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Invited Review

Adoptive cellular immunotherapy: NK cells and bone marrow transplantation

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Summary. Allogeneic bone marrow transplantation (BMT) has been increasingly used for the treatment of both neoplastic and non-neoplastic disorders. However, serious obstacles currently limit the efficacy and thus more extensive use of BMT. These obstacles include: graft-versus-host disease (GVHD), relapse from the original tumor, and susceptibility of patients to opportunistic infections due to the immunosuppressive effects of the conditioning regimen.Overcoming these obstacles is complicated by dual outcome of existing regimens; attempts to reduce GVHD by depleting T cells from the graft, result in increased rates of tumor relapse and failure of engraftment. On the other hand, efforts to increase graft-versus-tumor (GVT) effects of the transplant also promote GVHD. In this review, the use of natural killer (NK) cells to overcome some of these obstacles of allogeneic BMT is evaluated. Adoptive immunotherapy using NK cells after allogeneic BMT has several potential advantages. First, NK cells can promote hematopoiesis and therefore engraftment by production of hematopoietic growth factors. Second, NK cells have been shown to prevent the incidence and severity of GVHD. This has been shown to be at least partially due to TGF-ß, an immunosuppressive cytokine. Third, NK cells have been shown to augment numerous anti-tumor effects in animals after BMT suggesting a vital role of NK cells in mediating GVT effects. Finally, NK cells have been demonstrated to affect B cell recovery and function in mice. Therefore, understanding the mechanisms of beneficial effects of NK cells after BMT may lead to significant increases in the efficacy of this procedure.

Key words: NK cells, GVHD, GVT, Allogeneic BMT

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Introduction

Bone marrow transplantation (BMT) is currently used for patients with various malignant and non-malignant diseases including: severe anemias, acute and chronic leukemias, myelomas, and lymphomas, and its application is now extended to the treatment for some solid tumors such as breast cancer (Storb, 1995; Lebkowski et al., 1997; Ringden, 1997). However, serious problems currently limit the efficacy of BMT. Two primary sources of stem cells for transplantation are autologous, in which the host's own bone marrow or peripheral blood is used, and allogeneic, in which the host receives stem cells from another person. There are advantages and disadvantages associated with both autologous and allogeneic BMT. In autologous BMT, the level of engraftment is high, and the risk of graft-versus-host disease (GVHD) is low due to a lack of histocompatibility barriers. However, when autologous BMT is used for the treatment of cancer, the relapse rates from the original tumor is significantly higher compared to allogeneic BMT (Storb, 1995). In contrast, allogeneic BMT has the advantage of lower rate of tumor relapse due to a graft-versus-tumor (GVT) effect presumably mediated by immunocompetent donor T cells. However, GVHD caused by the same contaminating T cells in the graft and is a significant cause of morbidity (Ferrara and Deeg, 1991). T cell depletion from the graft reduces the incidence and severity of GVHD but also results in the higher rate of marrow graft failure, tumor relapse, and an increased occurrence of Epstein-Barr Virus (EBV) induced lymphomas (Storb, 1995). In addition, the conditioning regimen (i.e. total body irradiation and/or chemo-therapy) required for both autologous and allogeneic BMT to reduce the tumor burden and remove host cells capable of resisting the graft, leaves patients with severe immunosuppression and a susceptibility to opportunistic infections (Walter and Bowden, 1995). In spite of these drawbacks, the shortage of histocompatibility antigen (HLA) matched siblings among the patients who require BMT results in the increased use of marrow graft with

partial HLA matches or that from HLA-matched, unrelated donors. Therefore, it is essential to develop means to improve the efficacy of BMT. In the following sections, the issues relevant to GVHD and GVT effect and various efforts to improve the efficacy of BMT will be presented.

