Immune competence of cancer-reactive T cells generated de novo in adult tumor-bearing mice

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Running title: Immune competence of T cells generated in the presence of tumor antigens

Keywords: T cell recognition, immune tolerance, cancer immunity
Summary

The impact of timing of antigen introduction into fetus and neonates leads to the suggestion that pre-existing antigens are tolerogenic to immune competent cells generated thereafter. This hypothesis predicts that in cancer patients undergoing bone marrow transplantation, newly produced T cells with specificity for pre-existing tumor cells will be inactivated by the tumor antigens in the host. Since the effect of tumor cells on developing cancer-reactive T cells has not been investigated, we set out to systematically analyze the impact of tumor cells in the periphery on the development of tumor-reactive T cells in the thymus and their immune competence in the periphery. Our data demonstrate that in the host in which a tumor is established in the periphery, the cancer-reactive T cells develop normally, remain fully immune competent, become activated in the periphery and cause regression of large established tumor. The immune competence of T cells generated in an antigen-bearing host is also confirmed in a skin graft transplantation model.
Introduction

Classic studies by Medawar et. al. established a fundamental principle that antigens presented to an immature immune system are tolerogenic. This discovery forms the cornerstone of immunology, as it is the basic tenet of the clonal deletion theory proposed by Burnet, Talmage and Lederberg. Brestcher and Cohn formulated their two-signal theory in part based on the consideration that this process can be continuous throughout the life-span of animal. For antigens that are present throughout ontogeny, numerous studies have demonstrated the tolerogenic effect of pre-existing antigens on immune competent cells. What was less clear was the fate of self-reactive T cells produced de novo in adults to pre-existing antigens in the periphery, such as cancer antigens. Interestingly, studies with chimera mice suggested that for the same antigen and T cell combination, transgenic T cells specific for allogeneic antigen expressed in the skin were tolerized in neonatal, but not in adult mice.

In recent years bone marrow transplantation has become a promising cancer immunotherapy. Thus T cell tolerance in adult animals to antigens not expressed in the thymus is a fundamental issue considering the implications for immunity generated subsequent to bone marrow transplantation. In addition to a wealth of clinical data showing increased patient survival following bone marrow transplantation, it has also been demonstrated that tumor antigen-specific T cells...
have been produced after bone marrow transplantation\textsuperscript{10}. Likewise, donor cells transplanted into virally-infected baboons generated virus-specific CTL\textsuperscript{11}, however it remains unclear whether the antigen-specific T cells were derived from mature T cells in the bone marrow or from T cells produced \textit{de novo} in the antigen-bearing host.

In order to definitively address whether developing T cells in adult animals remain competent to peripheral tumor antigens, we investigated the development of transgenic T cells specific for tumor antigen P1A in tumor-bearing adult mice and analyzed their immune competence, antigen-induced activation and effector function. Here we report that cancer-specific cells produced \textit{de novo} in tumor-bearing mice survive thymic deletion and remain competent when encountering tumor cells causing the regression of large established tumors.

**Materials and methods**

**Experimental animals and cell lines** BALB/c and C57BL/6 mice were purchased from Charles River Labs (Wilmington, MA) under contract with the National Cancer Institute. BALB/c RAG-2\textsuperscript{(-/-)} and BALB/c nude mice were purchased from Taconic (Germantown, NY) and bred in our facility. Mice containing a transgenic T cell receptor for the tumor antigen P1A (P1CTL mice) have been described previously\textsuperscript{12}. Mice were housed in a specific-pathogen free environment.
Bone marrow transplantation Bone marrow from donor mice was obtained by flushing out marrow from the femur, tibia, and humerus. Red blood cells were lysed with ammonium chloride (Sigma, St. Louis, MO). Mature T cells were depleted by incubation with rat anti-mouse CD4 and CD8 mAbs, followed by goat anti-rat IgG Dynabeads (Dynal, Brown Deer, WI). T cells bound by Dynabeads were removed by magnetic separation, and T cell-depleted bone marrow was injected intravenously. For some treatment groups, T-depleted bone marrow from P1CTL mice was mixed with T-depleted bone marrow from BALB/c mice at a 3:2 ratio before injection.

