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New perspectives on the biology of acute GVHD

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Abstract

The use of allogeneic hematopoietic cell transplantation (HCT) has increased as new techniques have been developed for transplantation in patients who previously would not have been considered HCT candidates. However, its efficacy continued to be limited by the development of frequent and severe acute GVHD. The complex and intricate pathophysiology of acute GVHD is a consequence of interactions between the donor and host innate and adaptive immune responses. Multiple inflammatory molecules and cell types are implicated in the development of GVHD that can be categorized as:(1) triggers that initiate GVHD by therapy-induced tissue damage and the antigen disparities between host and graft tissue; (2) sensors that detect the triggers, that is, process and present alloantigens; (3) mediators such as T-cell subsets (naive, memory, regulatory, Th17 and natural killer T cells) and (4) the effectors and amplifiers that cause damage of the target organs. These multiple inflammatory molecules and cell types that are implicated in the development of GVHD have been described with models that use stepwise cascades. Herein, we provide a novel perspective on the immunobiology of acute GVHD and briefly discuss some of the outstanding questions and limitations of the model systems.

Keywords

acute graft-versus-host disease; adaptive immune responses; allogeneic hematopoietic stem cell transplantation

Introduction

Fifty years ago, Billingham[1] identified three prerequisites for the development of GVHD: (1) the presence of immunocompetent cells in the donor inoculum, (2) the inability of the recipient to reject the donor cells and (3) a histocompatibility difference between the donor and recipient. These experiments formed the foundation for all later hypotheses related to the pathogenesis of GVHD. Nearly two decades ago Korngold and Sprent[2] identified mature donor T cells as the fundamental cellular mediators of GVHD in an MHC matched minor

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Conflict of interest

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antigenic disparate allogeneic BMT. More recently, the critical role of host[3] and donor[4,5] APCs in the development of GVHD has been established. We now know that the fundamental interaction for induction of GVHD, as it is for all adaptive immune responses, is the interaction of donor T cells with APCs and that this interaction is regulated positively or negatively by a plethora of cytokines, chemokines and several immune cell subsets. However, the key mechanisms that lead to the maintenance and chronicity of the GVHD process remain largely unknown. Nonetheless, the cross talk between the multiple cells and inflammatory molecules makes our current understanding of acute GVHD daunting. We provide below a novel perspective based on the current information from experimental studies on the biology of acute GVHD. We would also like to note at the outset that, given the limitations of space and the brief nature of the review, we are unable to cite all the relevant studies.

Biology of acute GVHD

GVHD can be considered as a complex immune response that has gone awry and can be understood as a pathway that consists of (1) triggers, (2) sensors, (3) mediators and (4) effectors of GVHD.

Triggers for induction of GVHD

Similar to all immune responses, certain triggers are critical for induction of acute GVHD. These include:

Disparities between histocompatibility antigens.—Antigen disparity can be at the level of the MHC, that is, MHC mismatched or at the level of mHA, that is, MHC matched but mHA mismatched. In humans, the *MHC* gene of chromosome 6 encodes the HLAs.[6] The severity of acute GVHD is directly related to the degree of MHC mismatch.[7] In BMT that are MHC matched but mHA disparate, donor T cells still recognize MHC peptide derived from the products of recipient polymorphic genes, the mHAs.[8–10] The expression of mHAs is wide and variable. Thus, different mHAs might dictate variable phenotype, target organ involvement, development kinetics of GVHD and antitumor responses after allogeneic BMT.[11] Some mHAs, such as HA-1, HA-2, HB-1 and BCL2A1, are primarily found on hematopoietic cells, whereas others such as the H-Y antigens, HA-3, HA-8 and UGT2B17 are ubiquitous.[12] It is important to note that not all mHAs are equivalent in terms of inducing an immune response. They, the mHAs, have hierarchical immunodominance that also likely contributes to GVHD variability.[13] Furthermore, the impact of mHAs is likely to be expanded further by the process of epitope spreading, which is a process in which the target of an immune response extends from the intended antigen to other epitopes.[14]

Damage induced by conditioning regimens and underlying diseases.—Under most circumstances, the initiation of an adaptive immune response is triggered by the innate immune response. The innate immune system is triggered by certain exogenous and endogenous molecules. This is likely the case in the induction of acute GVHD. Toll-like receptors (TLR) have an essential role in innate immunity by recognizing conserved pathogen-associated molecular patterns (PAMPs) and initiating the cellular signaling

pathways that activate cytokine secretion, such as nuclear factor-κB.[15] The PAMPs, such as lipopolysaccharide (LPS), which is recognized by TLR-4, are released during the chemo- and radiotherapeutic conditioning regimens performed before the infusion of BMT donor cells.[16,17] In this way, the conditioning regimens amplify the secretion of proinflammatory cytokines such as IL-1, TNF-α,[17–19] IL-6[20] and other IFN family members in a process described as a 'cytokine storm'.

