MINIREVIEW

Graft-Versus-Host Disease in Experimental Allogeneic Bone Marrow Transplantation

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Bone marrow transplantation is currently used primarily in the treatment of aplastic anemia, high risk leukemia in first remission, and severe combined immunodeficiency (1, 2). This procedure is also potentially applicable to numerous other syndromes, including genetic, metabolic, and hematologic disorders, but only if the risk factors involved with transplantation can be significantly reduced. Graft-versus-host disease (GVHD) remains the most critical complication, particularly in allogeneic transplants for leukemia. Here, (approximately) 35–60% of patients exhibit moderate to severe acute GVHD; GVHD is the primary cause of death in 12–20% of these patients (3, 4). The skin, liver, gut, and lymphoid organs are the principal target organs for GVHD. Tissue damage often leads to immunodeficiency, which in turn leads to a wide range of opportunistic (pathogenic) infections. Approximately 20–45% of transplant recipients surviving for more than 180 days experience a chronic form of GVHD. This syndrome is not as life-threatening as acute GVHD, but can cause severe debilitation (5, 6).

GVHD is commonly defined as the consequence of transferring immunocompetent lymphocytes to hosts expressing foreign transplantation antigens. Overt GVHD is most prominent in immunosuppressed hosts. The intensity of GVHD correlates with the strength of the antigenic differences between the donor and host, and it has long been recognized that marrow transplantation across major histocompatibility complex (MHC) barriers generally leads to a fatal acute GVHD reaction (7). Consequently, since the 1970s most transplants have been limited to HLA-matched sibling donors. However, there are two main obstacles to this approach. First, despite MHC compatibility between the donor and recipient, there is still a high incidence of both acute and chronic GVHD (2–4). Second, a significant number of patients needing bone marrow transplants cannot be treated because of the lack of an HLA-matched donor. As more potential donors are entered into the international registry for marrow transplantation, this latter problem may be substantially reduced, although the use of unrelated donors creates additional problems (8).

The etiology and pathogenesis of GVHD in humans has long been the center of controversy. In the case of the acute and invariably fatal form of GVHD which occurs after HLA-mismatched marrow transplantation, there is general agreement that mature immunocompetent T cells in the marrow inoculum are the main cause of the disease. This concept is strongly supported by studies in rodents (9–11) and dogs (12). The acute GVHD seen after HLA-matched marrow transplantation is considered to be a reflection of mature donor T cells responding to non-HLA antigens of the host (13). The experimental basis for this concept has come largely from murine models (14, 15), where GVHD directed to defined minor histocompatibility (H) antigens can be prevented by elimination of contaminating T cells from the marrow inoculum. The relative contributions of $CD4^+$ and $CD8^+$ T cell subsets in the etiology of GVHD across either MHC or non-MHC barriers has been one of the primary aims of our research in recent years and will be discussed later in this article.

The implication of mature donor T cells in the marrow inoculum as the main cause of GVHD has

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stimulated attempts to purge the donor marrow inoculum of T cells before transplant. This approach has been undertaken at several clinical transplant centers around the world, using either anti-T cell monoclonal antibodies plus complement treatment or lectin-rosette removal to deplete T cells (16, 17). This approach is highly successful in reducing the incidence of GVHD (18). However, it is now clear that T cell depletion of marrow can cause a noticeable increase in the other serious complications of marrow transplantation, particularly graft failure, immunoincompetency (which leaves the patient open to opportunistic infections), and relapse of leukemia (17, 18). Failure of engraftment may involve an active radioresistant host cell reaction (either T cell or natural killer cell) which would normally be counteracted by the anti-host response of the donor T cells. This problem can be partly overcome by more intensive preconditioning protocols to eliminate these residual host components (17). In some cases, stem cell engraftment and development is facilitated by *in vivo* administration of recombinant lymphokines (17). In relation to opportunistic infections, marrow transplantation often involves an extended period of time after transplant during which the recipient is generally immunocompromised (19). In healthy hosts with an intact thymus, significant T cell immunity can develop within 4–6 weeks as new donor T cells begin to emerge from the thymus. However, the thymus is often only poorly functional as a consequence of the disease afflicting the patient or on account of the pre- and posttransplant conditioning regimens which generally involve immunosuppressive pharmacologic agents and/or irradiation. Prolonged immunodeficiency reflecting thymic dysfunction is exacerbated by manipulating the marrow to remove T cells and other immune cells (20, 21). As a result, the patient has little defense against opportunistic infections, and may be particularly susceptible to viral pathogens. Cytomegalovirus (CMV) infections are a particular concern and occur in approximately 50% of all allogeneic bone marrow transplant patients; CMV infections are more common when either the donor or host is seropositive (22). CMV-associated interstitial pneumonia develops in about 10–30% of all patients and results in >85% mortality (23). Eliminating seropositive blood products and the use of Ganciclovir and immunoglobulin are helpful but are only partly successful in counteracting CMV infection.

