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Are there effective new strategies for the treatment of acute and chronic GvHD?

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Abstract

The molecular biology and pathophysiology underlying graft-versus-host disease (GvHD) remains an area of intensive research. Understanding normal and abnormal developments of both the innate and adaptive immune systems are beginning to provide understanding of the details of relevant pathways. Recent work in gene-expression profiling suggests a critical next step in identifying broader patterns that will stand up to examination in independent data sets and provide a sturdy basis for targeted therapy and preventive measures against GvHD.

Keywords

acute graft-versus-host disease; chronic graft-versus-host disease; GvHD; gene expression profiling

INTRODUCTION

The best new strategy for treatment of both acute and chronic graft-versus-host disease (GvHD) is prevention. It has been known for years that T cells are the cause of GvHD in the acute setting. Eliminate T cells and there is no GvHD. However, without T cells, the risk of relapse is high; but where T cell populations are high, the risk of GvHD is high. Clinically, the goal is to maintain enough T cells to establish a good anti-leukemia effect, but not enough to cause GvHD. To do that, an understanding of the process of T-cell production and maturation is required. T cells can be approached in various ways: as CD4 and CD8 cells; as TH1, TH2, Tc1, and Tc2 cells; as naïve, memory, central memory, and effector memory cells; and as inflammatory, regulatory, or veto cells. Understanding what each subset of these cells represents in normal immune processes is basic to the goal of balancing the need for graft-versus-leukemia effect with the risk of GvHD.

DEVELOPMENT OF NORMAL T CELLS

The major morbidity of GvHD may arise from the fact that the immune system in a patient who has undergone bone marrow transplant is not normal. A normal immune system requires a normal marrow as the source of precursors and functioning secondary lymphoid organs including the thymus. These support both innate (neutrophils, monocytes, dendritic cells, and natural killer (NK) cells) and adaptive (T cells, B cells, and dendritic cells) immunity. Precursors from the marrow travel to the thymus and then to the thoracic ducts, circulate to the

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secondary lymphoid organs, go back to the thymus, and recirculate. The majority of the cells in the periphery are single positive CD4 or CD8 cells. For the first 2 decades or so of life, under normal steady state with good thymic output, there is a large peripheral naïve T-cell pool with polyclonal T-cell receptors. With age, the peripheral population shifts to more oligoclonal T cells and more of a memory phenotype (Figure 1).

DEVELOPMENT OF RESTRICTED T CELLS

When the normal peripheral T-cell pool is damaged by cancer chemotherapy, transplantation, infection, or stress, it must be reconstituted with naïve cells. However, for the T-cell population to return to normal, a functional thymus must be present. The functional status of the thymus is determined by age and how much damage was sustained during therapy, and dictates the quantity and quality of T-cell reconstitution. T-cell reconstitution in older adults and individuals with atrophied thymuses shifts the thymic output to peripheral expansion. The newborn and young adult will still have enough polyclonal T cells to expand peripherally. However, in older individuals, the polyclonal T cells or the T cell population that can expand peripherally to reconstitute that peripheral pool drops dramatically.

With age, the thymic microenvironment changes markedly. Thymic atrophy is expected and has been associated with an arrest in T-cell development at the CD3⁺, CD4⁺, and CD8⁺ stage. With age, the stem cell population also ages. Epithelium that expresses self peptides for regulation is lost. T-cell TCR gene rearrangement capacity fades.¹ Many cytokines—IL-1, IL-6, G-CSF, M-CSF, TGF α , TGF β , IL-7, LIF, OSM, and IL-15—some of which are prothymic while others are antithymic, are regulated in older patients. Sempowski and colleagues at Duke studied normal human thymic tissue grouped into quintiles by age—0-1 years, 2-10 years, 11-25 years, 26-49 years, and >50 years. They examined the thymic epithelium and perivascular space and then the T-cell receptor excision circle expansion by polymerase chain reaction (PCR) to quantitate thymopoiesis. Next they studied the cytokine gene expression. From very young children to older adults, the amount of thymic epithelium atrophy and the increase in perivascular fat tissue are clearly visible to light microscopy.² Older individuals do not have as much thymic cortex or medulla. Given that the usual older patient population with acute myeloid leukemia (AML) has very scant thymic tissue, the amount of functional thymic tissue left after cytotoxic chemotherapy or radiation is dramatically reduced.

