Role of MHC restriction in allogeneic immune responses to cancer

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1. Introduction

Cancer is a disease of genes, with a myriad of them showing altered expression, either contributing to the cause of cancer, or consequence of it. These changes in gene expression make many alterations in cell physiology like: - 1) self-sufficiency in growth signals, 2) insensitivity to anti-growth signals, 3) evasion of apoptosis, 4) limitless replicative potential, 5) sustained angiogenesis, and 6) tissue invasion and metastasis [1]. Oncogenesis, as a result of overactivity of growth factor receptors, cytokine receptors and oncoproteins, coordinates immune evasion [2]. Tumor cells display different antigens coupled with MHC I than the normal cells. Ideally our immune system employs a defense mechanism called 'immunological surveillance' to detect and destroy these cancer cells, a concept proposed by Elrich in 1909 [1, 2].There are several mechanisms of escape from the immune surveillance like immunoselection of tumor antigen-negative variants, the downregulation of MHC class I expression, suppressive T cells, and the elaboration of immunosuppressive cytokines and other factors [3]. Professional APCs are endowed with the ability of 'cross presentation' i.e., presentation of antigens on MHC class I molecules. Cross presentation can either lead to tolerance or to immunity. If antigens are obtained themselves alone, it leads to tolerance. If antigens are acquired with immunostimulatory signals, it leads to immunity. The phenomenon of cross presentation is essential for development of immunity to tumors [4]. Immunotherapy has long been viewed as an alternative to overcome the serious side effects of treatments like chemotherapy and radiotherapy. The immune system has the potential to either promote or delay tumor onset and progression, the effectiveness of immune surveillance and the efficacy of immunotherapy depend on the balance between these diametric opposites. In leukemia one treatment option is replacing the bone marrow. Often bone marrow infused from allogeneic donor to the patient induces a beneneficial graft vs leukaemia or to say in general graft vs tumor effect In this review we are exploring strategies, in context of MHC restriction to contain this graft vs host disease and at the same time retain a sustained anti-tumor[here mainly anti-leukemia] effect. Further the use of this principle in conjunction with immunobiotechnology approaches like siRNA and allogeneic therapeutic vaccines can be extended to solid tumors.

2. MHC Restriction And Alloreactivity

The term alloreactivity denotes the immunologic reactions that occur when tissues are transplanted between two individuals within the same species. T cells as a part of their maturation process undergo positive selection i.e., only those cells which are able to recognize self MHC survive. However, self MHC Restriction is not absolute on average. On an average 1-24% of T cells are able to recognize a foreign haplotype. Also average self MHC Restriction and average alloreactivity are inversely correlated [5]. Thymic positive selection and the mode of alloreactivity induction are the major independent factors determining the patterns of alloantigen recognition [6]. Homology between the selecting ligand and an alloantigen can influence the avidity of the T cell repertoire for the alloantigen [7]. The structural basis for the crossreactivity between different MHC alleles is the similarity in amino acid sequence of that part of the molecule predicted to make contact with the T cell receptor [TcR][8]. Alloantigen recognition can be peptide independent, peptide specific, or peptide dependent. After transplantation, host alloantigens may be presented directly by host APCs or be crosspresented by donor APCs[4]. Transplantation tolerance across major histocompatibility complex [MHC] occurs spontaneously in nature, as evidenced by the fact that pregnant females do not reject their conceptus [9]. HLA-G seems to get up-regulated in tumor cells that may favor their escape from anti-tumor immune responses [10].

3. Graft vs Host Disease

Acute GVHD is a major cause of mortality in allogeneic bone marrow transplantation. It is a potentially life-threatening immune attack against the tissues of an alloHCT recipient by mature donor T cells contained within the graft [11]. The syndrome manifests symptoms of diarrhea, weight loss, skin changes, and liver abnormalities. HLA proteins are essential to the activation of allogeneic T cells There are three prerequisites for the development of graft-versus-host disease [GVHD]: first, the presence of immunocompetent cells in the donor inoculum; second, the inability of the recipient to reject the donor cells; and third, a histocompatibility difference between the donor and recipient [12]. The immunobiology of GVHD can be explained in 3 stages-