In addition to LPS, there are other TLR ligands that can also enhance the induction of GVHD reaction. For example, regardless of whether recipients were exposed to irradiation or not, the addition of a TLR9 agonist enhanced GVHD mortality in an MHC mismatch murine model.[21] In another model of mixed chimera with 2C transgenic mice, the conditioning-related cytokine storm regulated tolerance negatively.[22]

Pathogen-associated molecular patterns are also recognized by certain non-TLR molecules such as nucleotide-binding oligomerization domain containing 2.[23] Emerging clinical data suggest that these molecules might also have an important role in the GVHD.[24,25]

In addition to the exogenous microbe-associated molecules, endogenous triggers as a consequence of damage, called damage-associated molecular patterns (DAMPs), might also have a critical role in GVHD.[26] In fact, the proinflammatory cytokines themselves might serve as DAMPs. It stands to reason that the type of damage (apoptosis vs necrosis), the specific DAMPs (proteases, the proteolytic products, ATP, ions, uric acid, HMGB1, S100 protein family, oxidized lipoproteins and so on) will be relevant, but much of that remains poorly understood. In addition, the role of nonpathogenic commensal bacteria and other nonmicrobial factors, such as allergens and irritants that are not a direct consequence of damage, also remains largely unknown.

Sensors of GVHD

The triggers that initiate an immune response have to be sensed and presented. APCs might be considered the sensors for acute GVHD (Figure 1). The APCs sense the DAMPs, present the MHC disparate or mHA disparate protein and provide the critical secondary (co-stimulatory) and tertiary (cytokine) signals for activation of the alloreactive T cells, the mediators of acute GVHD.

Pathways of antigen presentation.—APCs sense allodisparity through MHC and peptide complexes. DCs are the most potent APCs and the primary sensors of allodisparity.[27] Recipient DCs that have been primed by the conditioning regimen will process and present MHC and peptide complexes to donor T cells at the time of transplant.[3] At later time points, donor DCs may take over this role.[4,5] Langerhans cells are also sufficient for the induction of skin GVHD when all other APCs are unable to prime donor T cells.[28] Several different modes of antigen presentation have been described and are summarized in Figure 1. The immunological paradigm is that endogenous alloantigens are presented to $CD8^+$ T cells, whereas exogenous antigens are presented to $CD4^+$ T cells. In the case of hematopoietic cell transplant (HCT), recipient DCs present the endogenous and the exogenous antigens to donor $CD8^+$ and $CD4^+$ T cells, respectively. This process is known as the direct mode of Ag presentation. Exogenous antigens are also presented through the

classical cross-presentation and indirect pathways.[29] In the classical cross-presentation pathway, exogenous antigens are presented to donor $CD8^+$ T cells by MHC class I on donor DCs. In the indirect pathway, exogenous antigens are presented to donor $CD4^+$ T cells by MHC class II on donor DCs. Traditionally, the MHC class I and class II pathways were thought to be independent, but recent reports have shown that endogenous antigens, which are released by lysosomal ruptures through a mechanism call autophagy, can be presented to $CD4^+$ T cells through the class II pathway.[30] We now know that there is no predilection for allopeptides to be recognized by either $CD4^+$- or $CD8^+$-mediated presentation.

As noted earlier, DCs are important initiators of GVHD. Plasmacytoid DCs (pDCs) have received recent interest because of their tolerogenic role in an immature state. A subset of host pDCs that express the CCR9 receptor can suppress GVHD.[31] However, host pDCs have been shown to induce GVHD in a TLR-independent manner.[32] In addition, GVHD prevents the maturation of donor pDCs.[33] Thus, the role of pDCs in acute GVHD is still controversial and confirmatory studies about their functions are necessary before a therapeutic approach based on this mechanism can be contemplated.

Of interest, a recent report showed that both subsets of dermal DCs ($CD1a^+$ and $CD14^+$) are rapidly depleted and replaced by donor cells, whereas recipient macrophages persist weeks after GVHD. They induce cytokine expression in memory $CD4^+$ T cells and activation and proliferation of $CD8^+$ T cells, suggesting that they contribute to GVHD by sustaining the alloreactive responses of previously activated T cells.[34]

However, the kinetics of the switch from recipient to donor APCs, the contributions of different APCs subsets, the importance of direct alloantigen presentation and the magnitude of indirect alloantigen presentation in GVHD remain to be determined.