Pathology of GVHD

GVHD occurs in a patient undergoing allogeneic BMT due to contaminating donor T cells in the marrow graft attacking normal tissues of the host. To study the pathology and the mechanisms of GVHD, various experimental models are utilized. In particular, mouse models have been useful in understanding the progression of GVHD due to the availability of inbred strains with known genotypes. In mouse models, acute GVHD is induced by deliberately infusing MHC mismatched donor splenic T cells with the graft, and the severity of GVHD is measured by examining the major target organs. The extent of weight loss, skin lesions, and necrosis of gut and the liver (Table 1, Fig. 1) (Asai et al., 1998) resemble the manifestations of GVHD in clinical cases. Patients with acute GVHD experiences rash and eventually lesions in the skin and thus are at a high risk of infections (Gillman and Murphy, 1997). In the liver, venoocclusive disease occurs and results in susceptibility to viral hepatitis (Crawford, 1997). A significant necrosis of the epithelial cells and crypt abscesses in the gut are also associated with acute GVHD and leaves patients susceptible to bacterial and viral gastroenteritis as well as severe fluid loss (Mowat, 1997). In mice, weight loss is a common parameter assessed to monitor progression of the disease. In addition, pneumonitis may occur in the lung (Madtes and Crawford, 1997). Ultimately, in both mice and man, severe acute GVHD is often fatal due to

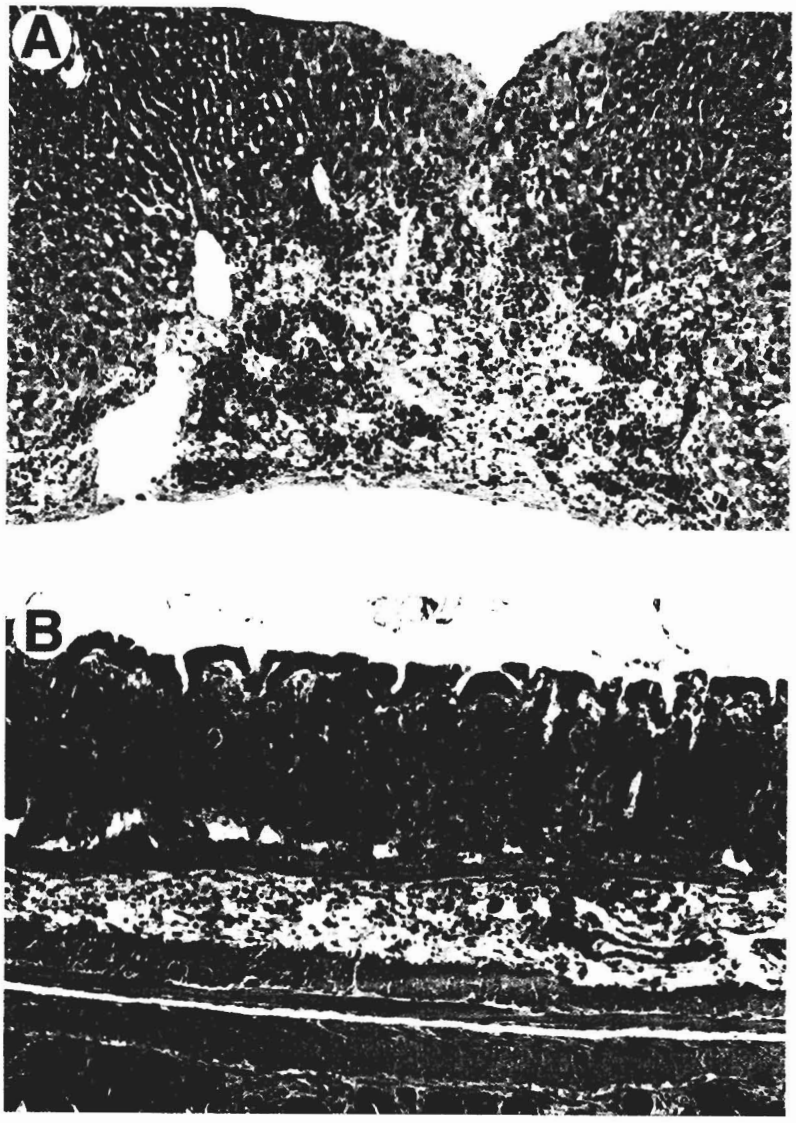

Table 1. Pathology of acute GVHD induced by lethal TBI and MHC class I disparity in mice.

