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Recipient lymphocyte infusion in MHC-matched bone marrow chimeras induces a limited lymphohematopoietic host-versus-graft reactivity but a significant antileukemic effect mediated by CD8⁺T-cells and natural killer cells

Lien De Somer,¹,² Ben Sprangers,¹ Sabine Fevery,¹ Omer Rutgeerts,¹ Caroline Lenaerts,¹ Louis Boon³, Mark Waer¹ and An D. Billiau¹

¹Laboratory of Experimental Transplantation, University of Leuven, Belgium; ²Division of Pediatrics, Department Woman and Child, University of Leuven, Belgium, and ³Bioceros BV, Utrecht, The Netherlands

Correspondence
An D. Billiau, Laboratory of Experimental Transplantation, Campus GHB O&NI box 811 Herestraat 49, 3000 Leuven, Belgium. Phone: international + 32.16.346022. Fax: international + 32.16.346035. E-mail: an.billiau@med.kuleuven.be

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**Background.** Challenge of MHC-mismatched murine bone marrow chimeras with recipient-type lymphocytes (recipient-lymphocyte-infusion, RLI) produces antileukemic responses in association with rejection of donor chimerism. In contrast, MHC-matched chimeras resist eradication of donor chimerism by RLI. Here, we investigated lymphohematopoietic host-versus-graft reactivity and antileukemic responses in the MHC-matched setting, which is reminiscent of the majority of clinical transplants.

**Design and Methods.** We challenged C3H→AKR radiation chimeras with AKR-type splenocytes (=RLI) and BW5147.3 leukemia cells. We studied the kinetics of chimerism using flowcytometry and the mechanisms involved in antileukemic effects using in vivo antibody-mediated depletion of CD8+ T and NK-cells, and intracellular cytokine staining.

**Results.** Whereas control chimeras showed progressive evolution towards high-level donor T-cell chimerism, RLI-chimeras showed a limited reduction of donor chimerism with delayed onset and long-term preservation of lower-level mixed chimerism. RLI chimeras nevertheless showed a significant survival benefit after leukemia challenge. *In vivo* antibody-mediated depletion experiments showed that both CD8+ T-cells and NK-cells contribute to the antileukemic effect. Consistent with a role for NK-cells, the proportion of IFN-γ-producing NK-cells in RLI-chimeras was significantly higher than in control chimeras.

**Conclusion.** In the MHC-matched setting, RLI elicits lymphohematopoietic host-versus-graft reactivity that is limited but sufficient to provide an antileukemic effect, and this is dependent on CD8+ T-cells and NK-cells. The data indicate that NK-cells are activated as a bystander phenomenon during lymphohematopoietic T-cell alloreactivity and thus support a novel type of NK involvement in anti-tumor responses after post-transplant adoptive cell therapy.
INTRODUCTION

Donor lymphocyte infusion (DLI) after allogeneic stem cell transplantation (alloHSCT) represents a successful strategy to induce or reinforce graft-versus-leukemia (GvL) responses. In the current understanding, donor T-cells and recipient antigen presenting cells (APC) in the lymphohematopoietic compartment play a critical role in initiating GvL responses, whereas donor APCs have been proposed to contribute by maintaining alloreactive and antitumor T-cell activity through cross-presentation of alloantigens and tumor antigens.(1-3) In clinical and experimental models, DLI-induced GvL is usually associated with conversion from mixed to full chimerism and in those cases where DLI is used to prevent relapse, this is also the actual objective.(4-8) Lymphohematopoietic graft-versus-host T-cell reactivity is therefore considered critical. The major problem associated with induction of GvL by DLI is the high risk of graft-versus-host disease (GvHD). The overall incidence of acute GvHD is 19–60%, with grade III–IV GvHD affecting 6–35% of patients. Chronic GvHD occurs in 33–61% of patients, and mortality rate attributable to GvHD is in the range of 6–11%.(9)

