Depletion of T lymphocytes from allogeneic bone marrow transplants successfully prevents the development of graft-versus-host disease (GvHD) but is associated with impaired engraftment, immunosuppression, and abrogation of the graft-versus-leukemia effect. We therefore explored the possibility of selectively eliminating alloreactive T cells by CD95/CD95L-mediated activation-induced cell death (AICD) in a major histocompatibility complex allogeneic murine model system. Activation of resting or preactivated T lymphocytes from C3H/HeJ (H-2b) mice was induced with irradiated BALB/cJ (H-2d) mouse-derived stimulators. Substantial decrease (≥ 80%) of proliferative and lytic responses by activated alloreactive T cells was subsequently achieved by incubating the mixed lymphocyte culture with an agonistic monoclonal antibody to CD95, and residual T cells recovered did not elicit alloreactivity upon challenge to H-2d. Depletion of alloreactive T lymphocytes by AICD was specific because reactivity to an I-A<sup>d</sup>–restricted ovalbumin (OVA) peptide by OVA-specific CD4<sup>+</sup> T cells mixed into the allogeneic T-cell pool and subjected to induction of AICD in the absence of OVA peptide could be preserved. Adoptive transfer of donor-derived allogeneic T lymphocytes, depleted from alloreactive T cells by AICD in vitro, in the parent (C3H/He) to F<sub>1</sub> (C3H/He × BALB/c) GvHD model prevented lethal GvHD. The results presented suggest that alloreactive T cells can effectively be depleted from allogeneic T cells by induction of AICD to prevent GvHD and might introduce a new strategy for the separation of GvH-reactive T cells and T cells mediating antiviral and possibly graft-versus-leukemia effects. (Blood. 2002;99:3041-3049)

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AICD specifically to a given alloantigen. In addition, depletion of activated alloreactive T cells prior to adoptive transfer of allogeneic T cells into F1 recipients prevents the occurrence of lethal GvHD, which invariably occurred in untreated controls.

The results presented suggest that elimination of alloreactive donor T lymphocytes following activation by CD95 death receptor–mediated depletion might provide one approach to modify the T-cell repertoire in allogeneic transplants and complement strategies to include pathogen-specific T lymphocytes and in vitro–generated tumor-specific T cells for immunotherapy in an allogeneic BMT.

Materials and methods

Mice and tumor cell lines

C3H/HeJ (H-2d) mice, BALB/cJ (H-2b) mice, (C3H/HeJ × BALB/cJ) F1 (H-2b×d) hybrid mice, and BALB/cJ OVA257-339-specific TCR transgenic mice originally obtained from Dr Loh and colleagues24 were bred and maintained at the Central Animal Facility of the University of Mainz. All mice strains were subjected to regular health status controls and were free of commonly tested viruses, parasites, and bacteria.

The animals were housed in sterilized microisolation cages with filter tops, and experimental procedures were performed according to German federal and state regulations and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

The age of mice to be used for experiments ranged from 8 to 14 weeks. For adoptive transfer experiments, only female F1 mice were taken as recipients.

The A20 tumor cell line represents a B-cell lymphoma derived from BALB/c mice. The thymoma cell line EL-4 was derived from C57BL/6 (H-2b) mice.

Media and reagents

The tumor cell lines A20 and EL-4 were propagated in RPMI 1640 medium (Biomed, Berlin, Germany) supplemented with 5% fetal calf serum (FCS). The tumor cell lines A20 and EL-4 were propagated in RPMI 1640 medium (Biomed) supplemented with 5% fetal calf serum (FCS), 10% horse serum (Gibco) and 1% antibiotic-antimycotic (Gibco). Culture medium for the induction of AICD in MLC assays

Cytolytic assays

A total of 2 × 10^6 A20 (H-2d) or EL-4 (H-2b) tumor cells was labeled with 100 μCi (3.7 MBq) of ^{51}Cr for 4 hours. After washing 3 times in PBS, labeled targets were placed at 1 × 10^3 cells per well in U-bottom microtiter plates (Nunc, Wiesbaden, Germany). Allogeneic CD90+ responder T cells (H-2b), preactivated in a primary MLC to H-2d spleen cells for 5 days, were washed 3 times and added to triplicate wells at varying effector-target ratios to be incubated for 5 hours. Maximum and spontaneous release were determined by incubation of targets with Triton X-100 (Sigma) or addition of media, respectively. ^{51}Cr activity in the supernatants taken 5 hours later was determined in a β-plate liquid scintillation counter (Wallace, Turku, Finland).