Bone marrow transplantation for leukemia presents a special problem. Here there is an inverse relationship between relapse rate and the presence of GVHD (17, 18, 24). The prevailing view is that, in addition to attacking host tissues in general, the donor T cells exert a graft-versus-leukemia reaction and thereby prevent re-emergence of the leukemia. T cell graft-versus-leukemia activity is difficult to demonstrate in animal models and it is still uncertain whether this reaction is directed to tumor-specific antigens per se (24). Thus, gaining an understanding of how the positive effects of graft-versus-leukemia might be separated from the negative effects associated with GVHD is an important goal in advancing the field of marrow transplantation.

T Cell Subsets

T cells of mice can be divided into two major subsets: H-2 class I-restricted (cytotoxic T lymphocytes, CTL) cells expressing the CD8 marker and H-2 class II-restricted (helper-type) T cells bearing the CD4 phenotype (25). Whereas, $CD4^+$ T cells respond to antigen autonomously, it is often argued that $CD8^+$ T cells function poorly *in vitro* unless supplemented with $CD4^+$ cells or their products, particularly interleukin 2 (IL-2). However, although there is ample evidence that IL-2 guides the clonal expansion of activated CTL, there are a number of situations in which resting CTL precursors can be induced to proliferate and differentiate in the absence of exogenous help. For example, purified unprimed $CD8^+$ cells can give high mixed lymphocyte reaction and cell-mediated lympholysis responses to H-2 class I differences *in vitro* without added help (26). Likewise, purified $CD8^+$ cells are fully capable of mediating various alloagressive functions *in vivo*, including skin graft rejection and lethal GVHD (27). These helper-independent responses are presumed to reflect that $CD8^+$ cells are capable of producing their own growth factors (28). Purified $CD4^+$ cells are also effective mediators of skin graft rejection and lethal GVHD, but only against class II determinants (27). This raises the question whether $CD4^+$ and $CD8^+$ cells use the same or different mechanisms to mediate *in vivo* functions such as GVHD and skin graft rejection. This is still unclear. Thus, although some of the functional properties of $CD4^+$ and $CD8^+$ cells are distinctly different, these cells nevertheless show a considerable overlap in the range of lymphokines they release and in certain functions, e.g., cytotoxic activity.

A summary of our work on the patterns of GVHD mediated by $CD4^+$ and $CD8^+$ cells is given below. The experiments employed highly purified T cell subsets prepared by combining negative selection with antibody plus complement and positive selection by panning over antibody-coated plates (27). Purified T cells were supplemented with T cell-depleted (anti-Thy-1 plus complement-treated) bone marrow, usually of donor origin, and were injected into hosts exposed to whole body irradiation. The host mice expressed either MHC (H-2 class I or II) or non-MHC (minor H) differences.

GVHD Across MHC Class II Barriers

$CD4^+$ T cells react poorly to class I differences but give strong responses to class II differences in several

assay systems, including *in vitro* and *in vivo* proliferation and skin allograft rejection (26, 27). In these assays, C57BL/6J (B6) $CD4^+$ T cells respond well to the class II-different bm12 (I-A different) mutant but fail to react to class I-different bm1 (H-2K-different) mutants. Likewise, the transfer of B6 $CD4^+$ cells causes lethal GVHD in heavily irradiated (1000 rads) (B6 × bm12)F_1 mice but not in (B6 × bm1)F_1 recipients. Interestingly, the mortality rates in irradiated (B6 × bm12)F_1 recipients of B6 $CD4^+$ cells show an inverse correlation with the number of T cells injected: small doses (10^5–10^6) cause severe mortality whereas high doses (2×10^7) often lead to long-term survival with only minimal symptoms of GVHD (29). The protection afforded by large doses of $CD4^+$ cells might be a manifestation of restoring the host's immune system, pathogens being kept at bay. Alternatively, the phenomenon could reflect a form of suppression resulting from overproduction of IL-2 (30). An interesting feature of GVHD mediated by $CD4^+$ cells is that lethal GVHD is only seen in hosts given heavy irradiation (>750 rads) (29). With lower doses of irradiation lethal forms of GVHD are rare, providing the mice are relatively free of intercurrent infections. This only applies if the mice are given donor T cells and donor marrow. If host marrow cells are substituted, the mice die rapidly from marrow aplasia, presumably because the host marrow cells are destroyed by $CD4^+$ cells with cytotoxic activity (29). This phenomenon probably reflects direct CTL activity rather than a bystander effect because marrow aplasia fails to occur when $CD4^+$ cells are injected along with a mixture of donor and host marrow.