Recent studies indicate that not only graft-versus-host, but also host-versus-graft lymphohematopoietic T-cell reactivity can participate in the effector phase of an anti-tumor response. The exploitation of antileukemic effects initiated and/or effectuated by recipient immune cells holds the invaluable advantage of not causing GvHD. In the clinic, 2 studies have reported on a group of patients in which loss of donor chimerism was still associated with a potent anti-tumor response.(10;11) Inspired by this clinical observation, the group of M. Sykes developed a MHC-mismatched mouse model where recipient lymphocyte infusion (RLI) induced an antileukemic effect.(12) This was associated with a strong lymphohematopoietic host-versus-graft reaction resulting in rapid loss of donor chimerism. The antileukemic effect was shown to be dependent on recipient CD4+ T-cells, recipient iNKT and RLI-derived CD8+ T-cells.(13;14)

Interestingly, we had previously shown that in AKR→C3H MHC-matched BM-chimeras such a challenge with RLI does not result in a loss of donor chimerism.(15) This is in contrast with the strong lymphohematopoietic graft-versus-host response that is generally seen after DLI in these chimeras(16) and also with the pronounced lymphohematopoietic host-versus-graft response seen after RLI in the MHC-mismatched model.(12) Thus, in MHC-matched chimeras, lymphohematopoietic alloreactivity elicited by RLI follows a particular course.

Here, we aimed to explore in more detail how adoptive cell therapy with recipient lymphocytes influences lymphohematopoietic T-cell alloreactivity -and possibly antileukemic responses- in the MHC-matched setting, which is representative of the majority of clinical transplants.(17) We found that RLI resulted in a limited and delayed-onset lymphohematopoietic host-versus-graft response with long-term preservation of mixed chimerism; this was nevertheless associated with a significant antileukemic response involving CD8+ T-cells and NK-cells.
DESIGN AND METHODS

Bone marrow transplantation
AKR and C3H mice were obtained from Harlan BV (Horst, The Netherlands). Recipient mice were given 9.5 Gray total body irradiation on day -1 and 5x10⁶ T-cell-depleted AKR or C3H bone marrow (BM) cells on day 0.(16) At indicated time points, BM-chimeras received an IV infusion of 50x10⁶ host-type AKR (RLI) or donor-type C3H (DLI) splenocytes. For leukemia survival studies, mice were challenged, 1 week after DLI or RLI, with 5x10⁶ BW5147.3 leukemia cells (AKR mouse lymphoma; ATCC, Rockville, MD).(16) All experiments were approved by the Ethical Committee for Animal Science of the K.U.Leuven.

In vivo cell depletion
Anti-asialoGM1 (Wako, Germany) Ab was administered IP (20 µl per mouse), twice weekly, from day 16 after allogeneic bone marrow transplantation (BMT) to deplete NK-cells. RLI-donor mice were given 2 doses of anti-asialoGM1 Ab at day-3 and day-1 before sacrifice. YTS169 anti-CD8 mAb (Bioceros BV, Utrecht, The Netherlands) was administered IP. (200 µg/mouse) to deplete CD8⁺ T-cells on day 28 and 29 after BMT and further continued twice weekly. RLI-donor mice were given 200 µg of anti-CD8 mAb on day -2 and day-1 before sacrifice.

Flowcytometry
Flowcytometry studies were performed on peripheral blood and spleen cells collected at indicated time points, using a FACS Canto (BD Biosciences, Belgium) and mAb against mouse Thy1.1, Thy1.2, CD3, CD4, DX5, IFN-γ (intracellular staining, according to manufacturer’s instruction) or the appropriate isotype control Ig (Serotec, BD Biosciences).