Co-stimulation.—APCs provide the critical co-stimulation signals for turning on the acute GVHD process. The interaction between the MHC/allopeptide complex on APCs and the TCR of donor T cells is insufficient to induce T-cell activation.[35] A second signal through T-cell co-stimulatory molecules and their ligands on APCs is required to achieve T-cell activation, proliferation, differentiation and survival.[36,37] An *in vivo* blockade of positive co-stimulatory molecules (CD28, ICOS, CD40, CD30, 4–1BB and OX40—those that activate T-cell response) reduces acute GVHD,[38–43] whereas a blockade of inhibitory signals (those that inhibit or exhaust T-cell response), such as programmed death-1 (PD-1) and CTLA-4, exacerbates acute GVHD in murine models.[44]

Interactions that enhance or inhibit the function of APCs.—As mentioned above, the inflammatory cytokines and DAMP ligands released during pretransplant conditioning regimens exert their effects as a third signal to enhance recipient APCs and donor T-cell interactions (Figure 2). In addition, various modulations of APCs can influence GVHD development. Recent data show that exposure to G-CSF shortly after HCT, in combination with a TBI conditioning regimen, significantly worsened GVHD in mice. TBI renders host DCs responsive to G-CSF by upregulating the expression of the G-CSF receptor. Stimulation of host DCs by G-CSF subsequently activates a cascade of events characterized by donor natural killer T cell (NKT cell) activation, IFN-γ secretion and CD40-dependent

amplification of donor CTL function during the effector phase of GVHD.[45] These data might explain the increased incidence of GVHD found in recipients receiving prophylactic G-CSF in the European BMT registry.[46]

DCs can be treated with several agents to render them tolerogenic. Histone deacetylase inhibitors such as suberoylanilide hydroxamic acid (SAHA) have been shown to reduce development of GVHD in murine models by STAT-3 acetylation, which controls indoleamine 2,3-dioxygenase-dependent DC functions.[47–49] SAHA is currently in clinical trials for the prevention of GVHD. Another potential therapeutic approach to modulate DC tolerance is the development of an antibody to the DC surface maturation antigen CD83; this antibody has been shown to prevent acute GVHD in a humanized mouse model with severe congenital immunodeficiency disorder.[50]

Recent experimental observations have also shown that several innate and adaptive immune cellular subsets negatively affect the functions of APCs (Figure 2). For example,

1. γδ T cells, which are innate immune cells commonly found in the gastrointestinal (GI) tract and skin. Host γδ T cells are associated with reduced APC activation and suppressed GVHD in MHC mismatched mouse models.[51]
2. Natural killer (NK) cells, which are inhibited by recognition of class I alleles on target cells through the killer Ig-like receptors, downregulate APC activation of T cells perhaps by directly killing APCs.[52–54]
3. Host NKT cells can negatively regulate APC interactions with donor T cells in an IL-4- and Th2-dependent manner.[55,56] In contrast, donor NKT cells activate this response.[45,57,58]
4. B cells: the role of B cells in acute GVHD is currently under investigation, particularly regarding the possible role in attenuating the disease. Mouse studies show that host B cells produce IL-10 following TBI and attenuate acute GVHD after allogeneic HCT by inhibiting recipient APC interactions with donor T cells.[59] In a study of 254 recipients of sibling donor transplants, the number of donor B cells in the graft correlated inversely with the cumulative incidence of grade II–IV acute GVHD.[60]
5. Geographic location: the interaction between the APCs and the donor T cells occurs primarily in the secondary lymph nodes, although *in vivo* imaging suggests that it also occurs in Peyer's patches.[61] However, although the secondary lymphoid organs are the likely location for the interaction of APCs and T cells, they are not obligatory for the development of GVHD.[62]

Mediators of GVHD

Donor T cells are the critical mediators of acute GVHD regardless of the type of antigen severity (Figure 2). Evidence suggests that alloreactive donor T cells consist of several subsets with different stimuli responsiveness, activation thresholds and effector functions. The alloantigen composition of the host determines which donor T-cell subsets differentiate and proliferate.

CD4$^+$ and CD8$^+$ T cells.—CD4 and CD8 are the co-receptors for MHC class II and class I receptors, respectively. As mentioned previously, in the majority of HLA matched HCT, acute GVHD may be induced by either CD4$^+$ or CD8$^+$ or both subset responses to mHAs.[63] The repertoire and immunodominance of the GVHD-associated peptides presented by MHC class I and class II molecules have not been defined.[64] One approach to retaining the beneficial GVL effects while eliminating the negative effects of GVHD is to deplete selectively subsets of donor alloreactive T cells in the hematopoietic cell inoculums using a TCR Vβ repertoire analysis with CDR3-size spectratyping.[65]