The key point emerging from these studies is that $CD4^+$ cells can be very potent mediators of lethal GVHD in class II-different combinations; $CD8^+$ cells, by contrast, cause no mortality with class II differences.

GVHD Across MHC Class I Barriers

In addition to exerting powerful proliferative responses and skin allograft rejection (29), purified B6 $CD8^+$ T cells are potent inducers of lethal GVHD in irradiated MHC class I-different (B6 × bm1)F_1 mice; $CD4^+$ cells cause no mortality. $CD8^+$ cells characteristically cause a chronic form of GVHD with most deaths occurring after 30–40 days. This syndrome is seen with hosts given even light irradiation (500 rads) and applies with a wide dose range of $CD8^+$ cells (from 10^5 to 2×10^7) (31). The chronic GVHD mediated by $CD8^+$ cells contrasts with the hyperacute GVHD elicited by $CD4^+$ cells responding to class II differences. Chronic GVHD is only seen when $CD8^+$ cells are transferred with donor marrow. When transferred with host marrow, $CD8^+$ cells cause rapid death from hematopoietic failure. This syndrome fails to occur when $CD8^+$ cells are transferred with a mixture of donor and host marrow. Like $CD4^+$ cells, $CD8^+$ cells thus seem to be able to mount a direct CTL response to host stem cells. Rapid mortality due to hematopoietic failure also occurs when $CD8^+$ cells are transferred without donor marrow to low-dose irradiated (500-rad) recipients; addition of donor B6 marrow prevents hematopoietic failure and the mice eventually succumb to chronic GVHD (31).

The pattern of development of chronic GVHD in heavily irradiated (B6 × bm1)F_1 mice injected with B6 $CD8^+$ cells reveals a curious biphasic effect. The mice typically become ill within the first 2 weeks after transplant, show a partial recovery over the next 2–3 weeks, and then relapse into severe illness with progressive deterioration until death. Why the hosts show a late exacerbation of disease is unclear. One possibility is that the donor $CD8^+$ cells eventually receive a boost of help (IL-2) when $CD4^+$ cells arise *de novo* in the host thymus. This possibility seems unlikely because the generation of new $CD4^+$ cells by the host thymus is very limited, probably because the thymus is damaged by GVHD. Moreover, thymectomized hosts or hosts given repeated injections of anti-CD4 MAb exhibit no significant differences in GVHD development relative to untreated mice. Despite this finding, it has been found that supplementing $CD8^+$ cells with small numbers of donor $CD4^+$ cells or IL-2 is able to potentiate GVHD and cause a significant reduction in survival time. These results suggest that both helper-dependent and helper-independent $CD8^+$ cells may mediate or contribute to GVHD. Exogenous help is not essential for GVHD induction but can significantly augment the intensity of the disease.

One final feature of GVHD mediated by $CD8^+$ cells should be mentioned. As discussed earlier, $CD4^+$ cells given in large doses can protect mice against anti-class II GVHD. The same finding applies to GVHD directed to class I differences. Thus, whereas small doses of $CD4^+$ cells potentiate the capacity of $CD8^+$ cells to elicit GVHD in class I-different hosts, supplementing $CD8^+$ cells with large doses of $CD4^+$ cells provides strong protection with a marked reduction in mortality rates (31). As for class II differences, the most likely explanation for this finding is that $CD4^+$ cells provide T helper function which allows the host to mount immune responses to opportunistic infections. This notion is in line with the observation that mice kept in a germ-free environment are comparatively resistant to lethal forms of GVHD (32).