Nude P1CTL bone marrow transplantation Athymic nude mice were bred with P1CTL mice to obtain F1 mice. F1 mice were screened for the presence of the TCR transgene by staining peripheral blood for the Vα8.3 TCR. F1 mice were bred together to produce F2 mice. Nude pups were screened for the presence or absence of the TCR transgene by PCR of tail DNA. Bone marrow from 3-4 week old TCR transgene-positive or transgene-negative mice was harvested as above. Red blood cells were lysed and bone marrow was injected intravenously.

Tumorigenicity experiments 1-5x10^6 J558 cells were injected subcutaneously in the lower abdomen. In experiments using mixed bone marrow from BALB/c and P1CTL donors, bone marrow was given at days 6 and 13 after tumor challenge. In the Nude P1CTL experiment, bone marrow was given in a single dose on day 2 after tumor challenge. In some experiments, mice were sacrificed
after one month to analyze spleen and thymus. Tumor size was measured in two perpendicular dimensions.

**Flow cytometry** Fluorochrome-labeled antibodies against cell surface markers and H-2L\textsuperscript{d} dimeric fusion proteins were purchased from BD Pharmingen (San Diego, CA).

**Allogeneic skin graft experiments** BALB/c mice were anesthetized and a 1-2 cm patch of flank skin was removed and replaced with a similar size patch of C57BL/6 skin. Grafts were bandaged for approximately one week, and mice possessing healthy intact grafts after at least 2 weeks were used for experiments. T-depleted bone marrow (prepared as above) or undepleted spleen cells from BALB/c mice were injected intravenously (bone marrow) or intraperitoneally (spleen cells) in two doses at one week intervals. Mice were observed for signs of graft rejection, and grafts were considered rejected when completely removed from mice.

**In vivo cytotoxicity assay** Spleen cells from BALB/c mice were pulsed with 10 \textmu g/ml of either P1A (LPYLGWLVF) or a control peptide (YPHFMPTNL) in the presence of either 5 mM or 0.5 mM of CFSE, respectively. After mixing at 1:1 ratio, the labeled cells were injected intravenously into recipients and spleen cells were harvested 4-6 hours later and analyzed by flow cytometry for the relative abundance of the CFSE\textsuperscript{hi} and CFSE\textsuperscript{low} populations.
Results

Tumor growth did not cause clonal deletion of tumor-specific T cells in the thymus

Since mice with a targeted mutation of RAG-2 lack endogenous T and B cells, tumors can be established in these mice that will not induce adaptive immune response prior to bone marrow transplantation. Taking advantage of this, we began by injecting plasmacytoma J558 into syngeneic RAG-2(-/-) mice. Once the tumors became palpable, the tumor-bearing mice were reconstituted with a mixture of T cell-depleted bone marrow from both syngeneic wild-type mice and transgenic mice expressing a TCR specific for the unmutated tumor antigen P1A\(^{12}\). As a control for the effect of tumor, we also generated chimera mice that were not tumor challenged.