Naïve and memory T cells.—T cells in murine models can be divided into naive (CD62L$^+$ CD44$^-$), central memory (CD62L$^+$ CD44$^+$) and terminally differentiated effector/effector memory (CD62L$^-$ CD44$^-$) subsets. Donor naïve CD62L$^+$ T cells are the primary alloreactive T cells that enhance the GVHD reaction, whereas the donor effector memory CD62L$^-$ T cells do not.[66,67] Interestingly, donor Tregs expressing CD62L are also critical to the regulation of GVHD.[68,69] We now know that it is possible to modulate the alloreactivity of naïve T cells by inducing anergy with co-stimulation blockade, deletion through cytokine modulation or mixed chimerism. Donor effector memory T cells that are non-alloreactive do not induce GVHD, yet are able to transfer functional memory[66] and mediate GVL.[70] In addition, lymphopenia-induced proliferation gives rise to cells that are similar to memory T cells and enhance the graft-vs-tumor effect after donor leukocyte injection.[71] In contrast, memory T cells that are alloreactive can cause severe GVHD.[72–74]

Tregs.—Distinct subsets of regulatory T cells (Tregs) exist: the naturally occurring CD4$^+$CD25$^+$ Tregs that express the Forkhead Box Protein P3 (FOXP3), CD4$^+$CD25$^-$IL-10$^+$ Tr cells, γδ T cells, double-negative T cells and NKT cells.[51,75–79] In mice, naturally occurring Tregs develop in the thymus, prevent autoimmunity and suppress the pathology that is inflicted by uncontrolled immune responses.[80] In mouse BMT models, naturally occurring donor-derived Tregs suppress the proliferation of conventional T cells, prevent GVHD and preserve GVL effects depending on the ratio of effector T cells to Tregs.[81–86] Furthermore, viral immunity is preserved in the presence of Tregs after allogeneic HCT.[87] The mechanisms for suppression in the context of GVHD remain completely unknown. Nonetheless, clinical trials exploiting the properties of Tregs for the suppression of GVHD are ongoing, but the isolation and expansion of human Tregs remains challenging and labor intensive.[88–90] These technical hurdles may be overcome by a recent study showing that combined CD4$^+$ donor lymphocyte infusion and low-dose recombinant IL-2 expands Tregs *in vivo* following allogeneic HCT.[91] Adaptive IL-10-secreting Tr1 also promotes tolerance in GVHD through IL-10 or TGF-β release or through possible contact-mediated inhibition of cell growth.[92]

Th subsets.—Based on the dominant cytokines that are produced on activation, T cells can be distinguished into various subsets such as Th1, Th2 and Th17 cells. The Th1 cytokines (IFN-γ, IL-2 and TNF-α) have been implicated in the pathophysiology of acute GVHD.[93–95] IL-2 production by donor T cells remains the main target of many current clinical therapeutic and prophylactic approaches, such as CYA, tacrolimus and MoAbs against the IL-2 and its receptor to control acute GVHD.[96,97] However, emerging data

indicate an important role for IL-2 in the generation and maintenance of $CD4^+CD25^+Foxp3^+$ Tregs, suggesting that prolonged interference with IL-2 may have an unintended consequence in the prevention of the development of long-term tolerance after allogeneic HCT.[98–101] Furthermore, studies by Sykes and colleagues[102–104] and others have shown that the role of Th1 cytokines is complex. For example, exogenous administration of IFN-γ or T cells from IFN-γ-deficient donors has shown a reduction and enhancement of GVHD. Recent studies also suggest that IFN-γ might have a differential role in the severity of distinct GVHD target organs.[105] Thus, whether Th1 cytokines exerts their effect as the regulators or inducers of GVHD severity might be contextual.

Several different cytokines that polarize donor T cells to Th2 such as IL-4, G-CSF, IL-18, IL-11, rapamycin and the secretion of IL-4 by $NK1.1^+$ T cells can reduce acute GVHD.[106–113] A recent study with donor T cells that lacked the ability to secrete all Th2 cytokines also showed greater GVHD severity.[114] However, the Th1 and Th2 subsets cause injury of distinct acute GVHD target tissues and some studies failed to show a beneficial effect of Th2 polarization on acute GVHD.[115]

The role of recently described IL-17-producing CD4 T cells (Th17) in GVHD is currently unclear. Initial studies showed that donor T cells deficient in IL-17A augmented Th1 differentiation and exacerbated acute GVHD.[116] In contrast, a second study using a similar model showed that IL-17 contributes to CD4-mediated GVHD without affecting the OS rates.[117] In addition, the differentiation of Th17 cells *in vitro* appears to cause lethal acute GVHD with severe cutaneous and pulmonary damage.[118] These conflicting results may be due to variations among the model systems, and further analyses are required to define the role of Th17 cells in GVHD.