Activation of Antigen Presenting Cells:- In the first phase, high-dose chemoradiotherapy causes damage to host tissues, including a self-limited burst of inflammatory cytokines such as tumor necrosis factor [TNF]-alpha and interleukin 1, described as cytokine storm. [13, 14] This affects host antigen presenting cells [APCs], by increasing their maturation and upregulation of costimulatory molecules/cytokines and helps fuel the alloreactive donor T cell response [15]. Donor T cell activation- is characterized by donor T cell interaction with host APCs and subsequent proliferation, differentiation and secretion of cytokines. Cytokines such as IL-2 and IFN-gamma enhance T-cell expansion, induce cytotoxic T cells [CTL] and natural killer [NK] cell responses and prime additional mononuclear phagocytes to produce TNF-alpha and IL-1 [16]. Nogueira-Martins MF proved that B-1 and B cells participate in alloimmune response. They observed a delay in rejection kinetics of B-1 deficient mice as compared to wild type mice [17]. Effector phase:- These inflammatory cytokines in turn stimulate production of inflammatory chemokines, thus recruiting effector cells into target organs [14.] The effector phase that leads to the GVHD target organ damage is a complex cascade of multiple cellular and inflammatory effectors that further modulate each others responses either simultaneously or successively. Effector mechanisms of acute GVHD can be grouped into cellular effectors [e.g., CTLs] and inflammatory effectors such as cytokines [14]. There is a major histocompatibility complex [MHC] restriction between P-HSCs and stromal cells; normal P-HSCs can proliferate and differentiate efficiently in collaboration with MHC class I-compatible stromal cells [18]. A successful allogeneic [allo] BMT can be executed by recruiting donor bone marrow stromal cells. This may include injection of whole bone marrow cells [BMCs] including stromal cells via the portal vein [PV] [19]. The antigen Stro1 is a marker for a pure primitive Mesenchymal stem cell subset. The Stro-1-enriched immunosuppressive effect is linked to increased gene expression for soluble inhibitory factors such as interleukin-8 [IL-8], leukemia inhibitory factor [LIF], indoleamine oxidase [IDO], human leukocyte antigen-G [HLA-G], and vascular cell adhesion molecule [VCAM1] [20]. MHC-related peptide-induced antigen-specific unresponsiveness represents a novel form of immunomodulation. Several groups have now shown that peptides derived from conserved regions of both class I and II MHC molecules may inhibit the auto- and alloimmune response in vitro. Rationally designed peptides that mimic the putative interaction site of CD4 and the MHC class II molecule have been shown to have significant benefits in animal models of experimental bone marrow transplantation [21].

4. Graft vs Tumor

The Graft-versus-Tumor [GVT] effect occurs if the malignant cells are the targets of the graft vs host reaction [22]. The target molecules involved in the allo-immune graft-versus-tumor reaction are tumor-specific antigens, tumor-associated antigens, and tissue- and cell-specific minor histocompatibility antigens. Recognition of minor histocompatibility antigens [mHAs] by donor T cells contributes to antitumor responses [23]. Alloreactive CD8+ T cells targeting minor histocompatibility antigens [MiHA] on malignant cells of the recipient play a pivotal role in graft-versus-tumor responses [24]. In T cell depleted HLA-haploidentical HCT, natural killer cells play a role [25]. The therapeutic effect of allogeneic hematopoietic stem cell transplantation [HSCT] aimed against haematological malignancies is attributed to graft-versus-leukemia effect that is dependent on donor T lymphocytes. Dissociation of GVL effects from GVHD has been the ultimate goal of allogeneic BMT in the treatment of hematologic malignancies [26]. MHC mismatched mixed chimeras are quite preferred against full chimeras for a robust GvL response. Also HLA mismatch combinations have been associated with decreased risk of relapse [27]. Diverse T-cell response specific for minor H and tumor-associated antigens expressed by CLL

predicts an effective graft-versus-leukemia response after NM-HSCT [28]. HLA-class II is predominantly expressed under noninflammatory conditions on hematopoietic cells. MHC alloreactivity and GVL are separable from GVHD when donor T cells are administered as delayed DLI. GVL effects of delayed DLI are dependent on cooperative interactions between donor CD4+ and CD8+ T cells [28, 29]. Certain tumor associated antigens which includes cancer-testis antigens like PRAME are overexpressed in many acute and chronic leukaemias. PRAME specific CTLs obtained from healthy bonors display a significant avidity as compared to patient's CTL [PRAME specific] and can generate a selective GvL effect after allogeneic stem cell transplantation. CTL administration may be used in sequence with peptide vaccines to maintain long-term immune surveillance [30].