ORGAN	PATHOLOGY
Gut	Crypt cell hyperplasia in small intestine
	Villous sloughing in small intestine
	Ulcer/erosion in large intestine
	Granulomatous inflammation in colon
	Proliferative colitis in colon
	Proliferative typhlitis in cecum
Liver	Subacute inflammation
	Oval cell hyperplasia
	Hepatocellular vacuolation
Skin	Chronic active inflammation
	Squamous hyperplasia
	Dermal melanosis
	Exudate crust
	Hyperkeratosis
	Ulcer/erosion

Fig. 1. A representative histological evaluation of mice receiving fully MHC-mismatched allogeneic BMT. The liver **(A)** and gut **(B)** from mice with acute GVHD were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. A, x 33; B, x 25

the damage in multiple organ systems.

It has been indicated that cytokines produced by both donor and recipient cells play a critical role in and contribute to the severity of GVHD. Total body irradiation (TBI), a conditioning regimen used for BMT, itself causes tremendous release of inflammatory cytokines that exacerbate GVHD. This can result in amplifying the activation and expansion of donor T cells that are sensitized to the host cells. For example, in mice, higher doses of TBI correlate with the increase in severity of and mortality due to GVHD (Hill et al., 1997). Furthermore, higher doses of TBI increase the level of inflammatory cytokines, TNF-α and IL-1, in the serum as early as 1 week after BMT and prime macrophages to respond to endogenous LPS resulting in increased production of inflammatory cytokines (Hill et al., 1997).

Since GVHD is cell-mediated process, the hypothesis that TH1-type cytokines such as IL-2 and IFN-γ, which activate T cells, are deleterious, and TH2-type cytokines (IL-4 and IL-10) are protective has been generally accepted (Krenger and Ferrara, 1996). However, several recent reports show conflicting results. While neutralization of IL-12 has been shown to protect animals from the disease suggesting that IL-12 has deleterious effects (Williamson et al., 1997), Sykes et al. demonstrated that high doses of IL-2 or IL-12 can be protective (Sykes et al., 1990, 1995a,b; Abraham et al., 1992). Similarly, there are reports indicating both deleterious and protective roles of IFN-γ (Brok et al., 1993, 1997; Krenger et al., 1996) as well as TH2 cytokines in GVHD (Blazar et al., 1995; Krenger et al., 1995, 1996a,b; Ushiyama et al., 1995). Additionally, when IFN-γ knockout (KO) mice are used as donors in allogeneic BMT, the recipient mice succumb to death much faster than the control mice from acute GVHD, suggesting that IFN-γ can be protective in acute GVHD (Murphy et al., 1998). Conversely, mice receiving allogeneic IL-4 KO cells survive longer than the control group suggesting that IL-4 may be deleterious in GVHD pathology (Murphy et al., 1998). It has been suggested that IFN-γ can be radioprotective (Gardner, 1998). Considering that the doses of TBI influence the level of inflammatory cytokines produced by the host cells, the roles of TH1 and TH2 cytokines play in onset and the severity of GVHD may depend on the extent of conditioning administered to the recipients. Indeed, preliminary results from experiments in which IFN-γ KO donor cells are given to sublethally irradiated hosts indicate that they protect from GVHD suggesting that the extent of conditioning markedly changes the role of a particular cytokine in the pathology of GVHD (manuscript in preparation).

Adoptive immunotherapy following BMT

One obvious goal in cancer therapies is to develop means to optimize GVT effects without exacerbating GVHD after BMT. One such approach has been systemic administration of cytokines such as IL-2, GM-CSF, and G-CSF. For instance, G-CSF and GM-CSF, both hematopoietic growth factors, have been used after autologous and allogeneic BMT to enhance engraftment of hematopoietic cells and resistance to opportunistic infections (De Witte et al., 1992; Saarinen et al., 1996). IL-2 has been given after autologous BMT to promote the immune reconstitution and to decrease the rate of tumor relapse. However, the reports on the efficacy of IL-2 treatment are conflicting (Favrot et al., 1989; Blaise et al., 1990; Higuchi et al., 1991; Attal et al., 1995; Raspadori et al., 1995; Robinson et al., 1996). Although systemic administration of these cytokines can be helpful, it has been hampered by inefficient pharmacokinetics (Ruef and Coleman, 1991; Petros et al., 1992; Robak, 1995; Morstyn et al., 1996) and subsequent toxicity at optimally effective doses.