Experimental model for induction of lethal GvHD

Acute and lethal GvHD was induced in a classical P−F1 model using C3H/HeJ mice (H-2b) or BALB/cJ mice (H-2b) as donor of T cells and (C3H/He × BALB/c) F1 mice (H-2b×d) as recipients. Following depletion of alloreactive T cells in bulk culture MLC in the presence of agonistic anti-CD95 antibody for 5 to 6 days as described above, remaining T cells were harvested to be purified by immunomagnetic separation on a MACS.
investigated the possibility of deploying AICD as a method for selective depletion of alloreactive T cells in an allogeneic mixed lymphocyte reaction. Naïve T cells isolated ex vivo from C3H/He (H-2\textsuperscript{k}) mice were highly purified into CD4\textsuperscript{+} or CD8\textsuperscript{+} T-cell subpopulations by immunomagnetic separation using the MACS technology and cultured together with irradiated unfractionated splenic stimulator cells from BALB/c (H-2\textsuperscript{d}) mice in the presence of increasing concentrations of soluble agonistic mAb (Jo2) to CD95 for 5 to 6 days. Jo2 has previously been shown to induce apoptosis in lymphocytes.\textsuperscript{25} Following stimulation to the H-2\textsuperscript{d} alloantigen, the proliferative responses of both CD4\textsuperscript{+} or CD8\textsuperscript{+} alloreactive T lymphocytes were reduced by up to 90% in the presence of anti-CD95 mAb in a dose-dependent fashion when compared with the proliferation of untreated allogeneic controls (Figure 1). Reduction of proliferative responses was specific for anti-CD95–mediated apoptosis of T cells, because responder cells stimulated in the presence of an anti-CD95 mAb-matched hamster Ig isotype control showed comparable responses to untreated allogeneic controls (Figure 1). Flow cytometry analyses of CD90\textsuperscript{+} responders isolated at daily time points during

**Results**

**Proliferative responses of alloreactive T cells are strongly reduced after induction of AICD by agonistic mAb to CD95**

Because both CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells undergo CD95-mediated activation-induced apoptosis after prolonged activation,\textsuperscript{20-23} we
stimulation with T-cell–depleted allogeneic stimulators (H-2k) in a 6-day bulk culture by immunomagnetic separation revealed an increased CD95 expression within the first 36 to 48 hours following activation and an increase of apoptotic CD4+ and CD8+ T lymphocytes upon treatment with agonistic anti-CD95 mAb as measured by annexin V and 7-aminoactinomycin D viability staining.

Both naive and preactivated T lymphocytes are equally susceptible to AICD

Because T cells have been reported to be initially resistant to AICD upon primary activation but elicit increasing susceptibility to CD95-mediated induction of apoptosis 4 to 5 days upon sustained TCR re-engagement, we further investigated the induction of AICD on naive versus preactivated T cells.

Following polyclonal activation in vitro by TCR cross-linking through coated anti-CD3 mAb (145-2C11) for 3 days, CD4+ T-cell blasts as well as freshly isolated resting CD4+ T lymphocyte mice were stimulated with alloantigen (H-2b) in a bulk culture MLC in the presence of agonistic mAb to CD95 for 5 days to be tested for residual response to the stimulating alloantigen in a mixed lymphocyte reaction. Upon induction of AICD, both primary stimulated (Figure 2A) or restimulated (Figure 2B) T-cell responders showed a marked decrease in their capacity to proliferate to allogeneic splenic stimulators regardless of their preactivation state (note the reduced proliferation of 85% both in Figure 2A and 2B on day 3) in contrast to their untreated responder controls, although with an earlier kinetic in the response to H-2b for anti-CD3–preactivated cells.

There results suggested that induction of AICD at least in an allogeneic situation upon repetitive stimulation of T lymphocytes with MHC alloantigen was not dependent on the preactivation state of the alloreactive T cells.