T-cell trafficking in GVHD targets.—Donor T cells migrate to the secondary lymphoid organs where they recognize alloantigens on either recipient or donor APCs, and become activated. They then exit the lymphoid tissues and traffic to the target organs where they cause tissue damage.[119] Although almost all tissues express alloantigens, the three main clinical target organs of acute GVHD are the skin, the GI tract[17] and the liver. The thymus is also a GVHD target organ.[120–123] The lung, although a major target of chronic GVHD, appears, if at all, to be a less common target of acute GVHD. The reasons for such selectivity of target organs are largely speculative. The spatiotemporal expression of cytokine and chemokine gradients might provide one explanation. Indeed, the trafficking of donor T cells into the GVHD target organs is chemokine dependent.[124,125] Chemokines including CCL2–5, CXCL2, CXCL9–11, CCL17 and CCL27 are overexpressed by the liver, spleen, skin and lungs during acute GVHD.[125] T cells expressing the CXCR3 and CCR5 receptors cause acute GVHD in the GI tract and liver.[126–128] Interestingly, CCR5 expression on Tregs has also been found to be a critical mediator of GVHD.[129] Integrins and their ligands are also implicated in donor T-cell trafficking into target organs.[119] The integrin α4β7 and its ligand MadCAM-1 are essential for homing of donor T cells to Peyer's patches and for induction of intestinal GVHD.[130] It is unlikely that a single chemokine or integrin accounts for the majority of GVHD effects as their roles are redundant and the trafficking of donor T cells has been shown to also depend on other factors such as the conditioning regimen and cytokine release.[131]

Effectors and amplifiers of GVHD

The effector phase that leads to GVHD target organ damage is a complex cascade that involves cytolytic cellular effectors such as CD8 CTLs, CD4 T cells, NK cells, and inflammatory molecules such as TNF-α, IFN-γ and reactive oxygen species (Figure 3).

Cellular effectors.—Cellular effectors require cell–cell contact to kill the cells of the target tissues through activation of the perforin/granzyme, Fas/FasL (CD95^{-} CD95L) or TNFR/TRAIL pathways. CD8 CTLs are the major effectors of GVHD.[132,133] Perforin and granzyme are stored in the cytotoxic granules of CTLs, secreted on recognition of target cells and induce lysis by perforation of target cell membranes. Fas clustering on the surface of target cells is induced by binding to FasL on CD8 T cells, resulting in the formation of death-inducing signal complex and triggering of apoptosis of target cells. Other CTL-killing mechanisms involve TNF death ligand receptor-triggered apoptosis by activation of the TNF/TNFR, TRAIL, TWEAK and LTβ/LIGHT pathways.[132–139] The CD4 effector T cells exerts their effect mainly through the Fas/FasL pathway and secondarily through the granzyme pathway.

Inflammatory effectors.—Inflammatory pathways do not require cell–cell contact to kill target cells. Cellular damage is amplified by inflammatory mediators including IFN-γ produced by T cells, TNF-α[140] and IL-1[141] produced by T cells and monocytes/macrophages, and nitric oxide (NO) produced by monocytes/macrophages.[142,143]

The role of several effector molecules that cause GVHD is being increasingly understood, whereas the effector pathways that are used for negatively regulating GVHD remain largely unknown.

Limitations of the models of GVHD biology

In addition to the perspective above, the biology of acute GVHD has also been summarized in stepwise cyclical models. Antin and Ferrara[144] first put forth the three-step model in 1992: (step 1) conditioning regimen-related damage and the release of endotoxins such as LPS, (step 2) donor T-cell proliferation and (step 3) target organ damage by effectors. This model has been, for the most part, supported by cumulative research to date. More recently, it has been refined by Blazar and colleagues[145] to include two additional steps: (step b, between 1 and 2) the induction of T-cell activation by their cognate ligands on the APCs in secondary lymphoid tissue and (step d, between 2 and 3) recruitment of other effector leukocytes (polymorphonuclear leukocytes, NK cells, monocytes).

Together, these are the current predominant models for understanding the biology of acute GVHD. Although they elegantly summarize the complex biology of GVHD, they are limited by reductionism and by the observations from a collection of two-dimensional data points. They can give an impression that GVHD is a linear, stepwise cascade that occurs in discrete stages. Thus, while useful, they leave little room for contextual information and do not illuminate the critical role of milieu, space and time. Clearly, the biology of GVHD is complex with multiple cells and proteins having distinct, overlapping or antagonistic roles in a feedback or feed-forward manner depending on the context. For example, as shown in

Figure 4, even a limited review of recent experimental data on various cellular subsets and cytokines and their interactions, when put together, leads to a complex network of interactions that would be hard to categorize into distinct stages. As such, an additional explanation must be sought to complement these models. One such approach would be to understand the biology of GVHD from a systems perspective based on multiscale models. Rather than dividing the complex biology of GVHD into its component parts and stages, it would be important to take an integrative systems biology approach that combines computational and mathematical modeling with direct experimentation to link the spatial and temporal scales. Such a model will be interactive and dynamic, in which the properties of a single cell are contingent on its relationship to other cells and the activities of many other molecules and cytochemokines within the network (Figure 4). The data that are available to date on the biology of GVHD can potentially be incorporated into a multiscale model that spans from the single cells to target organ systems. We anticipate that such a model will be a combination of agent-based model (single cells) and ordinary differential equations for the organ system. The principles and development of the robust software and the underlying mathematics for such an approach are time consuming and labor intensive. The generation of one such model is currently under development by our group (Reddy and Kirscher, unpublished observations). We posit that the development of such an integrative approach is imperative to provide a mechanistic explanation of GVHD that is both robust and contextual. This will require an interdisciplinary approach that melds cellular–molecular immunology, computational–bioinformatic sciences and applied mathematics.