We have previously demonstrated that more than 95% of transgenic T cells express high levels of transgenic TCR\(^{12,13}\), and therefore have the ability to bind to their cognate antigen. Based on this, we used the L\(^d\):P1A\(^-\) dimer to identify transgenic T cells\(^{14}\). As shown in Fig. 1a (upper panels), despite a 3:2 advantage of bone marrow from transgenic mice, the majority of T cells produced are from WT bone marrow as revealed by the fact that most of the thymocytes are L\(^d\):P1A\(^-\). To determine whether established tumors caused deletion of tumor-specific T cells, we compared the composition of cells derived from TCR-transgenic bone marrow in mice with or without pre-existing tumor. As shown in
Fig. 1a (lower panels), thymocytes from the non-transgenic donors have developed normally regardless of whether the recipients have tumor. Perhaps owing to deletion of P1CTL as a result of expression of P1A antigen in the thymic medullary epithelial cells, the phenotype of transgenic T cells is consistent with that of a partial clonal deletion, characterized by an increased proportion of CD4⁻CD8⁻ T cells and a decreased proportion of CD4⁺CD8⁺ T cells (Fig. 1a, lower panels). However, the profiles of thymocytes from tumor-free and tumor-bearing mice are indistinguishable (Fig. 1a, b). Moreover, in contrast to non-transgenic mature T cells, which consist of more CD4⁺CD8⁻ subset, there is a significant skewing toward CD8⁺CD4⁻ subset in both groups. This is consistent with a normal positive selection in both groups.

Since the P1A:Ld dimer has relatively low affinity for TCR in comparison with monoclonal antibodies, it is possible that it may not be able to detect P1CTL TCR at lower levels. To overcome this caveat, we also used anti-Vα₈.3 TCR antibody which provides about 10-fold stronger staining of the T cells. As expected, the anti-Vα₈.3 TCR antibodies marked T cells with broader levels of TCR/CD3 and thus a higher proportion of T cells. Nevertheless, the relative abundance of each subset was unaltered irrespective of which reagents were used to mark transgenic T cells (Fig. 1a). Therefore it appears that established tumors in the periphery have little impact on the development of cancer-reactive T cells in the thymus.

**Activation of T cells generated de novo in tumor-bearing mice**
It has been established that self-reactive T cells that exit the thymus are also controlled by peripheral mechanisms, including functional inactivation and activation-induced cell death.\(^6,15,16\). We compared the peripheral T cells from tumor-bearing and non-tumor bearing mice for their frequency and phenotype. As shown in Fig. 2a, the number of P1A-reactive T cells is significantly increased in tumor bearing mice. Since the number of transgenic T cells in the thymus is similar between tumor and non-tumor bearing hosts, it is likely that the T cells have expanded in the periphery. Consistent with this notion, essentially all splenic transgenic T cells in tumor-bearing mice exhibit a phenotype of activated T cells, as revealed by up-regulation of CD44 and down-regulation of CD62L (Fig. 2b).

Although we have depleted mature T cells from the bone marrow to barely detectable levels (data not shown), it remains possible that the cells responding to tumor antigens in the periphery are expanded from undepleted mature T cells. To rule out this possibility, we bred the P1CTL-transgene into the nude (\textit{nu/nu}) background and used the P1CTL\(^+\)nu/nu bone marrow cells to reconstitute tumor-bearing RAG-2\((-/-\)) hosts. As shown in Fig. 3, the bone marrow from young P1CTL\(^+\)nu/nu mice had no transgenic T cells.

Since both donor and recipient mice were incapable of producing mature T cells, all T cells in the chimera mice were produced \textit{de novo} after bone marrow reconstitution of the tumor-bearing host. One can therefore investigate the responsiveness of the T cells generated in the presence of tumors. In mice that received non-transgenic nu/nu bone marrow, all but one mouse had scarcely
detectable levels of P1A-reactive T cells. Expansion of T cells in this mouse can be caused by abnormal up-regulation of P1A antigen, which is expressed at low levels under normal circumstances \(^{12}\). However it is interesting to note that a high level of P1A-reactive T cells was found among tumor-infiltrating cells, although considerable variation was observed in different recipients (Fig. 4a). These data indicate that even in non-transgenic mice P1A-reactive T cells are generated \textit{de novo} and preferentially expanded in tumor-bearing hosts.