Recently, a strategy involving tumor-antigen vaccines and BMT has been attempted. In these studies, immunization of donor mice with tumor specific antigen before BMT and the tumor bearing hosts after BMT resulted in longer survival of the animals (Kwak et al., 1991, 1996; Hornung et al., 1995). Another avenue employed has been adoptive transfer of donor mononuclear cells following the transplant. Walter and colleagues have shown that transfusion of donor T cell clones specific for CMV promotes cellular immunity against CMV in patients after allogeneic BMT (Walter and Bowden, 1995). In addition, it has been demonstrated that infusion of buffy coat cells of donor origin decreases relapse rates (Collins et al., 1995; Porter and Antin, 1995; Tsuzuki et al., 1995; Bertz et al., 1998). Unfortunately, the decrease in tumor relapse in some of these studies is strongly associated with the increase in GVHD (Collins et al., 1995; Porter and Antin, 1995; Tsuzuki et al., 1995; Bertz et al., 1998). However, delayed lymphocytic infusions (DLI), administered well after the transplant has resulted in significant GVT with less GVHD. Another study has also shown that infusion of donor-derived polyclonal T-cell clones specific for EBV proteins enhance anti-tumor responses against EBV-induced or EBV-related lymphoma in patients who received T-cell depleted BMC (Rooney et al., 1998). Adoptive transfer of T cell clones specific for the defined minor H antigens could selectively increase T cell responses against leukemia (Warren et al., 1998). The accumulating results from adoptive immunotherapy using donor mononuclear cells after allogeneic BMT show that it is of critical importance to delineate and dissociate the mechanisms of GVHD versus GVT to develop effective means to overcome the obstacles associated with allogeneic BMT. In this regard, understanding the biology and potential role(s) of natural killer cells can bring some insights in order to exploit their use in immunotherapy (Murphy and Longo, 1997).

Natural killer cells

Natural killer (NK) cells were originally described

as lymphoid cells capable of lysing transformed or virally-infected cells without prior sensitization in vitro (Trinchieri, 1989). NK cells do not require previous exposure to antigens in context of MHC, as in the case with T cells, to carry out the effector functions, and thus their cytotoxic ability is defined as non-MHC restricted. However, as it will be discussed later, MHC class I expression on target cells plays an important role in NK cell cytotoxicity (George et al., 1997; Hoglund et al., 1997). NK cells do not rearrange either TCR α/ß or γ/δ genes or immunoglobulin genes (Trinchieri, 1989; Lanier et al., 1992). Human NK cells are characterized by the expression of CD56 (NCAM) and CD16 (FcγRIII) (Trinchieri, 1989). Similarly, mouse NK cells are also characterized by the pan expression of NK1.1 (Koo and Peppard, 1984) and DX5 (Ortaldo et al., 1998) as well as asialo GM1 (Yang et al., 1985) and FcγRIII (Perussia et al., 1989). These markers are not truly NK-specific in that a small subpopulation of CD4+ T cells also express NK 1.1 (Arase et al., 1993). However, NK cells can be defined as a distinct population that expresses certain markers (i. e. NK1.1 and FcγR) and lacks others (i.e. CD3). As both human and murine NK cells express intermediate affinity IL-2 receptor, they can be activated by IL-2 in vivo and in vitro. In addition, a number of cytokines such as IFN-α/ß and IL-12 can activate NK cells to proliferate and augment cytotoxicity (Chehimi et al., 1993). IL-15 has been described and shown to activate mature NK cells although its activity may be more important for the development of immature NK cells (Carson et al., 1994; Puzanov et al., 1997). Activated NK cells are potent producers of IFN-γ, which plays a critical role in some viral as well as bacterial infections (Biron and Gazzinelli, 1995; Biron, 1998). Additionally, there are accumulating data indicating that NK cells produce numerous other cytokines such as GM-CSF, G-CSF, TNF-α, TGF-ß, IL-1, and IL- 8 (Trinchieri, 1989).

One of the major effector functions of NK cells is direct cytotoxicity against various cell types. It has been shown that NK cells utilize cytolytic granules, including perforin and granzymes, which are released upon ligation of receptors (Trapani and Smyth, 1993). NK cells can also kill targets via Fas/FasL interaction (Oshimi et al., 1996).