GVHD Responses to Minor H Antigens

Although MHC differences provide the strongest stimulus for GVHD, transfer of untreated bone marrow cells across non-MHC barriers in mice often leads to severe GVHD. Lethal GVHD requires heavy irradiation of the host and is directly proportional to the number of T cells in or added to the donor marrow inoculum. Lethal GVHD is directed to multiple minor

H antigens and in some combinations, e.g., B10.BR → CBA/J, mortality rates approach 100% (14). The relative contribution of $CD4^+$ and $CD8^+$ cells to GVHD directed to minor H antigens varies according to the particular strain combination examined (33–35). In a recent study we compared the ability of purified donor $CD4^+$ and $CD8^+$ T cell subsets to mediate GVHD in six different MHC-matched, multiple minor H different strain combinations (34). $CD8^+$ T cells mediated lethal GVHD in each combination tested, with varying degrees of potency (in both incidence and median survival time). $CD4^+$ T cells alone failed to cause GVHD in four combinations, but led to severe GVHD in two other combinations; the GVHD-inducing potency of $CD4^+$ cells did not correlate with proliferation capacity in mixed lymphocyte reactions *in vitro* and was not enhanced by preimmunization of the donor mice.

Since minor H antigens are comparatively weak immunogens *in vitro* and are claimed to be heavily helper dependent, the question arises whether the capacity of purified $CD8^+$ cells to mediate lethal GVHD in minor H-different hosts depends upon exogenous help arising from contaminating $CD4^+$ cells of either donor or host origin. This possibility seems unlikely because injecting large doses of opsonizing anti-CD4 (GK1.5) MAb at time of transplant and/or at weekly intervals thereafter fails to reduce the severity of GVHD. In recent studies we have reexamined the helper dependency of $CD8^+$ CTL stimulated with either single or multiple minor H antigens *in vitro*, after priming *in vivo* (Wettstein PJ, Tews A, Korngold R, submitted for publication). For $CD8^+$ cells from B6 mice primed to either H-3, H-4, or H-Y single minor H antigens, generation of secondary CTL responses after *in vitro* exposure to these antigens was found to be helper independent. Thus, good responses were observed when the $CD8^+$ cells were cultured in the absence of $CD4^+$ cells and without added lymphokines. On the other hand, B6 $CD8^+$ CTL responses to BALB.B multiple minor H antigens were highly dependent upon the presence of $CD4^+$ cells in secondary culture; B10.BR anti-CBA CTL responses were partially dependent on $CD4^+$ cells. The relevance of these findings to GVHD induction is still unclear. Although the data suggest that some (but not all) anti-minor H antigen CTL responses are helper independent, it should be borne in mind that detection of CTL depends upon considerable expansion of the responding T cells. Such expansion might not necessarily be essential for GVHD induction.

The protective function of $CD4^+$ cells in anti-class II GVHD raises the question whether a high dosage of donor $CD4^+$ T cells can prevent mortality for GVHD directed to minor H antigens. This does not seem to be the case. Thus far in the B10.BR → CBA strain combination, in which $CD8^+$ T cells are potent mediators of disease and $CD4^+$ cells are inactive (34), a recent study showed that transfer of 2.5×10^7 donor $CD4^+$ T cells along with 2×10^6 $CD8^+$ cells and anti-Thy-1 plus complement-treated bone marrow caused no protection and in fact enhanced GVHD. Likewise, in the B10.D2 → DBA/2 combination, in which $CD4^+$ T cells are strong effectors for GVHD, increasing the dosage of purified donor $CD4^+$ T cells up to 2.5×10^7 resulted in a corresponding increase in the intensity of GVHD (36). Thus, the protective effect of $CD4^+$ cells does not seem to apply in MHC-matched situations. Until the mechanism of protection seen in the MHC-mismatched combinations is clarified, any possible clinical application of this phenomenon should be treated with caution.

We have recently compared the histopathology mediated by $CD4^+$ versus $CD8^+$ cells in minor H antigen-different combinations (41), using the combinations of C3H.SW → B6 (in which $CD8^+$ T cells alone cause lethal GVHD) and B10.D2 → DBA/2 (in which $CD4^+$ T cells are more potent effectors than $CD8^+$ T cells). In the first situation, B6 mice were irradiated (750 rads) and injected with a mixture of T cell-depleted C3H.SW bone marrow and 10^6 $CD8^+$ T cells; GVHD developed by the third week posttransplant and primarily involved the skin and liver, with only mild involvement of the gut. Dermal fibrosis was clearly evident between the fourth and fifth week, along with bronchiolitis, and most of the mice died by the end of the sixth week. Significantly, these histologic findings were only observed with donor $CD8^+$ T cells; donor $CD4^+$ cells caused no pathology. In the second combination, transfer of T cell-depleted B10.D2 bone marrow along with 10^6 $CD4^+$ T cells into irradiated (850 rads) DBA/2 mice resulted in similar dermal and hepatic changes, but with much more pronounced intestinal involvement, beginning around Days 30–35; epididymal lesions were seen but there were no prominent pulmonary lesions. A similar pattern of histopathology was seen in recipients of $CD8^+$ cells; tissue damage here was less marked than in mice given $CD4^+$ cells, which correlated with the lower mortality rates induced by $CD8^+$ cells than the $CD4^+$ cells in the B10.D2 → DBA/2 combination. Although these findings are based on only a limited number of strain combinations, the data suggest that $CD8^+$ and $CD4^+$ cells mediate quite similar patterns of tissue damage, with the exception of gut damage being more prominent in recipients of $CD4^+$ cells.