5. Minor Histocompatibility Antigens

Minor histocompatibility antigens [mHag] were originally identified as antigens causing graft rejection or graft-versus-host disease in human leukocyte antigen [HLA]-matched allogeneic transplantation [31]. Minor histocompatibility [H] antigens are polymorphic peptides that are presented on the cell surface by major histocompatibility complex class I or II molecules. These peptides are derived from any number of polymorphic genes throughout the genome that differ between the donor and recipient. These polymorphisms are often single nucleotide polymorphisms [SNPs], which result in amino acid sequence differences or differences in the expression of normal cellular proteins. In general, minor H antigen-encoding proteins are biallelic, encoding an immunogenic and a non-immunogenic allele. Individuals homozygous for the non-immunogenic minor H allele can develop immune responses to cells expressing the immunogenic minor H allele [32, 33]. The first mHA to be described were those encoded by the Y chromosome; male recipients of female allografts experience both a higher severity of GVHD and more effective GVT responses, presumably due to recognition of Y-chromosome-encoded mHA by the female donor T cells [25]. Moreover, minor histocompatibility allo-antigens are not subjected to self tolerance [34].

6. Strategies to Harness Graft vs Tumor Effect

6.1. Use of Immunomodulatory Cells

6.1.1 Use of NK cells

HLA disparity between hematopoietic stem cell [HSC] donor and recipient triggers NK-cell allorecognition. NK cell alloreactivity seems to reduce the risk of relapse in acute myeloid leukemia patients while improving engraftment and protecting against graft-vs.-host disease [GvHD][35]. The MHC constitutes a quality referential for the NK lymphocytes. A transformed somatic cell represses the expression of one or more of its class I HLA alleles, this absence of the self is perceived by the NK lymphocytes which proceed to its elimination through cytolysis [36]. AML and CML may be better target cells for NK cells than ALL because of their higher LFA-1 expression, and they may be better able to provide activation signals to the NK cells [37]. High resolution molecular HLA typing of recipient and donor, positive identification of donor KIR genes, and the functional assessment of donor NK clones can identify haploidentical donors who are able to mount donor-vs.-recipient NK alloreactions [35].

6.1.2 Role of NKT cells

NK-T cells are CD3+ cells that express a distinct V{alpha}24+ T-cell receptor [TCR] together with an NK cell marker [CD161] [37]. The expression of CD158b is downregulated with the occurrence of acute GVHD [38]. The alloreactions seem to be determined by the mismatched HLA class I ligands and their killer-cell immunoglobulin-like receptors [39]. NKT cells include a CD1d-reactive subset that influences immunity by rapidly producing large amounts of Th1 and/or Th2 cytokines. Shaulov A et al proved that granulocyte CSF treatment of allogeneic or autologous BM *in vitro* produced Th1 CD1d-reactive NKT cells which could stimulate anti-tumor responses [40]. Activation of NKT cells followed by activation of APCs and IL-12 production may lead to activation of NK cells and suppress GVHD in mismatched major histocompatibility complex combinations or may induce GVL effects [41].

6.1.3 Regulatory T cells

Regulatory function of these cells is dependent on TCR stimulation, and hence are said to be specific in the regulation of alloimmune responses [42]. Several Tr subsets have been identified within CD4+ T cell populations. They can be type 1 [Tr1], characterized by secretion of IL-10 and TGF-β with negligible production of IL-4 or Th3, characterized by secretion of IL-10 and TGF-β, with concomitant dependence on IL-4 for functional differentiation and yet another categoty of suppressor CD4+ T cells, identifiable by their coexpression of CD25 [43]. Th1 and Th2 responses activate two pathways of alloantigen specific Tregs that can mediate transplant tolerance, the two pathways being dependent on the set of cytokines secreted by Th1 or Th2 cells [44, 45]. Li AH proved that serum dereived from patients with ALL can convert CD4 [+] CD25 [-] T cells to CD4 [+] CD25 [+] Tregs, thus contributing to immunosuppresion [46]. Cao J etal engineered CD4 [+] CD25 [-] T cells to express Foxp3. Coinjection with donor bone marrow cells into the recipient resulted in minimized GVHD without compromising GvL [47]. Use of exvivo expanded regulatory T cells encoding CD90 and thymidine kinase suicide gene gives us a modular control to prevent uncontrolled immunosupression [48]. Recent studies demonstrated that Extracorporeal Photochemotherapy downregulates the immune response and induces tolerance by regulatory T cells.The basis of extracorporeal photopheresis is the reinfusion of leukocytes previously exposed to 8-methoxypsoralen [8-MOP] and ultraviolet A radiation [49].