In order to study the immune competence of P1A-reactive T cells, we analyzed the expression of cell surface markers and cytokine response of P1A-reactive T cells derived from P1CTL\(^+\)nu/nu bone marrow. As shown in Fig. 4b, in mice that received a mixture of both transgenic and non-transgenic nu/nu bone marrow, the priming of P1A-reactive T cells is suggested by the down-regulation of CD62L and up-regulation of CD44. To test whether transgenic T cells have been primed in the tumor-bearing host, we stimulated the spleen cells from tumor-bearing recipients with tumor antigenic peptide P1A35-43 and analyzed the synthesis of IFN\(\gamma\) following a short \textit{in vitro} stimulation. As shown in Fig. 4c, the transgenic T cells in the spleens were highly responsive to antigenic peptide as judged by the considerable production of IFN\(\gamma\).

The fact that tumor-specific T cells were primed \textit{in vivo} indicates that they have come into contact with tumor antigens. To determine the functionality of these T cells, we analyzed the tumor-infiltrating T cells from mice that received P1CTL\(^+\) nu/nu bone marrow cells. As shown in Fig. 4d (top, right), essentially all P1A-specific T cells exhibit an activated phenotype. Moreover, after P1A peptide
stimulation, a high percentage of tumor-infiltrating T cells were capable of producing IFNγ (Fig. 4d, bottom). Since the TIL remained immune competent in an antigen-rich milieu, contacting pre-existing antigen did not tolerize the newly produced T cells.

To determine whether T cells generated in tumor bearing mice are cytotoxic, we carried out an in vivo cytotoxicity assay comparing the efficiency of elimination of P1A- versus control-peptide pulsed syngeneic T cells. As shown in Fig. 5a, in RAG-2-deficient hosts, the P1A-pulsed and control peptide-pulsed targets are presented at approximately 1:1 ratio. A slight, but statistically insignificant reduction of P1A-pulsed target was observed in one of the naïve WT P1CTL transgenic mice (Fig. 5b). In the unchallenged recipients of T-depleted P1CTL bone-marrow, the ratio of control to P1A-pulsed targets is approximately 3:1, which is consistent with activation of P1CTL either by homeostatic proliferation or by endogenous P1A antigen. Most remarkably, in tumor bearing mice the ratio has increased to 54:1. These data provide clear evidence that tumor growth activates, rather than silences T cells generated de novo in tumor-bearing hosts.

Transplantation of transgenic P1CTL bone marrow provides therapeutic effect to tumor-bearing mice

An important issue is whether tumor-specific T cells generated de novo in tumor-bearing hosts are capable of causing the rejection of tumor cells. As shown in Fig. 6a, with a single dose of bone marrow, we observed a substantial
survival advantage and delay in tumor growth in mice receiving P1CTL+ nu/nu bone marrow, compared to mice receiving non-transgenic nu/nu bone marrow. The efficacy of the bone marrow cells is comparable to that of mature T cells, as we and others have reported 14,17-19.

**Transplantation of T-depleted bone marrow causes rejection of established allogeneic skin graft**

Previous work by Alferink et al. 9 demonstrated that transgenic T cells were neither tolerized nor activated in adult hosts that exclusively expressed specific antigen in the skin. To test whether polyclonal T cells developed in hosts bearing established allogeneic grafts are immune competent, we transferred T-depleted bone marrow cells from immune competent BALB/c mice into syngeneic BALB/c RAG-2-/- mice bearing an established skin graft from C57BL/6 mice. As shown in Fig. 7, graft rejection was observed in all chimera mice that received T-depleted bone marrow cells. The significant delay in rejection in comparison to the mice that received allogeneic spleen cells is consistent with the time frame required for the maturation of T cells. Thus, immune competence of the T cells generated in an antigen-bearing host is not restricted to those that are specific for tumor antigen.
Discussion

Our data demonstrate that T cells generated de novo in antigen-bearing hosts are activated rather than inactivated. Our results provide a new explanation for the substantial clinical benefit of bone marrow transplantation into cancer patients. Previous studies demonstrated the function of donor T cells following bone marrow transplantation, although it is less clear in these earlier studies whether the antigen-specific T cells were derived from mature T cells in the bone marrow or from T cells produced de novo in the antigen-bearing host. Using bone marrow from T cell-deficient hosts, we have demonstrated unequivocally the immune competence of antigen-specific CD8 T cells generated in tumor-bearing hosts.