Concluding remarks

Substantial progress has been made in our understanding of the immunobiology of acute GVHD. The underlying mechanisms of GVHD have emerged as a complex network of immune interactions where the key players are the naïve T cells, the host and donor APCs, CTLs and regulatory T cells, along with new players such as pDCs, B cells and Th17 cells (see Figure 4). Acute GVHD primarily affects the recipient immune system in addition to the skin, the GI tract and the liver. It is likely that our understanding of the immunobiology of acute GVHD will be further refined by focusing research on defining the determinants of target organ specificity, the GVHD antigenic peptide repertoire and immunodominance, the effects of tissue damage end products such as extracellular matrix breakdown products resulting from necrosis/apoptosis, the role of sterile inflammation, the contribution of the microbiome to GVHD induction, and the contributions of neovascularization and immunosuppression induced by the GVHD (see Table 1). More importantly, it would be critical to develop robust computational and mathematical tools to put all of the emerging experimental data into a multiscale model that links intracellular molecular interactions with intercellular behavior to target organ systems.

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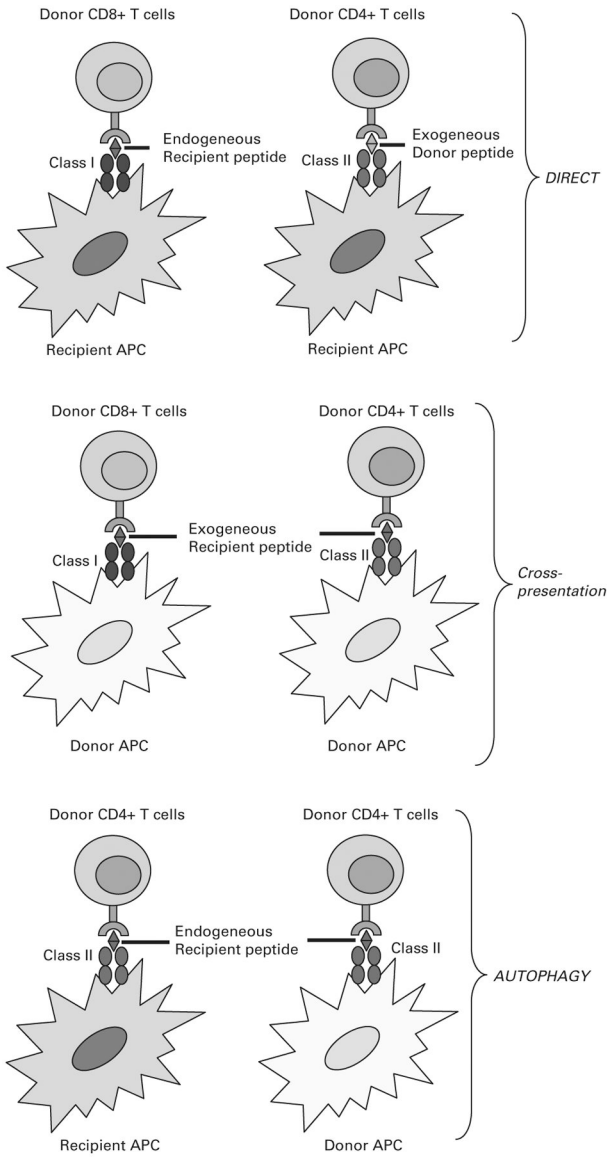

Figure 1.

Pathways of antigen presentation in GVHD. The direct and cross-presentation pathways of antigen presentation. Endogenous antigens released by autophagy can be presented to $CD4^+$ T cells through a novel MHC class II pathway.