One of the first descriptions of NK effector functions came from the observation of their ability to reject bone marrow allografts in lethally irradiated mice (Cudkowicz and Bennett, 1971a). Moreover, NK cells were shown to mediate a unique process called "hybrid resistance" in which F1 hybrid recipients reject parental bone marrow graft while accepting solid tissue (Cudkowicz and Bennett, 1971b; Bennett, 1987). Thus, it has been proposed that NK cells may play a role in homeostasis of hematopoiesis. However, the mechanisms involved in hybrid resistance and the rejection of bone marrow allografts are yet to be delineated. While it is possible that NK cells may see some other determinants in addition to MHC class I ligand on hematopoietic cells and mediate cytotoxicity against BMC via perforin and/or FasL, it has been shown that NK cells do not kill BMC in vitro (Aguila and Weissman, 1996). Furthermore, perforin knockout mice and *gld* mice that are defective in FasL (Baker et al., 1995) expression and yet can mediate BMC rejection indicating that other mechanisms such as production of appropriate cytokines may be involved in regulating hematopoiesis. Additionally, it appears that the environmental conditions in which the experimental mice are housed may be an important factor contributing to the rejection of the graft (Bennett et al., 1998).

Understanding the role of NK cells in hematopoiesis has become more complicated by now a growing list of receptors whose expression defines NK cell subsets. In mice, these receptors are C-lectin type molecules and members of Ly-49 family (Ryan and Seaman, 1997); in human NK cells, there exist killer immunoglobulin-like receptors (KIR) which belong to immunoglobulin superfamily (Reyburn et al., 1997). These receptors have different specificities for MHC class I molecules, and some of their cytoplasmic portion include tyrosine based motifs, immunoreceptor tyrosine-based inhibitory motif (ITIM), which deliver an inhibitory signal when the receptors bind their ligands (Ryan and Seaman, 1997; Reyburn et al., 1997). Existence of the subsets of NK cells characterized by expression of various inhibitory receptors can provide some insights with regard to the mechanism of the rejection of bone marrow allografts by NK cells. Bone marrow cells which lack the expression of MHC class I molecules specific for certain Ly-49 receptors expressed on NK cell subsets results in the absence of a negative signal and thus rejection of the graft occurs. Furthermore, there appears to be a regulatory mechanism among the subsets of NK cells such that the presence or absence of certain subsets can influence the functions of other subsets (Raziuddin et al., 1996, 1998). In addition, there are increasing number of studies demonstrating that negative signals generated via binding of MHC class I molecules by inhibitory receptors down-regulate cytotoxicity mediated by NK cell subsets expressing the receptors in vitro (George et al., 1997). Recently, it has been reported that negative signals received through Ly-49 receptors also inhibit cytokine production by NK cells in vitro (Ortaldo et al., 1997). Interestingly, in mice, at least two of the Ly-49 receptors, Ly-49D and Ly-49H, have been shown to lack ITIM in their cytoplasmic domains (Smith et al., 1998). Instead, they associate non-covalently with another transmembrane protein, DAP12, which contains ITAM, and thus cross-linking of these receptors results in generation of activating signals (Smith et al., 1998; George et al., 1999). Recently, such activation receptors were identified in human NK cells with NKG2C being shown to bind DAP12 and result in activating signals (Lanier et al., 1998). Therefore, it appears that the intricate balance between the signals generated through these receptors play a critical part in NK cell functions. As NK cells do not appear to reject solid tissue allografts

yet mediate anti-tumor effects, it is possible that they may be of potential use as an immunotherapy in cancer.

The role of NK cells in GVHD and GVT after BMT

The efforts to dissect the role of NK cells in GVHD has been hampered by the difficulty in obtaining pure population of NK cells without contaminating T cells. Previously, it has been shown that depletion of cells expressing the glycoprotein asialo GM1 (ASGM1), which includes NK cells, by treatment of mice with anti-ASGM1 antibody resulted in reduced GVHD in incidence and severity (Charley et al., 1983). It also has been reported that ASGM1+ cells, as well as NK activity, as assessed by cytotoxicity, can be detected from the lesions during acute GVHD (Ghayur et al., 1987, 1988). However, activated T cells and macrophages also express ASGM1 and thus the absence of these cell types in reducing the severity of GVHD cannot be excluded. More recently, there has been a report that depletion of NK1.1+ cells from donor BMC significantly reduced the incidence of GVHD and increased the survival of mice receiving the allograft (Ellison et al., 1998). However, the effect of depletion of NK1.1+ cells could only be seen when donor mice were pretreated with an agent that induced interferon alpha-beta, and thus it is difficult to interpret the data in relation to clinical scenarios.

