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Filtering lymphocytes may decrease the need for immunosuppression in solid organ transplantation

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ABSTRACT

Organ transplantation has become very important for patients with irreversible organ diseases. The transplanted organ is foreign to the host and, therefore, it induces a complex immune response of the patient. Therefore, Immunosuppressive agents are usually required to suppress both specific and nonspecific immunity and prevent allograft rejection in recipients who undergo organ transplantation. Of the late years, newer immunosuppressive agents with non-overlapping toxicities have been used in combinations in order to provide better patient and graft survival. However, these medications are associated with significant adverse effects that impact quality of life and sometimes long-term survival of the patient. Adverse effects can differ between the immunosuppressants, but many result from the overall state of immunosuppression. Strategies to manage immunosuppressant adverse effects often involve minimizing exposure to the drugs while balancing the risk for rejection. However, to prevent rejection of the transplanted organ, there may be unproven approaches other than immunosuppressive drugs. Filtering lymphocytes by a specific filter with respect to their size can be an alternative way. Our hypothesis was concerning of if such a filter could manage this and take the place of these drugs.

Introduction and background

Immunobiology of solid organ transplantation

The immunobiology of solid organ transplantation is a very complex system which primarily involves the response to donor antigens. In organ transplantation, the principal target of the immune response to the graft is the Major Histocompatibility Complex (MHC) molecules. These proteins are expressed on the surface of the donor cells and are the main antigenic determinants of graft rejection [1]. T-cells recognize antigen which is presented by the antigen-presenting cell (APC) in the form of peptide bound to MHC proteins. This costimulatory receptor/ligand interaction is the first event that initiates the effector mechanisms of the immune response. The activation of costimulatory pathways are required for T-cell entry into the cell cycle [2,3]. After T-cells are activated, they undergo clonal expansion under the influence of mitogenic growth and differentiation factors, such as interleukin-2 (IL-2). These activated T-cells then induce CD8 positive T-cell mediated cytotoxicity, help B cells for antibody production and assist macrophages to induce delayed type hypersensitivity responses [4].

There are at least two different pathways of allorecognition which leads to generation of distinct allospesific T-cells. *Direct pathway* is the first one in which host T-cells recognize intact allo-MHC molecules on the surface of the donor cell. Direct allorecognition by T-cells of intact surface MHC is thought to be the dominant pathway involved in the early alloimmune response. Thus, direct allorecognition is of major importance in acute allorejection [5]. Acute allograft rejection is also mediated by Type 1 helper T-cells (CD4 positive) which produce IL-2, interferon-gamma and induce macrophage activation leading to delayed type hypersensitivity responses [6]. In the *indirect pathway*, T-cells recognize processed alloantigen presented as peptides by self-APCs. For indirect allorecognition, the basic mechanism involved in allograft rejection is that donor MHC molecules are shed from the graft, taken up by recipient APCs, and then presented to T-cells [7]. So, *allopeptide-reactive T-cells* take part during both acute and chronic rejection.

Immunosuppressive therapy in renal transplantation in adults

Almost all kidney allograft recipients require immunosuppressive therapy to prevent rejection and loss of the allograft [8]. The

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optimal regimen, including induction therapy remains controversial. A large number of controlled randomized trials and meta-analyses indicate that induction therapy consisting of biologic antibodies (specific anti-lymphocyte or interleukin-2 receptor antibodies) plus conventional immunosuppressive agent therapy is superior to conventional agent therapy alone in reducing kidney allograft rejection and allograft failure [9–12]. One exception of immunosuppressive therapy is the recipients of two haplotype-identical living related allografts. Due to their markedly decreased immunologic risk of acute rejection, they do not generally require induction therapy with antibodies [13].

Chronic immunosuppression is an essential part of the organ protection. So, other than recipients of HLA two-haplotype allografts and HLA identical allografts from a monozygotic twin, it is generally recommended to administer a maintenance regimen consisting of triple immunosuppression therapy with a calcineurin inhibitor, an anti-metabolite and prednisone [14–17]. Although an adequate level of immunosuppression is required to dampen the immune response to the allograft, the level of chronic immunosuppression is slowly decreased over time to reduce the overall risk of infection and malignancy. Because, these risks correlate directly with the degree of overall immunosuppression [18].

Diameter of blood cell types

A typical human *erythrocyte* which is much smaller than most other human cells, has a disk diameter of approximately 6.2–8.2 μm. It has also a maximum thickness of 2–2.5 μm and a minimum thickness in the centre of 0.8–1 μm [19]. These cells have an average volume of about 90 fL with a surface of about 136 μm^2, and can swell up to a sphere shape containing 150 fL, without membrane distension. The membrane of red blood cell plays many roles in regulating their flexibility, surface deformability, adhesion to other cells and immune recognition. These functions are highly dependent on its composition, which defines its properties [20]. Plasma membranes of the aging erythrocytes undergo some changes over time. These changes made them susceptible to selective recognition by macrophages and subsequent phagocytosis in the reticuloendothelial system (spleen, liver and bone marrow) [21].

Platelets or *thrombocytes* are small (2–3 μm in diameter), irregularly shaped clear cell fragments which are derived from fragmentation of precursor megakaryocytes. Activated platelets form pseudopods on their surface and change in shape to become more spherical. Thus they assume a stellate shape. Old platelets are destroyed by phagocytosis in the spleen and by Kupffer cells in the liver [22].