Induction of AICD in alloreactive T cells is specific for the stimulating alloantigen

To evaluate whether alloantigen-specific T-cell depletion by AICD would retain T-cell specificities to defined nonalloantigens, we chose to add I-Ak-restricted OVA243-251-specific CD4+ T cells isolated from BALB/c OVA-TCR transgenic mice to allogeneic responder T cells from BALB/c mice (H-2b) at different ratios. These T cells should not be activated in the absence of OVA and thus not be susceptible to AICD. Following culture with splenic stimulators from F1 mice (H-2b) in the presence of cross-linked anti-CD95 mAb, remaining responder T cells were then challenged with F1 stimulators as well as with BALB/c-derived spleen cells presenting OVA peptide.

As expected from the above findings, restimulation to H-2k alloantigen in vitro resulted in an impaired proliferative response by these T cells compared with untreated controls (Figure 3A, in vitro). The proliferation of untreated T cells to H-2k observed in Figure 3 was truly allospecific because T cells from the OVA transgenic mice were shown not to cross-react with H-2b (data not shown). In contrast, proliferation of the responder cells to OVA was preserved in both anti-CD95–treated and untreated T-cell responder populations (Figure 3A, in vitro).

In addition, challenge of purified CD4+ and CD8+ T cells isolated ex vivo from F1 recipients 6 days after adoptive transfer of a mixture of OVA-specific T cells and allogeneic T-cell responders of BALB/c donors either left untreated or depleted of alloreactive cells by AICD into irradiated F1 mice confirmed the findings obtained in vitro. Again, the response of pretreated responders to H-2k alloantigen was reduced in contrast to untreated controls (Figure 3B, ex vivo), but stimulation of OVA-specific T cells by OVA peptide–presenting BALB/c spleen cells appeared not to be significantly different (Figure 3B, ex vivo) in both responder populations.

These results strongly suggested that induction of AICD using the agonistic mAb Jo2 to CD95 was specific in depleting alloreactive T cells to a given alloantigen while retaining T cells specific for non-MHC antigens like OVA.

Abrogation of cytolytic responses of alloreactive T cells after induction of AICD

We further tested whether induction of AICD by anti-CD95 mAb would also reduce the cytolytic responses of alloreactive T cells. Following stimulation of purified CD90+ responder T cells from C3H/He mice with irradiated allogeneic splenic stimulators from BALB/c mice in the presence of anti-CD95 mAb (1 µg/mL) in a bulk culture MLC for 5 days, remaining T lymphocytes were recovered from culture after treatment and tested in a cytolytic assay using chromium-labeled A20 cells as targets. The capacity of residual CTLs to kill A20 targets was reduced by at least 80% compared with untreated alloreactive control T cells, which exhibited 50% to 60% specific lysis at an effector–target ratio of 3:1.

Figure 2. Both resting and preactivated T lymphocytes are susceptible for AICD. Strongly decreased proliferation of responders after induction of AICD independent of whether alloreactive T cells had been subjected to anti-CD95 treatment during primary or secondary stimulation. Small resting CD4+ T cells (H-2k) or CD4+ T cells preactivated by TCR cross-linking using anti-CD3 mAb (145-2C11) for 3 days were stimulated with irradiated allogenic splenic stimulators (H-2b) in bulk culture in the presence of agonistic anti-CD95 mAb for 5 days. Residual T cells collected from these cultures were then tested for alloreactivity to the H-2b alloantigen in a 5-day MLC. Results are expressed as mean ± SD of triplicate wells and show 1 of at least 3 representative experiments. (A) Alloreactivity of responders primarily stimulated to H-2k alone (■) or in the presence of anti-CD95 mAb (■). (B) Alloreactivity of anti-CD3-preactivated responders after restimulation to H-2b alone (■) or in the presence of 1 µg/mL anti-CD95 mAb (■). Syntigeneic controls are shown without (□) or with (●) induction of AICD.
irradiated (6.5 Gy [650 cGy]) within 6 to 8 hours prior to
H-2 dxk ) murine GvHD model.

