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The anti-tumor effect of allogeneic bone marrow/stem cell transplant without graft vs. host disease toxicity and without a matched donor requirement?

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Summary The anti-tumor immune response that occurs in allogeneic bone marrow/stem cell transplant (BMT) settings is capable of eradicating tumors that are resistant to chemotherapy/radiation treatment. This anti-tumor immune response, known as the graft vs. tumor (GVT) effect, is the most effective immunotherapy treatment ever discovered. Unfortunately, the clinical application of GVT is severely limited due to the intimate association of GVT with the extremely toxic and often lethal side-effect known as graft vs. host disease (GVHD). It is a major research focus in the field of BMT to develop methods to separate the beneficial GVT effect from the detrimental GVHD toxicity. However, due to the intimate association of these effects, attempts to limit GVHD also have a tendency to limit the GVT effect. We propose a new concept for harnessing the power of the GVT effect without the toxicity of GVHD. Rather than trying to separate GVT from GVHD, we propose that these naturally coupled effects can 'mirrored' onto the host immune system and maintain their intimate association. The 'mirror' of GVHD is a host rejection of a graft (HVG). As rejection of an allograft would not be toxic, an HVG effect coupled to a host vs. tumor (HVT) effect, the 'mirror' of the GVT effect, would provide the anti-tumor effect of BMT without GVHD toxicity. In the 'mirror' setting, the HVT effect must occur against syngeneic tumors, while in the BMT setting the GVT effect occurs in the allogeneic setting. Previous attempts to elicit syngeneic anti-tumor immunity using therapeutic tumor vaccines have had disappointing results in the clinic due to the influence of tumor immunoavoidance mechanisms. We propose that the 'danger' signals that are released as a result of GVHD in the allogeneic BMT setting serve as an adjuvant to the GVT effect disabling tumor immunoavoidance. The chemotherapy/radiation conditioning prior to transplant is a required initiating event to the coupled GVT/GVHD effects. The conditioning releases 'danger' signals that mediate this adjuvant effect. To imitate this immunological event in immunocompetent, non-conditioned patients we propose that infusion of freshly activated, polyclonal CD4+ memory Th1 cells which express CD40L on the cell surface will stimulate a HVT/HVG 'mirror' effect, providing a

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non-toxic means to elicit the effective immune-mediated anti-tumor effect of BMT without the GVHD toxicity and without the requirement for a matched donor.

Background

The immune response that occurs in allogeneic bone marrow/stem cell transplant procedures (BMT) is a curative therapy for many types of cancers, but is intimately coupled to a toxic and often lethal immune response called graft vs. host disease (GVHD), which limits its clinical application. In order to mitigate the severity of GVHD toxicity, BMT procedures usually require a donor that is a close HLA match to the recipient. This requirement further limits the clinical application of BMT.

The term graft-vs.-tumor (GVT) effect is used herein to describe the immune response which destroys tumors and conserves a state of continued remission following BMT. A major research emphasis in the BMT field is to discover methods to separate the beneficial GVT effect from the toxic GVHD effect [1]. However, the attempts to separate these effects have proven difficult, because the detrimental GVHD effect is intimately associated with GVT [2,3]. This association can be explained because both the GVHD and the GVT effects are mediated by donor T-cells [4]. Therefore when T cells are purged from the bone marrow graft, this effectively prevents GVHD, but also eliminates the GVT effect [5].

We propose a new approach to the problem of preserving the GVT effect and eliminating the GVHD effect. Rather than try to separate the coupled GVT/GVHD immune responses that occur in BMT procedures, we propose that these coupled effects can be 'mirrored' onto the host immune system. The 'mirror' of the GVT effect would be a host vs. tumor effect (HVT) and the 'mirror' of GVHD would be a host vs. graft (HVG) rejection effect.