Immunodominance in GVHD Responses to Minor H Antigens

Studies on the *in vitro* generation of CTL in the C57BL/6By (B6/By) anti-BALB.B $H\text{-}2^b$-matched strain combination (37) have indicated that responses to individual minor H antigens are subject to the phenomenon of immunodominance. Although B6/By and

BALB.B strains express many different minor H antigens, CTL responses appear to be directed to only a few target antigens; these antigens were defined by using a panel of target cells from CXB recombinant inbred (RI) strains, which express different composites of the original BALB minor H antigens. The results indicate that there are two immunodominant minor H cytotoxic T cell-target (CTT) antigens in the B6/By anti-BALB.B response, CTT-1 and CTT-2 (Table 1). Second-order dominant antigens, CTT-3 and CTT-4, become prominent as targets only in the absence of CTT-1 or CTT-2 antigens. These data indicate that a hierarchy of T cell responses to minor H antigens exists *in vitro*; immunodominance might reflect competition for MHC sites at the antigen-presenting cell level or might be a manifestation of immunoregulation at the T cell level.

The immunodominance seen for CTL generation raises the question whether a similar hierarchy of immunodominant minor H antigens is operative in GVHD (38). This question was addressed by transferring B6/By T cells plus T cell-depleted bone marrow to lethally irradiated hosts, using BALB.B or various CXB RI strains as recipients. The results are interesting in that immunodominance for induction of GVHD seems to involve a different hierarchy of minor H antigens than for CTL generation *in vitro* (Table 1). The most striking finding is that RI strains expressing the strongest CTT antigens, e.g., CXB G (CTT-1 and CTT-2) and CXB K (CTT-1), cause only a very low incidence of GVHD, even when primed T cells are transferred. Instead, RI strains expressing the second order CTT-3 and CTT-4 antigens (and/or other antigens yet to be defined) provide the strongest stimulus for GVHD induction.

The discordance in the patterns of minor H antigens eliciting GVHD versus *in vitro* generation of CTL could have several explanations. Since unseparated T cells were used for GVHD induction, one possibility is that the effector cells for GVHD were largely $CD4^+$ cells (which have poor CTL activity) rather than $CD8^+$ cells. A second possibility is that some minor H antigens show a restricted tissue distribution; for example, CTT-1 and CTT-2 might be expressed only on lymphocytes (concanavalin A-treated blast cells were used as CTL targets *in vitro*) and thus be inappropriate stimulators for inducing GVHD *in vivo*. Third, in a GVHD setting, responses to the strong CTT-1 and CTT-2 antigens might generate suppressor cells specific for these antigens, leaving other (second order) antigens to be the targets for GVHD. Fourth, an entirely different set of minor H antigens might be subject to immunodominance *in vivo*. Indications for the existence of second order dominant antigens in GVHD induction was provided by the observation that CXB E donor T cells can cause lethal GVHD in either CXB G or BALB.B recipients, suggesting that in the absence of recognition of the immunodominant antigens expressed in CXB E mice (e.g., the CTT-3 and/or CTT-4 antigens) there is now a response to another set of antigens (possibly the CTT-1 and CTT-2 antigens) (38). On the other hand, CXB E T cells do not induce GVHD in irradiated B6/By recipients, which makes it unlikely that B6/By background minor H antigen differences that may exist between the CXB E and CXB G strains are responsible for the GVHD in CXB G mice. These findings raise the question of why the transfer of B6/By T cells to irradiated CXB G hosts fails to cause GVHD directed to the same second order dominant antigens that are recognized by CXB E donor T cells? Although this question has yet to be resolved, the observations would seem to place a major component of the immunodominance effect at the level of the donor T cells (either involving repertoire differences or immunoregulation), rather than merely on the competition between minor H antigens for MHC binding sites on antigen-presenting cells.