Figure 3. Depletion of alloreactive T cells by AICD to the H-2d alloantigen is specific. Purified CD4+ responder T cells from BALB/c mice (H-2d) mixed with OVA-specific CD4+ T cells derived from BALB/c (H-2d) at a 1:1 ratio were stimulated with spleen cells from F1, mice (H-2dm) in the absence or presence of anti-CD95 mAb (1 μg/mL) cross-linked by protein G (2 μg/mL) in a bulk MLC for 5 days. Remaining T cells were then challenged in vitro (A) with syngeneic spleen cells ( ), H-2dm alloantigen ( ), or with H-2d stimulators presenting OVA peptide ( ). In addition, remaining T-cell responders from primary cultures were transferred into irradiated F1 recipients. On day 6 after transfer, splenic responders recovered from these recipients ex vivo (B) were tested for reactivity to syngeneic spleen cells ( ), H-2dm alloantigen ( ), as well as to OVA peptide ( ). Shown are stimulation indices from cultures harvested on day 6. Results are expressed as mean ± SD of triplicate wells and represent 1 of 2 reproducible experiments. (A) In vitro restimulation of responder T cells depleted of alloreactive cells by AICD during primary MLC resulted in a decreased reactivity to H-2dm compared with alloreactive T cells left untreated. However, proliferative responses of OVA-specific T cells stimulated with OVA peptide were comparable between anti-CD95–pretreated T cells and untreated controls. (B) Alloreactivity of responders isolated ex vivo after adoptive transfer of pretreated T lymphocytes to H-2dm was strongly impaired in contrast to untreated controls. Again, specific and comparable OVA responses could be measured in both responder populations regardless of initial depletion by AICD or no treatment.

Figure 4. Cytolytic response of alloreactive T cells after induction of AICD. A representative experiment showing impaired capacity of allogenic CTLs to lyse H-2d-expressing A20 target cells compared with H-2d by AICD ( ● ) in comparison to untreated activated allogeneic control cells ( ○ ). (A) Lytic activity of anti-CD95–treated and untreated alloreactive T cells after 2, 10, and 60 minutes of incubation with H-2d target cells. (B) Conventional dose–response curve of the cytolytic activity of AICD alloreactive T cells. Values shown represent 1 of 3 representative experiments. (C) Alloreactive T cells were treated with anti-CD95 mAb cross-linked by polyclonal anti–hamster Ig prior to adoptive transfer of purified CD4+ ( Figure 5A ) or CD8+ ( Figure 5B ) allogeneic T cells resulted in the amelioration of acute GvHD in F1 recipients. In this experimental group all recipient mice survived and were free of clinical GvHD, as did control mice that received syngeneic spleen cells. Prevention of GvHD was stable and lasted until day 120, when the monitoring was terminated.

In recent experiments we also used this P→F1 model for allogeneic BMT in which 2×10^7 T-cell–depleted BM cells of H-2d origin were transplanted together with 2.5×10^8 purified allogeneic (H-2d) CD90+ T cells or T cells subjected to anti-CD95 treatment prior to transfer. These studies appear to confirm the results described above because F1 recipients that received pre-treated donor T lymphocytes did not develop GvHD, in contrast to F1 BMT controls.

Histopathological examination of sections taken from skin, small intestine, and spleen at different time points after adoptive transfer confirmed the survival data: apoptotic cells in the basal layer of epidermis and inflammation in the cryptic epithelium within the small intestine due to infiltrating lymphocytes were clearly detectable in mice injected with unmodified donor T cells, in contrast to F1 recipients that had received T cells depleted of alloreactive lymphocytes ( Figure 6 ). Moreover,

Recipients of allogeneic T cells depleted from host reactivity by AICD do not develop GvHD