Mixed lymphocyte reaction
3x10⁵ MACS-isolated CD4⁺T-cells isolated from RLI-chimeras and control chimeras were stimulated with 1x10⁶ MACS-isolated CD11c⁺DC (Miltenyi Biotec, The Netherlands) isolated from naive AKR and C3H mice, in a final volume of 200µl/well in a flat-bottomed 96-well plate for 5 days at 37°C and 5%CO₂. Cultures were harvested after a 16 hour-pulse with 1mCi [3H]TdR. Results are expressed as stimulation index (mean counts per minute of stimulated cells/means counts per minute of non-stimulated cells).

Statistical analysis
The Mann-Whitney U test was used to estimate the level of statistical significance of differences between groups of data. The Log-rank test was used to estimate the level of significance of differences in survival (p<0.05 was considered as evidence for statistical significance; Bonferroni correction was applied when multiple comparisons were performed).
RESULTS

Recipient lymphocyte infusion induces a late-onset and partial decrease in donor T-cell chimerism

First, we determined the kinetics of donor chimerism in peripheral blood taken at regular time intervals from chimeras given RLI on day 21 (RLI-chimeras) and chimeras not given RLI (control chimeras). In these experiments, all chimeras remained clinically healthy and survived long-term (not shown), indicating that RLI is a safe procedure. Control chimeras showed progressively increasing donor T-cell chimerism reaching high-level donor T-cell chimerism at week 10 after BMT (mean 72.6 ± 1.3 SE, n=11) and remaining stable until the end of follow-up (day 220). In chimeras given RLI at week 3, donor T-cell chimerism also followed a progressive increase, similar to that of control chimeras, until week 6; at this time point chimerism first stabilized and from week 8 onwards, it progressively decreased to a lower level of mixed chimerism at week 16 (mean % 36.9 ± 2.0 SE, n=12), which was maintained long-term (Figure 1A). We conclude that RLI elicits a slow and limited host-versus-graft T-cell response, as evident from the late-onset and partial decrease in donor T-cell chimerism.

We further documented that RLI elicited in vivo T-cell alloreactivity prior to the stabilisation and decrease of donor chimerism: in in vitro MLR assays, CD4+ T-cells obtained from RLI-chimeras on day 35 after BMT mounted a limited but clear proliferative response against donor and host antigens (Figure 1B).

The limited lymphohematopoietic host-versus-graft reactivity provoked by RLI is associated with a significant antileukemic effect

Next, we showed that the limited lymphohematopoietic host-versus-graft alloreactive T-cell response elicited by RLI is sufficient to elicit an antileukemic effect. RLI and control chimeras were challenged with BW5147.3 leukemia cells on day 28 after BMT. Animals were inspected daily and follow-up was terminated on day 130.

Whereas control chimeras showed 100% mortality from leukemic disease between day 37 and 63 after BMT, RLI-chimeras showed a significant survival benefit with 64 % mortality (occurring between day 53 and 130), and 36% long-term survival (p=0.01, log-rank test). In a selected experiment, we included a group of mice treated with DLI and leukemia challenge, and conform previous studies,(16;18) the DLI-challenged group showed 66% long-term survival after leukemia challenge (Figure 2).(16)

The antileukemic effect of RLI requires a bone marrow graft of allogeneic origin and a sufficient level of allogeneic donor chimerism

Having demonstrated that in vivo alloreactivity accompanies the antileukemic effect, we further documented the prerequisites for RLI to generate an antileukemic effect.

First, we challenged syngeneically transplanted AKR ->AKR chimeras with RLI on day 21 and with leukemia cells on day 28. In these animals an antileukemic effect could not be observed (mortality 100% by day 98 after BMT in both groups)
(Figure 3B). Next, we administered RLI at an early time point, i.e. day 7 after BMT, and challenged these mice with BW5147.3 leukemia cells on day 14. In contrast to the day-21-RLI effect on leukemia-free survival, chimeras given RLI on day 7 did not exhibit a survival benefit over controls (93.3% mortality by day 130 after BMT in both groups) (Figure 3C). Studies of the kinetics of chimerism revealed that RLI in the early post-transplant period prevented progressive engraftment, but on the other hand did not lead to complete graft rejection (mean donor T-cell chimerism on day 21 in RLI-day-7 chimeras was 9.8%± 0.6 SE, remaining stable until end of follow-up, whereas in control chimeras this was 26.8’%±4.2 SE (n=9), with a further progressive increase to 85.5%±2.3 SE (n=9) at day 70 after BMT) (Figure 3D). Finally, RLI administered to naive AKR mice or to AKR mice given total body irradiation only (without BMT) failed to generate an antileukemic effect (Figure 3A).