6.2 MiHag-based immunotherapy

Genotypic disparity of SNP between transplantation donors and recipients in allogeneic haematopoeitic cell transplantation gives rise to mHag as non-self antigens for both the donor and the recipient [31]. The strategies for this therapy include adoptive cellular immunotherapy with mHAg-specific T cells; mHAg peptide, protein, mRNA or DNA vaccination selective immunodepletion of GVHD-instigating T cells; and mHAg-specific TCR gene transfer [50]. HA-1, a prototypic autosomal mHag derived from single nucleotide dimorphism [HA-1[H] versus HA-1[R]] in the HMHA1 gene. The HA-1[H] peptide is restricted by HLA-A2 and is immunogenic in HA-1[R/R] into HA-1[H] transplants [51]. The mHag HA-1 is aberrantly expressed in cancers of most entities albeit not in some solid tumors where hypermethylation in the promoter region inhibits its transcription. Hambach L, etal used DNA hypomethyating drug 5-aza-2'-deoxycytidine that induced HA-1 expression only and sensitized the tumor cells for adoptive immunotherapy [52]. HA-1 and HA-2 variability are not associated with the presence of cancer. So determination of HA-1 and HA-2 variability can be an important parameter for the selection of allogeneic stem cell donors, in particular for patients affected by hematologic malignancies without a tumor-specific molecular marker [53]. Meunier MC etal carried out an analysis of proliferative dynamics and persistence of H7[a] specific tumor cells after adoptive transfer and concluded that it may be advantageous to target MiHAs with a restricted tissue distribution in order to promote persistence of memory T cells and thereby minimize the risk of cancer recurrence [54]. Another popular potential therapeutic target is LRH-1 encoded by the gene P2X5. P2X5 is not expressed in prominent graft-versus-host-disease target tissues such as skin, liver and gut but highly expressed in malignant cells. De Rijke et al. found that the P2X5 gene of the HSCT recipient contained a deletion of a single nucleotide, resulting in a frameshift. HSCT donor T cells might be able to recognize peptides derived from the recipient's P2X5 gene product fragment following the frameshift [55, 56]. Stumph AN et al identified four 4 new HLA-DR-restricted minor histocompatibility antigens, which could be recognized by T cells only on hematopoietic cells. They have suggested that CD4+ T cells administered late after alloSCT may selectively confer GVL without GVHD [57]. Vaccines that contain the patient's tumor cells which express the patient's miHAs, are also another alternative [58].

6.3 Donor lymphocyte infusions

Donor lymphocyte infusions [DLI] often are used after allo-SCT to augment the graft-versus-tumor effect [59]. DLI is being used as prophylaxis after SCT for patients with a high risk of relapse due either to advanced disease stage or in conjunction with T-cell-depleted grafts. For patients with relapsed chronic-phase CML, DLI is dramatically effective without other therapy. In case of an advanced-phase CML or acute leukemia, remission rates are low and often of short duration, hence DLI is therefore often given in conjunction with cytoreductive chemotherapy [60]

Allospecific donor Tc2 cells result in reduced GVHD, and mediate a significant GVL effect [61]. A state of mixed chimerism is superior to complete donor chimerism because host-type APCs facilitate a DLI-induced GVL effect without severe GVHD [62].

6.4 Allodepleted T cells

An alternative approach to overcome the problem of alloreactivity is to selectively deplete the T-cell product of alloreactive cells expressing activation markers in response to alloantigen. Global T cell depletion has high mortality chances due to viral infections and disease relapse. Hence selective T cell depletion is preferred. Ge X etal proved that use of CD134-allodepleted grafts may improve allogeneic SCT by reducing GVHD without loss of pathogen-specific and leukemia-specific immunity. Also compared to other markers CD134 is superior because of its negative baseline expression and rapid upregulation after activation [63]. Selective T cell depletion of activation marker CD25 with an immunotoxin directed against it is also widely investigated [64]. To further increase the efficacy of the technique Tey SK etal, used a suicide gene, inducible caspase 9 [iCasp9], to transduce allodepleted T cells, permitting their destruction should administration have adverse effects. The gene could be induced by a small molecule dimerizer, and it could induce more than 90% of apoptosis [65]. Another recent strategy is infusion of photo-allodepleted T cells. In a mixed lymphocyte reaction, alloantigen-stimulated T cells uptake 4, 5-dibromorhodamine methyl ester [TH9402] which preferentially localizes in mitochondria and when exposed to 500- to 600-nm wavelength visible light delivered through the Theralux device [Kiadis Pharma, Amsterdam, The Netherlands] becomes highly cytotoxic through oxidative damage [66].