Our data also indicated that it is necessary to achieve a high frequency of antigen-specific T cells to reject rapidly growing tumors. Since the technical barrier of genetic reprogramming has been overcome recently, antigen-specific T cells can be produced en masse in tumor-bearing hosts to convey protection against established tumors.

The absence of tumor-induced clonal deletion, as reported here, differs from the clonal deletion in the setting of neonatal tolerance. Thus, previous studies using viral antigen and viral superantigens demonstrated that neonatal tolerance often involves clonal deletion in the thymus. One potential explanation is that the antigens in the peripheral tissues in neonates may be
expressed or presented in the thymus, while those produced in the adult mice are not. Such a difference in antigen expression may explain why tumors established in adults do not cause clonal deletion.

Previous work by Aferink et al.\textsuperscript{9} demonstrated that T cells produced in the adult host are ignorant of allogeneic antigens expressed as a transgene. On the other hand, our data demonstrates that tumor antigens and allogeneic skin grafts are capable of priming T cells. The fact that substantial numbers of tumor-reactive T cells (around 7\% of CD8 T cells) can be detected in tumor-bearing mice reconstituted with non-transgenic\textit{nu/nu} bone marrow indicate that the T cells have undergone substantial clonal expansion. Moreover, in another non-transgenic model we show that newly generated T cells cause complete rejection of MHC-mismatched skin grafts. As such, the naïve antigen-specific T cells were vigorously primed. These differences between our results and the conclusions of Aferink can be attributed to differences in experimental design. First, Aferink et al. expressed the allogeneic MHC transgene by a keratin promoter. Therefore the allogeneic MHC (H-2K\textsuperscript{b}) is expressed in non-hematopoietic cells. Since the alloantigen is not cross-presented, it will likely be presented to T cells by non-hematopoietic cells. In contrast, the P1A antigen is readily cross-presented, as we have reported\textsuperscript{25}. Secondly, an elegant study by Barker and Billingham\textsuperscript{26} has established a critical role for afferent lymphatics in skin graft rejection. In this regard, transgenically expressed K\textsuperscript{b} on nonhematopoietic cells also differs immunologically from the alloantigen expressed naturally in the skin that contains Langerhans cells which migrate into lymphoid organs to cause T cell priming.
Taken together, our main conclusion of immune competence of cancer-reactive T cells produced in tumor-bearing mice demonstrates the utility of stem-cell based immunotherapy. This approach differs from T-cell adoptive therapy in an important way, as stem cells can continuously supply immune competent cells that are educated, but not tolerized in the cancer-bearing host.

Acknowledgement

We thank Lynde Shaw for secretarial assistance. This work is supported by grants from National Institutes of Health and Department of Defense Prostate Cancer Program.
References


a. Thymus- No Tumor  Thymus- Tumor

b. CD3^+/L^d::P1A^- summary

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CD3^+/L^d::P1A^+ summary

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Fig. 1. Development of P1A-reactive T cells in RAG-2-deficient chimera mice reconstituted with a mixture of bone marrow cells from BALB/c and BALB/c P1CTL. RAG-2(-/-) BALB/c mice were injected with either PBS or J558 tumor cells subcutaneously. Six days later, when the tumors were palpable, a mixture of T-depleted BALB/c P1CTL and WT BALB/c bone marrow cells (3:2) were injected intravenously in two doses at a one week interval. At three weeks after the second injection, when tumor rejection was observed in the periphery, the mice were sacrificed and the T cell subsets in the thymus were analyzed by four-color flow cytometry using Ld:P1A dimer, and anti-CD3, CD4 and CD8. a. Representative FACS profiles of thymocytes from control (left panels) and tumor-bearing (right panels) mice are shown. The profiles of non-transgenic T cells are shown on the left of each group, while that of the transgenic T cells, as revealed by their specific binding to the P1A:Ld dimer or with anti-Vα8.3 TCR antibody, are shown on the right. b. Summary of the T cell subsets among polyclonal (left panel) and transgenic P1A-reactive T cells (right panel) in tumor-bearing and unchallenged mice. Graphs depict the mean ± SEM of 6-7 mice per group, and are representative of two independent experiments.
a. No Tumor