Taken together, these experiments show that a BM graft of allogeneic origin and a sufficiently high level of donor chimerism are critical prerequisites for RLI to induce an antileukemic effect.

The antileukemic effect of recipient lymphocyte infusion involves CD8+ T-cells and NK-cells

The observation that RLI elicits a limited lymphohematopoietic host-versus-graft T-cell response while producing a significant antileukemic effect suggested that in addition to T-cells, also non-T-cells - in particular NK-cells - may take part in the antileukemic effector mechanism. To investigate the role of CD8+ T-cells and NK-cells in the antileukemic effect of RLI, we administered anti-asialoGM1 and anti-CD8 antibodies in vivo: in order to obtain complete depletion of these CD8+ T-cells and NK-cells, both the RLI-donor mice and BM recipients were given depleting antibody treatment.

In these experiments, RLI-chimeras showed a significantly better survival rate after leukemia challenge than did control chimeras (p=0.011, log-rank test) (Figure 4A). The removal of CD8+ T-cells led to a significant reduction of the survival benefit relative to RLI-chimeras (p=0.003, log-rank test). When asialoGM1+ cells were depleted, this resulted in an even more pronounced reduction of survival benefit (p=0.00001, log-rank test) (Figure 4A).

In the current C3H->AKR strain combination, due to the lack of expression of the NK1.1-marker, we chose anti-asialoGM1-Ab to deplete NK-cells. In addition to NK-cells this antibody targets asialoGM1+CD8+ T-cells, a minor subset of CD8+ T-cells, of which the significance is incompletely understood. AsialoGM1+CD8+ T-cells have been reported to correspond with naive, antiviral or alloreactive CD8+ T-cells.(19-21) We documented the evolution of chimerism in anti-CD8-mAb and anti-asialoGM1-Ab treated mice, and found that the characteristic chimerism changes disappeared in both treatment groups (Figure 4B-C). We conclude that the abrogation of the lymphohematopoietic host-versus-graft response after anti-CD8-mAb treatment is due to the removal of alloreactive CD8+ T-cells, and since similar effects was seen after anti-asialoGM1-Ab treatment, that asialoGM1+CD8+ T-cells represent the alloreactive CD8+ T-cell subset. These data imply that
effects seen with anti-asialoGM1 antibody treatment may—at least in part—be attributed to depletion of this CD8⁺ T-cell subpopulation. However, the survival of asialoGM1-depleted chimeras was significantly worse than of CD8-depleted RLI-chimeras (p=0.006, log-rank test). These data indicate that the RLI-induced antileukemic effect in BM-chimeras is dependent not only on CD8⁺ T-cells, but, importantly, it also involves asialoGM1⁺ cells that are not CD8⁺ T-cells, in casu NK-cells. Consistent with this, using intracellular flow cytometry, we documented that 10 days after RLI, the proportion of IFN-g-producing NK-cells in RLI-chimeras was significantly higher than that in control chimeras (mean 12.7%±1.2 SE in RLI-chimeras (n=5) versus 6.7%±0.5 SE in control chimeras (n=4), p= 0.02, Mann-Whitney U) (Figure 5).