Unlike GVHD, HVG rejection would be a non-toxic event. Coupling this non-toxic HVG effect to a host mediated anti-tumor effect (HVT), the 'mirror' of the GVT effect of BMT procedures, would enable the same anti-tumor effect of BMT without the toxicity of GVHD. Since graft rejection would be a required part of a coupled HVT/HVG response, it would not be necessary to use an allograft from an HLA-matched donor to elicit the effect. In fact, it would be preferable to have a completely mismatched HLA donor in order to ensure that the graft is rejected.

While the ability to elicit a HVG rejection response upon infusion of allogeneic cells can be readily envisioned, the coupling of HVG to a HVT effect is not as apparent. In the 'mirror' setting, the host and the tumor are syngeneic (autochthonous) as opposed to being allogeneic in the BMT setting. Therefore, if the GVT effect is mediated exclusively through an allogeneic recognition mechanism rather than a tumor-specific mechanism, it would not be possible to 'mirror' this anti-tumor effect onto the host immune system where the immune response is autochthonous to the tumor.

However, there is evidence to support that the GVT effect is not exclusively an allogeneic immune response. Donor-derived tumor-specific CTL have been shown to develop in the host after BMT and contribute to the GVT effect [6,7].

However, past immunotherapy protocols aimed at stimulating a HVT immune attack against cancer have had very disappointing results in the clinic [8]. This is thought to be due to the ability of tumors to evoke several mechanisms to avoid an immune attack [9].

By contrast, in the allogeneic BMT setting, the immune response that develops in the grafted immune system is capable of overcoming these tumor immunoavoidance mechanisms. The transplanted immune system in these patients develops an anti-tumor specific immune response [10] that is a proven curative therapy for hematological malignancy [11—13] and also has shown application for the treatment of solid tumors [14—16]. The GVT effect provides long-term remission through the development of Type 1 tumor-specific immunity [17].

The success of allogeneic BMT procedures to elicit curative GVT anti-tumor specific immunity in the graft in conjunction with GVHD, and the failure of immunotherapy protocols to elicit HVT curative anti-tumor specific immunity in the host without GVHD suggests that GVHD serves as an adjuvant to the anti-tumor effect.

Tumors actively produce Type 2 cytokines as a strategy to avoid immune elimination [18]. Therefore, switching the cytokine environment in cancer patients from Type 2 domination to Type 1 is an essential requirement for eliciting anti-tumor immunity [19]. GVHD causes a 'cytokine storm' of endogenous Type 1 cytokines [20,21] that serve to adjuvant an anti-tumor immune response by suppressing Type 2 immunity, promoting development

of protective Type 1 immunity, as well as down regulating a variety of other tumor immunoavoidance mechanisms [22].

The 'mirror effect' hypothesis

Based on the assumption that the detrimental GVHD effect serves as an adjuvant to the beneficial GVT effect of BMT, our 'mirror effect' hypothesis maintains that the 'mirror' of these effects can be elicited from the host immune system rather than the graft. The 'mirror' of the GVT effect is a host-vs.-tumor (HVT) effect which is coupled to a non-toxic rejection of an allogeneic graft, a host-vs.-graft (HVG) effect, which serves as an adjuvant to the HVT effect.

Our 'mirror effect' hypothesis proposes that reversing the direction of the intimately related GVT/GVHD effects originating in the graft to the 'mirrored' HVT/HVG effects that originate in the host, provides a strategy for mediating the anti-tumor effect of allogeneic BMT procedures without GVHD toxicity.

Evidence of a 'mirror effect'

The direction of the GVHD effect is spontaneously reversed in patients with chimeric immune systems after allogeneic BMT that experience a graft rejection (loss of engraftment). Significantly, a portion of patients that lose engraftment enjoy sustained remissions of advanced hematological malignancies [23]. This suggests that the HVG rejection effect can be coupled to a HVT effect.

An animal model has confirmed this 'mirror effect' observation demonstrating that host rejection of a grafted immune system (HVG) can lead to significant host-mediated anti-tumor immune responses against resident autologous tumors (HVT) [24]. The HVT anti-tumor effect in this model was correlated with the intensity of the HVG rejection response and was found not to be due to bystander killing of tumor cells or by alloactivated host T cells [24,25].