b. No Tumor

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<th>% of splenic Va8.3CD8+ cells</th>
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Fig. 2. Activation of P1A-reactive T cells in the periphery of tumor-bearing mice. Spleen cells from the chimera mice reconstituted with bone marrow from BALB/c P1CTL and BALB/c mice as described in the Fig. 1 legend were analyzed for the phenotype and number of P1A-specific T cells. a. Expansion of P1A-specific T cells in the spleens of tumor-bearing mice. Spleens from chimera mice were stained with anti-CD8, and L^d^:P1A dimer or anti-Vα8 TCR. FACS plots are representative profiles of cells within the lymphocyte gate. Graph depicts mean ± SEM of 6-7 mice per group. This trend of expanded P1A-specific T cells in tumor-bearing mice is representative of two independent experiments. 
b. Phenotype of P1A-specific T cells from tumor-bearing and non-bearing mice. Data shown are CD44 and CD62L profiles of gated CD8^+^Vα8 TCR^+^ T cells. A representative profile from each group is presented on the left, while the graph on the right depicts the mean ± SEM of 3 mice per group, and is representative of two independent experiments.
Fig. 3. P1CTL nu/nu bone marrow do not have mature transgenic T cells.

One-month old P1CTL nu/nu bone marrow cells were stained with anti-CD8, anti-Vα8.3 TCR, or isotype controls. WT P1CTL bone marrow was used as positive control. Data indicate a complete lack of T cells in the P1CTL+ nu/nu bone marrow.
**a. nu/nu bone marrow**

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**b. nu/nu + P1CTL+ nu/nu bone marrow**

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**Graph**

- **Legend:**
  - %Ld:P1A+ among CD8+ T cells
  - Spleen: 1% (n=11), 6.9% (n=12), 0.57% (n=6)
  - Tumor Spleen: 1% (n=11), 6.9% (n=12), 0.57% (n=6)

**Statistical Significance:**

- p = 0.0020
c. nu/nu + P1CTL$^+$ nu/nu bone marrow

![Image 1](#)

![Image 2](#)

![Image 3](#)

![Image 4](#)

**Fig. 4.** Immune competence of T cells produced de novo in tumor bearing mice. RAG-2-deficient BALB/c mice were challenged with $5 \times 10^6$ J558 tumor cells or PBS as control. Two days later, the mice received 500 Rad of irradiation and infusion of nu/nu bone marrow cells or a mixture of bone marrow cells from P1CTL$^+$ nu/nu and nu/nu mice. The recipient mice were sacrificed at the fourth week and tumor-specific T cells analyzed for their phenotype and response to P1A antigenic peptide. a. High frequency of P1A-specific T cells among the
tumor-infiltrating lymphocytes (TIL) of nu/nu bone marrow recipients. Left panels
show representative FACS profiles depicting highly expanded tumor-specific T
cells among the TIL (top left panel) but not in the spleen (bottom left panel).
Middle panels depict control stains. A summary graph of P1A-reactive CD8 T
cells among the splenocytes and TIL of nu/nu recipients is shown in the right
panel. b. Enhanced activation of P1A-specific CD8 T cells. Cell surface
phenotype of P1A-reactive and non-reactive CD8 T cells in the spleens of tumor-
bearing mice reconstituted with a 4:3 mixture of bone marrow from nu/nu and
P1CTL⁺nu/nu mice. c. Cytokine response of spleen cells in tumor-bearing mice.
Spleen cells from tumor-bearing mice reconstituted with a mixture of nu/nu and
P1CTL⁺nu/nu bone marrow were stimulated with P1A (left panels) or control
peptides (right panels) in the presence of Golgi blocker for 6 hours and stained
with anti-IFNγ antibodies after fixation and membrane permeabilization. Data
shown are gated CD8⁺ cells and are representative of 3 independent
experiments. d. Accumulation and immune competence of P1A-specific T cells
in the tumors. Top panels show accumulation (left) and activation status (right)
of tumor reactive T cells, while the lower panels show cytokine response to tumor
antigenic peptide P1A. Tumor single-cell suspension from chimera mice
reconstituted with P1CTL⁺nu/nu bone marrow were stimulated with P1A or
control peptides in the presence of Golgi blocker for 6 hours and stained with
anti-IFNγ antibodies after fixation and membrane permeabilization. Cytokine
response is shown for gated CD8+ T cells. T-depleted splenocytes from
syngeneic wild-type mice were used as APC. All data presented in this figure have been repeated 2-3 times.