The role of NK cells in GVHD may also depend on the particular time-point in which they are assessed. NK cells produce inflammatory cytokines such as TNF-α and IL-1 that can worsen ongoing GVHD. In addition, NK cells can produce IFN-γ at high levels, which can increase the expression of MHC alloantigens on the host. On the other hand, it has been shown that NK cells can also produce cytokines that are inhibitory to immune functions. For instance, NK cells have been demonstrated to inhibit generation of cytotoxic T lymphocyte (CTL) precursors in mixed lymphocyte culture via TGF-ß (Yamamoto et al., 1994). Similarly, it has been shown that activated NK cells can suppress GVHD in mice following allogeneic BMT (Asai et al., 1998). In this study, lethally irradiated C57BL/6 ($H2^b$) mice received allogeneic BMC (BALB/c, $H2^d$) along with IL-2 activated NK cells from C. B-17 severe combined immunodeficient (SCID) ($H2^d$) mice. SCID mice do not rearrange TCRα/ß or immunoglobulin genes and thus lack B and T cell functions, but are an excellent source of NK cells. The results demonstrated that IL-2 activated NK cells did not induce GVHD after allogeneic BMT. Moreover, donor-type NK cells inhibited GVHD in mice that received allogeneic splenocytes with BMC to induce acute GVHD (Asai et al., 1998). Further studies addressing the mechanism of inhibition of GVHD by IL-2 activated donor type NK cells indicated that the protection from GVHD was due to TGF-ß, as mice concurrently treated with anti-TGF-ß monoclonal antibody succumbed to GVHD (Asai et al., 1998). It should be noted that the protective effects of IL-2 activated NK cells were critically dependent on the timing of the adoptive transfer. Administering IL-2 activated NK cells to recipient mice later than three days after allogeneic BMT worsened GVHD (Murphy and Longo, 1997). It is possible that IL-2 treatment at a later time point following allogeneic BMT may activate donor T cells that are already responding to host antigens, and/or it may be due to the inflammatory cytokines produced by the IL-2 activated NK cells affecting the target tissues.

This critical element of timing offers some insights into the role of NK cells in GVHD. It appears that IL-2 activated donor type NK cells can prevent induction of