Similarly, changes in signal joint T-cell receptor delta (TCRD) excision circle (sjTREC) generation can be documented. sjTRECs are episomal circles of TCRD DNA in newly produced T cells and can be assayed as a molecular marker of recent thymic emigrants or long-lived naïve T cells.³ For T-cell receptors to rearrange their alpha beta chain, a delta chain that sits in the middle of the chain needs to be excised. This process can be followed with PCR techniques to give a quantitative measure of new thymic immigrants. For example, children with severe combined immune deficiency (SCID) have no detectable TRECs before transplantation and after transplant they show normal detectable levels of TREC.⁴ Over an 80 year life span, normal individuals demonstrate about a three log drop in detectable TREC-positive cells.⁵

RECONSTITUTING THE IMMUNE SYSTEM AFTER TRANSPLANTATION: THE KEY TO AVOIDING ACUTE GvHD?

As described above, a degree of progressive thymic atrophy is normal with age. The thymic epithelial space decreases; the perivascular space expands with the adipocytes; the overall thymic output of naïve cells significantly drops. Thymopoiesis persists up to at least the eighth decade of life, but the available functional tissue shrinks and is very susceptible to damage by chemotherapy or radiation. Reconstituting the immune response following transplantation

improves outcome. It is also a factor in whether the patient will develop GvHD. Patients do not in fact reconstitute their immune function properly after transplant, which is perhaps the major factor in developing GvHD.

One of the areas our group at Duke has been studying is whether the stem progenitor cells can be pushed to the thymus to increase peripheral T cells. Part of this effort is determining whether depletion of a group of naïve T cells in a transplant population is possible. Because the naïve T-cell population is associated with GvHD, the overall immune function may be improved by their depletion. We found that if the CD62L lymphocyte population in mice was depleted, the animals did very well after grafting whereas the untreated mice or those with the naïve population had a much more rapid onset of GvHD.⁶ The histology with memory cells only did not look very different from the histology with T-cell-depleted grafting, whereas in the naïve tissue, cellular infiltration was clearly visible.

Based on this, we examined whether immune recovery could be improved by preventing GvHD.⁶ In a congenic transplantation mouse model, strains of CD45.1 and CD45.2 from black mice were transplanted into a white mouse and examined by flow cytometry to analyze T-cell recovery and the source of the cells.⁷ As shown in Figure 2, depending on which quadrant of the graph the cells fell into, it could be determined which mouse the T cells were from, and whether they were bone-marrow-derived host cells, or the expanded peripheral T cells. Transplanting memory cells alone resulted in a strong and rapid rise in the CD4 and CD8 cells (upper-right quadrant) compared to the T-cell-depleted upper-left quadrant. These results suggest that there is enhanced engraftment of the donor cells plus a more rapid recovery of the immune system in the absence of GvHD as well as the ability to reconstitute peripheral T-cell populations.^{6,7} CD62L-negative T cells appear to promote new T-cell generation by enhancing engraftment through overcoming host resistance. This promoting effect requires alloantigen recognition and is not mediated by veto effect. CD62L-negative T cells appear to actually promote functional immune recovery in vivo.⁷

CHRONIC GvHD

The incidence of chronic GvHD appears to be increasing with the rising number of grafts overall, but particularly in older patients. Pavletic and colleagues at the NIH have focused on gaining consensus about approaches to chronic GvHD,⁸ but the etiology and optimal therapy for GvHD remain elusive.