To verify the efficacy of depleting alloreactive T cells by AICD in vitro, we investigated the potential of residual T cells to evoke a GvHD in the parent C3H/He (H-2k)→F1 (BALB/c × C3H/He, H-2k× ) murine GvHD model. Naïve allogeneic CD90+ donor T lymphocytes or CD90+ T cells primed to H-2k BALB/c spleen cells in vivo 14 days prior to isolation from donor mice were cultured with irradiated BALB/c-derived splenic stimulators in bulk MLC in the presence of Jo2 mAb for 5 to 6 days. Remaining responders were separated into CD4+ or CD8+ donor T cells by immunomagnetic separation to be injected into the tail vein of F1 recipients irradiated (6.5 Gy [650 cGy]) within 6 to 8 hours prior to transfer. During the preparation of T-cell responders, extensive care was taken to wash out excessive anti-CD95 mAb. Furthermore, viability and purity of cell preparations was assessed by flow cytometry as described in “Materials and methods.” A dose response of donor T cells depleted of alloreactivity or left untreated was performed, and F1 mice that received 1×10^6 to 5×10^6 in vivo–primed and in vitro–restimulated donor T lymphocytes invariably developed an acute GvHD between days 9 to 18 after transfer depending on the amount of T cells injected. GvHD was clinically characterized by rapid weight loss, severe diarrhea, and hunched posture, and all mice died within 20 days after T-cell transfer, as shown for injection of 2.5×10^6 T cells per recipient in Figure 5.

Surprisingly, adoptive transfer of donor T lymphocytes depleted of alloreactive donor cells by soluble mAb to CD95 alone also led to the development of lethal GvHD ( Figure 5 ). However, depletion of activated alloreactive T cells using anti-CD95 mAb cross-linked by polyclonal anti–hamster Ig prior to adoptive transfer of purified CD4+ ( Figure 5A ) or CD8+ ( Figure 5B ) allogeneic T cells resulted in the amelioration of acute GvHD in F1 recipients. In this experimental group all recipient mice survived and were free of clinical GvHD, as did control mice that received syngeneic spleen cells. Prevention of GvHD was stable and lasted until day 120, when the monitoring was terminated.

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The significant incidence of GvHD following transfer of donor T lymphocytes contained in the stem cell graft to recipients remains as a major caveat in allogeneic BMT, although various strategies for the prevention have been proposed.2-9 In particular, T-cell depletion is undoubtedly a very efficient method to prevent GvHD; however, it increases the risk of severe infections caused predominantly by Epstein-Barr virus26 and cytomegalovirus,10 engraftment failure,11 and relapse of leukemia.12,13 An ideal strategy for a specific and individualized immunotherapy would thus aim for selective elimination of host-reactive T lymphocytes but would leave all other T-cell specificities recognizing Epstein-Barr virus and cytomegalovirus or leukemia-restricted (minimal) histocompatibility antigens and leukemia antigens unaffected. Various experimental means, including elimination of alloreactive T cells by immunoconjugating idiotypic mAbs to CD25 mAb (as described by Datta et al14 and followed by others8) or via the early T cell activation antigen CD69,27 have been employed. Furthermore, suicide gene transfer into donor T lymphocytes,4,5 induction of energy by blocking costimulatory signals necessary for optimal T-cell activation,5,7 and the administration of cytokines to modify T-cell effector responses9,28 have been reported. These methods, however, affect either primarily T-cell subpopulations6,7 or all T cells regardless of their specificity.4,5 They also appear not to be very effective on the negative selection of alloreactive T cells,14 technically quite complex,27 or limited by the development of resistant T lymphocytes transduced with the HSV-Tk suicide gene to ganciclovir.29 Alternatively, the in vitro generation of alloreactive T cells conferring GvL reactivity without causing GvHD has been described.16,19

We explored the possibility of separating alloreactive T cells from antiviral T-cell specificities and possibly GvL-conferring T lymphocytes using the CD95/CD95L-mediated AICD as a strategy to deplete GVH-reactive T lymphocytes by apoptosis. AICD via the CD95 system has been demonstrated to occur in T-cell hybridomas as well as in human and murine T cells following activation by TCR cross-linking or by antigen and is thought to represent an important mechanism to limit the clonal expansion of activated T cells at the down phase of an immune response.20-22,30,31 AICD also plays a major role in the maintenance of peripheral T cell tolerance.20,32 Mature T cells acquire susceptibility to AICD after prolonged activation.20-22 This process requires repetitive TCR re-engagement by antigen or anti-TCR/CD3 complex antibodies, is dependent on cell cycle progression caused by interleukin-2,23,33 and results in apoptosis of antigen-specific T cells but not of resting T cells.20,22,23

