially by the infusion of NK cells [12]. Technologies that consistently produce 5-log depletion of T cells with minimal loss of stem cells are needed.

A number of methods for T cell depletion have been investigated. Mostly, these have included monoclonal antibody-based negative selection techniques, separation based on lectin-mediated agglutination, and physical separation by size and density. The majority of these methods result in depletion of 2-4 $\log_{10}$ of T cells, which is not sufficient to prevent severe GvHD in unrelated donor or haplotype-mismatched SCT. Furthermore, these methods frequently result in the loss of more than half of hematopoietic progenitors as assessed by granulocyte monocyte colony forming units (CFU-GM) or CD34+ cells.

More recently, positive selection of CD34+ cells has been used to eliminate T cells. The first positive CD34 selection system developed for clinical scale purification of CD34+ cells was the **Ceprate SC stem cell collection system** (Cellpro, Bothell, WA). Using an avidin-biotin immunoadsorption technique, biotinylated anti-CD34 antibody-labeled stem cells are passed through a column containing avidin-coated beads. Unbound cells are washed away, and then the bound CD34 cells are eluted using mechanical agitation. The extent of T cell depletion achieved with this system is approximately 3 $\log_{10}$, which is insufficient for abrogating GvHD in the haplotype-mismatched transplant setting, and the mean CD34 recovery has varied between 31-79% [13-15]. Additional steps, including further depletion T cells by rosetting with sheep red blood cells have been used but are extremely tedious and result in significant loss of stem cells.

More recently, immunomagnetic methods of cell selection have been developed. The **Isolex 300i** cell selection technology (Baxter, Irvine, CA) involves specific binding of target cells by a mouse anti-CD34 monoclonal antibody, 9C5. The target cells-9C5 complexes are then captured by sheep anti-mouse IgG-coated paramagnetic ***microspheres*** (diameter 3-4 μm). Unbound CD34- cells are removed by magnetic washes on the Isolex Magnetic Cell Separator. In a final step, target cells are released from the 9C5-microspheres by a peptide that binds competitively to the 9C5 monoclonal antibody. This method is reported to achieve a mean CD34+ cell recovery between 41-69% [16-18]. T cell depletion is between 3 and 4 $\log_{10}$ [19], thus resulting in T cell doses in the allograft lower than those suggested to cause acute GvHD in the T-depleted stem cells in the HLA-matched setting. However, a higher level of T cell depletion is required in the haplotype-mismatched setting. A recent up-grade of software (version 2.5) is reported to improve the extent of T cell depletion to a median of 4.5 $\log_{10}$, with a range of 3.5 to 4.8 $\log_{10}$ [20]. However, to further improve T cell depletion, a negative selection step has been added following positive CD34 selection. In this modification, CD4 and CD8 monoclonal antibodies bound to microspheres are automatically added to the primary chamber at various times indicated by the device to bind residual contaminating T cells and retain them in the magnetic field. Combined with +/- cell selection technology has, this device is been reported to result in median 5.1 (range 4.4-5.6) $\log_{10}$ T cell depletion, with median 57% (range 39%-68%) CD34+ recovery [20]. The currently available Isolex 300i version 2.0 with +/- technology uses anti-CD2 for the negative selection step; the performance of this antibody compared to the combination of anti-CD4 and anti-CD8 has yet to be determined. None of the Isolex 300i devices are currently approved for T cell depletion in the United States.

The other available immunomagnetic positive CD34 selection technology is the **CliniMACS** device. In this system, the stem cell product is incubated with a monoclonal anti-CD34 antibody conjugated to iron-dextran ***nanobeads*** (50-100 nm). After washing, the bound cells are captured by passage through a tube with a ferromagnetic core, attached to a permanent magnet. The tube is washed to remove unbound cells. After removal of the magnet, the retained cells are eluted and passed through a newly prepared tube. The reported mean T cell depletion is 4 $\log_{10}$ [21,22], with a reported median CD34+ cell recovery of 71% (range 24% to 105%) [22]. The CliniMACS device is not currently approved in the United States.

Overall, the major limitation of the available technology, the Isolex 300i with +/- selection and the CliniMACS, is the significant loss of CD34+ cells associated with the procedure. Up to 50% of stem cells may be lost during T cell depletion. When it is considered that successful engraftment in this setting may require infusion of $\geq 10\times10^6$ CD34+ cells/kg, a total of 20×10^6 CD34+ cells will need to be mobilized and collected. In a study of 112 normal donors mobilized in a standard manner with granulocyte-colony stimulating factor (G-CSF), the median number of CD34+ cells collected per apheresis procedure was 7.58 (range 2.05-27.96) $\times10^6$/kg recipient weight [23]. This means that in more than half of donors, several apheresis procedures, and in some cases, several mobilization attempts will be required to achieve the required cell dose. Optimizing the cell separation procedure to achieve 4-5 $\log_{10}$ T cell depletion with minimal loss of CD34+ cells (e.g. >90% recovery) will result in major cost saving and comfort to the donors.

SELECTION OF SPECIFIC LYMPHOCYTE SUBSETS: While depletion of T cells can eliminate GvHD, the process is also associated with delayed immune reconstitution post-transplant with a consequent increased risk of infection. Cell selection techniques may also allow the depletion of specific subsets of T cells that mediate GvHD (alloreactive T cells) while preserving other T cells are also under investigation.

Natural killer cells are lymphocytes that do appear to mediate GvHD, but have potent alloreactive activity against malignant cells. There is growing interest in the use of purified NK cells for cellular therapy. A major obstacle to the development of treatment protocols of adoptive immunotherapy with NK cells has been the lack of clinical scale technology for the isolation of these cells from peripheral blood. There is currently no commercially available device for the clinical scale isolation of NK cells, and most clinical protocols have used lymphokine activated killer (LAK) cells, which are >90% polyclonal nonspecific T cells. Recently, Frohn et al. have used the SuperMACS, a large scale CD56 column designed for laboratory research use, for the purification of NK cells from allogeneic donors for adoptive therapy of 11 patients with renal cell carcinoma [24]. They were able to infuse 1.02±0.265x109 cells, with NK cell purity of 85-95%. However, a significant number of residual T cells remained in the cellular product, which would have been capable of causing severe GvHD in an HLA-mismatched setting. Large scale isolation of NK cells from haploidentical donors for posttransplant immunotherapy has also been reported by Koehl et al. who initially depleted T cells with magnetic bead conjugated CD3 antibodies using the CliniMACS, followed by a second step with magnetic bead conjugated CD56 antibody [25]. Although NK cell purity was reported to be high at 96.2±4.4% (range 91.0-98.1%), recovery of NK cells was suboptimal at 45.9±11.7%. CD3 cell contamination was <0.08%. Overall, there is need a major need to develop clinical scale systems for the rapid and efficient isolation of NK cells for adoptive immunotherapy.

Other application include the selection of CD34 cells for ex-vivo expansion, the selection of lymphoid cells for expansion of T cell clones reactive against certain infectious agents (e.g. cytomegalovirus and Epstein-Barr virus), and selection of cells for the generation of dendritic cells for vaccine generation. Many of these are still in the preclincial phase of development. Overall cell selection techniques that result in a favorable balance between the depletion of unwanted cells without the concomitant loss of important cell populations will greatly enhance the field of cellular therapy.

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