There are several different types of white blood cells. Although they all have many things in common, they are all different in form and function. The presence of granules is a major distinguishing feature of the *leukocytes* and they are often characterized as granulocytes or agranulocytes [23]:

Table 1
The sizes of the various blood cell types.

Type		Diameter (μm)
Leucocytes and their % in adults	Neutrophil (62%)	10–12
	Eosinophil (2.3%)	10–12
	Basophil (0.4%)	12–15
	Lymphocyte (30%)	Small lymphocytes 7–8 large lymphocytes 12–15
	Monocyte (5.3%)	7.7–9.9
	Macrophage is a monocyte derivative	Approx 21 and sometimes as great as 60–80
Erythrocytes		6.2–8.2
Thrombocytes		2–3

- Granulocytes (polymorphonuclear leukocytes): Granulocytes characterized by the presence of differently staining granules in their cytoplasm when viewed under light microscopy. These granules are membrane-bound enzymes that act primarily in the digestion of endocytosed particles. There are three types of granulocytes which are named according to their staining properties: *neutrophils*, *basophils* and *eosinophils*. Their diameters are 10–12 μm, 12–15 μm and 10–12 μm, respectively.
- Agranulocytes (mononuclear leukocytes): Agranulocytes characterized by the apparent absence of granules in their cytoplasm. Although the name implies a lack of granules, these cells do contain non-specific azurophilic granules, called as lysosomes. These cells are *lymphocytes, monocytes*, and *macrophages*. Monocytes have a diameter of 7.7–9.9 μm while macrophages have a diameter of approximately 21 μm (sometimes as great as 60–80 μm). Diameter of small lymphocytes is 7–8 μm and diameter of large lymphocytes is 12–15 μm. The sizes of the various blood cell types was shown in Table 1.

Hypothesis: rationale for filtering lymphocytes

In our hypothesis, we designed to place a filter into the artery of transplanted kidney either at the time of transplantation or via percutaneous route afterwards. This filter is not like the vena cava inferior filters which are particularly used to prevent life-threatening pulmonary emboli. Because, the shape of the filter must be compatible to the blood flow. Being conical or helical in shape may be the most appropriate ones in order not to disrupt the blood's laminar flow. It could be composed of synthetic material that the most artificial vascular grafts are made or an autologous vessel could be available. Filter must also have the pores which could discriminate blood cells according to their size. If the pores of the filter have a maximum diameter of about 7 μm, it could allow to pass through for only erythrocytes and thrombocytes. Besides, any monomorphonuclear or polymorphonuclear leukocytes could not able to leak through the pores as the smallest one had the diameter of 7 μm.

There may be red blood cells which are larger than 7 μm in the blood vessels. However, erythrocytes have the capacity of flexibility so as to pass through the pores which are smaller than their size. The membrane of erythrocyte behaves as a viscoelastic solid, since it is capable of undergoing large elastic extensions and completely recovers its initial shape. So, the property of its membrane deformability provides them to pass through the narrow openings, such as fenestrations in the splenic cords.

Mononuclear cells of the innate immune system consist of lymphocytes and monocytes. Monocytes are also known as macrophages after they migrate from the bloodstream and enter tissue. Large granular lymphocytes include natural killer cells while small lymphocytes are T and B cells. They are all an important component in the immune system to fight infection and adapt to pathogens. They recirculate continually from blood to lymphoid tissues, awaiting contact with antigen presenting cells. Once they have identified a pathogen, the cells generate specific responses that are tailored to maximally eliminate specific invaders. When monocytes and lymphocytes are activated, they both become larger than their normal size. Therefore, the filter could allow mononuclear cells to pass through while diverting the activated T lymphocytes.

T cells are critical in the regulation of both cellular and humoral effector mechanisms. They regulate the activities of B lymphocytes and other cells participating in immune responses. They are especially activated in response to specific pathogens that replicate intracellularly (e.g., bacteria) and cells exhibiting aberrant differentiation (e.g., neoplasms). T cell mediated immunity also destroys allogeneic cells in graft rejection. In our hypothesis, the activated

T cells could not able to pass through the filter. However, this blockage certainly does not mean that all the lymphocytes are being captured by the filter. Of course, very small lymphocytes could leak to circulation of the transplanted organ. So, an adequate level of immunosuppression is still required to reduce the immune response.

Conclusion

The risk of infection in the organ transplant patient is determined by state of immunosuppression. Obstructing the T lymphocytes passage by such a filter and lessening their amount in the target organ, may decrease the necessity of immunosuppression. So, size could provide sufficient discrimination between cells that provoke or cause transplantation rejection and those cells that are needed to maintain health of the transplanted organ. As a result, instead of immunosuppression of the whole body, only the required organ is able to be selectively immunosuppresed. The effectiveness and safety profile of this filter is not established, and in general, they could only be test in some high-risk scenarios. For example, if there is a contraindication for immunosuppression or a serious complication caused by immunosuppressive drugs, this filter can be an alternative method in order to save time.

Conflict of interest statement

The authors declare that they have no conflicts of interest in relation with this paper.

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