One interesting line of research in GvHD explores the role of the B-cell activating factor, BAFF/BLyS. This is part of the tumor necrosis factor (TNF) family and is a key regulator of B-cell homeostasis. High levels of BAFF are found in patients with autoimmunity. In healthy individuals with normal levels of BAFF, B cells with low-affinity self-reactive receptors fail to develop. High levels of BAFF rescue these low-affinity B cells from apoptosis. The current hypothesis is that these then may become auto-reactive cells, that is, high levels of BAFF induce differentiation of memory B cells into autoantibody-producing cells. Not surprisingly, this work has sparked a lot of interest recently in the potential role of these autoantibodies in chronic GvHD. Work done at Dana-Farber in Boston has shown that patients developed antibody specific for H-Y, a minor histocompatibility antigen.⁹ The development of these H-Y antibodies correlates with chronic GvHD,¹⁰ and there is some data to suggest that perhaps rituximab has some efficacy in steroid-refractory GvHD, especially of the skin.¹¹

Along the same lines, work done by Schultz KR and colleagues suggests that soluble BAFF levels in transplanted patients with GvHD and control patients who were transplanted but have no GvHD are not different.¹² However, compared to healthy controls, transplant patients with and without GvHD have somewhat elevated BAFF levels, and those with GvHD have higher

levels than those without GvHD. These findings suggest that BAFF levels may influence the ability to develop the autoantibodies associated with GvHD.

Another interesting line of inquiry is whether innate immunity plays a significant role in the development of GvHD. Toll-like receptors (TLR) are part of innate immunity capacity, particularly in the recognition of bacterial and fungal cellular components. TLRs are widely preserved across living systems, and over the last few years, a host of different types of TLRs have been identified. TLR2 is activated by bacterial lipoarabinomannan (LAM), lipoprotein (BLP), and peptidoglycans (PGN).¹³ TLR2 is also responsible for recognition of the yeast cell-wall.¹⁴ TLR4 is primarily activated by bacterial lipopolysaccharide (LPS) and lipoteichoic acid (LTA).¹⁵ TLR5 recognizes bacterial flagella and activates the NFkappaB pathway.¹⁶ LR9 is responsible for the recognition of CpG islands of bacterial DNA.¹⁷ More recently, data have shown that NOD2/CARD15 mutations are a marker for Crohn's disease, but exploratory work by Ernst Holler and colleagues suggests that some of these variants may be associated with the onset of chronic GvHD and with treatment-related mortality, especially when combined.¹⁸ In addition, when looking at the cumulative risk of bronchiolitis obliterans (BO) as a manifestation of chronic GvHD, any variant in NOD2/CARD15 was associated with a much higher risk of developing BO compared to wild type. This work is still very preliminary and requires confirmation in an independent data set, but opens new lines of thinking about the molecular pathophysiology of GvHD.

CONCLUSION

There is still an enormous amount that is not known about the molecular biology underlying GvHD, but exploration of the development and derangement of both the innate and adaptive immune systems is beginning to provide interesting details of relevant pathways and polymorphisms. Recent work in gene expression profiling suggests a critical next step in identifying broader patterns that will stand up to examination in independent data sets and provide a sturdy basis for targeted therapy and preventive measures against GvHD.

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Figure 1.

Most peripheral T cells are single positive CD4 or CD8 cells. For the first 20 years of life, people tend to have a large peripheral naïve T-cell pool with polyclonal T-cell receptors. With people age, the peripheral population shifts to more oligoclonal T cells and more of a memory phenotype.

Figure 2.

In a congenic transplantation mouse model, strains of CD45.1 and CD45.2 from black mice were transplanted into a white mouse and examined by flow cytometry to analyze T-cell recovery and the source of the cells. Based on which quadrant of the graph the cells fell into, it could be determined which mouse the T cells were from and whether they were bone-marrow-derived, host cells, or the expanded peripheral T cells.