Using an (MHC) allogeneic model system we first demonstrated the requirements for induction of AICD in vitro. Secondly, we used this physiologic mean to deplete alloreactive T cells from an allogeneic T-cell repertoire. Upon stimulation of purified naive T lymphocytes from C3H/He mice with BALB/c mouse–derived allogeneic splenic stimulators in the presence of an agonistic mAb (Jo2) to CD95 during a primary MLC proliferation of both CD4+ or CD8+-alloreactive T cell was substantially (≥ 80%) decreased in comparison to control alloreactive responses. Reduction of proliferative responses was mediated by the agonistic anti-CD95 mAb Jo2 23 in a dose-dependent fashion. Seemingly, the capacity of CTLs to lyse allogeneic target cells was also strongly impaired after depletion of alloreactive responders by CD95-mediated AICD. Flow cytometry analysis of responder T cells performed at

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**Discussion**

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**Figure 5. Depletion of alloreactive T cells by AICD prevents acute GvHD in a murine P–F1 GvHD model.** Depicted are survival curves representing 3 independent experiments in which F1 recipients (n = 10) received 2.5 × 10^6 purified viable parental donor T lymphocytes. T cells from C3H/He mice (H-2k) previously primed to H-2d alloantigen in vivo were restimulated with H-2d-expressing BALB/c stimulator cells and depleted from alloreactivity by AICD. Following adoptive transfer of treated T lymphocytes into irradiated (6.5 Gy [650 cGy]) female F1 hosts, recipients did not develop acute GvHD, in contrast to mice injected with untreated allogeneic control cells. (A) Depletion of transferred CD4+ T cells from host reactivity by induction of AICD into F1 recipients prevents development of acute GvHD (P < .05). In contrast, transfer of activated untreated control cells (v) or donor T lymphocytes depleted of alloreactive responders by soluble anti-CD95 mAb (o) led to rapid and lethal GvHD within 20 days after transfer (P < .05). Controls shown received equivalent numbers of syngeneic spleen cells (□). (B) The same experiments as described in panel A but performed with allogeneic CD8+ T cells are presented.
different time points during MLC with T-cell–depleted stimulator cells revealed that activated alloreactive T lymphocytes up-regulated expression of the activation markers CD69 and CD25 as well as CD95 in a time-dependent fashion and became susceptible for AICD when cultured with agonistic anti-CD95 mAb. Induction of AICD upon activation of alloreactive T cells with one particular alloantigen was specific and did not affect I-Ad–restricted T lymphocytes expressing the transgenic αβ-TCR (DO11.10) specific for the OVA233–349 peptide upon titration into the experimental system. Moreover, OVA-specific T cells could be restimulated from F1 recipients after adoptive transfer of a mixture of OVA–T cells and allogeneic responders pretreated by AICD. Thus, depletion of alloreactivity by AICD retained functional T-cell specificities to defined nonalloantigens.

We then established a classical parent (C3H/HeJ or BALB/cJ) F1 murine model. F1 models have become an invaluable tool in a number of adoptive transfer and BMT studies on GvHD and GvL because recipients cannot evoke any host-versus-graft effects, in contrast to models using 2 different mice strains. Our model also allows us to use CD95L-deficient C3H/gld donor T cells as controls for current studies on the GvL effect of anti-CD95–pretreated T cells.

Figure 6. Histologic examination of GvHD. Induction of lethal acute GvH reactions after adoptive transfer of allogeneic donor T lymphocytes or donor T cells depleted of alloreactivity by AICD was examined histologically and by immunohistology as described in “Materials and methods.” On day 6 after transfer, 2 mice from each group were killed and tissue from skin, liver, spleen, small intestine, and lymph nodes was fixed or embedded. Depicted are sections of skin (A) and small intestine (B) (magnifications of 100 μm and 200 μm) stained by hematoxylin and eosin, which show cells in the basal layer of the epidermis undergoing apoptosis and lymphocytic infiltrations into the cryptic epithelium of small intestine following adoptive transfer of allogeneic donor cells. In addition, immunohistology (shown in red) revealed massive expansion of CD4+ T cells and, albeit to a lesser extent, of CD8+ T cells in spleen (C) isolated from F1 recipients that had received untreated allogeneic donor T cells. In contrast, no significant GvHD histopathology could be detected in recipients administered donor T lymphocytes subjected to AICD prior to transfer.