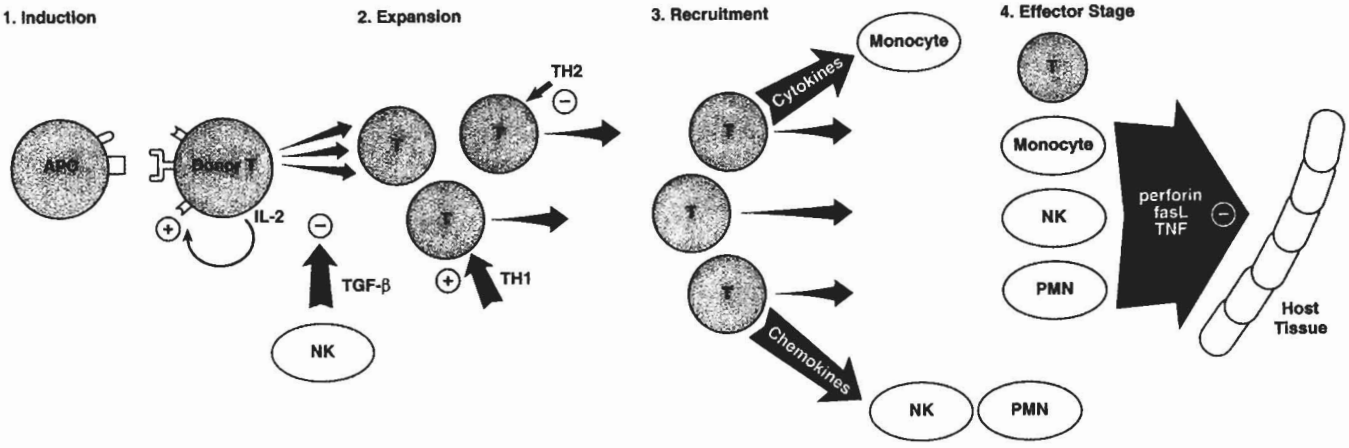

Fig. 2. Dual effects of IL-2 activated NK cells in GVHD. At the induction phase of GVHD, where donor T cells are sensitized by host cells, IL-2 activated NK cells can protect the host by production of immunosuppressive cytokines such as TGF-ß to inhibit the expansion of the sensitized donor T cells. However, if IL-2 activated NK cells are introduced after the induction phase, IFN-γ produced by NK cells can enhance the production of other TH1 cytokines, which augment donor T cell responses (expansion and recruitment). Therefore, together with enhanced donor T cell responses and other inflammatory cytokines, NK cells can exacerbate GVHD via the mechanisms such as perforin, fasL, and TNF (effector stage).

GVHD at least in partvia induction of TGF-ß at the initiation of GVHD when donor T cells are sensitized by host cellular antigens. However, once the alloreactive T cells begin to respond and inflammatory cytokines are produced, NK cells no longer can protect the host from GVHD and contribute to the pathology of GVHD (Fig. 2). Therefore, the possible application of adoptive transfer of IL-2 activated donor NK cells as an approach to reduce GVHD in clinical settings should be reviewed carefully. It may be necessary to transfer activated NK cells with a T cell depleted marrow graft. NK cells should still provide protection from GVHD that may result from residual T cells and may enhance engraftment and immune recovery.

Because of the ability of NK cells to lyse various transformed cell lines in vitro, it has been hypothesized that the major role of NK cells is in immunosurveillance to remove spontaneously arising tumor cells. Although there is no direct evidence of immunosurveillance by NK cells, there is a correlation between NK activity and longer period of remission and increased duration of survival in some human studies (Schantz et al., 1987). In addition, reduction in NK activity is correlated with tumor relapse (Pross et al., 1984). Anti-tumor effects of NK cells have been reported especially in mice with metastatic tumors (Trinchieri, 1989). It has been reported that GVT effects can be mediated by $ASGM1^+$ cells, which include NK cells, after allogeneic BMT without the evidence of GVHD (Okunewick et al., 1995). Furthermore, it has been demonstrated that adoptive transfer of IL-2 activated donor NK cells along with allogeneic BMT can significantly increase the survival of mice with advanced colon carcinoma (Asai et al., 1998). Therefore, IL-2 activated donor type NK cells appear to exert protective effects on both GVHD and GVT, and more importantly, this model provides a means to dissociate the mechanisms of GVHD from GVT.

NK cells and immune reconstitution after BMT

Although NK cells have been mainly described for cytotoxic effector function, reports indicating the regulatory role of NK cells during immune responses have also been accumulating. For example, activation of NK cells in vivo with either polyinocynic-polycytidylic acid [poly (I:C)] or certain tumor cells can increase antigen specific IgG2a without affecting other antibody isotypes during T-dependent (TD) and T-independent (TI) antibody responses (Wilder et al., 1996; Koh and Yuan, 1997). This increase in IgG2a depends on the presence of NK cells as depletion of NK cells abrogates the response. Since IFN-γ is a major inducer of switching the immunoglobulin isotype to IgG2a (Snapper and Paul, 1987), IFN-γ produced by activated NK cells appears to play an essential role in regulation of antibody responses.

In light of the fact that activated NK cells produce cytokines that can not only modulate immune responses (i. e. IFN-γ) but also promote hematopoietic recovery after allogeneic BMT (i.e. GM-CSF and G-CSF), adoptive transfer of IL-2 activated donor NK cells have been used to examine their role in immune reconstitution. Previous studies indicate that adoptive transfer of IL-2 activated donor NK cells promote hematopoietic engraftment as demonstrated by increases in hematopoietic colony formation by BMC and splenocytes in vitro after allogeneic BMT in mice (Murphy et al., 1992a,b). It also increases the rate of B cell development and the degree of donor chimerism in bone marrow (Murphy et al., 1992). In order to delineate the effects of NK cells on immune reconstitution and function, lethally irradiated C57BL/6 ($H2^b$) mice were given IL-2 activated adherent lymphokine activated killer (ALAK) cells. The ALAK cells were prepared from B and T cell-depleted BALB/c ($H2^d$) splenocytes and BMC and thus >90% NK cells. One day after the adoptive transfer of donor-type ALAK cells, mice received allogeneic donor BMC. In addition, systemic administration of IL-2 was performed for three consecutive days beginning on the day of ALAK cell transfer. At various days post-BMT, splenocytes from these mice were stimulated with the mitogens LPS or Concanavalin A. The results indicated that the proliferative responses of splenocytes from mice given donor ALAK cells with BMT were significantly higher compared to those from control mice (Table 2). The

Table 2. Mitogenic responses of splenocytes at day 21 post-allogeneic BMT[a].