DISCUSSION

Strategies that exploit host-versus-graft lymphohematopoietic T-cell reactivity for the induction of antileukemic effects hold the theoretical advantage of avoiding GvHD. Conversely, such strategies carry the risk of complete graft rejection, as evident from clinical observations and from the findings in a recently published MHC-mismatched mouse model.(10-12) In the current study, we showed that in a model of MHC-matched allogeneic BMT, which is representative of 50-85% of all clinical transplants, RLI is not associated with a complete loss of donor chimerism. We observed a slow and limited lymphohematopoietic host-versus-graft response that was however associated with a significant antileukemic effect dependent on CD8⁺ T-cells and NK-cells. These data indicate that RLI in an MHC-matched, multiple miHC-mismatched setting holds the advantage of preserving mixed chimerism and transplant tolerance, and reveals a novel role for NK-cells in the antileukemic effect of adoptive cell therapy.

Looking at the kinetics of donor chimerism as a reflection of lymphohematopoietic host-versus-graft reactivity, we found that in the MHC-matched setting, RLI elicits a late-onset and only partial decrease of chimerism. This chimerism evolution is in contrast with the rapid conversion from mixed to full donor chimerism following DLI in the same experimental model(18) and with strong lymphohematopoietic alloreactivity generally seen in patients after DLI therapy.(4-8) We postulate that following lethal irradiation, infused healthy donor BM cells have a competitive advantage over residual host-type BM cells and that adoptively transferred donor lymphocytes reinforce this effect, leading to a rapid conversion from mixed to full donor chimerism. Moreover, in the context of DLI non-tolerant donor T-cells are confronted with abundant host APCs since donor chimerism at day 21 only amounts to ±15% which results in extensive donor T cell activation and total elimination of recipient hematopoietic cells. In contrast, in the case of RLI, non-tolerant recipient T-cells, infused at a similar time point will encounter a small number of donor APCs only. Our data are also in discrepancy with the rapid loss of donor chimerism following RLI in the MHC-mismatched setting, which is probably due to the strong mismatch in MHC-antigens. We postulate that the minor mismatch in transplantation antigens explains why in our model the
chimerism changes are slow to appear and result in a partial rejection of donor cells.

In vitro, CD4⁺ T-cells of RLI-chimeras generated a distinct proliferative response against donor and host antigens. Whereas the anti-donor response can be attributed to the reactivity of the non-tolerant T-cells from the RLI-inoculum against donor antigens, the anti-host response indicates that donor T-cells in the chimera mount an alloresponse upon encounter with additional host APCs from the RLI-inoculum. This may explain the biphasic evolution of donor chimerism after RLI, with an initial further increase (donor-anti-host) and a subsequent decrease (host-anti-donor).

Despite the limited lymphohematopoietic host-versus-graft response, RLI in the MHC-matched setting elicits a significant antileukemic response. In concordance with the concept that lymphohematopoietic alloreactivity is critical for antileukemic responses, we determined firstly that this RLI-antileukemic effect could only be elicited in mice given irradiation and a bone marrow graft of allogeneic (and not syngeneic) origin. Secondly, RLI in the early post-transplant period, when donor lymphohematopoiesis and the amount of donor APCs is very low, did not produce an antileukemic effect.

From the significant reduction in leukemia-free survival after anti-CD8-mAb treatment, we conclude that CD8⁺ T-cells play a role in the protection of an RLI-chimera against challenge with leukemia. This is conform the findings in the MHC-mismatched setting model where the anti-tumor response involved RLI-derived CD8⁺ T-cells.(13) The observation that the characteristic chimerism changes were abrogated in anti-CD8-mAb and anti-asialoGM1-Ab-treated chimeras indicates the critical role of alloreactive CD8⁺ T-cells and identifies asialoGM1⁺CD8⁺ T-cells as alloreactive T-cells, consistent with previous report by others.(20) Interestingly, a recent report showed asialoGM1⁺CD8⁺ T-cells to have a central memory phenotype and exhibit early IFN-γ production, leading the authors to propose a critical role in Th1-mediated immunity such as in tumor immunity.(19)