An experimental method was also developed that enabled the changing of direction of the GVT/GVHD effects from graft mediated GVT/GVHD effects to host mediated HVT/HVG effects and provided further support for the 'mirror effect' mechanism. In chimeric tumor-bearing mice the infusion of donor lymphocytes caused donor immune cell rejection of the host immune system and conversion to full donor chimerism which elicited GVT/GVHD effects. Whereas, infusion of host lymphocytes derived from syngeneic mice caused rejection of the allogeneic donor cells and elicited HVT/HVG effects, including development of tumor-specific Type 1 immunity in the host [26]. The magnitude of the GVT vs. HVT anti-tumor effects observed were not significantly different, however the HVT effect occurred without toxicity [27].

These experiments provide proof-of-concept that the 'mirror effect' represents a novel strategy for preserving the curative GVT effect of allogeneic BMT without GVHD toxicity by providing support for the concept that an allogeneic rejection response is coupled to an anti-tumor effect regardless of whether the effectors are allogeneic or syngeneic to the tumor. Further, these experiments support that an allogeneic rejection provides an adjuvant effect in support of Type 1 anti-tumor immunity and the disabling of tumor immunoavoidance.

However, translation of these mouse experiments to elicit the HVT/HVG effects in the clinic would be limited to patients that previously underwent an allogeneic BMT and presented with both a chimeric immune system and tumor recurrence. Lymphocytes would need to be harvested from these patients and then subsequently purged of tumor cells so they could be used for infusion in order to initiate the rejection of the donor immune system (HVG) and elicit coupled host-mediated anti-tumor immunity (HVT) [28].

It would be more desirable to elicit the 'mirror effect' in patients that had not undergone an allogeneic BMT procedure and did not have a chimeric immune system. Can the 'mirror effect' that has been shown to occur in the chimeric setting be elicited in the immunocompetent, tumor-bearing host?

Infusion of allogeneic lymphocytes in a tumor-bearing host that has not received pre-conditioning chemotherapy/radiation treatment to enable allogeneic BMT engraftment (non-chimeric host), results in rejection of an allogeneic graft (HVG) without any anti-tumor effect (HVG without HVT). By contrast, the same HVG allogeneic rejection response is coupled to an HVT anti-tumor effect when it occurs in the chimeric setting. What is present in the chimeric host during allogeneic rejection that enables a coupled anti-tumor effect and is missing in the non-conditioned host that prevents the coupling of an anti-tumor effect to the reject?

Common mechanism of GVT/GVHD and HVT/HVG

The presence of large amounts of Type 1 cytokines seems to be the missing component when comparing allogeneic rejection in a chimeric setting vs.

rejection in an immunocompetent setting. Both the GVT/GVH and HVT/HVG effects that occur in chimeric hosts are associated with a massive, systemic Type 1 cytokine release that is absent in the allogeneic rejection response that occurs in the non-chimeric setting [24,26].

In the chimeric setting, either syngeneic host or donor lymphocyte infusions elicit an anti-tumor effect coupled with an allogeneic rejection. Type 1 cytokine release has been shown to be as readily induced by a GVH reaction as it is by a HVG reaction in chimeric mice [29]. Of the Type 1 cytokines, IFN-γ appears to play a critical role [30].

In support, it was shown that HVT/GVT anti-tumor effects are not elicited in IFN-γ deficient mice receiving IFN-γ deficient lymphocyte infusions, however the HVG/GVH rejection effects could still occur [24]. Therefore, the response to allogeneic rejection in IFN-γ deficient mice is the same observed in the non-chimeric host, supporting that the missing mechanism required for anti-tumor activity coupling to an allogeneic rejection response is related to lack of Type 1 cytokine release.