TREATMENT	LPS (25 μg/ml)	ConA (5 μg/ml)
PBS	3.762±1.435[b]	3.742±1.519
IL-2	5.027±1.814	7.156±781
ALAK[c]±IL-2	16.611±2.185[d]	33.013±7.508[e]

[a]: lethally irradiated C57BL/6 mice were given $1x10^7$ BALB/c BMC; [b]: proliferative responses were determined by uptake of ^{3}[H]-Thymidine in 4 day cultures, the values represent geometric means of responses of splenocytes from 3 separate mice ± SEM in cpm; [c]: donor ALAK cells were prepared by activating T and B cell-depleted BALB/c splenocytes and BMC with IL-2 for 6-7 days; [d]: using Welch's approximation test, p=0.001 compared to IL-2 treated group; [e]: using Welch's approximation test, p=0.009 compared to IL-2 treated group.

Table 3. Total serum Ig production post-allogeneic BMT[a].

TREATMENT	IgG1 (μg/ml)[b]		IgG2a (μg/ml)	
	Day 9	Day 14	Day 9	Day 14
PBS	82.8±30.17	53.2±10.99	3.85±2.83	4.30±1.74
IL-2	87.9±22.98	62.9±15.03	2.83±2.05	8.34±6.32
ALAK[c]+IL-2	94.6±22.98	62.9±13.18	11.74±2.48[d]	10.95±2.94

[a]: lethally irradiated C56BL/6 mice were given $4x10^6$ BALB/c BMC; [b]: total serum immunoglobulin levels were determined by ELISA; c: donor ALAK cells were prepared by activating T and B cell-depleted BALB/c splenocytes and BMC with IL-2 for 6-7 days. [d]: the level of serum IgG2a in ALAK treated group (p<0.001) compared to PBS and IL-2 control groups. The p values were obtained using student t-test.

analysis of total serum immunoglobulin (Ig) levels showed that adoptive transfer of donor ALAK cells resulted in increased level of serum IgG2a without affecting other isotypes compared to those in control group at day 9 followed by comparable levels of IgG2a at day 14 and 21 post-BMT (Table 3). These results suggest that the increase in serum IgG2a may be due to the effect of ALAK cells on donor plasma cells present in bone marrow. Although a further functional analysis of donor ALAK cell transfer on specific immune responses in vivo is needed, the results are encouraging that NK cells may have beneficial effects on immune recovery after allogeneic BMT.

It has been demonstrated that NK cells play an important role in early resistance to viral, bacterial, and fungal infections (Biron and Gazzinelli, 1995). In many of the studies, IFN-γ produced by NK cells at early time points during the infection is essential in amplifying the immune response through induction of IL-12 (Biron and Orange, 1995; Biron, 1998). Adoptive transfer of donor ALAK cells, therefore, may augment the host immune response to the opportunistic infections by promoting the recovery immune cells and production of cytokines that are protective. With regard to enhancement of immune response, it would be important to examine the role of NK cell subsets as they are characterized by expression of inhibitory and/or activation receptors. It would be of interest if adoptive transfer of certain subsets of ALAK cells, bearing different Ly-49 receptors can promote immune responses to a greater extent than whole populations of NK cells.

Conclusions

In order to enhance the efficacy of allogeneic BMT for the treatment of cancer, it is essential to augment GVT effects without increasing GVHD, and various efforts have been made to achieve this objective. These efforts include the use of systemic cytokine administration, tumor antigen-vaccine therapy, and adoptive cellular immunotherapy of donor mononuclear cells. Additionally, experimental animal models of adoptive transfer of activated donor NK cells have been explored. These studies show that activated donor NK cells prevent GVHD and enhance GVT effects as well as engraftment of donor BMC and immune recovery. Further investigations of the optimal conditions for the use of activated donor NK cells may provide an effective means to enhance the efficacy of allogeneic BMT.

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