We found that also NK-cells contributed to the RLI-induced antileukemic effect. This was evident from the finding that the survival of asialoGM1-depleted RLI-chimeras was significantly worse than that of CD8-depleted RLI-chimeras. In addition, we documented that RLI led to an increase in IFN-γ-expressing NK-cells relative to control chimeras. The role of NK-cells in antileukemic responses after transplantation is increasingly being acknowledged.(22) particularly in the setting of KIR-ligand mismatched alloHSCT, where they might contribute to anti-tumor effects through killing on the basis of ‘missing-self’. (23;24) In contrast, in this study, we work in a MHC-matched transplantation model and NK-cells from naive AKR and C3H mice are not able to lyse BW5147.3 or C3H and AKR blasts in vitro (not shown). We therefore postulate that the lymphohematopoietic alloreactivity provoked by adoptively transferred non-tolerant T-cells provides a cytokine environment that—as a bystander phenomenon—activates NK-cells, thereby providing them with the capacity to recognize and lyse recipient-type tumor cells. This corresponds with the in vitro phenomenon known as lymphokine-activated killer cells.(25-27) This contribution of NK-cells to the RLI-
induced antileukemic effect is supported by preliminary findings in the MHC-mismatched model showing increased expression of CD69 by NK-cells after RLI.(14) It should be noted that in the current experimental set-up, in vivo antibody treatment may also interfere with a protective mechanism present in chimeras independently of RLI. In this respect, we noted that allogeneic BM chimeras show a significantly better survival after leukemia challenge as compared to naive recipient mice, whereas such a protective effect was not seen for syngeneically transplanted chimeras. Studying the mechanisms responsible for this effect, we were able to demonstrate that MACS-purified CD8+ T-cells, isolated from day-28 chimeras failed to lyse BW5147.3 cells ex vivo (51Cr release assay, data not shown), arguing against a role for CD8+ T-cells in the protection of allogeneic chimeras. In contrast, MACS-purified NK-cells from allogeneic chimeras, but not those from syngeneic chimeras or naive donor- or host-type mice exhibited pronounced cytotoxic reactivity against recipient-type tumor cells ex vivo (12% specific lysis in allogeneic chimeras relative to 0% in syngeneic chimeras, 0% in naive AKR and 0.4% in naive C3H mice (data from 1 of 3 experiments, not shown). When taking these data into account, we conclude that the reduction in leukemia-free survival of anti-asialoGM1-Ab-treated RLI-chimeras is–at least in part–due to interference with the NK-dependent protection present in allogeneic chimeras independently of RLI. On the other hand, these observations further support our hypothesis that lymphohematopoietic T-cell reactivity gives rise to NK-cell activation: in particular, we postulate that alloreactive CD4+ T-cells, when producing lymphohematopoietic graft-versus-host reactivity during engraftment, provide cytokines that activate NK-cells, thereby providing these NK-cells with anti-tumor activity. Reportedly, the dose of anti-asialoGM1-Ab used in the current study depletes NK-cells, but does not remove NKT-cells in vivo, owing to the low expression levels of asialoGM1 in these cells(28;29) suggesting that in our model iNKT-cells do not play a central role in RLI-induced anti-tumor immunity, as opposed to their role in the MHC-mismatched model as recently reported by Saito et al.(14) Interactions between NK-cells and CD8+ T-cells have been reported.(30-34) It has been shown that NK-cells can induce proliferation and/or differentiation of CD8+ T-cells into cytolytic effector T-cells,(32-34) and that CD8+ T-cells can become activated via IL-12, produced by DCs in response to IFN-g producing NK-cells.(30;31). Whether or not such interactions contribute to the RLI-effect remains to be determined. In conclusion, in the MHC-matched setting, RLI elicits lymphohematopoietic host-versus-graft reactivity that is limited but sufficient to provide an antileukemic effect. Long-term mixed chimerism is preserved and may offer a platform for additional GvL-inducing therapy with DLI. The antileukemic effect is dependent on CD8+ T-cells and NK-cells: the data indicate that NK-cells are activated as a bystander phenomenon during lymphohematopoietic T-cell alloreactivity and thus support a novel type of NK involvement in anti-tumor responses after post-transplant adoptive cell therapy.
Whereas the current study was performed in mice having mixed chimerism, in clinic, patients often evolve to full donor chimerism; we postulate that RLI in such patients, due to the abundance of donor-type APCs, would result in similar, if not stronger, lymphohematopoietic T-cell alloreactivity and bystander activation of NK-cells. Finally, insight in the immune effects of RLI may open interesting perspectives for the treatment of therapy-resistant solid tumors. Clinical data exist that provide a scientific background for the use of alloHSCT in the treatment of renal, colon and ovarium carcinoma and pancreatic tumors.(35-38). In this setting, where avoidance of GvHD is a particular objective, RLI may activate potent antitumor effects, specifically for tumors that are NK sensitive.