Why does a Type 1 cytokine storm occur during allogeneic rejection in the chimeric setting and not in the setting of an immunocompetent host? The answer seems to be related to the tissue damage caused by the chemotherapy/radiation conditioning used in the BMT setting and absent in the setting of allogeneic rejection in the immunocompetent host.

GVHD initiating event

The immunopathologic cascade of events leading to acute GVHD has been described as a three phase process [31]. The first phase involves tissue injury and release of Type 1 cytokines caused by chemotherapy and irradiation administered to patients as conditioning for allogeneic BMT. Tissue damage causes translocation of bacterial lipopolysaccharide (LPS) and release of proinflammatory Type 1 cytokines.

The importance of this initiating event is supported by evidence that low levels of tissue injury in the conditioning phase results in lower incidence of GVHD [32]. In the non-conditioned host, no tissue damage is present and thus this may explain the failure of an allogeneic rejection response (HVG) to elicit the sustained Type 1 cytokine release that is predicted to be necessary in order to link HVT/HVG responses. Therefore, in order to elicit the 'mirror effect' in non-conditioned hosts, it will be necessary to mimic the translocation of LPS and Type 1 cytokine release without using chemotherapy/radiation conditioning.

How to elicit the 'mirror' effect

The inflammatory initiating event

The first step in the initiation of the cascade of events that leads to GVT anti-tumor activity and GVHD in allogeneic BMT is the conditioning of the host. Chemotherapy/radiation causes tissue damage and the release of LPS, a potent activator of innate immunity, elicitor of Type 1 cytokines and promoter of DC maturation to produce IL-12. Innate immune activation is a requirement for induction of effective adaptive immunity, in which IL-12-producing mature DC play a pivotal role as master regulators of the immune system [33]. However, the tissue damage and release of LPS is also the cause of GVHD. Therefore, in order to elicit the 'mirror' of GVT/GVHD effects in the host immune system without causing GVHD side-effects, it will be necessary to substitute this conditioning with a non-toxic stimulatory event that has the same mechanism of action as LPS.

DC maturation and production of IL-12 is a key step in the cascade of events required for initiation of Type 1 anti-tumor immunity. Production of IL-12 by DCs requires 2 signals, including a priming signal supplied by IFN-γ and a maturation signal. The maturation signal can be provided by LPS, but the same signal can also be provided by CD40L [34]. Thus, we propose that the inflammatory initiating event can be provided by CD40L.

Hypothesis

An infusion of polyclonally activated, allogeneic CD4+ memory Th1 cells which express CD40L (CD154) and produce Type 1 cytokines (e.g., IFN-γ, GM-CSF, and TNF-α) to an immunocompetent, tumor-bearing host will elicit a non-toxic host vs. graft (HVG) rejection coupled to a host vs. tumor (HVT) immune response that will be the 'mirror' of the highly effective GVT anti-tumor immune response that occurs in allogeneic bone marrow transplant procedures (BMT) without the associated GVHD toxicity.

Discussion

Replacing the signal provided by LPS with a CD40L signal is a strategy for initiating host-mediated anti-tumor immunity that is the 'mirror' of GVT, without host conditioning. CD40L can be delivered by ex vivo activated CD4+ memory (CD45RO+) cells. CD4+ memory cells with a Th1 phenotype can be

produced ex vivo from precursors found in the peripheral blood under good manufacturing practice (GMP) conditions [35]. CD4+ memory cells can rapidly express CD40L upon activation from pre-formed storage [36]. This expression of CD40L starts between 1 and 2 h, peaks at 6 h, and remains at a high level for >20 h [37]. If the CD4+ memory cells are terminally differentiated to the Th1 phenotype, they will produce IFN-γ after activation. If the CD4+ memory Th1 cells are allogeneic to the host, they will stimulate a host-mediated allorejection response which will upregulate CD40L on host T-cells [38,39]. Thus, activated CD4+ memory Th1 cells that express IFN-γ and CD40L, provide the initiation event, allogeneic rejection response, Type 1 cytokine storm and the two signals necessary to cause maturation of DC to produce IL-12.