Table I. Immunodominance in the GVHD Response to Minor H Antigens in the B6/By → BALB.B and CXB RI Strain Combinations

Irradiated recipient strain	CTL target antigens *in vitro*	GVDH mortality
BALB.B	All	++++
CXB E	CTT-3 and CTT-4	++++
CXB G	CTT-1 and CTT-2	–
CXB I	CTT-1 or -2 and CTT-3 or -4	+++
CXB J	CTT-3 or CTT-4	+++
CXB K	CTT-1	–

Selective T Cell Depletion

The dominant involvement of $CD8^+$ T cells in the etiology of GVHD directed across either an MHC class I or multiple non-MHC minor H barrier is now well established. $CD4^+$ cells do induce GVHD in certain minor H antigen different combinations, but this is the exception rather than the rule. Therefore, for HLA-matched donor-host combinations, the question arises whether it might be beneficial to selectively deplete the marrow of $CD8^+$ cells. The inclusion of mature $CD4^+$ cells in the transferred inoculum might provide some degree of immunocompetence and allow the host to mount significant responses to pathogens, especially to viruses such as CMV. The inoculum of $CD8^+$ cell-depleted marrow could be supplemented with presensitized helper $CD4^+$ T cells and B cells; these cells could be prepared either from presensitized donors or from unprimed donor cells sensitized to viral antigens in tissue culture. This scenario, of course, would depend on the ability of HLA typing to clearly define donor-host incompatibilities, and, as in the case of HLA-

identical matches, on methods to predict whether the particular donor $CD4^+$ cells will cause GVHD directed to the non-HLA antigens. Although these latter methods are not yet available, we are encouraged by two recent observations: (i) a clinical trial in which selective $CD8^+$ T cell depletion in HLA-matched situations did not lead to a high incidence of GVHD, nor when it did occur was it of high severity (39); and (ii) a study of cloned T cells derived from HLA-matched GVHD patients, in which $CD8^+$ T cells specific for non-HLA antigens were always present, but $CD4^+$ cells were far more limited in their presence (40). Both of these reports indicate that $CD4^+$ T cells may not be highly reactive in GVHD to non-HLA antigens, but this notion is certainly in need of more expansive investigation.

To assess the validity of the selective T cell depletion approach, we have investigated whether minor H antigen-different C3H.SW $CD4^+$ T cells and B cells from murine CMV (MCMV) primed donors can protect irradiated B6 mice against a lethal dose of MCMV challenge (Korngold R, Shanley J, Shearer G, unpublished data). In control experiments with syngeneic (B6 → B6) mice, we have found strong protection against MCMV challenge provided that both $CD4^+$ cells and B cells are transferred to the irradiated hosts. Protection is only observed with primed donor cells, and $CD4^+$ T cells by themselves are ineffective. Survival correlates with the presence of significant serum anti-MCMV antibody titers at Day 7 after challenge and the absence of detectable replicating virus in the lungs. Protection thus seems to depend upon early production of antibody. Preliminary results with irradiated B6 mice given allogeneic C3H.SW donor cells presensitized to MCMV are very encouraging. As expected, selectively depleting the transferred cells of $CD8^+$ cells prevents GVHD. The key finding is that the remaining MCMV-primed lymphocytes ($CD4^+$ cells and B cells) are highly efficient at protecting the irradiated recipients from viral challenge. These data are still preliminary and optimum conditions for obtaining protection against MCMV have yet to be clarified. In terms of clinical application, it will be important to determine if the donor cells can be effectively primed to MCMV *in vitro*.

Summary

Considerable progress has been made in defining the relative contributions of $CD4^+$ and $CD8^+$ cells to GVHD. Studies in mice have shown that, in isolation, each T cell subset is able to induce lethal GVHD in irradiated hosts. For hosts differing at the MHC (H-2), $CD4^+$ cells cause GVHD directed to H-2 class II antigens whereas $CD8^+$ cells produce GVHD to H-2 class I antigens. With H-2-matched hosts expressing multiple minor H antigens, induction of lethal GVHD is largely under the control of $CD8^+$ cells. Which particular minor H antigens provide the targets for GVHD in mice is still unclear. Clarifying this question is complicated by the finding that the target antigens for GVHD do not necessarily correlate with the targets for cytotoxicity measured *in vitro*; moreover, immunodominance occurs when T cells are exposed to multiple minor H antigens *in vivo*. In terms of clinical application, there is a need to devise animal models for improving the success of HLA-matched bone marrow transplantation. Selectively depleting the marrow inoculum of $CD8^+$ cells and preimmunizing the donor against viral pathogens are two procedures which are currently under study.

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