Figure 7. Reduced in vivo expression of donor T cells depleted from host reactivity by AICD. Shown by flow cytometry analysis is a very low expansion of allogeneic CD4+ or CD8+ T cells (H-2K) depleted of alloreactive responders (B) in contrast to significantly increased populations of untreated allogeneic donor CD4+ and CD8+ T cells (A) in F1 hosts 10 days after transfer. Monitoring the expansion of donor-derived T cells was performed on CD4+ and CD8+ T cells isolated from spleen and lymph node suspension by immunomagnetic separation and stained for H-2K and H-2D to discriminate single-positive donor-derived (H-2K) T cells from F1-specific (H-2K) T lymphocytes.
In line with the results obtained in vitro, we could show by adoptive transfer of donor-derived T lymphocytes that F1 recipients that received allogeneic T cells, depleted from alloreactive responders by AICD in vitro, did not develop a lethal GVHD. In contrast, mice receiving untreated allogeneic T-cell blasts displayed all clinical signs of acute GVHD and died within 20 days after transfer. Recent experiments performed on lethally irradiated F1 mice and receiving BM cells together with pretreated donor T cells also did not yet result in the induction of GVHD compared with controls.

Additionally, histopathological examinations of skin and small intestine sections taken from F1 mice with GVHD revealed apoptotic cells in the basilar lamina of epidermis and infiltration of lymphocytes into cryptic epithelium of small intestine. The monitoring of allogeneic donor cells in the host by flow cytometry also supported the findings described, because 5-fold expansion of alloreactive T cells was exclusively seen in mice receiving untreated allogeneic T lymphocytes. In contrast, very low host-reactive cells were detected in mice that had received allogeneic cells depleted of alloreactivity. Moreover, splenic cells isolated from F1 recipients 6 days after adoptive transfer of both anti-CD95–pretreated OVA-specific T cells and donor T cells could respond to OVA peptide, whereas proliferation to H-2k was reduced.

In summary, these results strongly suggest that depletion of alloreactive T lymphocytes by AICD prevents GVHD and retains functional specificities of non-GVH–reactive donor T cells for engraftment.

Although we cannot exclude that a low number of alloreactive T cells might escape induction of AICD because it has been reported that some T cells escape CD95-mediated apoptosis to become memory cells,34 the numbers of remaining alloreactive T cells in our GVHD model appear to be below a clinically relevant threshold. In fact, considering the diverse repertoire of alloreactive T-cell specificities that might confer a GVH effect, it might be conceivable to tolerate a very low number of, for example, defined alloreactive T-cell subpopulations. Because CD4+ T helper 1 (Th1)–type T cells have been described to be more susceptible to CD95-mediated AICD than their Th2 type counterparts,35 depletion of alloreactive T cells by AICD also polarizes residual CD4+ GVH-reactive T lymphocytes toward a Th2-type (data not shown), which additionally might protect against clinical manifestation of acute GVHD.36,37 Moreover, because the CD95/CD95L system has been documented by several groups to play an important role in the pathogenesis of GVHD18,39 and blockade of this pathway appears to ameliorate GVHD without impairing GVL effect,40 it remains to be elucidated whether the elimination of alloreactive T lymphocytes by the induction of AICD results in the modifications of allogeneic donor T lymphocytes suitable for preserving a GVH effect in the absence of a clinically manifested GVHD.

In conclusion, we have established an (MHC) allogeneic model system that allows elimination of alloreactive T cells by the induction of CD95-mediated AICD. Transfer of a modified allogeneic donor T-lymphocyte repertoire into a P→F1 model demonstrated that depletion of alloreactive T cells was sufficient for preventing a lethal GVH in F1 recipients. Thus, utilizing AICD for elimination of alloreactivity in allogeneic transplants might represent a suitable strategy for the enrichment of T cells that mediate an antiviral as well as a GVL effect.

Further studies on the phenotypic and functional characterization of the residual allogeneic T lymphocytes as well as their capacity to confer a GVH effect are in progress. They will help us assess the utility of AICD to optimize clinical BMT.

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References


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