Fig. 5. In vivo cytotoxicity of T cells produced in tumor-bearing hosts. 

*RAG-2*−/− mice were challenged with tumor cells or left unchallenged. Two days later, the mice were reconstituted with T-depleted bone marrow cells from P1CTL.
mice. At 4 weeks after reconstitution, they received an i.v. injection of a mixture of P1A-pulsed (CFSE$^{\text{hi}}$) and control peptide-pulsed (CFSE$^{\text{lo}}$) syngeneic BALB/c spleen cells (5x10^6 each). The spleens were harvested and analyzed by flow cytometry for distribution of fluorescence. Data shown are dot plots, showing the forward scatters and fluorescence intensity. CFSE$^{\text{hi}}$ and CFSE$^{\text{lo}}$ cells are gated as indicated. The number shown in the gates are the % of gated cells, while the number shown underneath the gates are means and SD of the ratios of CFSE$^{\text{lo}}$/CFSE$^{\text{hi}}$ cells. The data indicate drastically stronger cytolysis of P1A-pulsed targets by CTL in tumor-bearing hosts. The recipients used were: a. Control RAG-2-deficient mice without tumor challenge; b. P1CTL mice without tumor challenge; c. Unchallenged RAG-2-deficient mice reconstituted with T-depleted P1CTL bone marrow; d. Tumor challenged RAG-2-deficient mice reconstituted with T-depleted P1CTL bone marrow. Data shown are representative of two independent experiments involving a total of 3-5 mice per group. Two-sided student t-tests indicate very significant difference between the cytolysis found in tumor-bearing host vs all other groups (P<0.001). None of the other differences are statistically significant.
Fig. 6. Bone marrow therapy of established tumors. a. Delayed tumor growth in RAG-2-deficient mice receiving bone marrow from P1CTL+ nu/nu mice compared to those receiving bone marrow from nu/nu mice. The growth kinetics of tumors is shown in the left panel while the survival of tumor-bearing mice (as defined by time to reach tumor size of 4,000 mm$^3$) is shown in the right panel. Growth kinetics are representative of 3 independent experiments, while the survival data of tumor-bearing mice are cumulative data from two independent experiments. b. Mixture of T-depleted bone marrow cells from P1CTL transgenic mice and WT mice (3:2 ratio) causes regression of large established tumors. Mice shown are those described in Fig. 1 legend. In both a and b, the schedule of bone marrow cell injection are indicated by arrows.
Fig. 7. T-depleted allogeneic bone marrow cells cause rejection of pre-established skin grafts. RAG-2-deficient mice were transplanted with skin grafts from C57BL/6j mice. Survival of the skin grafts was monitored over a 2-3 month period. Kaplan-Meier curves depict skin graft survival in mice treated with either T-depleted bone marrow (n=6, closed squares) or undepleted spleen cells (n=7, open circles). The rejection of allogeneic skin grafts after treatment with T-depleted bone marrow has been observed in two independent experiments.
Immune competence of cancer-reactive T cells generated de novo in adult tumor-bearing mice

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