6.5 Use of dendritic cells

Dendritic cells [DCs] are able to orchestrate innate and acquired immunity. Li YL et al pulsed bone marrow derived dendritic cells with tumor lysates to induce immunity against gastric cancer ex vivo. These cells were cultured with cytokines GM-CSF, IL-4, and TNFalpha to induce their maturation. The cells cultured with cytokines for 8 days gained the capacity to stimulate allogeneic T cells [67]. Kaneno R et al showed that noncytotoxic concentrations of chemotherapeutic agents do not induce apoptosis of DCs, but directly enhance DC maturation and function. They showed that 5-aza-2-deoxycytidine, methotrexate, and mitomycin C increased the ability of human DCs to stimulate proliferation of allogeneic T lymphocytes [68]. Wilde et al cotransfected autologous dendritic cells with allogeneic MHC molecule and a tumor associated antigen. The allo-restricted peptide specific T cells had superior capacity to recognize tumor cells and higher functional avidity [69]. A subset of human DC, termed DC-10 that express high levels of HLA-G and ILT4, secrete high amounts of IL-10, and induce allospecific Tr1 cells *in vitro* via an IL-10-dependent ILT4/HLA-G pathway. IL-10, HLA-G, and ILT4 may also be involved in Tr1-cell induction *in vivo* [70]. CCR9[+] plasmacytoid DCs seem to be potent inducers of regulatory T cell function and suppressed antigen-specific immune responses both *in vitro* and in vivo, including inhibiting acute graft-versus-host disease induced by allogeneic CD4[+] donor T cells in irradiated recipients [71].

6.6 Using fusion therapy

Cao DY et al fused autologous DCs from patients with hepatocellular carcinoma [HCC] to an allogeneic HCC cell line [HepG2]. These fusion cells co-expressed tumor-associated antigens [TAAs] and DC-derived costimulatory and MHC molecules. Both CD4 [+] and CD8 [+] T cells were activated by the fusion cells. These induced cytotoxic lymphocytes were able to kill autologous HCC. Such cross priming for shared tumor antigens can boost the prospects of adoptive immunotherapy [72].

6.7 Use of cytokines

Alloreactive donor-derived T cells from recipients of allogeneic BMT express little IL-7R. Posttransplant IL-7 administration to recipients of an allogeneic BMT enhances lymphoid reconstitution without aggravating GVHD while preserving GVL [73]. Multipeg-G-CSF treatment of donor induces greater levels of progenitor cell, myelogenous, and iNKT cell expansion, while inducing protection from GvHD. iNKT cells, significantly augment CD8+ T cell–mediated cytotoxicity and GVL effects after transplantation. Thus GVL and GVHD can be further separated

after allogeneic stem cell transplantation by mobilization with a multiple-pegylated G-CSF molecule [74]. IL-11 induces polarization of donor T cells toward type-2 cytokine response by inhibiting the secretion of TNF-α and IL-12 by host macrophages. Thus it can L-11 promotes leukemia-free survival after allogeneic BMT by reducing GVHD while maintaining GVL activity [75].

6.8 Use of drugs and metabolites

Rapamycin can increase the ratio of CD4[+] CD25[+] regulatory T cells *in vitro* and prolong the graft survival time obviously after adoptive immunity, and these effects are enhanced by low-dose of IL-2 [76]. Aspirin-treated dendritic cells have the nuclear factor K-B [NFKB] signaling pathway inhibited, modified cytokine production, reduced expression of co-stimulatory molecules [CD40, CD80, and CD86], increased expression of immunoglobulin-like transcript-3 [ILT3] with de novo generation of regulatory T cells. Unlike immature dendritic cells, aspirin-treated DCs have the potential to control unwanted immune-responses such as the indirect pathway of allo-recognition that drives chronic allograft rejection [77, 78].