Authorship and Disclosures
LDS, BS, SF and ADB designed and performed all experiments and analyzed all data. OR and CL provided technical assistance. LB provided the anti-CD8 mAb. LDS, BS and ADB wrote the manuscript. MW oversaw the study and critically revised the manuscript.
The authors reported no potential conflicts of interest.

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**Figure 1. Evolution of donor T-cell chimerism and *in vitro* T-cell response of chimeric CD4⁺ T-cells 2 weeks after RLI.**

(A) Evolution of peripheral blood donor T-cell chimerism in animals receiving an allogeneic BMT only (AlloBMT), compared to animals receiving an allogeneic BMT and RLI on day 21 (AlloBMT+RLI). Results are shown from a total of 11 ‘alloBMT’ mice and 12 ‘alloBMT+RLI’ mice, from 2 identically designed experiments. Results are presented as mean % ± SE.

(B) At day 35 after BMT, CD4⁺ T-cells were isolated from chimeras given RLI at day 21 (n=6) and control chimeras (n=5) and stimulated *in vitro* with CD11c⁺ DCs isolated from C3H (donor) or AKR (host) mice. The proliferative response from individual mice is shown. P=0.04 between the RLI-chimeras and control chimeras for the proliferative response against donor antigens and p=0.006 between the RLI-chimeras and control chimeras for the proliferative response against host antigens as tested by Mann-Whitney U. Data from 2 identical independent experiments are shown.

**Figure 2. Antileukemic effects of RLI in C3H→AKR chimeras.**

The Kaplan-Meier leukemia-free survival is shown of C3H→AKR chimeras following challenge with BW5147.3 on day 28 after allogeneic BMT in mice given DLI on day 21 days after allogeneic BMT (AlloBMT+DLI, n=6), in chimeras given RLI 21 days after allogeneic BMT (AlloBMT+RLI, n=11) and in control chimeras nöt given cell therapy (AlloBMT, n=9). Results are shown from 2 identically designed experiments. *p=0.01 between AlloBMT+RLI and AlloBMT as tested by the Log-Rank test.

**Figure 3. Prerequisites for the antileukemic effect after RLI.**

(A) Kaplan-Meier leukemia-free survival after leukemia challenge on day 28 in allogeneic BM-chimeras given RLI on day 21 after BMT (AlloBMT + RLI, n=5), in naive AKR mice given RLI (Naive AKR + RLI, n=6) and in mice given RLI after TBI only, without an allogeneic BM graft (TBI + RLI, n=4). Results are shown from 1 experiment. p=0.03 between AlloBMT+RLI and Naive AKR+RLI, p=0.04 between AlloBMT+RLI and TBI+RLI as tested by the Log-Rank test.