CD40L (CD154) expressed on the surface of allogeneic memory CD4+ Th1 cells is expected to interact with CD40 constitutively expressed on host hematopoietic progenitors, epithelial and endothelial cells, and all APC, DC, activated monocytes, activated B lymphocytes, follicular DCs and NK cells [40,41]. CD40L is one of the strongest inducers of Th1 responses and CD40L stimulation abrogates the suppressive effect of Treg cells [42]. CD40L also activates innate NK cells [41]. CD40-CD40L activation of DC leads to maturation and up-regulation of co-stimulatory molecules [43] and production of large amounts of IL-12 [44].

CD40L also has been shown to have direct antitumor effects both by suppressing tumor growth and by inducing extensive tumor death [45–47]. CD40L activation can also enhance CTL-mediated lysis of tumors [48].

If the activated CD4+ memory cells are terminally differentiated Th1 cells, they will also produce IFN-γ that can enhance the release of Type 1 cytokines during CD40:CD40L interaction [49], contributing to the development of a sustained Type 1 cytokine storm. Tumor regression was found to correlate with dominating Type 1 cytokine levels [50]. In addition, the Type 1 cytokines can non-specifically activate host T-cells which will express CD40L. Host T-cells that are activated in the rejection of the allogeneic CD4+ memory cells also will express CD40L. Further, the allogeneic CD4+ memory cells and the host activated T-cells can interact to activate host CTL. T cells activated by CD40L can develop high and specific cytotoxic activity against cancer cells and cause active production of Type 1 cytokines (IFN-γ, TNF-α, IL-2).

CD4+ memory Th1 cells also produce large amounts of GM-CSF when activated. GM-CSF induces production of Type 1 cytokines by human PBMC, T lymphocytes, and APC and down-regulates Type 2 cytokine expression, promotes differentiation of monocytes into DC with a preferential expansion of DC1 (IL-12-producing DC) and activation of NK activity [51]. Increased circulating DC1 levels are correlated with improved survival and reduced cancer relapse after allogeneic BMT [52] and increased IL-12 levels are associated with improved relapse-free survival [53]. In addition, intratumoral IL-12 acting through increased IFN-γ both activates CD8+ CTL and purges the tumor of Treg cells [38].

The significance of CD4+ memory cells expression of GM-CSF, IFN-γ and CD40L is highlighted by reports that vaccination of cancer patients with autologous tumor cells injected with bystander cells engineered to express GM-CSF and CD40L results in recruitment and activation of DC, and formation of tumor-specific T-cell responses [54] and autologous tumors injected with either IFN-γ or GM-CSF as an adjuvant results in recall immunity and increased survival [55].

Host rejection of the allogeneic CD4+ memory Th1 cells should cause the host to develop Type 1 immunity against the alloantigens. Therefore, additional injections of the allogeneic cells in primed individuals can recall the original inflammatory cascade and serve as a booster to promote the required sustained Type 1 cytokine environment. In addition, injury to tumor tissue by techniques such as cryoablation prior to infusion of the allogeneic CD4+ memory cells is a strategy to increase Type 1 cytokine release [56] and target activated T-cells to the tumor.

If proven, the 'mirror' effect would enable the separation of the beneficial graft vs. tumor (GVT) effect of BMT from the detrimental graft vs. host (GVH) toxicity, which currently limits the clinical application of this procedure. The 'mirror effect' would also eliminate the requirement for a tissue matched donor. As the 'mirror effect' requires rejection of the CD4+ memory Th1 cells, the greater the mismatch, the stronger the rejection response. The stronger the rejection response, the lower the risk of GVHD. In addition, the ability of the 'mirror effect' to potentially downregulate tumor immunoavoidance mechanisms would make activated CD4+ allogeneic memory Th1 cell therapy a powerful adjuvant to any immunotherapy strategy, especially therapeutic cancer vaccine strategies.

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