6.9 Allogeneic therapeutic vaccines

Complex whole cell-derived vaccines have given clinically superior responses compared to vaccines containing well-defined antigens, such as peptides or gangliosides; however, well-defined vaccines are theoretically more desirable because of their reproducibility [79]. Allogeneic HSCT followed by vaccination with irradiated tumor cells engineered to secrete GM-CSF generates a potent antitumor effect without worsening the toxicities of graft-versus-host disease [GVHD][80]. KGF-treated allo-BMT recipients have an improved ratio of T effector cells to regulatory T cells. KGF supports thymic epithelial cells and increases thymic output of naive T cells. Hence KGF can function as a potent vaccine adjuvant [81]. Nizar Habal et al developedCancerVax, an allogeneic tumor cell vaccine at the John Wayne Cancer Institute [JWCI]. This vaccine consists of three live human melanoma cell lines chosen for their wide range of tumor-associated antigens [TAA] and major histocompatibility complex antigens. The immune response to CancerVax can cross-react with nonvaccine tumor cells expressing some of the same immunogenic TAA, such as gangliosides [GD2, GM2, GD3, and GM3], glycoproteins [fetal antigen, TA90], and/or proteins [MAGE-1, MAGE-3] [82].

6.10 Use of siRNA

STAT3 has been described as an oncogene involved in tumor progression while its expression in immune cells is associated with cancer tolerance. STAT3 inhibition in tumor cells, using siRNA strategies, decreases invasion, angiogenesis, and reduces tumor spreading [83]. Lukemic blasts express HLA-E, which is an inhibitory ligand for CD94+/NKG2A+ NK cells. Tansfusion of *in vitro* cytokine/Hsp-70 peptide-stimulated NK cells might provide an immunotherapeutic option for treating patients with Hsp70 membrane-positive, HLA E membrane-negative tumors [84] siRNA directed against HLA-E in HLA-E positive leukemic blasts can be a option for this treatment. To induce an effective and long-lasting anti-leukemic T cell response, however, CD4+ T cells are necessary to provide help to CTLs upon activation by APCs[both in autologous and allogeneic settings]. Leukemic blasts express invariant chain intracellularly, as well as HLA-DR [DR] and CLIP at the plasma membrane, which facilitates them to escape immune surveillance by circumventing leukemia-specific CD4+ T cell recognition. Use of Ii-siRNA directed against CLIP can induce a long lasting anti-leukemic response [85].

7. Umbilical cord blood transplantation

Owing to less severe graft vs host disease umbilical cord blood transplantation is seen as an alternative to bone marrow transplantation. Cytokine immunotherapy using IL-15 simultaneously modulates several immune compartments and thus holds promise for facilitating post-transplant recovery and augmenting antitumor effect without aggravating GVHD in the setting of UCB transplantation [86]. Tanaka J et al expanded cytotoxic CD94-expressing CD8 T cells from CD4-depleted cord blood using an immobilized anti-CD3 monoclonal antibody with concomitant enrichment of CD4[+]CD25[+] regulatory T cells were expanded from a CD4-enriched fraction using anti-CD3/CD28 monoclonal antibody-coated Dynabeads and cytokines. They proposed that

these expanded cytolytic CD94-expressing CD8 cells might be able to induce a graft-vs-leukemia effect without enhancing graft-vs-host disease, and CD4[+]CD25[+] cells might be able to suppress allogeneic responses, including graft-vs-host disease and graft rejection after cord blood transplantation [87]. It was observed that partial HLA matching of UCB units is preferred since it elicits an enhanced graft vs leukemia effect in acute leukemia patients [88]. UCBT for acute leukemia in CR from KIR-ligand-incompatible donors is associated with decreased RI and improved LFS and OS [89].