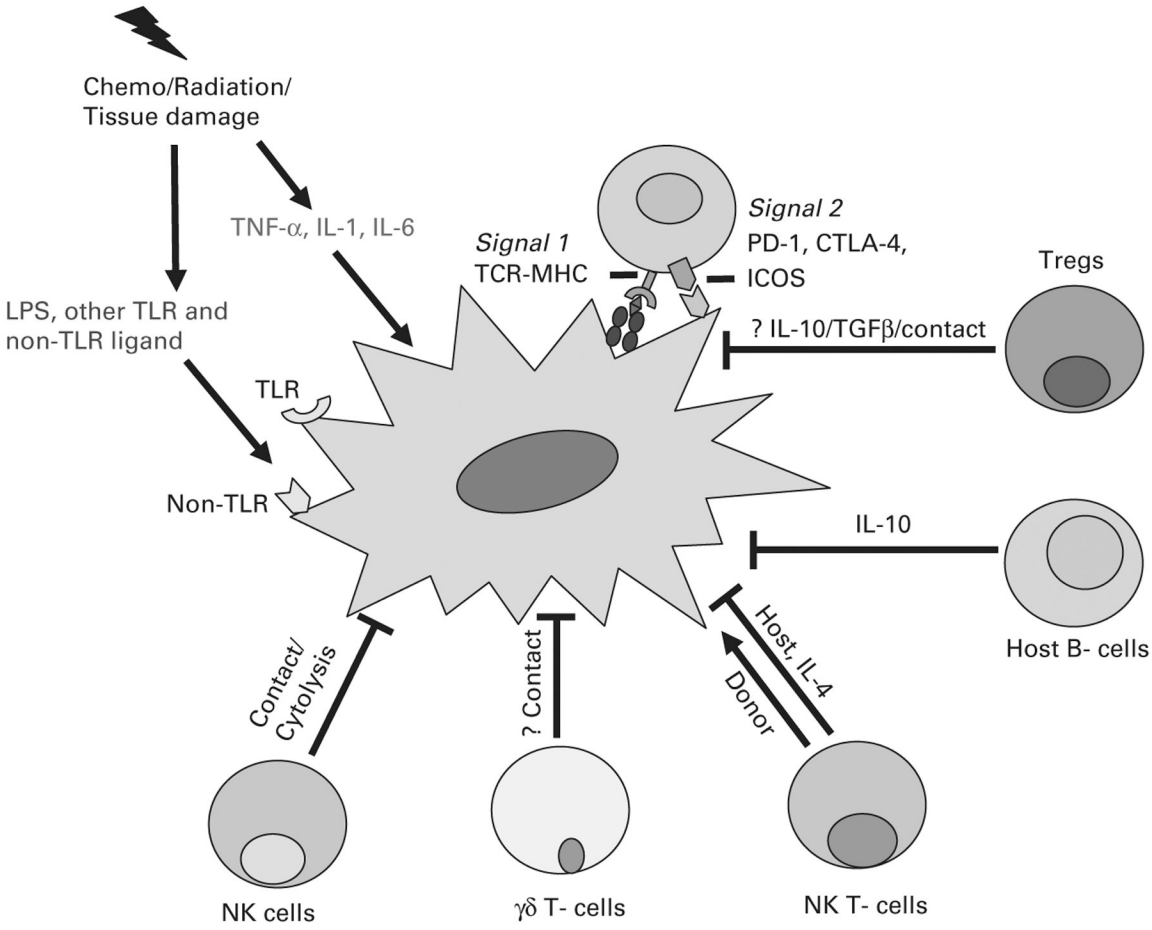

Figure 2.

GVHD sensors, (APC)-mediators and (T cell) interactions. The critical interaction for induction of GVHD is the activation of its primary mediators, donor T cells by the primary sensors, the professional APCs. This interaction is enhanced or negatively regulated by a plethora of other immune cell subsets, cytokines and chemokines.