(B) The Kaplan-Meier leukemia-free survival is shown after BW5147.3-challenge of syngeneic AKR BM-chimeras given RLI on day 21 after BMT (SynBMT+RLI, n=12), of syngeneic AKR BM-chimeras nöt given RLI (SynBMT, n=11) and of naive AKR mice (Naive AKR, n=6). Results are shown from 2 identically designed experiments. NS, not significant: p=0.9 between SynBMT + RLI and SynBMT as tested by the Log-Rank test.

(C) Kaplan-Meier leukemia-free survival after leukemia challenge on day 14 after allogeneic BMT in allogeneic BM-chimeras given RLI on day 7 after BMT (AlloBMT + RLI d7, n=15) and in allogeneic chimeras nöt given RLI (AlloBMT,
n=12). Results are shown from 3 identically designed experiments. NS, not significant: p= 0.35 between AlloBMT+ RLI d7 and AlloBMT as tested by the Log-Rank test.

(D) Evolution of donor T-cell chimerism in allogeneic C3H→AKR chimeras (AlloBMT, n=9) and in allogeneic chimeras challenged with RLI on day 7 (AlloBMT+RLI d7, n=9). Results are shown from 2 identically designed experiments. Results are expressed as % donor T-cell chimerism and presented as mean % ± SE.

Figure 4. Effects of anti-asialo-GM1Ab and anti-CD8 mAb treatment on leukemia-free survival and evolution of donor T-cell chimerism in allogeneic chimeras given RLI.

(A) Kaplan-Meier leukemia-free survival after leukemia challenge on day 28 in allogeneic BM-chimeras given RLI on day 21 after BMT (AlloBMT + RLI, n=35), in allogeneic BM-chimeras (AlloBMT, n=36), in RLI-chimeras depleted of CD8+ T-cells (RLI-chimeras+aCD8, n=13), RLI-chimeras depleted of asialoGM1+ cells (RLI-chimeras+anti-asialoGM1, n=22) and naive AKR mice (naive AKR, n= 30). Results are from a total of 7 experiments. p=0.011 between AlloBMT+RLI and AlloBMT, p=0.003 between AlloBMT+RLI and RLI-chimeras+aCD8, p=0.00001 between AlloBMT+RLI and RLI-chimeras+anti-asialoGM1, p=0.006 between RLI-chimeras+aCD8 and RLI-chimeras+anti-asialoGM1 and, p=0.00002 between AlloBMT and Naive AKR as tested by the Log-Rank test. Animals were followed until day 130.

(B) Evolution of peripheral blood donor T cell chimerism in control chimeras (AlloBMT, n=7), chimeras given RLI at day 21 after BMT (AlloBMT + RLI, n=8) and RLI chimeras given anti-CD8 mAb (RLI chimeras + aCD8, n=8). Results are from 2 identically designed experiments and are presented as mean % ± SE.

(C) Evolution of peripheral blood donor T cell chimerism in chimeras given RLI on day 21 after bone marrow transplantation (AlloBMT + RLI, n=8) and RLI chimeras given anti-asialoGM1 Ab treatment (RLI chimeras + anti-asialoGM1, n=11). Results are from 2 identically designed experiments and are presented as mean % ± SE.
Figure 5. IFN-gamma expression by NK cells in control and RLI chimeras. Intracellular IFN-gamma expression was determined using flowcytometry on splenic DX5+ cells of control chimeras and RLI chimeras, on day 10 after RLI. (A) Representative histograms of IFN- gamma expression in a RLI-chimera and a control chimera; the percentage IFN- gamma + cells is indicated as determined using isotype control antibody staining. (B) Mean IFN- gamma expression in RLI chimeras (n=5) and control chimeras (n=4). *p=0.02 as tested by Mann-Whitney U.
Figure 1
Figure 2
Figure 3

A

B

C

D

Proportion of surviving animals (%) vs Days after BMT

Proportion of surviving animals (%) vs Days after BMT

Proportion of surviving animals (%) vs Days after BMT

% of donor T-cell chimerism vs Days after BMT
Figure 4
Figure 5