8. Discussion

The host environment is a major factor influencing the cellular mechanisms of GVL. After transplantation of haploidentical hematopoietic stem cells and infusion of donor T cells, leukemic cells can escape from the donor's antileukemic T cells through the loss of the mismatched HLA haplotype [90]. The failure to identify an HLA null allele as a non-expressed variant in the stem cell transplantation setting may result in an HLA mismatch. This stimulates allogeneic T cells thus ending up in graft-vs-host disease. Some HLA alleles have truncated polypeptides as their translation product, which thus might act as minor histocompatibility antigens. Because the prevalence of HLA null alleles may be around 0.3% or even higher, an optimal screening strategy for HLA null alleles should be explored [91]. Human leukocyte antigen [HLA] recombination, particularly multiple recombinations can produce novel haplotypes, thereby complicating donor-recipient selection and possibly inducing severe graft-vs-host disease [GVHD] after nonfully matched allogeneic hematopoietic stem cell transplantation [92]. Worthley DL etal identified 18 patients with solid organ neoplasia that developed in female recipients of male allogeneic stem cell transplants [93]. The above issues need to be considered, in view of optimization of bone marrow transplantation. Superior immune plasticity of cord blood [CB] grafts that allows for less stringent HLA matching is especially valuable in the face of a persistently growing need for unrelated donor transplants. CB may become a frontline hematopoietic stem cell [HSC] source for transplantation, especially in children with leukemia. However, the technique needs to be a lot optimized for transplantation into adults. CB treated patients seem to have lesser neutrophil and platelet counts than the BM transplant patients. UCB transplant patients are more likely to die of infection [94]. Immunomodulation of the allogeneic bone marrow, seems to be the key for maximal separation of GvHD and GvL. Stem cell mobilization of donor bone marrow with G-CSF polarizes conventional alphabeta T cells toward a TH2 pattern of cytokine production, Studies done to date suggest that if GVHD is CD4 dependent and GVL effects are mediated by CD8+ CTL and NK cell, then G-CSF mediated separation of GVH and GVL will be most marked.[95]The transfer of donor Th2-type cells may be an important strategy for regulating GVHD Delayed donor Th2 cell infusion permits a graft-versus-tumor [GVT] effect to occur with subsequent amelioration of established graft-versus-host disease [GVHD][96]. Significant enthusiasm has emerged for manipulating Treg either ex vivo or *in vivo* for clinical benefit. Allospecific T cells can have effector or regulatory functions, and the relative proportions of the two populations activated following alloantigen presentation are two of the factors that determine the clinical outcome [97]. Tregs present in 1:1 ratio with effector T cells do not block T cell activation but seem to decrease the early proliferation responsible for aGVHD [98]. A certain critical level of host B cells seems to reduce the graft vs host disease through IL-10 mediated mediated inhibition of alloreactive T cell expansion [99]. The above observations make us think, what could be an ideal allogeneic donor bone marrow? Probably a marrow with helper T cells skewed towards Th2 profile, greater levels of NK, NKT and importantly regulatory T cells and regulatory dendritic cells. This may require pretreatment of donor bone marrow with requisite cytokines like multipegylated G-CSF, IL-4, IL-11 as well as requisite drugs and metabolites. This can be followed by siRNA based strategies to regulate cytokine profile in the recipient. At the same time reduced intensity conditioning of recipient can help in reducing GvHD. To keep a sustained GvL, post transplantation, delayed Th2 infusion and allogeneic therapeutic vaccines have a great potential. Similarly *in vivo* transfer of allogeneic MHC genes [100,69] into tumors can further help in maintaining a threshold GvL. Optimization of this former technique as well as its extension to solid tumors need to be explored. There is a critical bottleneck in identifying high-affinity TCR specificities needed to treat different malignancies which is the reason, for shift of focus towards APC cells [69]. Also donor T cells clones specific for pathogens like *Aspergillus*, and *Vyto megalovirus* can significantly reduce

the incidence of infections [101]. Minor H antigens have a broader clinical outcome, both in haematopoietic stem cell transplantation and in solid organ transplantation [102].

9. Conclusion and Future Prospects

An optimal bone marrow transplantation program with not just minimal GvHD but a sustained anti-leukemic effect, post transplantation is the need of the hour, for leukemia patients, especially for adults and old. There is a significant scope for insigtfull research into utilization of tumor specific antigens, siRNA based and *in vivo* efficient transfection strategies punctuated with increased understanding of MHC restriction between donor [especially immunomodulatory cells] and recipient cells. Also we cannot deny underlining the potential of allogeneic therapeutic vaccines. The wait for finding a suitable donor bone marrow is long. Further developments in peripheral and cord blood stem cell transplantation can help in proving as an alternative for leukemia patients, at least in children. Also the extension of techniques based on the principle of alloreactivity with enhanced understanding of miHA in context of MHC restriction; need to be explored for solid tumors.

Abbreviations

GVH- graft versus host
GVHD- GVH disease
GVL- graft versus leukemia
AML- acute myeloid leukemia
TCR- T cell receptor
MLR- mixed leukocyte reaction
IT- immunotoxin

Reference

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