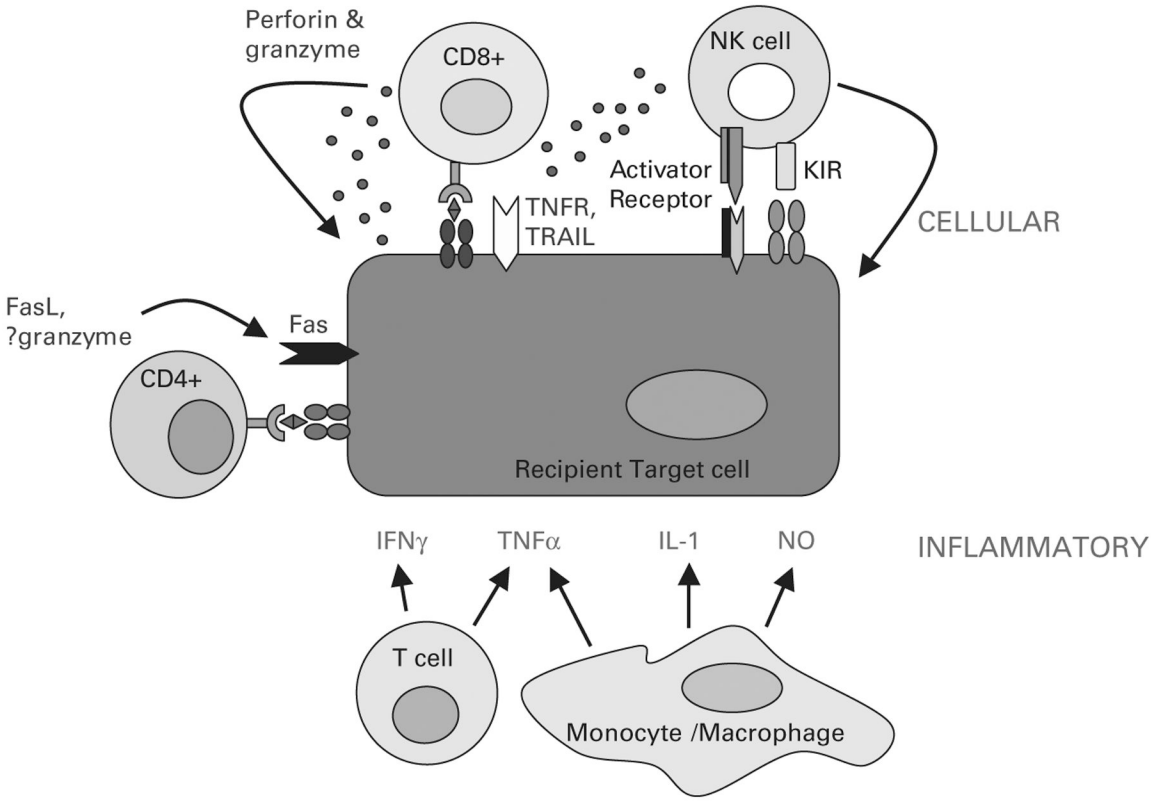

Figure 3.

GVHD effectors and amplifiers. Target organ apoptosis is induced both by (**a**) cellular effectors such as $CD4^+$ and $CD8^+$ effector T cells that induce epithelial cell apoptosis through the perforin/granzyme pathway or by the Fas/FasL pathway and (**b**) by inflammatory effectors such as IFN-γ, TNF-α, IL-1 cytokines and nitric oxide (NO) secreted not only by the effector T cells but also by a variety of other cells such as activated macrophages.

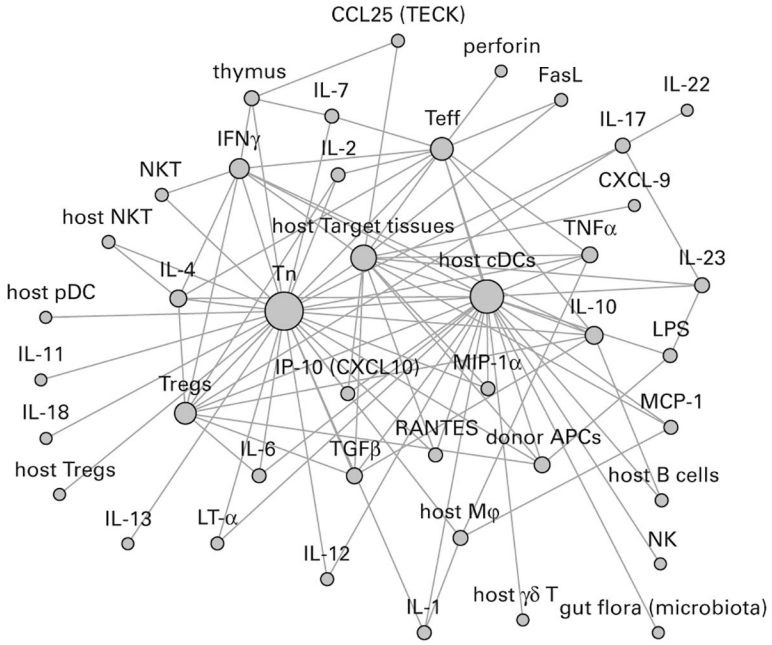

Figure 4.

Network of immune cell interactions in GVHD. Complex interactions exist between the various factors that contribute to the pathophysiology of GVHD. The lines between the nodes represent interactions and the node size is proportional to the number of factors interacting with the node. The network was created using Cytoscape version 2.6.3.[146]

Table 1

Some future research directions

1	Impact of type of damage (apoptosis vs necrosis of different cellular subsets), specific DAMPs, sterile inflammation and complement system.
2	Contribution of host gut microbiome and the donor immune status.
3	Determine GVHD antigenic repertoire, identify immunodominant antigens.
4	Understand the role of both professional (DCs, macrophages) and semi-professional (B cells) donor and host APC subsets, the relevance and mechanisms of cross-presentation.
5	The mechanisms and role of donor Th differentiation, regulatory, memory T cell, and NK cell subsets.
6	Roles of specific effector pathways in causing distinct target organ damage.
7	Determinants of target organ specificity, the role of mechanisms of repair and neovascularization in the severity of damage.
8	Mechanisms and consequence of immunosuppression induced directly by GVHD.
9	Development of an integrated systems approach for understanding the biology of GVHD.